Conf-9110330--1
BNL-47079

A PHASE 1 BIODISTRIBUTION STUDY OF p-BORONOPHENYLALANINE

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BNL--47079

DE92 007606

INTRODUCTION

The United States Food and Drug Administration (FDA) has approved an Investigational New Drug (IND) Exemption for the boron-containing amino acid *p*-boronophenylalanine (BPA). The IND application was based upon pre-clinical studies carried out at Brookhaven National Laboratory that included: 1) the demonstration of selective accumulation of boron in a murine melanoma (Coderre, 1987); 2) the report of successful boron neutron capture therapy in a murine melanoma following BPA administration (Coderre, 1988); and 3) the toxicology/histopathology reports following oral administration of BPA to mice and rabbits (Coderre, 1990). The IND application also included a review and bibliography of the Japanese results with BPA both in animals and in humans. Originally approved for studies in patients with melanoma, the BPA IND was amended to include patients with glioblastoma or breast cancer following the report that BPA selectively delivered boron to tumors other than melanoma (Coderre, 1990).

The IND Sponsor is A. Meek, MD, Chairman, Department of Radiation Oncology, State University of New York, Stony Brook, NY. Other participating investigators in the BPA Phase 1 biodistribution study include:

1. T. Nowak, MD, Department of Neurosurgery, State University of New York, Stony Brook, NY.
2. S. Packer, MD, Chief of Ophthalmology, Department of Ophthalmology, North Shore University Hospital, Manhasset, NY.
3. D. Wazer, MD, R. Zamenhof. PhD, Department of Therapeutic Radiology, and S. Saris, MD, Department of Neurosurgery, New England Medical Center, Boston, MA.
4. G. Rogers, MD, Department of Dermatology, University Hospital, Boston, MA.
5. R. Gahbauer, MD, Chairman, Department of Radiation Oncology, Ohio State University, Columbus, OH.
6. Z. Fuks, MD, A. Houghton, MD, Department of Radiation Oncology, Memorial Sloane-Kettering Cancer Center, New York, NY.
7. J. Coderre, PhD, Medical Department, Brookhaven National Laboratory, Upton, NY.

The objectives of the Phase 1 BPA biodistribution study are as follows:

Objective 1. To establish the safety of orally administered BPA as determined by monitoring of patient's vital signs and by clinical analysis of blood before and after BPA administration.

DISCLAIMER

Objective 2. To establish BPA pharmacokinetics by monitoring the rates of boron absorption into and clearance from the blood and the rate of urinary excretion of boron.

Objective 3. To measure the amount of boron incorporated into human tumors (melanoma, glioma, and breast carcinoma) using samples obtained at surgery or biopsy.

This report presents the results obtained from the first thirteen patients entered into the study. Three additional glioblastoma patients have been studied recently at Stony Brook, the tissues are still being analyzed.

MATERIALS AND METHODS

BPA. The ^{10}B-enriched L-BPA used for the first four patient studies was prepared at the Medical Department, Brookhaven National Laboratory. The BPA synthesis was carried out as described by Snyder (1958), with some minor modifications (Coderre, 1987). The D and L enantiomers of BPA were resolved enzymatically as described by Roberts (1980). The Callery Chemical Company, Callery, PA, has obtained a Drug Master File with the FDA for the preparation of patient grade ^{10}B-enriched L-BPA. All patient studies now utilize the Callery BPA. BPA is administered to patients orally as a slurry of the crystalline, free amino acid in water or fruit juice.

Boron Analysis. Boron analysis was performed by measuring the 478 keV photons produced during $^{10}B(n,\alpha)^{7}Li$ reactions (Fairchild, 1986). Samples up to 1.0 g can be analyzed in 200 s with an error (±1 SD) of ≈15% at the lower limit of detection, ≈1 μg ^{10}B, ≈10% at ≈5 μg ^{10}B, and ≈5% when the amount of ^{10}B in the sample is 10 μg or greater. Calibration of the prompt-gamma analytical system for ^{10}B determination was performed on each day of measurement using US National Bureau of Standards ^{10}B-enriched boric acid. Patient samples from studies carried out at the New England Medical Center or at the Boston University Hospital were analyzed at the Massachusetts Institute of Technology by either a prompt gamma technique patterned after (and cross-calibrated with) the facility at BNL or by a high resolution track etch method (Zamenhof, 1991).

RESULTS AND DISCUSSION

BPA Dose Escalation. Sixteen patients have been entered into the BPA Phase 1 biodistribution study as of September 1991. Data for the first thirteen patients have been analyzed and are included in this report. Table 1 lists the tumor types studied to date. The ocular melanoma patients were from North Shore University Hospital (Dr. Packer) the glioblastoma patients were from the New England Medical Center (Dr. Saris) and the cutaneous melanoma patients were from University Hospital (Dr. Rogers). In the thirteen patients studied to date there were no BPA-related effects on patient's vital signs (monitored for two hours post-administration) or on blood clinical chemistries at any dose level. The dose escalation schedule was recommended by the staff at the FDA. To put the low initial doses in perspective, the doses routinely

given to mice, rats and rabbits in studies at BNL range from 500 to 750 mg BPA/kg body weight. The doses used in the toxicity/histopathology studies submitted to the FDA utilized oral doses of BPA that ranged up to 5000 mg/kg body weight with no observable compound-related effect (Coderre, 1990).

Blood Pharmacokinetics. For pharmacokinetic modeling, a one compartment oral absorption model was used, assuming first order absorption into and first order elimination from a volume of distribution. The volume (V) of the compartment was calculated as 65% of body weight (an estimate of total body water). The following equation was fit to the experimental data,

$$y = \left(\frac{FD}{V}\right)\left(\frac{a}{a-b}\right)\left(e^{-bt} - e^{-at}\right)$$

where: y = the boron concentration (μg ^{10}B/g) in blood at time t
D = The administered dose in mg ^{10}B (given orally in a single dose)
V = the volume of distribution (total body water, 65% of body weight)

F = the absorbed fraction of the administered dose
a = the first order rate constant for absorption into V
b = the first order rate constant for elimination from V.

The blood boron concentrations, y, were determined by the prompt gamma technique; D, and V were based upon the individual patient's weight. The variables F, a, and b were fit to the experimental data for each patient by successive iteration using the curve fitting function in the graphics software package SigmaPlot 4.0. Table 2 lists the patient weight, the parameters V and D as well as the variables F, a, and b derived from the curve fitting for all thirteen patients. Also shown in Table 2 are the half times for absorption of ^{10}B into ($t_{\frac{1}{2}}$ abs) and elimination of ^{10}B from ($t_{\frac{1}{2}}$ elim) the volume of distribution, V. The last row in Table 2 provides the mean ± SD for the pharmacokinetic parameters and variables. As an example, Figure 1 shows the blood data and the plot of the fitted curve for patient SN13.

An important variable that will be monitored closely as the BPA biodistribution study proceeds is F, the fraction of the oral dose actually absorbed from the gastrointestinal tract. In animal tumor models (mice, rats and rabbits) as the amount of BPA contained in a single oral dose was increased, a point was reached beyond which no additional accumulation of ^{10}B in the tumor was observed (J. Coderre, unpublished data). It was inferred that the absorptive ability of the gastrointestinal tract was saturated. However, two properly timed oral doses have proven to be additive with respect to loading of the tumor with ^{10}B, while maintaining the same tumor-to-normal-tissue ^{10}B ratios (Coderre, 1991, 1991a). With the exception of patient SZ11, the F values in Table 2 appear to be decreasing as the BPA dose increases. If the trend continues, alternative dosing schedules will be evaluated.

Urine Pharmacokinetics. Figure 2 shows the cumulative excretion of ^{10}B in urine as a function of time for the first five patients studied. Each sample was collected separately and the boron content analyzed at BNL by the prompt gamma method. The fraction of the administered dose (in mg ^{10}B)

recovered in the urine within 48 hours was 61% for patient VZ1 (open squares), 34% for patient BW2 (closed triangles), 42% for patient LR3 (open triangles), 19% (only 19 hours collection) for patient MS4 (closed circles), and 65% for patient ES5 (open circles). Urine was collected in two 24-hour pools (not graphed) from patients SZ11 and JB12; the fractions of the administered dose recovered were 38% and 41%, respectively. These values for the amount of ^{10}B recovered in urine are in relatively good agreement with the values for F, the fraction of the administered dose absorbed, derived from the blood data (Table 2).

Uptake of ^{10}B in Human Tumors. Table 3 lists the time between BPA administration and surgery, the concentration of ^{10}B in tumor and the concentration of ^{10}B in blood at the time of surgery for the thirteen patients studied to date. Figure 3 shows the ^{10}B concentrations in tumor samples from patients that received 90 mg BPA/kg or more (patients 6-13 in the study, see Table 3). For three patients, multiple samples were analyzed from a single surgical specimen; all data points are plotted. Thus, the three inverted triangles at 6.5 hrs are from patient RB10, the two filled circles at 8 hrs are from patient JR7 and the two filled circles at 9.5 hrs are from patient JM8. The blood simulation line was obtained by plotting the solution to the one compartment oral absorption model

$$y=(FD/V)(a/a-b)(e^{-bt}-e^{-at})$$

using the mean values for F, V, a and b obtained from the fitting of all patient's individual blood data (see Table 2), and 189 mg/kg for D. The actual tumor-to-blood ^{10}B concentration ratios for each individual patient can be obtained from Table 3. The cutaneous melanoma samples and the glioblastoma samples (which were needle biopsies) were analyzed by the high-resolution alpha track radiographic technique at MIT. The ocular melanoma samples were analyzed by the prompt-gamma method at BNL.

SUMMARY

The three study objectives stated in the Introduction above have been met to varying degrees.

Objective 1. There was no BPA-related effect on vital signs (monitored for two hours post-administration) or on blood clinical chemistries at all dose levels.

Objective 2. Quantitative data was obtained regarding a) the boron levels in the blood; b) the rate of boron excretion in the urine; and c) the total amount of boron excreted in the urine over a 48 hour period. Using these data, the pharmacokinetics of BPA absorption and excretion have been successfully modeled using a one compartment model with first order absorption and first order elimination. The apparent half-times for absorption into, and clearance from, the volume of distribution were 1.5 and 12.4 hours, respectively. The half-time for appearance of boron in the urine was approximately 9 hours. The fraction of the administered dose of boron that was recovered in the urine within a 48 hour period, ranged from 34 to 65%.

Objective 3. Boron concentrations in tumor samples following BPA doses below 90 mg/kg were below the detection limit of the BNL boron analysis facility. Reliable values for boron concentrations in tumor were obtained for the last seven patients studied (2 glioblastoma, 2 ocular melanoma, and 3 cutaneous melanoma). The data points were widely scattered and too few to enable any prediction of the time course of boron accumulation in the tumor following BPA administration. Some very encouraging boron concentrations in tumor and tumor-to-blood boron concentration ratios were obtained for individual patients (glioblastoma: 11.2 ppm, tumor/blood ratio = 7:1; cutaneous melanoma: 11.3 ppm, tumor/blood ratio = 6:1).

CONCLUSIONS

1) Oral BPA is safe in humans at the 189 mg/kg level.

2) Additional biodistribution studies are needed to determine the time course of the tumor uptake and clearance of BPA for each tumor type.

3) Higher single doses or multiple doses will be required to obtain therapeutically useful levels of boron in the tumor.

ACKNOWLEDGEMENTS

The author thanks Peggy Micca for invaluable assistance including the prompt-gamma boron analyses performed at BNL. This work was supported in part by a grant from the National Cancer Institute (CA42446) to J.A.C., and by the Office of Health and Environmental Research of the U.S. Department of Energy under contract DE-AC02-76CH00016. Accordingly, the U.S. government retains a nonexclusive, royalty-free license to publish or reproduce the published form of this contribution, or allow others to do so, for U.S. Government purposes.

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FIGURE LEGENDS

Figure 1. ^{10}B concentration (μg $^{10}B/g$) in blood as a function of time following BPA administration (data points). The solid line is the fit of a one compartment oral absorption pharmacokinetic model to the experimental data (see text). The data shown are an example for one patient (SN13) to illustrate the rapid uptake of boron into ($t_{\frac{1}{2}}$ = 1.3 hours) and slower clearance of boron from ($t_{\frac{1}{2}}$ = 23.3 hours) the blood.

Figure 2. Cumulative excretion of ^{10}B in urine versus time post-administration for patients VZ1 (open squares), BW2 (filled triangles), LR3 (open triangles), MS4 (filled circles), and ES5 (open circles). The data are plotted as the running total (each new point contains the sum of all previous points).

Figure 3. ^{10}B concentration (μg $^{10}B/g$) in tumor as a function of time after BPA administration. The solid line is a simulation of the ^{10}B concentration in blood at the 189 mg BPA/kg dose level obtained by plotting the solution to the one compartment oral absorption model using the mean values for the pharmacokinetic parameters (see Table 2).

TABLE 1

Patient Information

Patient	Tumor Type	Dose mg BPA/kg	Dose mg BPA/m^2
VZ1	ocular melanoma	27	1000
BW2	ocular melanoma	54	2000
LR3	ocular melanoma	54	2000
MS4	ocular melanoma	54	2000
ES5	ocular melanoma	90	3340
LF6	cutaneous melanoma	90	3340
JR7	glioblastoma	90	3340
JM8	glioblastoma	135	5010
ES9	cutaneous melanoma	135	5010
RB10	cutaneous melanoma	135	5010
SZ11	ocular melanoma	189	7014
JB12	ocular melanoma	189	7014
SN13	cutaneous melanoma	189	7014

TABLE 2

Blood Pharmacokinetic Parameters

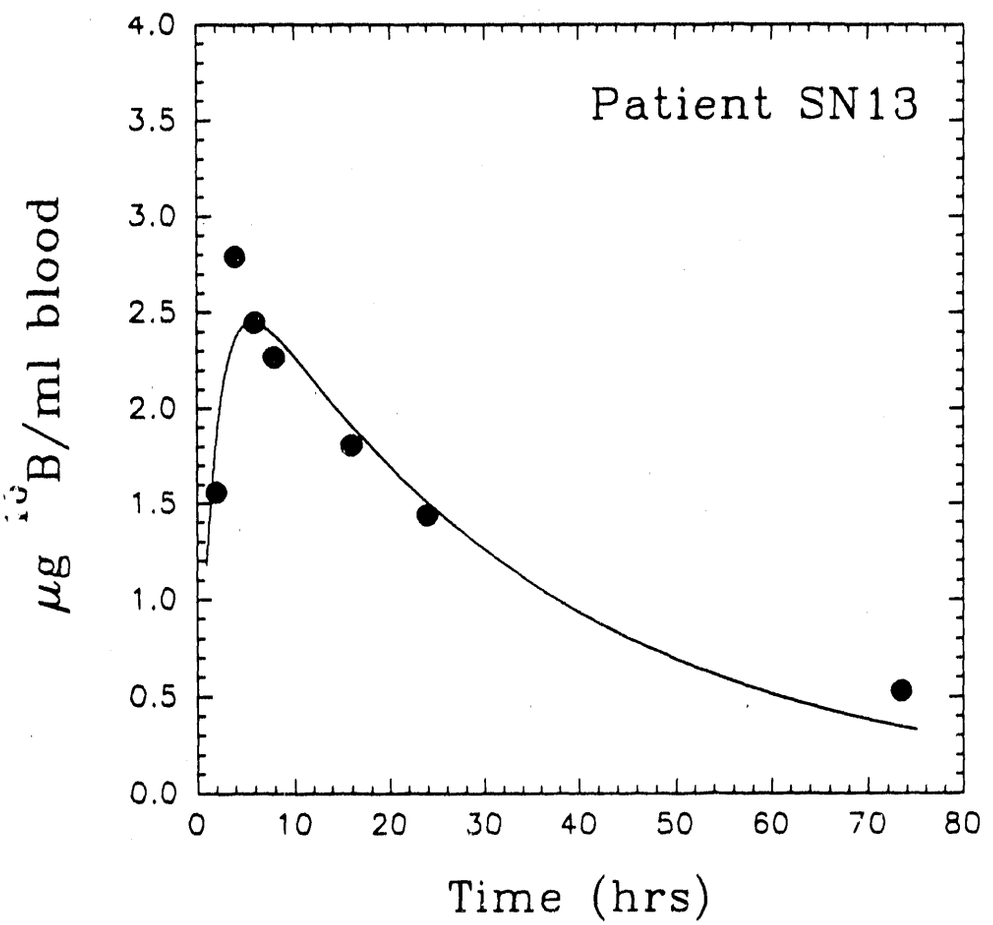
Patient	wt. (kg)	V (l)	D ($mg^{10}B$)	F (%)	a (hr^{-1})	b (hr^{-1})	$t_{1/2}$ abs (hr)	$t_{1/2}$ elim (hr)
VZ1	81.0	52.7	107.2	51.18	1.00	0.05	0.69	14.87
BW2	95.0	61.8	251.4	74.12	0.18	0.18	3.87	3.84
LR3	86.0	55.9	227.6	60.07	1.60	0.04	0.43	16.31
MS4	81.8	53.2	216.4	47.19	0.75	0.08	0.92	8.37
ES5	45.0	29.3	198.4	58.55	0.58	0.09	1.19	7.41
LF6	64.5	41.9	284.4	35.80	0.81	0.05	0.85	14.56
JR7	91.0	59.2	401.3	36.60	0.40	0.08	1.72	8.49
JM8	75.0	48.8	496.1	16.71	0.44	0.04	1.56	16.46
ES9	63.6	41.3	420.7	26.18	0.92	0.04	0.75	15.61
RB10	70.4	45.8	465.7	23.28	0.81	0.05	0.86	15.10
SZ11	70.9	46.1	656.6	58.51	0.22	0.09	3.17	7.57
JB12	62.3	40.5	577.0	38.28	0.40	0.07	1.74	9.28
SN13	93.0	60.5	861.3	20.41	0.53	0.03	1.31	23.26
mean ±SD	75.3 ±14.5	49.0 ±9.4		42.07 ±17.8	0.66 ±0.38	0.07 ±0.04	1.47 ±1.01	12.39 ±5.33

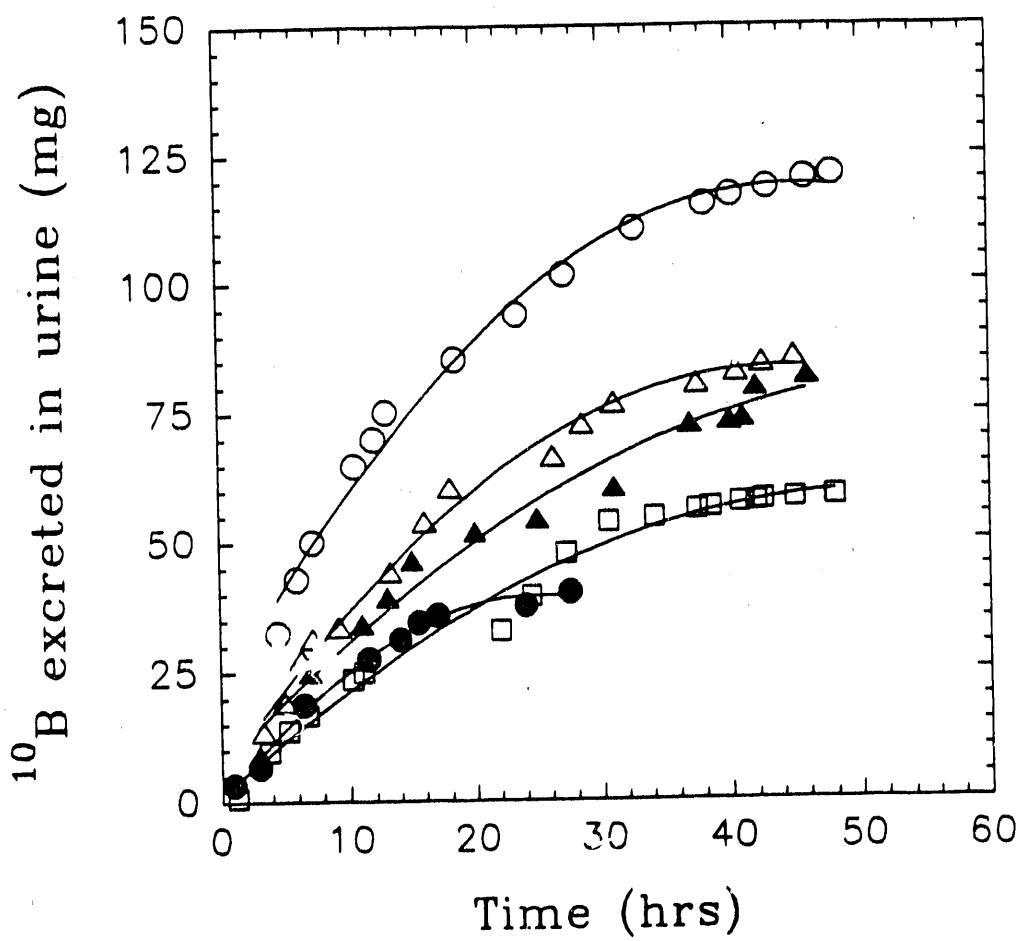
TABLE 3

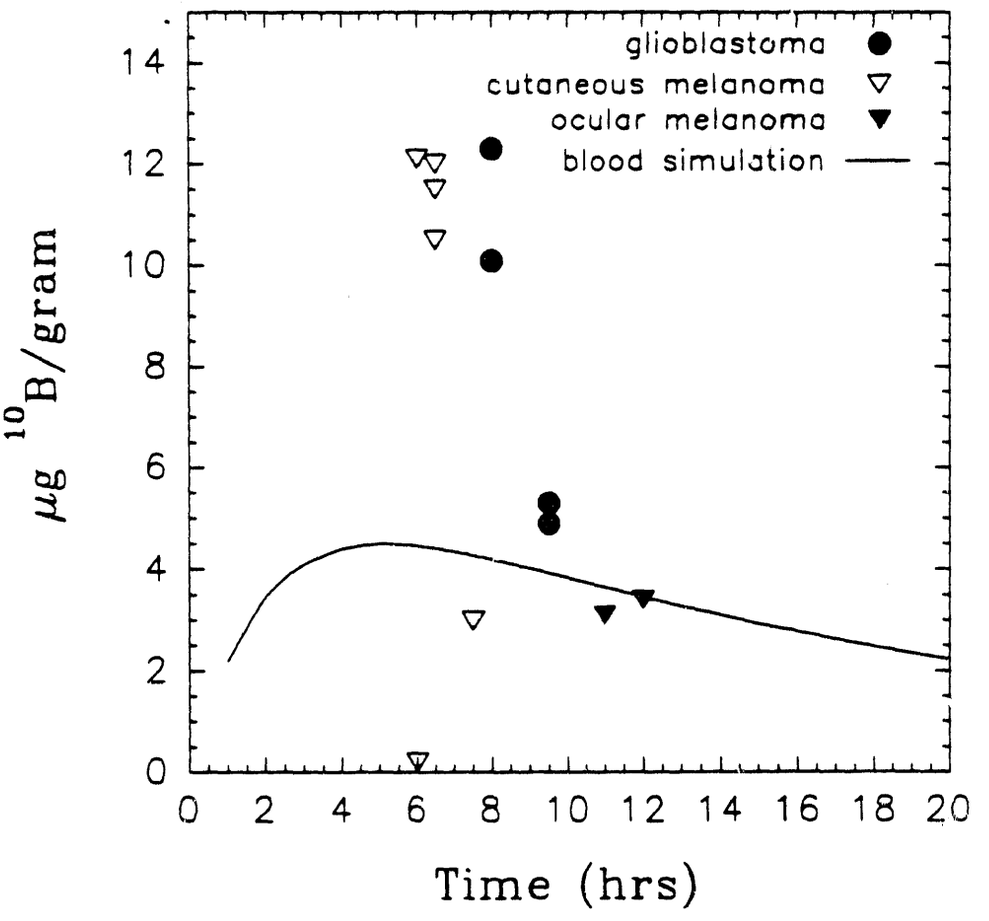
^{10}B Concentrations in Tumor and Blood

Patient	Tumor Type	Dose (mg/kg)	Time[a] (hrs)	^{10}B in Tumor (μg ^{10}B/gram)	^{10}B in Blood[b] (μg ^{10}B/gram)
VZ1	ocular melanoma	27	12	ND[c]	<1.0
BW2	ocular melanoma	54	18	ND	<1.0
LR3	ocular melanoma	54	16	ND	1.2
MS4	ocular melanoma	54	17	ND	<1.0
ES5	ocular melanoma	90	15	ND	1.4
LF6	cutaneous melanoma	90	6	0.2	2.1
JR7	glioblastoma (III/IV)	90	8	10.1;12.3	1.6
JM8	glioblastoma	135	9.5	4.9;5.3	1.2
ES9	cutaneous melanoma	135	6	12.1	2.2
RB10	melanoma (axillary node metas.)	135	6.5	10.5;11.5;12.0	1.8
SZ11	ocular melanoma	189	12	3.4	2.5
JB12	ocular melanoma	189	11	3.1	3.8
SN13	cutaneous melanoma	189	7.5	3.0	2.3

[a] Elapsed time between BPA administration and surgery.
[b] ^{10}B concentration in blood at the time of surgery.
[c] ND, not detected.


Patient SN13
μg ¹⁰B/ml blood
Time (hrs)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
80


150
125
100
75
50
25
0
0
10
20
30
40
50
60
^{10}B excreted in urine (mg)
Time (hrs)


glioblastoma
cutaneous melanoma
ocular melanoma
blood simulation
μg ^{10}B/gram
0
2
4
6
8
10
12
14
0
2
4
6
8
10
12
14
16
18
20
Time (hrs)

END

DATE FILMED

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