

## Carbon Beam Therapy Overcomes the Radiation Resistance of Uterine Cervical Cancer Originating from Hypoxia

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**Abstract Purpose:** High linear energy transfer (LET) particles are believed to decrease tumor radiation resistance originating from hypoxia. However, no proof of this effect has been provided by clinical trials and related clinical research. Hence, we investigated the radiation biological aspects of high LET carbon beam therapy on cervical cancer.

**Experimental Design:** This study involved 49 patients with stage IIIb bulky and stage IVa cervical cancer treated with high LET carbon beams between October 1995 and June 2000. Oxygen partial pressure ( $pO_2$ ) was measured by using a needle-type polarographic oxygen electrode.

**Results:** The 4-year disease-free survival rates of patients with  $pO_2 \leq 20$  mm Hg (hypoxic tumor) and  $pO_2 > 20$  mm Hg (oxygenated tumor) before treatment were 37% and 21%, respectively. The local control rates of hypoxic and oxygenated tumors before treatment were 58% and 54%, respectively. The disease-free survival rates of hypoxic and oxygenated tumors assessed by oxygen status at the 5th day of irradiation were 33% and 32%, respectively. The local control rates of hypoxic and oxygenated tumors at the 5th day were 60% and 58%, respectively. There was no significant prognostic difference between hypoxic and oxygenated tumors.

**Conclusion:** The similar disease-free survival and local control rates between hypoxic and oxygenated tumors before and during treatment indicated that the role of the tumor oxygenation status was not so important in local control in carbon beam therapy. These results indicated that high LET carbon beam irradiation might reduce the radiation-resistant nature stemming from tumor hypoxia.

High linear energy transfer (LET) particle therapy has various advantages in terms of radiobiological effects as well as dose distribution and has been expected to offer a therapeutic advantage over conventional photon therapy. The biological advantages of high LET radiation, including neutrons and heavy-charged particles, compared with low LET radiation-like photons, are summarized as a decreased oxygen enhancement ratio (OER), diminished capacity for sublethal and potentially lethal damage repairs, and diminished cell cycle-dependent radiosensitivity (1). However, there is no convincing clinical evidence that high LET beams decrease the OER and successfully control hypoxic tumors, whereas the fast neutron therapy for various types of malignant tumors has been tried for more than four decades (2–6).

The existence of hypoxic cells is well recognized as one of the major factors affecting resistance against radiation therapy and local failure (7–9). Recently, some investigators have directly evaluated hypoxic cells in tumors by the use of special electrodes (10, 11), thereby proving the presence of hypoxic tumor cells in human cancers. Furthermore, a comparison of tissue oxygen distribution and clinical prognosis showed that low intratumoral oxygen partial pressure ( $pO_2$ ) of uterine cervical cancer is a strong prognostic factor of poor survival in not only radiation therapy but also surgical treatment (11). Moreover, we showed that the reoxygenation status of tumors influenced the local response of radiation therapy for cervical cancer (12). We considered that these various procedures and findings highlight the necessity of a subsequent analysis using high LET particle therapy.

Heavy-charged particle radiation therapy for cancer treatment started at the National Institute of Radiological Sciences in June 1994 using carbon ions that were generated by the heavy-ion medical accelerator in Chiba (13). Phase I and II clinical trials for uterine cervical cancer were started from June 1995 (14). The experimental report of our carbon beams for use in clinical trials showed that OER of carbon beams (70 keV/n) was 2.0 for cultured cell lines and 1.6 for inoculated murine tumors, indicating a distinct advantage in OER (15, 16).

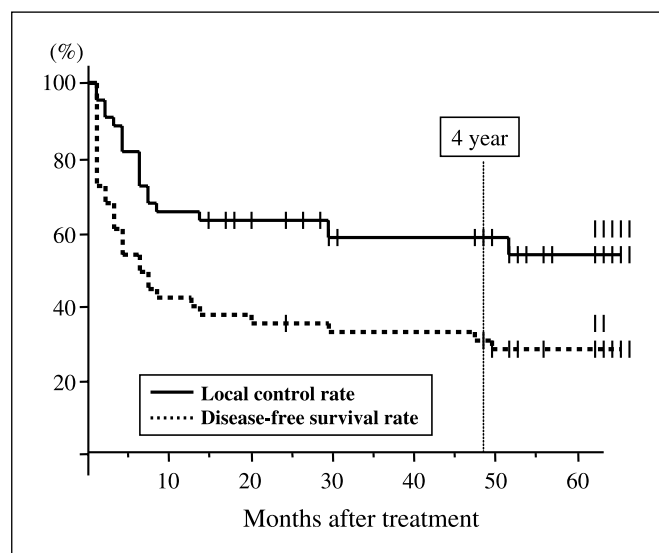
Hence, in the present study, we comparatively investigated the tumor  $pO_2$  status before and during both carbon beam and photon beam therapies for cervical cancer and evaluated whether high LET carbon beams reduce the radiation-resistant

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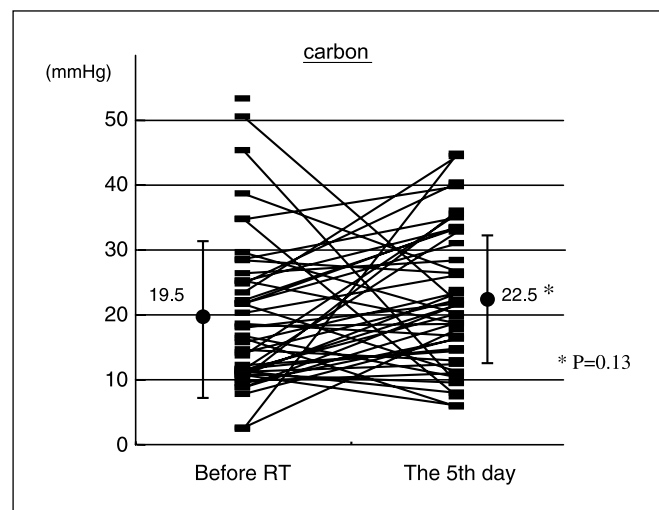


**Fig. 1.** Disease-free survival rate and local control rate of the patients treated with carbon beams.

nature originating from hypoxia of the tumors and also whether hypoxic tumors are effectively controlled by high LET beams compared with photon beam therapy. This is the first clinical attempt to elucidate evidence that high LET beams actually decrease OER and successfully control hypoxic tumors.

### Materials and Methods

**Treatment facility.** Heavy-ion medical accelerator in Chiba is the first heavy ion accelerator specially dedicated to medicine in the world, and its design variables are based on the radiological requirements (17). When applying radiation therapy, Bragg's peak of monoenergy is spread out at various degrees to create a spreadout Bragg's peak to cover tumors



**Fig. 2.** Tumor pO<sub>2</sub> distributions before and at the 5th day of the patients treated with carbon beams. Mean  $\pm$  SD pO<sub>2</sub> of tumors before treatment and at the 5th day of irradiation were  $19.5 \pm 11.6$  and  $22.5 \pm 10.7$  mm Hg, respectively. There was no significant difference between them ( $P = 0.13$ ).

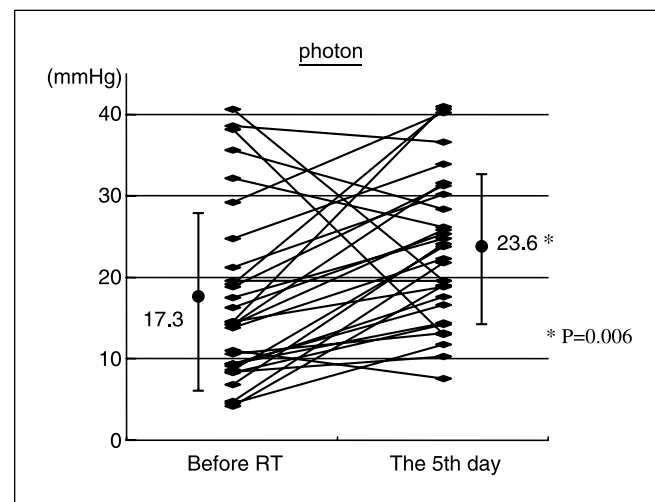
with biologically equivalent dose distribution for use in treatment planning. To express particle radiation doses in terms comparable to megavoltage X-rays, the beam's acute relative biological effectiveness value was used to calculate the "equivalent radiation dose," details of which are cited elsewhere (17). Clinical relative biological effectiveness was set at 3.0 based on previous experimental data and the results of fast neutron treatment at National Institute of Radiological Sciences (17).

**Purpose of the clinical trial.** A major objective of this trial was to determine the optimal fraction dose for the pelvic field and to assess the following end points: (a) acute normal tissue tolerance to carbon ions to determine optimal fraction dose; (b) late normal tissue tolerance to carbon ions to determine total safety dose; and (c) local tumor response, survival, and quality of survival.

**Patient characteristics.** During ~5 years from October 1995 to June 2000 (except between July 1998 and February 1999 due to a physician shortage), 49 patients with cervical cancer consecutively treated with carbon beams were eligible for this study (mean and median age,  $57.3 \pm 10.5$  and 56.0 years, respectively). Because this was a phase I and II study, patients with a very advanced stage or deemed hopeless for curing with conventional radiation treatment were entered into this study. They consisted of 42 squamous cell carcinomas, 1 adenosquamous cell carcinoma, and 6 adenocarcinomas. Of these, 33 patients were stage IIb and 14 were stage IVa disease, with most tumors invading to the bladder, except one with rectal invasion. All the patients were followed up for a minimum of 4 years or until death. Mean and median follow-up periods were 39 and 27 months (range, 4-104 months). The numbers of patients with relatively small tumors ( $\leq 100$  mm<sup>3</sup>) and bulky tumors ( $>100$  mm<sup>3</sup>) were 19 and 30, respectively.

As a control for high LET carbon therapy, 30 patients with uterine cervical cancer who received photon therapy at our hospital between September 1995 and November 1999 were comparatively analyzed. The numbers of patients with stages IIb, III, and IVa disease were 10, 16, and 4, respectively. Mean and median follow-up periods were 35 and 27 months, respectively (range, 4-98 months).

**Carbon beam therapy.** The energy of carbon beams used was 350 to 400 MeV. The treatment protocol consisted of a fixed total number of fractions and treatment time of 24 fractions over 6 weeks with 4 fractions weekly. Anteroposterior and posteroanterior ports were used for 16 fractions over 4 weeks to cover the cervical tumor and pelvis lymph node chains. Additional 8 fractions over 2 weeks were given with lateral opposing ports as a boost for only macroscopic cervical tumors.



**Fig. 3.** Tumor pO<sub>2</sub> distributions before and at the 5th day of the patients treated with photons. The mean pO<sub>2</sub> at the 5th day was  $23.6 \pm 9.1$  mm Hg, significantly higher than the  $17.3 \pm 10.8$  mm Hg pO<sub>2</sub> before treatment ( $P = 0.006$ ).

**Table 1.** pO<sub>2</sub> status of the patients treated with carbon beams

Carbon beam treatment	pO <sub>2</sub>		P
	≤20 mm Hg	>20 mm Hg	
Before treatment			
Stage II	2	0	0.30
Stage III	19	13	
Stage IV	6	6	
Total	27	19	
At the 5th day			
Stage II	2	0	0.37
Stage III	13	18	
Stage IV	5	8	
Total	20	26	

The treatment was initiated with a fraction dose of 2.2 Gy equivalent dose, with the fraction doses being increased step-by-step. The total dose began with 52.8 Gy equivalent dose and was increased up to 72.8 Gy equivalent dose. Each dose group consisted of at least five patients.

**Photon therapy.** Control patients were treated with a combination of external and high-dose rate intracavitary irradiation. Details of the protocol have been reported elsewhere (18). Briefly, patients were given external whole pelvic irradiation with a dose of 1.8 Gy per fraction, five times weekly, to a total dose of 30.6 Gy. This was followed by a central shielding pelvic field, with a dose of 2 Gy per fraction, five times weekly, to a total dose of 20 Gy. Along with the central shielding irradiation, these patients also received intracavitary irradiation with a remote after-loading system using either cobalt-60 or iridium-192 sources. They received four sessions (once weekly) with a fraction dose of 5.5 to 6.0 Gy at point A, with the total doses ranging from 22 to 24 Gy.

**pO<sub>2</sub> monitoring.** All of the 49 patients giving their informed consent of the measurement of pO<sub>2</sub> were measured either before radiation therapy or at 6 hours after the fifth irradiation (5th day) or both. pO<sub>2</sub> of cervical cancer was measured by needle-type polarographic oxygen electrode (POE-10N, Intermedical, Tokyo, Japan) and a pO<sub>2</sub> monitoring machine (P100, Intermedical). Details of this electrode have been reported elsewhere (19, 20). This electrode is made of very thin platinum and is coated with three layers of macromolecule membrane; it allows the measuring of almost any area. Damage to tissue is minimal because the diameter of this electrode is only 200 μm. Calibration of the pO<sub>2</sub> monitor was done before every measurement according to the calibration protocol. The electrode was injected and placed at a 1-cm depth beneath the tumor surface under direct vision. Where feasible, measurements were done at five or more points of a tumor where nonnecrotic and viable tumor cells were present before radiation therapy and at 6 hours after the 5th day.

**Statistical analysis.** The patients were divided into two groups according to the tumor pO<sub>2</sub> status (i.e., pO<sub>2</sub> ≤ 20 mm Hg and pO<sub>2</sub> > 20 mm Hg), because the radiation resistance of tumors is regarded to appear when tumor pO<sub>2</sub> is around or less than 20 mm Hg (21). The Kaplan-Meier products-limit method was used to estimate the probability of overall survival and local-control survival. The log-rank test was used for statistical analysis of differences. The data of multivariate analysis for local control were assessed with Cox proportional multivariate analysis. To determine the correlation coefficient between changes in tumor pO<sub>2</sub> status and clinical stage, the Spearman rank test was applied.

**Informed consent.** Status of the disease of each patient, treatment methods, and their merits and demerits were explained carefully and precisely by the attending physicians. On this basis, informed consent

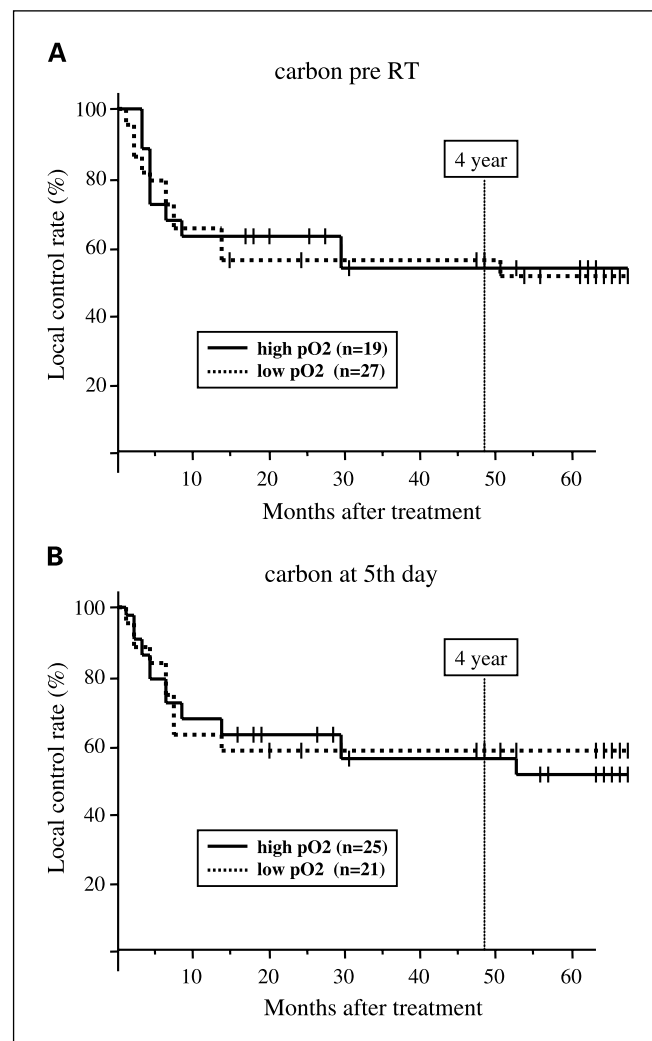
was obtained from the patients and their families. As for tumor biopsy and pO<sub>2</sub> measurement, explanation of the measurement was provided to all patients eligible for this study, and one patient disapproved of the measurement of pO<sub>2</sub> in this way. Informed consent was obtained from all the 49 patients of this study.

## Results

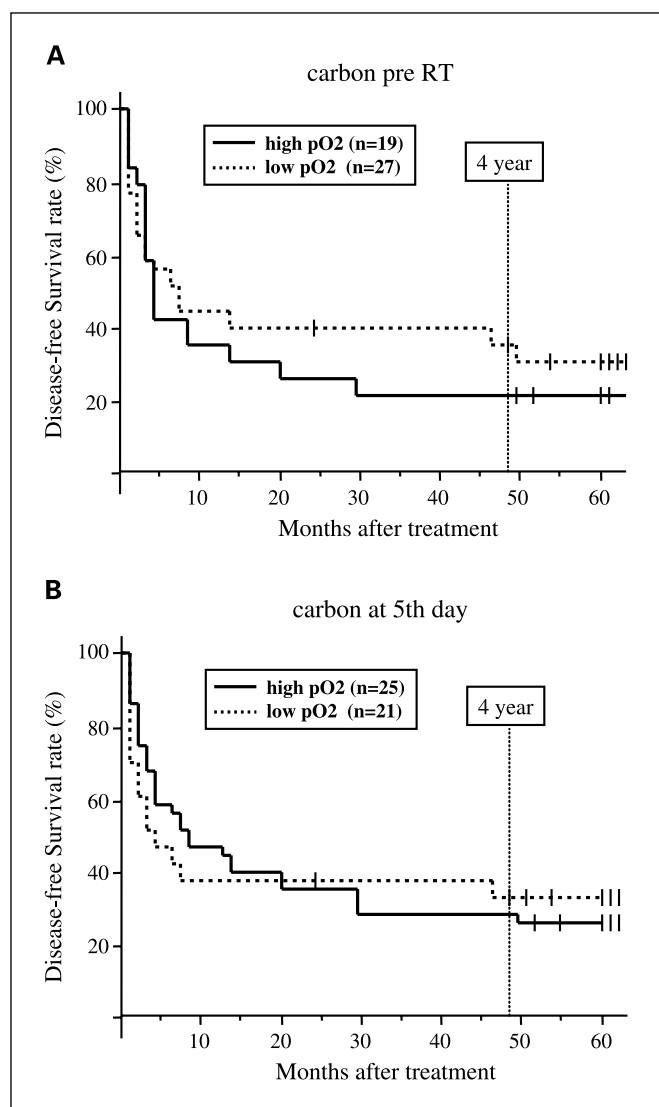
**Survival and local control.** The 4-year disease-free survival rate and local control rate of the 49 patients were 30% and 56%, respectively, as shown in Fig. 1.

**Intratumoral pO<sub>2</sub> measurement.** Forty-nine patients treated with carbon beams and 30 patients with photon beams were eligible for assessment of tumor pO<sub>2</sub> status.

Figure 2 shows tumor pO<sub>2</sub> distributions before treatment and at the 5th day of the patients treated with carbon beams. Mean ± SD and median pO<sub>2</sub> of tumors before treatment and at the 5th day of irradiation (8.8-12 Gy equivalent dose; 5th day) were 19.5 ± 11.6 and 16.6 mm Hg and 22.5 ± 10.7 and 21.6 mm Hg, respectively. There was no significant difference between them (P = 0.13).



**Fig. 4.** Local control curves of patients treated with carbon beams according to tumor pO<sub>2</sub> status before treatment (A) and at the 5th day (B).



**Fig. 5.** Disease-free survival curves of patients treated with carbon beams according to tumor pO<sub>2</sub> status before treatment (A) and at the 5th day (B).

Figure 3 shows tumor pO<sub>2</sub> distributions before treatment and at the 5th day of the patients treated with photons. Median pO<sub>2</sub> before treatment and at the 5th day were 14.2 and 24.0 mm Hg, respectively. The mean pO<sub>2</sub> at the 5th day was 23.6 ± 9.1 mm Hg, significantly higher than the 17.3 ± 10.8 mm Hg pO<sub>2</sub> before treatment (*P* = 0.006). The increment of tumor pO<sub>2</sub> at the 5th day from that before carbon beam therapy was similar to the increment of tumor pO<sub>2</sub> at the 5th day of photon therapy.

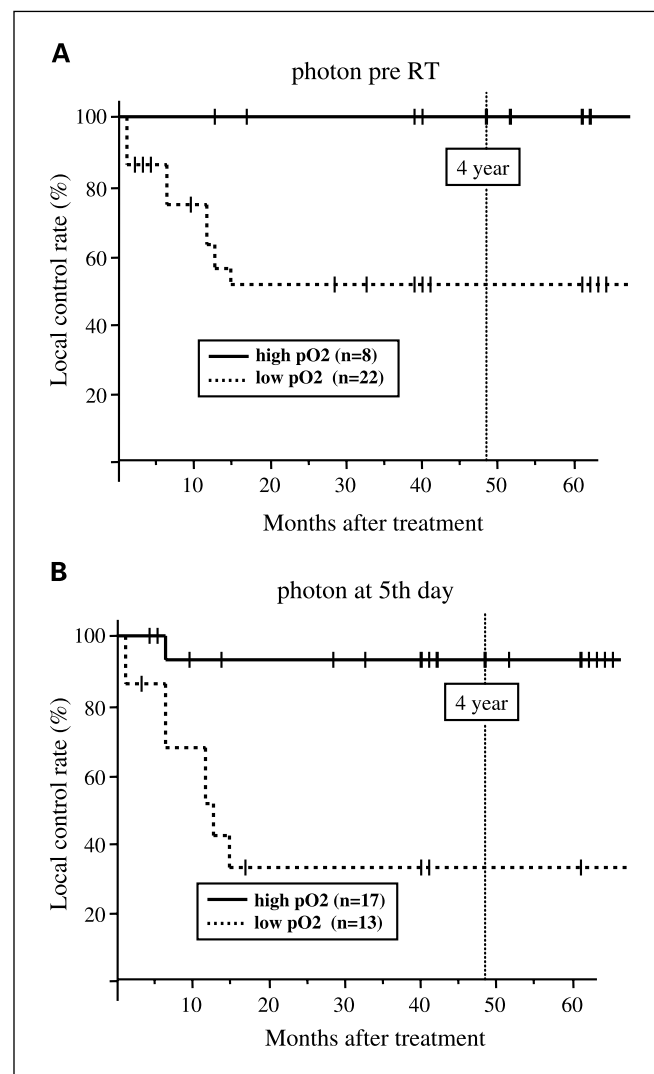
Table 1 shows the pO<sub>2</sub> status of tumors treated with carbon beams according to clinical stages. There was no significant difference in patient characteristics between pO<sub>2</sub> > 20 mm Hg and pO<sub>2</sub> ≤ 20 mm Hg, although carbon beams treated very advanced tumors.

Figure 4 shows local control curves of patients treated with carbons according to tumor pO<sub>2</sub> status before treatment (Fig. 4A) and at the 5th day (Fig. 4B). The 4-year local control rate of patients with pO<sub>2</sub> > 20 mm Hg before treatment was 54%, close to the 58% of the patients with pO<sub>2</sub> ≤ 20 mm Hg,

with the difference not being significant (*P* = 0.89). In the measurement of pO<sub>2</sub> at the 5th day, the 4-year local control rate of patients with pO<sub>2</sub> > 20 mm Hg was 58%, similar to the 60% of patients with pO<sub>2</sub> ≤ 20 mm Hg (*P* = 0.82).

Figure 5 shows the disease-free survival curves of patients treated with carbon beams according to the tumor pO<sub>2</sub> status before treatment (Fig. 5A) and at the 5th day (Fig. 5B). The 4-year disease-free survival rate of patients with pO<sub>2</sub> > 20 mm Hg before treatment was 21%, lower than the 39% of the patients with pO<sub>2</sub> ≤ 20 mm Hg. However, there was no significant difference between them (*P* = 0.60). In the measurement of pO<sub>2</sub> at the 5th day, the 4-year disease-free survival rates of patients with pO<sub>2</sub> > 20 mm Hg and pO<sub>2</sub> ≤ 20 mm Hg were 32% and 33%, respectively, without significance (*P* = 0.95).

Figure 6 shows the local control curves of all patients treated with photons according to the pO<sub>2</sub> status before treatment (Fig. 6A) and at the 5th day (Fig. 6B). The 3-year local control rate of the patients with pO<sub>2</sub> > 20 mm Hg was 100%, higher than the 52% of patients with pO<sub>2</sub> ≤ 20 mm Hg, a significant difference (*P* = 0.035). In the measurement of the pO<sub>2</sub> at the



**Fig. 6.** Local control curves of all patients treated with photons according to the pO<sub>2</sub> status before treatment (A) and at the 5th day (B).

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**Table 2.** Multiple regression analysis for local control

Variables	Standard regression coefficient	P
Age ( $\leq 50$ vs $>50$ y)	0.110	0.51
Stage (II, III vs IV)	0.061	0.72
Tumor volume ( $\leq 100$ vs $>100$ mm <sup>3</sup> )	-0.197	0.25
pO <sub>2</sub> status before treatment ( $\leq 20$ vs $>20$ mm Hg)	0.155	0.38
pO <sub>2</sub> status at the 5th day ( $\leq 20$ vs $>20$ mm Hg)	-0.074	0.67

5th day, the 3-year local control rate of the patients with pO<sub>2</sub> > 20 mm Hg was 93%, significantly higher than the 34% of patients with pO<sub>2</sub>  $\leq$  20 mm Hg ( $P = 0.001$ ).

Table 2 shows the multiple regression analysis for local control according to clinical features, including the pO<sub>2</sub> status of patients treated with carbon beams before treatment and at the 5th day. For both survival and local control, none of the clinical features was an independent prognostic variable.

## Discussion

Maruyama et al. reported superior results of neutron brachytherapy to conventional photon therapy in stage III cervical cancer patients (22). The reason for the superiority was attributed to the biological advantage of high LET and the concentrated dose distribution by the use of brachytherapy. The report indicated that, if high LET beams and superior dose distribution can be combined for advanced cervical cancer treatment, local control improvement could be expected. The present study was the first trial of heavy-charged particle therapy for cervical cancer that provides both high LET biological effect and superior dose distribution. The short-term survival rate and local control rate of the present study were relatively good, considering the very advanced stage of disease (23).

The radiation effect under oxygenated condition is about thrice higher than under anoxic condition, and the existence of hypoxic cells is well recognized as one of the major factors contributing to resistance against radiation therapy and local failure (7–9). High LET radiation can eradicate tumors more effectively under hypoxic condition compared with photon therapy. The OER of neutrons is 1.7 and that ratio of the present carbon beams is  $\sim 1.6$  to 2.0, changing depending on the degree of beam spreadout (16). Although patients are treated with effective dose prescription, hypoxic tumors may not be effectively eradicated with high LET beams because the low oxygen effect can remain after normalization of high LET beams. However, there is no clinical report on neutron beam therapy for various cancers that confirmed the benefits of small OER of high LET beams.

Recently, some investigators have evaluated hypoxic cells in cervical cancers using a polarographic oxygen needle electrode (Eppendorf, Hamburg, Germany; refs. 8, 10). Pretreatment intratumoral pO<sub>2</sub> measured with the Eppendorf electrode is reported to be an important prognostic factor in some cancer

patients treated with radiation therapy. Nordmark et al. reported the relationship between tumor hypoxia and poorer tumor response to radiation therapy in patients with head and neck cancers (9). As for carcinoma of the cervix, there are some reports about the relationship between pretreatment intratumoral pO<sub>2</sub> and prognosis (24–26). Hockel et al. reported that pretreatment intratumoral pO<sub>2</sub> of uterine cervical cancer is an important prognostic factor not only for radiation therapy but also for surgical treatment (25). They used a value of median pO<sub>2</sub> of 10 mm Hg as cutoff between oxic and hypoxic conditions, whereas the present cutoff value was a mean pO<sub>2</sub> of 20 mm Hg at five points or more in a tumor because we used a different pO<sub>2</sub> measurement apparatus. Hence, the assessment of the oxygenated condition of tumors in our study was somewhat different from that of other studies. The hypoxic tumors of the present study were thus defined based on hypoxic conditions at 1-cm depth from the surface. We consider that the mean pO<sub>2</sub> value of the points correlated with relative hypoxic condition of the tumors. Furthermore, Wong et al. reported that there was no significant difference in the mean values of median pO<sub>2</sub> and hypoxic proportion between the proximal, middle, and distal thirds of tumors (27). The cutoff value of 20 mm Hg was selected because local control rates of the two groups were seen to deviate most significantly by statistical analysis.

In the present study, longer follow-up confirmed a similar trend between pretreatment pO<sub>2</sub> and local control in patients treated with photon beams. This means that pretreatment intratumoral pO<sub>2</sub> can be suggested to be an indicator of local control as well as tumor malignant progression. There is a possibility that patients with high pO<sub>2</sub> included more patients with lower clinical stage and smaller tumor volume compared with patients with low pO<sub>2</sub>. However, multivariate analysis showed that there was a significant correlation between local control and pO<sub>2</sub> status at 9 Gy (12). In addition, some authors have reported that lower pretreatment intratumoral pO<sub>2</sub> is related to the probability of worse local control (28–30). In the present study, however, patients with hypoxic tumor, when treated with carbon beams, showed a similar local control rate to those without hypoxic tumor, and the relationship between pretreatment intratumoral pO<sub>2</sub> status and local control was not found. This is the first confirmation that high LET radiation can eradicate tumors under hypoxic condition as effectively as tumors under oxic condition.

The “reoxygenation” of tumor during radiation therapy is one of the effective functions of fractionated radiation therapy. Recently, reoxygenation in radiation therapy was reported in cervical cancer and head and neck cancer (31–33). We showed very recently that there was a significant increase in pO<sub>2</sub> 1 week after the initiation of conventional radiation therapy (12). Although the present study did not show a significant reoxygenation in cervical cancers treated with carbon beams, Ando et al. reported that accelerated reoxygenation was generated after carbon beam irradiation in inoculated murine tumor (15). In the present study, the increase in mean pO<sub>2</sub> at the 5th day in cervical cancers treated with carbon beams was evidence of reoxygenation after carbon beam therapy. However, the increment of tumor pO<sub>2</sub> at the 5th day in carbon beam therapy was similar to the increment of tumor pO<sub>2</sub> in photon therapy. Consequently, the present study did not confirm accelerated reoxygenation compared with photon therapy.

We have shown that patients with reoxygenation of cervical cancers showed significantly better local control with conventional X-ray treatment (12), and we confirmed the same trend by longer follow-up in the present study. In carbon beam treatment, however, reoxygenated tumors did not show any significantly better local control than their hypoxic counterparts in the present study (Fig. 4B). This result strongly suggested that the tumor-oxygenated condition did not affect radiation sensitivity of tumors in carbon beam therapy.

Bulky tumors are considered to have a large fraction of hypoxic tumor. The present study also showed that tumor volume was not a significant variable for prediction of either for disease-free

survival or local control. Especially, very advanced bulky stage IVa patients successfully achieved local control and good prognosis (23). These results together with the present results of less oxygen effects of carbon beam therapy suggested that the biological advantages of high LET particles of a lower OER may effectively overcome radiation-resistant anoxic tumor cells of bulky tumor.

In conclusion, we showed the intratumoral pO<sub>2</sub> status during carbon beam therapy for cervical cancer. The status had no influence on either disease-free survival or local control probability. In other words, the relatively good local control for hypoxic tumors, similar to that for oxyc tumors, was obtained in patients treated with carbon beam therapy.

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