cyclophosphamide had little effect on survival time or on tumor growth. Adriamycin and vinblastine increased survival time by >25% and adriamycin appears to produce some reduction in tumor size. The two nitrosoureas, methyl-CCNU and BCNU, are highly effective in reducing tumor size and have produced a >25% increase in life-span to date.

[Cancer Chemother Rep Part 2, vol 5:145-149, 1975]

Human renal cell carcinoma, if inoperable or with widespread metastases, yields very few long-term survivors. Five-year survival rates of <5% have been reported in cases of metastatic disease (1,2). The disease is unpredictable; it may progress, remit, and, in very rare cases, undergo spontaneous regression (3,4). Spontaneous regressions of pulmonary and bone lesions after radical nephrectomy have been reported, possibly indicating a hormonal or immunologic control mechanism (5). Removal of the primary renal tumor in the absence of metastasis has resulted in 3-year survival rates of 45% and 5-year survival rates of 34%. These long-term survival rates were not affected by chemotherapy (5). Generally, standard chemotherapy has not been effective against metastatic renal cell carcinoma (6). Carter has reported on the nearly total failure of chemotherapy against renal cell carcinoma; only five standard agents have been evaluated and none were active.[5] Hormonal therapy has been reported to produce both favorable as well as poor results (7,8).

[1]Supported by contract N01-CM-81021 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[2]The tumor was generously supplied to us, in intrarenally transplanted donor mice, by Dr. Gerald P. Murphy, Roswell Park Memorial Institute, Buffalo, NY.

[3]Life Sciences Research Division, IIT Research Institute, Chicago, Ill.

[4]*Reprint requests to:* Mr. Alan M. Shefner, Life Sciences Research Division, IIT Research Institute, 10 West 35th St, Chicago, Ill 60616.

[5]Carter SK. Report of the Associate Director. *In* Report of the Division of Cancer Treatment, NCI, 1974. Bethesda, Md, NCI, 1974. vol 1, pp 6.1-6.11.

Few animal models for renal cell carcino
been described. In the past year we have be
ing a murine renal cell carcinoma mode
which was developed and characterized by
and Hrushesky (9). The host is the BALB
The tumor originated as a spontaneous re
cal adenocarcinoma of the granular cell ty
develops after intrarenal (ir), im, ip, iv, and
plantation. It is quite slow-growing, with
vival time depending upon the site of ino
The purpose of our study is to characteriz
vival response of the animals after various
inoculation (particularly ir and sc), to s
growth patterns of the tumor, and to dete
response of this tumor to selected chemoth
agents leading to the possible utilization of
tem for specialized drug testing.

METHODS

Male and female BALB/c mice, at least
old, have been used with no differences note
mor growth or survival time.

For ir transplant a tumor cell suspension
pared. Our procedure for preparing and inoc
the renal tumor is essentially that descr
Murphy and Hrushesky with the exception
transplant the tumor under the capsule of
kidney (9). After the donor animal is killed,
mor mass is aseptically removed and separat
the remaining kidney tissue. The tumor is
and, using a glass tissue grinder, a 1:10 hom
is prepared with McCoy's medium contain
fetal bovine serum, 100 units/ml of penicil
100 μg/ml of streptomycin. The tumor prep

the tumor cell homogenate as prepared above.

CHARACTERISTICS OF THE MODEL

Murphy and Hrushesky (9) have characterized the murine renal cell carcinoma model in terms of the kinetics of tumor growth and metastasis using different transplantation routes and in terms of survival of transplanted mice. The tumor arose spontaneously as a renal cortical adenocarcinoma and was passaged sc approximately every 35 days. The tumor grows equally well when passaged into male or female BALB/c mice. Swiss mice challenged with as many as 1×10^6 tumor cells show no evidence of tumor growth. The metastatic index and tumor weights of recipient BALB/c mice inoculated ir with tumor suspensions ranging from 10^5 to as low as 50 viable cells correspond directly to the number of cells inoculated. Small localized tumors were found in 100% of the mice transplanted with 50 cells. Large tumors developed in 100% of recipient mice transplanted with 10^5 cells when the injection route was ir, ip, iv, im, or sc. The metastatic index was high only after ir transplant. Ir transplanted mice survived for an average of 46 days, while the median survival times were >60-75 days when other injection routes were used.

By Day 21 after ir transplant, all animals had metastatic tumor and 50% were grossly palpable. Tumor weight doubling time was approximately 7 days during the period of 21-35 days after inoculation.

Renal tumor growth was enhanced by the administration of testosterone or diethylstilbestrol but was unaffected by medroxyprogesterone, a progestational agent. Cell-free extracts of renal tumor did not produce tumor growth in 20-hour-old BALB/c mice who were ip inoculated or in 6-week-old BALB/c males or Swiss mice who were ir inoculated.

In our laboratory we have carried the tumor through 13 generations by ir transplant. The median survival times (MST) for generations three to 13 average 45.3 days and are in good agreement with the findings of Murphy and Hrushesky (9). All recipi-

weights were calculated using the formula:

$$\frac{\text{length(mm)} \times (\text{width[mm]})^2}{2}$$

In the period of 21-42 days after implant, t
for a doubling of tumor mass is approxim
days.

The calculated weights for the two sc
groups are generally in the same range, v
homogenate-inoculated mice having somewh
er tumors (tables 1 and 2). For both groups,
a wide spread in tumor size at each interva
group of 60 homogenate-inoculated mice, on
had a tumor which was palpable but not mea
through Day 56. We had hoped that the size
tumors would be more consistent when a ho
ous cell-suspension inoculum was used, but
not yet been demonstrated.

In addition to the wide variability in size,
major problem with both of the sc implant
tumor ulceration which often occurs as ea
weeks when the tumors are still quite small
velops in essentially all of the mice befor
This makes progressive tumor measurement

TABLE 1.—Calculated tumor weights of sc inoculated ren
homogenate (60 mice)

Day after implant	Weight (g)	
	Range of mean tumor weights of cage groups*	Overall range
23	>0.039-0.289	>0.0-0.527
28	>0.087-0.484	>0.0-0.827
34	0.174-0.899	>0.0-1.521
40	0.304-1.495	>0.0-2.458
49	0.379-3.805	>0.0-5.054
53	0.398-4.034	>0.0-5.596
56	0.510-3.837	>0.0-6.700

*5 mice per cage group.

39	0.169-2.160	0.900
42	0.267-3.092	1.090
48	0.329-2.246*	1.168
54	0.350-2.769	1.500

*Mouse with 3.092-g tumor died on Day 48.

or impossible and introduces the factor of bacterial infection.

We have also inoculated one group of mice ip with 0.5 ml of the 1:10 renal tumor homogenate which yields an MST of 34.7 days and 100% deaths by Day 58. This was approximately a five times larger cell inoculum than that used by Murphy and Hrushesky who reported >50% survivors at 60 days with ip inoculation of 10^5 tumor cells (9).

CHEMOTHERAPEUTIC TRIALS

Chemotherapeutic trials in this model have been quite limited. Based on clinical reports of activity against metastatic renal cell carcinoma, Hrushesky and Murphy tested CCNU and vinblastine against murine renal cell carcinoma (10). CCNU had yielded objective remissions in 20% of the patients in a study by Mittelman et al (11), and vinblastine had produced an approximately 30% rate of objective response (5). Hrushesky and Murphy evaluated the response to drug therapy by three criteria: tumor weight, metastasis, and survival time. Both drugs were given ip every seventh day (q7d) starting on Day 7 after tumor implant; in some experiments the drugs were given q7d starting on Day 21 when the renal tumor is palpable and metastasis has begun.

Tumor weight, as determined by autopsy at 40 days, was significantly reduced by both drugs when treatment was started on Day 7. The effect was dose related. A 75% reduction in tumor size with CCNU and a 95% reduction in tumor size with vinblastine were the best responses as compared to controls.

Lung metastases were determined microscopically after autopsy at 40 days. Both drugs were given q7d starting on Day 7. With CCNU given at 1.0 mg of drug/animal/injection (40 mg/kg), 80% of the animals had distant metastases at Day 40, and with

survival time, with the early treatment g
better than the delayed treatment. The
response was a >100% increase in mean
time (45 days for the controls and 92 days
vinblastine-treated group).

We currently have seven drugs on test in t
cell carcinoma system in ir tumored mice. Ble
adriamycin, and hydroxyurea were given on
schedule on Days 7-15. Vinblastine, cyclop
mide, methyl-CCNU, and BCNU were administ
starting on Day 7 for five doses. All were t
three dose levels. Each test group consiste
mice and a control group of 30 mice was
though the drug trials are still in progress
control MST = 40.0 days), the data in table
certain conclusions to be drawn.

Bleomycin, hydroxyurea, and cyclophos
were ineffective in producing increases in
times at the doses and regimens used in this
ment. Cyclophosphamide did appear to exer
fect on tumor growth during the period
treatment. Adriamycin showed some ther
effects at all dose levels that were tested.
highest dose tested, 1.0 mg/kg/day, all ten m
still alive at Day 50 and nine appeared to be
somewhat smaller tumors by palpation.

Vinblastine was not as effective in our dr
as it was in the report of Hrushesky and
(10) although the same dosages and treatmen
ules were used. The two lower doses (0.2 and
kg) did not increase the MST appreciably (1
7%). At the highest dose, 0.8 mg/kg, nine m
survive but appear to be bearing large tur
the study of Hrushesky and Murphy (10)
weights were determined in animals killed at
5 days after the last drug dose. Our comm
tumor size refer to palpable masses some
after the last drug treatment. Thus the diffe
the effect on tumor weight may be an indic
escape of residual tumor from drug control
differences in survival time found at lowe
cannot be similarly explained.

As of Day 50, the two nitrosoureas, methy
and BCNU, were the most effective drugs use

Hydroxyurea qd Days 7-15	100-25	All doses ineffective; tumor growth similar to controls
Vinblastine, q7d × 5	0.8-0.2	Highest dose, T/C >125%; 9 mice surviving; tumors generally smaller than in controls until treatment ceased
Cyclophosphamide, q7d × 5	50-12.5	All doses ineffective; tumors smaller than in controls during period of drug treatment
Methyl-CCNU, q7d × 5	24-6	All doses effective; 16 of 26 survivors have no palpable tumors; animals appear generally healthy
BCNU, q7d × 5	24-6	Highest dose toxic; 19/20 mice surviving at lower doses; 16/19 have no palpable tumors; all appear very healthy

*MST for controls (30 mice) was 40.0 days.

study. Toxicity was evidenced at the highest dose used with each compound, 24 mg/kg/injection. At the two lower doses of methyl-CCNU and BCNU, 39 of 40 mice were still alive at Day 50. In addition, 26 of these mice had no palpable tumor. The BCNU mice appear to be in good health as do those treated with methyl-CCNU at 12 mg/kg/injection. Whether the response to these two nitrosoureas will prove to be greater than that found by Hrushesky and Murphy with CCNU remains to be determined.

CONCLUSIONS

The renal cell carcinoma animal model developed and characterized in BALB/c mice by Murphy and Hrushesky (9) may prove to be a useful system for the selection of chemotherapeutic agents with activity against a slow-growing solid tumor of renal cell origin. The tumor can be transplanted by a variety of routes and drug effects can be measured on tumor size, extent of metastases, and survival time. The ir system has the advantages of a slow-growing solid tumor of specific renal origin, a system which mimics the human disease state including predictable metastases, and as a model using a bilateral organ, it is an ideal situation for surgical adjuvant stu addition, the 45-day average MST is worka lowing for the initiation of therapy at vari ease stages. Whether the spectrum of drug a in this model system will result in informati can be used in the development or selection o for treatment of human clinical disease ca foretold from the limited drug trials that ha conducted to date.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis ethyl)-1-nitroso-
Bleomycin: NSC-125066; [2,4'-bithiazole]-4-carboxylic a aminoethyl)-, monohydrate
CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1- ethyl)-3-cyclohexyl-1-nitroso-
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahyd ide, monohydrate
Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1
Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; u chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-
Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vi blastine, sulfate (1:1), monohydrate

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of metastatic renal cell carcinoma. JAMA 225:32-35, 1

Vol. 5, No. 1, December 1975