Table 2. Profile of hepatocellular carcinoma patients population

	Phase I	Phase II
No. of patients	12	19
Gender		
Male	9	14
Female	3	5
Age (years)		
Median	63	67
Range	56–78	56–77
Performance status		
0	11	7
1	1	12
Viral marker		
Hepatitis C antibody+	7	7
Hepatitis B antigen+	2	5
Previous treatment		
Surgical resection	4	10
Percutaneous ablation therapy	3	3
Transcatheter arterial chemoembolization	5	8
Transcatheter arterial infusion	3	5
Radiation therapy	1	2
None	3	3
Child–Pugh classification		
A	8	17
B	4	2
UICC tumor stage[a]		
III	4	6
IVa	3	1
IVb	5	12
Portal vein tumor thrombosis		
(+)	5	4
Extrahepatic metastasis		
Lymph node	5	7
Lung	0	6
Bone	0	3
Adrenal gland	0	1
Peritoneum	0	1
None	7	6

[a]The International Union Against Cancer, 6th edition.

patients received transcatheter arterial infusion with cisplatin, one patient received salvage TACE because of HCC rupture during the follow-up period, one patient received salvage radiofrequency ablation because of rapid growth of HCC that needed control and one patient received immnunotherapy.

Table 3. Toxicity

Toxicity grade	Phase I part									Phase II part		
	Level 1 ($n = 3$)			Level 2 ($n = 6$)			Level 3 ($n = 3$)			Level 2 ($n = 19$)		
	1–2	3	4	1–2	3	4	1–2	3	4	1–2	3	4
Hematological toxicity												
Leukopenia	2	1	0	0	2	0	0	1	1	4	9	3
Neutropenia	0	1	0	0	2	0	0	0	2	4	11	2
Thrombocytopenia	1	1	0	0	0	0	1	0	0	4	1	0
Anemia	0	0	0	1	0	0	0	0	0	1	0	0
Non-hematological toxicity												
Nausea	3	0	0	0	0	0	2	0	0	3	0	0
Anorexia	0	0	0	2	0	0	1	0	0	3	0	0
Elevated bilirubin	2	0	0	0	1	0	1	0	0	6	0	0
Hypoalbuminemia	1	0	0	0	0	0	0	0	0	1	0	0
Fatigue	0	0	0	0	0	0	1	0	0	1	0	0
Hyperpigmentation	0	0	0	0	0	0	0	0	0	1	0	0
Constipation	0	0	0	0	0	0	0	0	0	1	0	0
Elevated creatinine	0	0	0	0	0	0	0	1	0	0	0	0
Elevated AST	0	0	0	1	0	0	0	0	0	2	1	1[a]
Elevated ALT	0	0	0	1	0	0	0	0	0	1	2	1[a]
Liver dysfunction	0	0	0	0	0	0	0	0	0	0	0	1[a]

AST, aspartate aminotransferase; ALT, alanine aminotransferase.
[a]Death related to adverse event.

Toxicity

Table 3 summarizes the toxicities observed in the patients. At the recommended dose (level 2), the major Grade 3–4 hematological toxicities were leukopenia (63.2%) and neutropenia (68.4%). The most common non-hematological toxicities were elevated serum total bilirubin level (31.6%), elevated AST level (26.3%), elevated ALP level (26.3%) and anorexia (21.1%); however, no Grade 3–4 non-hematological toxicities were observed. One patient died of hepatic failure due to hepatitis B virus (HBV) reactivation.

Efficacy

Of the 19 patients who were administered the recommended dosage, 18 died during the follow-up period. All of the 19 patients administered the recommended dosage were evaluable for tumor response; of these, 1 patient achieved partial response (PR), with an overall response rate of 5.3% (95% CI, 0.0–26.0%). Eight patients (42.1%) had stable disease and 10 patients (52.6%) had progressive disease. The 1-year survival rate, median overall survival, median progression-free survival and time to progression were 26.3%, 8.4

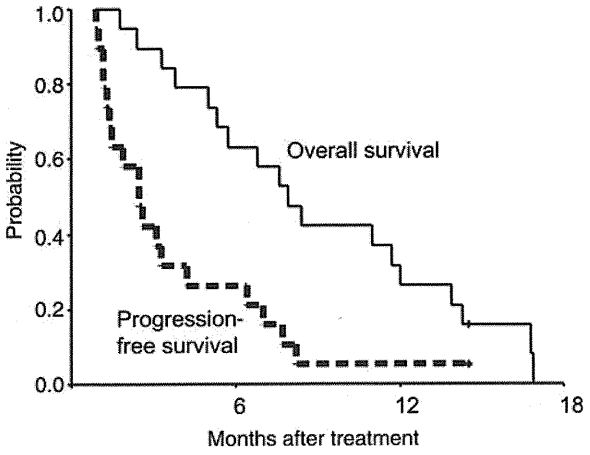


Figure 1. Overall survival and progression-free survival in 19 patients at the recommended dose. Tick marks indicate censored cases.

months (95% CI, 5.4–11.4) and 2.5 months (95% CI, 1.5–3.5), respectively (Fig. 1).

DISCUSSION

Systemic chemotherapy for unresectable HCC is recognized as an important treatment modality, because some patients who have recurrent or very advanced disease are not suitable candidates for effective local treatments such as surgical resection, liver transplantation, local ablation therapy and TACE. Many patients with HCC have underlying chronic liver disease and impaired hepatic function, increasing the toxicity of standard doses of many chemotherapeutic agents and causing difficulty in delivering combination chemotherapies. The results, in terms of the therapeutic efficacy, of investigation of cytotoxic agents for advanced HCC have been disappointing, with few agents have yielded response rates of over 20%, and no cytotoxic agents have produced convincing survival benefits in the Phase III setting (26–28).

In Japan, only five anticancer agents, UFT, adriamycin, cytarabine, mitomycin and 5-FU, had been approved for the systemic chemotherapy of HCC by the Ministry of Health, Labor and Welfare of Japan before sorafenib has been approved. Among these drugs, the results of multiagent regimens containing both a fluoropyrimidine and an anthracycline antibiotic have shown favorable results for advanced HCC (22–24). Thus, it was expected that the combination of mitoxantrone and UFT (UFM regimen) would have effective anticancer activity, and we conducted a Phase I/II study to evaluate this regimen.

In the Phase I part, we determined the recommended dose of mitoxantrone as 8 mg/m^2 on day 1 and of UFT as 300 mg/m^2 from days 1 to 21 of a 28-day cycle. The DLTs observed at Level 3 were Grade 4 neutropenia (two patients) and Grade 3 creatinine elevation (one patient).

Patients with HCC tend to experience more severe myelosuppression and hepatic toxicity than those with other malignant diseases, because most have underlying cirrhosis, which is usually associated with compromised hepatic function, leukopenia and thrombocytopenia (24). In 19 patients treated at the recommended dose level, the most frequently encountered toxicities were leukopenia and neutropenia, which are well-known toxicities of the two drugs. When compared with that in trial of mitoxantrone or UFT for other malignancies, Grade 3 or 4 hematological toxicities occurred more frequently (29–31). However, these toxicities were reversible and generally well tolerated in patients with advanced HCC, except for one case of treatment-related death; this patient developed hepatic failure due to HBV reactivation, because no antiviral drug for HBV infection, such as lamivudine or entecavir, was given. This is a well-recognized complication in patients with HBV infection who received immunosuppressive therapy or chemotherapeutic agents (32,33). Thus, patients with HBV infection should receive prophylactic antiviral treatment before chemotherapy.

In the current study, 1 of the 19 patients showed a PR (response rate, 5.3%). However, the rate of progressive disease was 52.6%. In addition, the result of median time to progression was only 2.5 months. Those results were unfavorable when compared with those reported from other clinical trials (8,21–23). Therefore, this regimen is considered to be ineffective and cannot be recommended for use in clinical practice. There were several reasons for this negative result. One of the reasons was the number of anticancer drugs in the regimen. A regimen containing two drugs may have little activity, and three or more drugs may be needed to obtain activity against HCC, because many of the regimens that have been shown to exert anticancer effect against HCC contain three or more drugs. The other reason was the recommended doses of the drugs in this regimen. We set the criteria of DLT which had included Grade 4 neutropenia or leukopenia. Two patients experienced DLT based on these criteria. However, both recovered soon, with only observation. Therefore, the criteria may be too strict, although the two drugs have been used at these recommended doses for other malignancies. It may be possible to set higher dose levels to obtain higher antitumor effect.

Recently, increasing knowledge of the molecular pathogenesis of HCC as well as the introduction of molecular-targeted therapies has created an encouraging trend in the management of HCC. Combination regimens consisting of molecular-targeted agents such as sorafenib and cytotoxic agents have been reported as promising regimens for patients with advanced HCC and other malignancies (34–37). The UFM regimen itself has little antitumor activity, but the result may be useful in the setting of future clinical trials of cytotoxic agents used in combination with molecular-targeted agents.

In conclusion, the recommended dose was mitoxantrone at 8 mg/m^2 and UFT at 300 mg/m^2/day. A combined chemotherapy with mitoxantrone and UFT appeared to show little activity in patients with advanced HCC, although this regimen was generally well tolerated. These findings do argue against the use of this regimen in clinical practice.

Acknowledgements

The authors thank Ms Kayo Takei and Ms Keiko Kondo for their devoted work and support.

Funding

This study was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare, Japan.

Conflict of interest statement

None declared.

References

1. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. *Int J Cancer* 1999;80:827–41.
2. Parkin DM, Bray F, Ferlay J. Global cancer statistics 2002. *CA Cancer J Clin* 2005;55:74–108.
3. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001;35:421–30.
4. Llovet JM, Real MI, Montaña X, Planas R, Coll S, Aponte J, et al. Arterial embolization or chemoembolization versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomized controlled trial. *Lancet* 2002;359:1734–9.
5. Takayasu K, Arii S, Ikai I, Omata M, Okita K, Ichida T, et al. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. *Gastroenterology* 2006;131:461–9.
6. Forner A, Hessheimer AJ, Isabel Real M, Bruix J. Treatment of hepatocellular carcinoma. *Crit Rev Oncol Hematol* 2006;60:89–98.
7. Thomas MB, Zhu AX. Hepatocellular carcinoma: the need for progress. *J Clin Oncol* 2005;23:2892–9.
8. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378–90.
9. Falkson G, Moertel CG, Lavin P, Pretorius FJ, Carbone PP. Chemotherapy studies in primary liver cancer: a prospective randomized clinical trial. *Cancer* 1978;42:2149–56.
10. Tetef M, Doroshow J, Akman S, Coluzzi P, Leong L, Margolin K, et al. 5-Fluorouracil and high-dose calcium leucovorin for hepatocellular carcinoma: a phase II trial. *Cancer Invest* 1995;13:460–3.
11. Kim SJ, Seo HY, Choi JG, Sul HR, Sung HJ, Park KH, et al. Phase II study with a combination of epirubicin, cisplatin, UFT, and leucovorin in advanced hepatocellular carcinoma. *Cancer Chemother Pharmacol* 2006;57:436–42.
12. Fujii S, Ikenaka K, Fukushima M, Shirasaka T. Effect of uracil and its derivatives on antitumor activity of 5-fluorouracil and 1-(2-tetrahydrofuryl)-5-fluorouracil. *Jpn J Cancer Res* 1978;69:763–72.
13. Pazdur R, Lassere Y, Diaz-Canton E, Bready B, Ho DH. Phase I trials of uracil–tegafur (UFT) using 5 and 28 day administration schedules: demonstration of schedule-dependent toxicities. *Anticancer Drugs* 1996;7:728–33.
14. Baker SD, Diasio RB, O'Reilly S, Lucas VS, Khor SP, Sartorius SE, et al. Phase I and pharmacologic study of oral fluorouracil on a chronic daily schedule in combination with the dihydropyrimidine dehydrogenase inactivator eniluracil. *J Clin Oncol* 2000;18:915–26.
15. Takiuchi H, Ajani JA. Uracil–tegafur in gastric carcinoma: a comprehensive review. *J Clin Oncol* 1998;16:2877–85.
16. Tokyo Liver Cancer Chemotherapy Study Group. Phase II study of co-administration of uracil and tegafur (UFT) in hepatocellular carcinoma. *Jpn J Clin Oncol* 1985;15:559–62.
17. Ishikawa T, Ichida T, Sugitani S, Tsuboi Y, Genda T, Sugahara S, et al. Improved survival with oral administration of enteric-coated tegafur/uracil for advanced stage IV-A hepatocellular carcinoma. *J Gastroenterol Hepatol* 2001;16:452–9.
18. Lai CL, Wu PC, Chan GC, Lok AS, Lin HJ. Doxorubicin versus no antitumor therapy in inoperable hepatocellular carcinoma. A prospective randomized trial. *Cancer* 1988;62:479–83.
19. Durr FE. Biologic and biochemical effects of mitoxantrone. *Semin Oncol* 1984;11:3–10.
20. Colleoni M, Nole F, Di Bartolomeo M, de Braud F, Bajetta E. Mitoxantrone in patients affected by heptatocellular carcinoma with unfavorable prognostic factors. *Oncology* 1992;49:139–42.
21. Yoshida T, Okazaki N, Yoshino M, Ohkura H, Miyamoto K, Shimada Y. Phase II trial of mitoxantrone in patients with hepatocellular carcinoma. *Eur J Cancer Clin Oncol* 1988;24:1897–8.
22. Ellis PA, Norman A, Hill A, O'Brien ME, Nicolson M, Hickish T, et al. Epirubicin, cisplatin and infusional 5-fluorouracil (5-FU) (ECF) in hepatobiliary tumours. *Eur J Cancer* 1995;31:1594–8.
23. Leung TW, Tang AM, Zee B, Lau WY, Lai PB, Leung KL, et al. Factors predicting response and survival in 149 patients with unresectable hepatocellular carcinoma treated by combination cisplatin, interferon-alpha, doxorubicin and 5-fluorouracil chemotherapy. *Cancer* 2002;94:421–7.
24. Ikeda M, Okusaka T, Ueno H, Tekezako Y, Morizane C. A phase II trial of continuous infusion of 5-fluorouracil, mitoxantrone, and cisplatin for metastatic hepatocellular carcinoma. *Cancer* 2005;103:756–62.
25. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205–16.
26. Johnson PJ. Hepatocellular carcinoma: is current therapy really altering outcome? *Gut* 2002;51:459–62.
27. Palmer DH, Hussain SA, Johnson PJ. Systemic therapies for hepatocellular carcinoma. *Expert Opin Investig Drugs* 2004;13:1555–68.
28. Nowak AK, Chow PK, Findlay M. Systemic therapy for advanced hepatocellular carcinoma. *Eur J Cancer* 2004;40:1474–84.
29. Onyenadum A, Gogas H, Kosmidis P, Aravantinos G, Bafaloukos D, Bacoyiannis H. Mitoxantrone plus gemcitabine in pretreated patients with metastatic breast cancer. *J Chemother* 2006;18:192–8.
30. Onyenadum A, Gogas H, Markopoulos C, Bafaloukos D, Aravantinos G, Mantzourani M, et al. Mitoxantrone plus vinorelbine in pretreated patients with metastatic breast cancer. *J Chemother* 2007;19:582–9.
31. Furuse J, Okusaka T, Ohkawa S, Nagase M, Funakoshi A, Boku N, et al. Early phase II study of uracil–tegafur plus doxorubicin in patients with unresectable advanced biliary tract cancer. *Jpn J Clin Oncol* 2006;36:552–6.
32. Foont JA, Schiff ER. Avoid the tragedy of hepatitis B reactivation in immunosuppressed patients. *Nat Clin Pract Gastroenterol Hepatol* 2007;4:128–9.
33. Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. *Hepatology* 2007;45:1056–75.
34. Richly H, Schultheis B, Adamietz IA, Kupsch P, Grubert M, Hilger RA, et al. Combination of sorafenib and doxorubicin in patients with advanced hepatocellular carcinoma: Results from a phase I extension trial. *Eur J Cancer* 2009;45:579–87.
35. Zhu AX. Development of sorafenib and other molecularly targeted agents in hepatocellular carcinoma. *Cancer* 2008;112:250–9.
36. Richly H, Kupsch P, Passage K, Grubert M, Hilger RA, Voigtmann R. Results of a phase I trial of BAY 43-9006 in combination with doxorubicin in patients with primary hepatic cancer. *Int J Clin Pharmacol Ther* 2004;42:650–1.
37. Dal Lago L, D'Hondt V, Awada A. Selected combination therapy with sorafenib: a review of clinical data and perspectives in advanced solid tumors. *Oncologist* 2008;13:845–58.

ELSEVIER

Int. J. Radiation Oncology Biol. Phys., Vol. 79, No. 5, pp. 1479–1486, 2011

0360-3016/$–see front matter

doi:10.1016/j.ijrobp.2009.12.048

CLINICAL INVESTIGATION **Liver**

DOSE–VOLUME HISTOGRAM ANALYSIS OF THE SAFETY OF PROTON BEAM THERAPY FOR UNRESECTABLE HEPATOCELLULAR CARCINOMA

MITSUHIKO KAWASHIMA, M.D.,*† RYOSUKE KOHNO, PH.D.,* KOHEI NAKACHI, M.D.,‡ TEIJI NISHIO, PH.D.,* SHUICHI MITSUNAGA, M.D.,‡ MASAFUMI IKEDA, M.D.,‡ MASARU KONISHI, M.D.,§ SHINICHIRO TAKAHASHI, M.D.,§ NAOTO GOTOHDA, M.D.,§ SATOKO ARAHIRA, M.D.,† SADAMOTO ZENDA, M.D.,* TAKASHI OGINO, M.D.,* AND TAIRA KINOSHITA, M.D.§

Divisions of *Particle Therapy and Radiation Oncology, Research Center for Innovative Oncology, †Radiation Oncology, ‡Hepatobiliary and Pancreatic Medical Oncology, and §Hepatobiliary and Pancreatic Surgery, National Cancer Center Hospital East, Kashiwa, Japan

Purpose: To evaluate the safety and efficacy of radiotherapy using proton beam (PRT) for unresectable hepatocellular carcinoma.

Methods and Materials: Sixty consecutive patients who underwent PRT between May 1999 and July 2007 were analyzed. There were 42 males and 18 females, with a median age of 70 years (48–92 years). All but 1 patient had a single lesion with a median diameter of 45 mm (20–100 mm). Total PRT dose/fractionation was 76–cobalt Gray equivalent (CGE)/20 fractions in 46 patients, 65 CGE/26 fractions in 11 patients, and 60 CGE/10 fractions in 3 patients. The risk of developing proton-induced hepatic insufficiency (PHI) was estimated using dose-volume histograms and an indocyanine-green retention rate at 15 minutes (ICG R15).

Results: None of the 20 patients with ICG R15 of less than 20% developed PHI, whereas 6 of 8 patients with ICG R15 values of 50% or higher developed PHI. Among 32 patients whose ICG R15 ranged from 20% to 49.9%, PHI was observed only in patients who had received 30 CGE (V30) to more than 25% of the noncancerous parts of the liver (n = 5) Local progression-free and overall survival rates at 3 years were 90% (95% confidence interval [CI], 80–99%) and 56% (95% CI, 43–69%), respectively. A gastrointestinal toxicity of Grade ≥2 was observed in 3 patients.

Conclusions: ICG R15 and V30 are recommended as useful predictors for the risk of developing PHI, which should be incorporated into multidisciplinary treatment plans for patients with this disease. © **2011 Elsevier Inc.**

Hepatocellular carcinoma, Proton beam radiotherapy, Dose–volume histogram, Radiation tolerance of the liver.

INTRODUCTION

Recent improvements in diagnostic imaging and radiotherapy (RT) techniques have made high-dose radiotherapy a safe and effective treatment for selected patients with unresectable hepatocellular carcinoma (HCC) (1). Charged-particle radiotherapy can potentially deliver considerably larger doses of RT to liver tumors, with greater sparing of normal tissues, and proton beam radiotherapy (PRT) for HCC using aggressively high total and fractional RT doses has been investigated during the last 2 decades. The results have shown local control rates ranging from 75% to 96% and overall survival (OAS) rates exceeding 50% at 2 years in groups of patients that include those who had HCC tumors of ≥5 cm in diameter (2–4). HCC has a high propensity for venous invasion, which is frequently associated with multiple tumors within resected specimens (5–9). In this context, the extent of resection was determined while considering potential tumor spread via portal blood flow and the necessity of preserving a functional liver reserve (5, 7, 10). Even in preselected patients who underwent hepatectomy, more than 50% of tumors with diameters greater than 4 cm demonstrated microscopic vascular invasion (8, 11). Consequently, it will become more crucial to consider the influence of vascular invasion on undetectable tumor dissemination at the periphery of the gross tumor in RT for unresectable HCC.

Given the high probability of obtaining local control by using PRT, an appropriate definition of the clinical target volume (CTV) according to patterns of tumor spread and patients' functional liver reserves is extremely important in order to maximize the therapeutic ratio. Ideally, the entire portal segment that contains HCC nodules should be covered within the CTV when the tumor shows macro- or microscopic vascular invasion. This requires a considerably larger

Reprint requests to: Mitsuhiko Kawashima, 6-5-1, Kashiwanoha, Kashiwa, Chiba, Japan, 277-8577. Tel: 81-4-7133-1111; Fax: 81-4-7171-4724; E-mail: mkawashi@east.ncc.go.jp

Conflict of interest: none.

Received Nov 24, 2009, and in revised form Dec 21, 2009. Accepted for publication Dec 21, 2009.

irradiated volume even with PRT, partly because of unavoidable uncertainty in treatment planning without using intraoperative ultrasonography (7). Another possible way to eradicate satellite HCC nodules, which are disseminated via portal blood flow, is transarterial chemoembolization (TACE). Currently, the standard treatment for patients with unresectable HCC that is not amenable to local ablation therapy is TACE instead of best supportive care (12). The OAS rate at 3 years after TACE ranges from 32% to 47% in patients with stage III cancer and with liver damage A to B, according to the staging system used in a nationwide cohort study conducted by the Liver Cancer Study Group of Japan (13). Considering that the tumoricidal effect of TACE in HCC with vascular invasion is frequently incomplete (13), a significant benefit of adding PRT to TACE would be expected. However, presently, there has been no robust evidence supporting this concept. Before we examine the validity of targeting the entire anatomical portal segment containing HCC in a multidisciplinary approach that includes PRT, practical methods to estimate the safety of PRT according to the dose–volume histogram (DVH) should be established in patients who have various levels of severity of liver dysfunction. Findings from our previous study consisting of 30 patients suggested that the risk of proton-induced hepatic insufficiency (PHI) could be predicted by the indocyanine green clearance test and the retention rate at 15 minutes (ICG R15) in combination with DVH parameters (14) such as percentages of hepatic noncancerous portions receiving doses of >30 cobalt-Gray-equivalent (CGE) (3). We have subsequently accumulated data from additional patients in clinical practice. The clinical results were evaluated, and we have again used the DVH analysis to examine the relationship between probability of PHI and dose–volume parameters.

METHODS AND MATERIALS

Patients

Patient eligibility was reported previously (3); in brief, they were required to have uni- or bidimensional measurable HCC nodules of ≤10 cm in maximum diameter on computed tomography (CT) and/or magnetic resonance imaging (MRI) without evidence of extrahepatic tumor spread. All patients had a white blood cell count of ≥2,000/mm^3; a hemoglobin level of ≥7.5 g/dl; a platelet count of ≥25,000/ mm^3; and adequate hepatic function (total bilirubin, ≤3.0 mg/dl; alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase of <5.0× normal; no ascites). Patients who had multicentric HCC nodules were not considered as candidates for PRT, except for those who fulfilled the following two conditions: (*1*) multiple nodules could be encompassed within a single clinical target volume; and (*2*) lesions other than those of the targeted tumor were judged to be controlled with prior surgery and/or local ablation therapy. This retrospective study was approved by the institutional ethics committee, and written informed consent was obtained from all patients.

Treatment Planning

ICG R15 was measured in all patients to quantitatively assess the hepatic functional reserve. Serological testing for hepatitis B surface antigen and anti-hepatitis C antibody was done. All patients were judged to be unresectable by expert hepatobiliary surgeons at our institution, based on the patient's serum bilirubin level, ICG R15, and expected volume of resected liver (10). Percutaneous fine-needle biopsies were performed for all patients unless they had radiologically compatible, postsurgical recurrent HCC (3).

Treatment methods were published previously (3). In brief, gross tumor volume (GTV) was defined using a treatment-planning CT scan, and CTV and planning target volume (PTV) were defined as follows in all but 2 patients: CTV = GTV + 5 mm, and PTV = CTV + 3 mm of lateral, craniocaudal, and anteroposterior margins. CTV encompassed the entire volume of the right lobe in 1 patient who had a tumor of 4 cm in diameter that broadly attached to the bifurcation of the right anterior and posterior portal veins. In this patient, right portal vein embolization was done to facilitate compensatory hypertrophy of the left lobe for expected surgery. However, the patient was finally judged to be unresectable, and PRT was selected. Another patient was treated with a CTV encompassing the entire right anterior portal segment because a tumor of 2 cm in diameter had invaded the bifurcation of the right anterosuperior and anteroinferior portal vein associating with daughter HCC at the right anterosuperior portal segment. The beam energy and spread-out Bragg peak (15) were fine-tuned so that a 90% isodose volume of the prescribed dose encompassed the PTV.

Forty-six patients received PRT to a total dose of 76 CGE in 3.8 CGE once-daily fractions, four to five fractions in a week. Another 3 patients underwent 60 CGE /10 fractions/2 weeks, depending on availability of the proton beam. Eleven patients whose PTV encompassed the gastrointestinal wall received 65 CGE in 2.5 CGE /fraction, five fractions per week. All patients were treated using a 150- to 190-MV proton beam. The relative biological effectiveness of our proton beam was defined as 1.1 (16). No concomitant treatment such as TACE, local ablation, or systemic therapy was allowed during or after the PRT, unless a treatment failure was detected. Both scanning of CT images for treatment planning and irradiation by the proton beam were done during the exhalation phase using the respiration-gated irradiation system and intrahepatic fiducial markers as previously reported (3).

Outcomes

Death from any cause was defined as an event in calculation of OAS, whereas tumor recurrences at any site or patient deaths were defined as events in disease-free survival (DFS). An increase of the tumor diameter within the PTV was defined as local progression, and patients who died without evidence of local progression were censored at the time of last radiographic examination. Adverse events were reviewed weekly during the PRT regimen by means of physical examination, complete blood count, liver function tests, and other biochemical profiles as indicated. The severity of adverse events was assessed using the National Cancer Institute common terminology criteria for adverse events, version 3.0. After completion of PRT, reviews that monitored disease status, including CT and/or MRI examinations and long-term toxicity, were done at a minimum frequency of every 3 months in all 60 patients. The percentages of hepatic noncancerous portions (entire liver volume minus gross tumor volume) receiving CGE doses of >0 (V0), ≥10 (V10), ≥20 (V20), ≥30 (V30), ≥40 (V40), and ≥50 (V50) were calculated using PRT planning software (PT-PLAN/NDOSE System, Sumitomo Heavy Industries Ltd., Tokyo, Japan), and their influence on the outcomes were analyzed (3). Time-to-event analyses were done using Kaplan-Meier estimates from the start of PRT. The differences between time-to-event curves were evaluated with the log-rank test. Multivariate analyses were performed with Cox's proportional hazards model.

RESULTS

Patients

A total of 60 patients with HCC underwent PRT in our institution between May 1999 and July 2007. Approximately 1400 patients with HCC were newly presented to our institution during this study period and about 35%, 30%, 25%, and the remainder primarily treated with hepatectomy, TACE, percutaneous local ablation, and other treatments, respectively. Therefore 60 patients in this study corresponded to approximately 4% of overall, or 7% of patients with unresectable HCC. Patient characteristics at the start of PRT are listed in Table 1. All patients had underlying chronic liver disease. One patient had a history of schistosomiasis, and another patient had autoimmune hepatitis as the cause of liver cirrhosis. Five additional patients were diagnosed with liver cirrhosis caused by non-B, non-C hepatitis. A total of 24 patients received PRT as the first treatment for their HCC. Ten patients had postsurgical recurrences, 22 patients received unsuccessful local ablation and/or TACE to the targeted tumor, and 4 patients underwent successful local ablation to a tumor other than the target prior to PRT. Histological confirmation was not obtained in 1 patient who had a tumor with typical radiographic features compatible with HCC (3). Six patients had HCC nodules of ≤3 cm in diameter; however, they were not considered candidates for local ablation therapy because of the tumor locations, which were in close proximity to the great vessels or the lung.

Table 1. Characteristics of patients

Characteristics	No. of patients (%)
Age (years)	
Median	70
Range	48–92
Gender	
Male	42 (70)
Female	18 (30)
ECOG performance status	
0–1	57 (95)
2	3 (5)
Viral markers	
Hepatitis B surface antigen-positive	3 (5)
Hepatitis C antibody-positive	49 (82)
Both positive	1 (2)
Both negative	7 (12)
Child-Pugh classification	
A	47 (78)
B	13 (22)
C	0
% patients with pretreatment ICG R15 values	
<20	20 (20)
20–40	25 (55)
40–50	7 (12)
≥50	8 (13)
Tumor size (mm)	
Median	45
Range	20–90
20–50	42 (70)
>50	18 (30)
Macroscopic vascular invasion	
Yes	42 (70)
No	18 (30)
Morphology of primary tumor	
Single nodular	45 (75)
Multinodular, aggregating	9 (15)
Diffuse	5 (8)
Portal vein tumor thrombosis	1 (2)
Serum alpha-fetoprotein level (IU/mL)	
<300	41 (68)
≥300	19 (32)
Histology	
Well-differentiated	15 (25)
Moderately-differentiated	28 (47)
Poorly-differentiated	7 (12)
Differentiation not specified	9 (15)
Negative (radiological diagnosis only)	1 (2)
Prior treatment	
None	24 (40)
Surgery	10 (17)
Local ablation/TACE	26 (43)

Adverse events during PRT

All patients completed the treatment plan. Prolongation of the overall treatment time for more than 1 week occurred in 4 patients: treatment of 3 patients was extended due to availability of the proton beam machine, and 1 patient's treatment was extended because of fever associated with grade 3 elevation of total bilirubin that spontaneously resolved within a week. A total of 14 patients experienced transient grade 3 leukopenia and/or thrombocytopenia without infection or bleeding that necessitated treatment. In addition, 8 patients experiencing grade 3 elevation of transaminases without clinical manifestation of hepatic insufficiency maintained good performance status. PRT was not discontinued for these patients; nevertheless, these events spontaneously resolved within 1 to 2 weeks.

Estimation of the risk of PHI by DVH analysis

Development of hepatic insufficiency presented with anicteric ascites and/or asterixis within 6 months after completion of PRT in the absence of disease progression was defined as PHI. Eleven patients, all of whom received a total PRT dose of 76 CGE, developed PHI at 1 to 6 months (median, 2 months) after completion of PRT without elevation of serum bilirubin and transaminases of more than threefold above normal levels. DVHs for hepatic noncancerous portions were drawn according to pretreatment ICG R15 values (Fig. 1A–C). Results showed that all 20 patients with ICG R15 of <20% were free of PHI, regardless of the DVH, for 2 to 94 months (median, 44 months). On the other hand, 6 of 8 patients with pretreatment ICG R15 values of ≥50% died of PHI with (n = 3) or without (n = 3) evidence of HCC recurrence at 2 to 15 months (median, 8 months). There was no obvious relationship between DVH and development of PHI in these 8 patients, as shown in Fig. 1C.

Among 32 patients whose ICG R15 values ranged from 20% to 49.9%, 5 patients developed PHI. The V0 to V50 in these 32 patients are shown in Fig. 2. Differences in distributions of these DVH parameters between patients who did

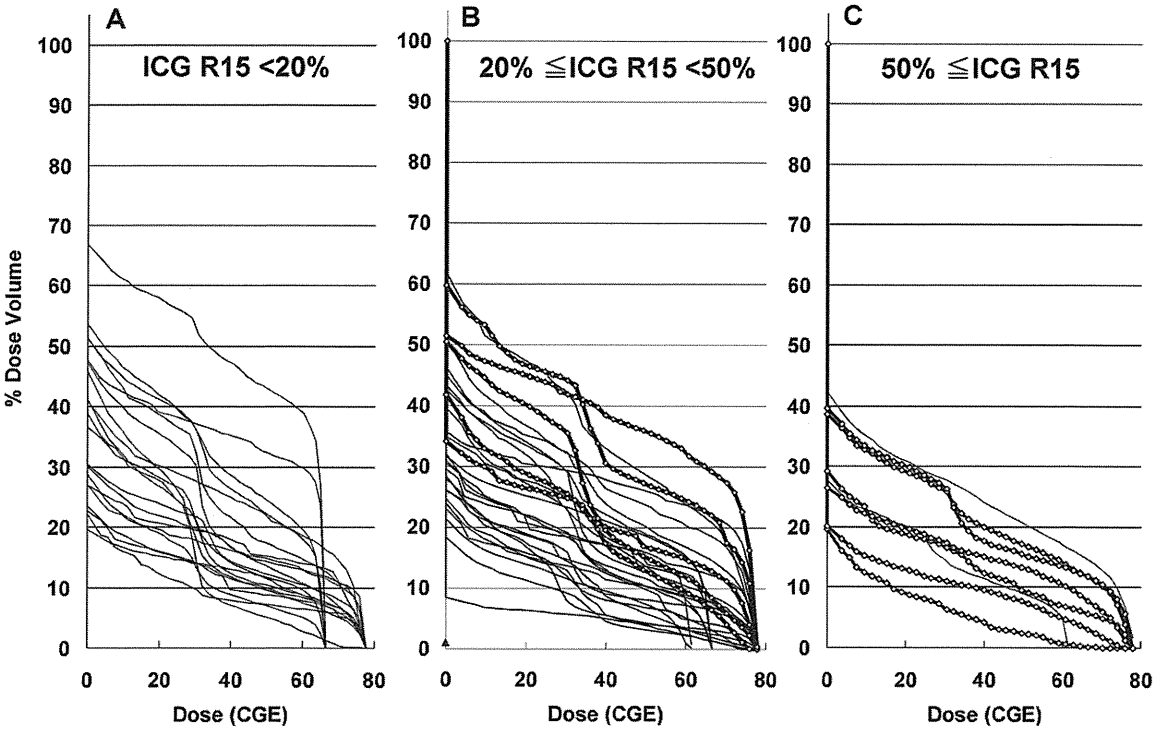


Fig. 1. DVH are shown for all patients according to their pretreatment ICG R15 values, as noted in each panel. Thick lines with rhomboid symbols represent DVHs for patients suffering from hepatic insufficiency within 6 months after completion of PRT.

and did not develop PHI were statistically significant, with p values of 0.012 in V0, 0.009 in V10, 0.012 in V20, 0.006 in V30, 0.016 in V40, and 0.024 in V50 (Mann-Whitney U test). The lowest p value was observed in the difference at V30. Among 32 patients whose ICG R15 values ranged from 20% to 49.9%, none of the 21 patients whose V30 were <25% experienced PHI, whereas 5 of 11 patients (45%) whose V30 was ≥25% developed PHI (p = 0.037, Mann-Whitney U test). The incidence of PHI was 2/25 (8%) in Child-Pugh class A patients, whereas PHI incidence was 3/7 (43%) in class B patients in this group of 32 patients (p = 0.218, Mann-Whitney U test). Of 5 patients who experienced PHI, 1 died at 8 months without evidence of HCC recurrence. PHI spontaneously resolved in 4 patients; 2 patients died of intrahepatic recurrence at 22 and 71 months, respectively; 1 patient died of brain metastasis at 8 months; and 1 patient was alive and disease free at 50 months. In both of the patients who survived for more than 4 years despite development of PHI, the pretreatment functional liver reserve was Child-Pugh class A and ICG R15 was less than 40%. On the other hand, all 3 patients who experienced PHI and died within 2 years had Child-Pugh class B liver functions. Relationships between ICG R15 and V30 according to occurrence of PHI in Child-Pugh class A and B patients are shown in Fig. 3a and b, respectively.

Other serious adverse events

Three patients experienced a gastrointestinal toxicity grade of ≥2. One patient developed hemorrhagic duodenitis associated with anemia at 2 months after completion of 76 CGE/20 fractions/30 days of PRT. The dose administered to the duodenum was estimated to be 50 to 80% of the prescribed dose. Bypass surgery was attempted to alleviate the symptoms; however, this patient died of postoperative hepatic failure at 6 months. Two patients received 65 CGE/26 fractions of PRT, with the entire circumference of the gastrointestinal walls covered within the PTV. One of these 2 patients experienced grade 3 hemorrhagic ulcer at the ascending colon, within the PTV. The patient was managed successfully with right hemicolectomy at 10 months; however, the patient

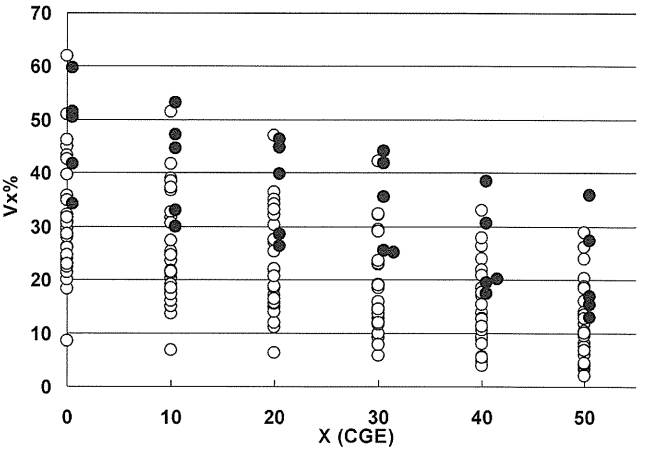


Fig. 2. Distribution of V0 to V50 in DVHs for 32 patients whose pretreatment ICG R15 values ranged from 20% to 49.9%. Open circles represent values for patients who did not experience PHI, whereas closed circles represent those who developed PHI.

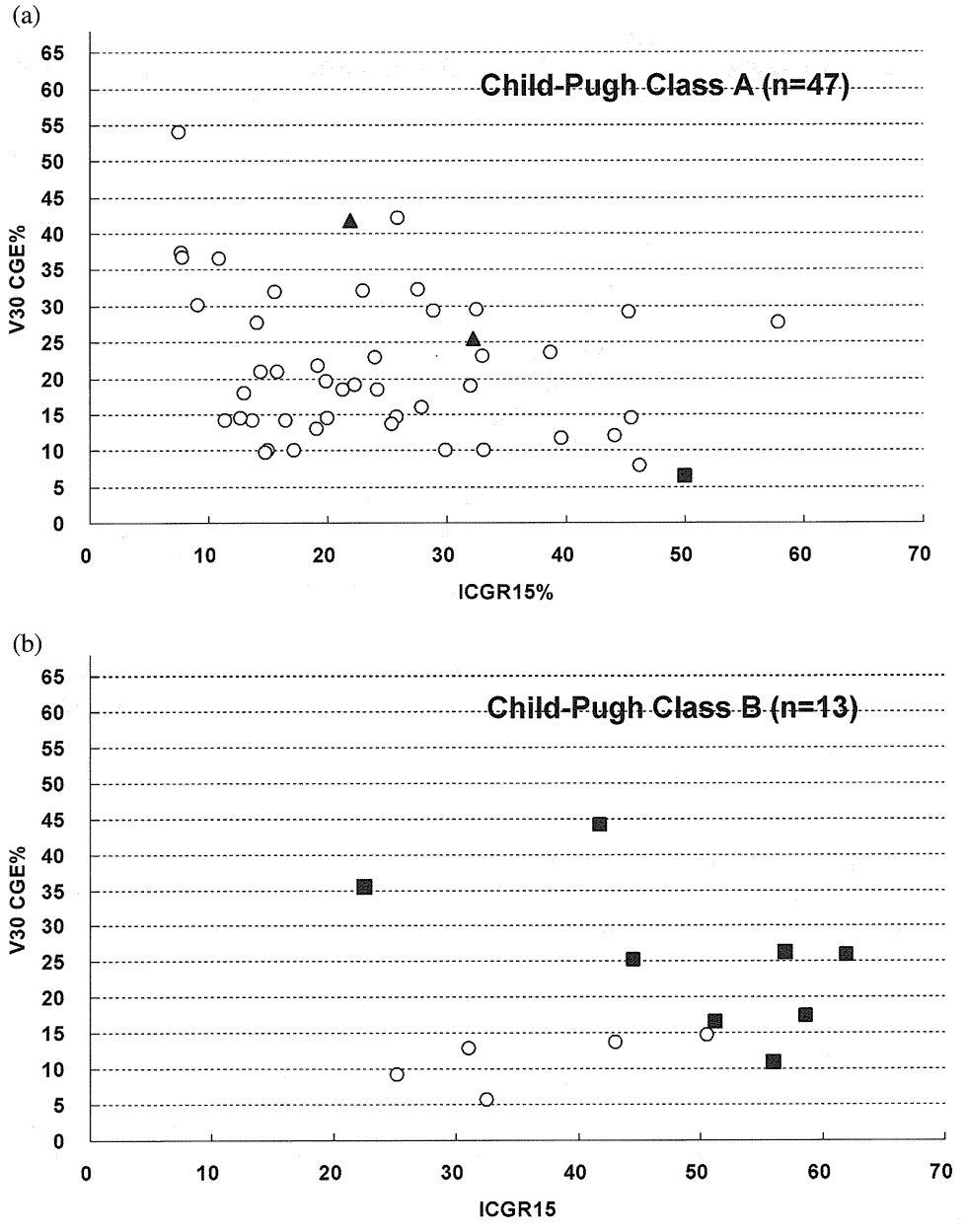


Fig. 3. Scattergram of V30 in each patient who had pretreatment liver functions classified as Child-Pugh class A (a) and class B (b), as shown in each panel, according to the ICG R15 value. Open circles represent values in patients who did not experience PHI. Closed squares represent those who developed PHI and died within 2 years with ($n = 5$) or without ($n = 4$) disease recurrence. Closed triangles represent those who experienced transient PHI and survived for more than 4 years after commencement of PRT.

died of local recurrence and subsequent hepatic failure at 23 months. The other patient developed grade 2 esophagitis within the PTV at 7 months. Repetitive balloon dilatations were required to alleviate the patient's dysphagia; however, the patient was alive without disease and taking a normal diet at 30 months. There were no other observations made of adverse events of Grade ≥3 in any of the patients.

Tumor control and survival

At the time of analysis in August 2009, 42 patients had already died because of intrahepatic recurrence in 27, nodal recurrence in 1, distant metastasis in 3, hepatic insufficiency without recurrence in 9, comorbidity in 1, and senility in 1. Forty of these 42 patients had been free from local progression until death; the durations ranged from 2 to 77 months (median, 20 months). Two patients who experienced local progression died subsequently. A total of 15 patients were alive at 25 to 92 months (median, 43 months) without local progression. Three patients were alive at 49, 53, and 94 months, respectively, after salvage treatment for local progression, using local ablation in 2 and TACE in 1 A total of 37 patients achieved complete disappearance of the primary tumor at 1 to 50 months (median, 10 months) post-PRT. Eighteen patients had residual tumor masses on CT

and/or MRI for 2 to 44 months (median, 21 months) until the time of death or last follow-up visit without local progression. The local progression-free (LPF) rates at 3 and 5 years were 90% (95% confidence interval [CI], 80%–99%) and 86% (95% CI, 74%–98%), respectively.

Of 5 patients who experienced local progression, 3 patients underwent 65 CGE/26 fractions, and 2 patients received 76 CGE/20 fractions of PRT. All 3 patients who received 60 CGE/10 fractions were free from local progression at 6, 30, and 51 months, respectively. LPF rates at 3 and 5 years for 46 patients who received 76 CGE/20 fractions were 97% (95% CI, 92%–100%) and 93% (95% CI, 83%–100%), respectively. LPF rates at 3 years for 11 patients who underwent 65 CGE/26 fractions of PRT were 56% (95% CI, 16%–95%) and was worse than that in patients who received 76 CGE/20 fractions with statistical significance ($p = 0.005$).

A total of 32 patients developed intrahepatic tumor recurrences that were outside of the PTV at 1 to 62 months (median, 20 months). Nine of these tumors occurred within the same segment of the primary tumor. Nodal recurrence at the hepatoduodenal ligament and distant metastasis were observed as the first sites of failure in 2 and 3 patients, respectively. In addition to the above-mentioned five deaths from PHI or postsurgical mortality, 4 patients died of hepatic failure because of underlying liver disease at 17 to 23 months, and 2 patients died from other reasons (comorbidity or senility) without evidence of HCC recurrence. Seven patients remained alive and disease free at 27 to 51 months (median, 30 months). The median survival time for all 60 patients was 41 months, and actuarial OAS rates at 3 and 5 years were 56% (95% CI, 43%–69%) and 25% (12%–39%), respectively. DFS rates at 3 and 5 years were 18% (95% CI, 7%–29%) and 4% (95% CI, 0%–12%), respectively, as shown in Fig. 4. Two Child-Pugh class A patients who underwent PRT with the CTV covering the entire right lobe or right anterior portal segment were alive and disease free at 50 and 26 months, respectively. The former patient had a pre-PRT ICG R15 of 22% and received a V30 of 42% and experienced transient PHI that resolved spontaneously; the latter patient, whose corresponding parameters were 8% and 37%, respectively, did not experience PHI.

Factor analysis

Univariate analyses revealed that factors related to functional liver reserve and occurrence of PHI had significant influence on OAS ($p < 0.05$). Liver function (Child-Pugh class A or B) and prior treatment (none or recurrent) were independent and significant prognostic factors ($p < 0.002$), and occurrence of PHI had marginal significance ($p = 0.011$) by multivariate analysis, as shown in Table 2. The DFS rate at 3 years for 24 patients who had no prior treatment for HCC was 35% (95% CI, 14%–56%), whereas DFS for the remaining 36 patients was 7% (95% CI, 0%–17%) ($p = 0.011$). In Child-Pugh class A patients, OAS at 3 and 5 years for those who had no prior treatment ($n = 17$) was 76% (95% CI, 56%–97%) and 59% (95% CI, 33%–86%), respectively, and 63% (95% CI, 45%–80%) and 25% (95% CI, 7%–42%), respectively, for 30 patients with recurrent tumor ($p = 0.060$). In Child-Pugh class B patients, the 2-year OAS for patients without PHI ($n = 5$) was 80% (95% CI, 45%–100%), while 8 patients who developed PHI died within 2 years with ($n = 5$) or without ($n = 3$) HCC recurrence ($p = 0.009$).

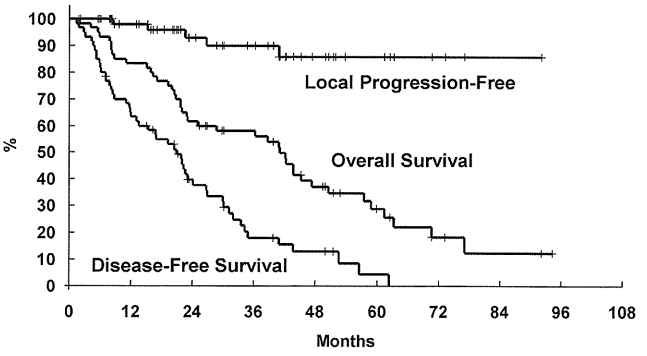


Fig. 4. Kaplan-Meier estimation of local progression-free survival, OAS, and disease-free survival rates for all 60 patients.

DISCUSSION

The promising tumoricidal effect of PRT using aggressive escalation of total and fractional doses, which has been repeatedly reported previously, was reproduced in this study (3, 4). The estimated actuarial local progression-free rate within the PTV in patients receiving 76 CGE/20 fractions exceeded 90% at 3 years. DFS at 3 years for patients who underwent PRT as an initial treatment ($n = 24$) was 35%, and, among them, OAS at 3 years was 76% in Child-Pugh class A patients ($n = 17$). These results are comparable to those observed after surgical treatment (17). Although the number of patients was small, these data indicate that appropriate local control with PRT may provide survival benefit in adequately selected patients with unresectable HCC. The fact that 9 of the 32 intrahepatic HCC recurrences occurred within the same anatomical portal segments showed that it should still be possible to improve the progression-free rate by defining the CTV so it covers undetectable tumor spread via the portal blood flow.

As shown in Fig. 3, no patient who had ICG R15 of less than 20% experienced PHI. In addition, only Child-Pugh class A patients with pre-PRT ICG R15 of less than 40% survived for longer than 4 years despite development of PHI. One of them underwent systematic portal segmental irradiation with the CTV covering the entire right lobe, and the details for this patient will be reported separately. On the other hand, all patients who had pre-PRT liver functions classified as Child-Pugh class B and/or ICG R15 of 40% or higher died within 2 years when they developed PHI. This suggests that the role of systematic portal irradiation requiring a large irradiated volume should be pursued further in Child-Pugh class A patients with favorable ICG R15 values; otherwise, the CTV should be confined to the GTV with adequate margins. Furthermore, in patients who have ICG R15 of 50% or

Table 2. Factors related to overall survival

Factor	No. of patients	% of OAS at 3 years (MST, months)	Univariate *p* value	Multivariate *p* value, hazard ratio (95% CI)
Age				
<70	29	55 (41)	0.660	0.087
≥70	31	61 (42)		0.52 (0.24–1.10)
Gender				
Male	42	62 (41)	0.332	0.194
Female	18	44 (42)		0.62 (0.29–1.30)
Tumor size (mm)				
<50	36	66 (44)	0.178	0.070
≥50	24	46 (23)		0.54 (0.28–1.05)
Pretreatment ICG R15				
<40%	45	67 (44)	0.002	
≥40%	15	33 (15)		
Child-Pugh classification				
A	47	68 (45)	<0.001	<0.001
B	13	23 (15)		0.19 (0.07–0.50)
Serum alfa-fetoprotein level (IU/mL)				
<300	41	61 (42)	0.617	0.618
≥300	19	53 (39)		0.83 (0.39–1.74)
PHI				
No	49	65 (44)	0.001	0.011
Yes	11	18 (9)		0.29 (0.11–0.76)
% of patients receiving V30				
<25%	40	57	0.724	
≥25%	20	60		
Total dose = 65 Gy				
Yes	11	44 (29)	0.646	0.185
No	49	61 (42)		1.88 (0.73–4.76)
Prior treatment				
None	24	67 (47)	0.112	0.002
Recurrence	36	53 (36)		0.32 (0.15–0.66)

Abbreviations: OAS = overall survival; MST = median survival time; CI = confidence interval; PHI = proton-induced hepatic insufficiency.

higher, the indication for PRT should be considered with extreme caution to prevent life-threatening PHI, as shown in Fig. 3.

Results of this retrospective study showed 56% OAS at 3 years in all patients and 68% in 47 Child-Pugh class A patients. All of them were judged strictly as unresectable and not amenable to local ablation. Therefore, a survival benefit of adding PRT to TACE could be expected, which should be tested in randomized trials. Suitable candidates for such a study may be patients who have unresectable HCC of >4 cm in diameter (*i.e.*, a high probability of microscopic vascular invasion) or who show macroscopic vascular invasion, which is amenable to selective segmental TACE as a curative treatment. Nevertheless, before developing that kind of randomized study, data should still be compiled regarding the safety and patterns of failure after PRT combined with TACE while ICG R15 and V30 are taken into account. Preliminary results of hypofractionated stereotactic body radiotherapy for patients with relatively small primary or metastatic liver tumors showed 70% to >90% of objective response rates and 20 or more months of median survival time (1, 18–20). Mature data regarding the relationship between oncological outcomes and tumor characteristics, as well as functional reserve of the liver, are needed to optimize cost-effectiveness of localized, high-dose RT using X-ray or charged particles for treatment of this disease. Nonetheless, RT should have no role in preventing multifocal tumorigenesis, which will be continuously encountered by multidisciplinary approaches (21).

The risk of developing serious gastrointestinal sequela after PRT is another important issue to consider in patients who have HCC located adjacent to the digestive tract. We attempted once-daily fractionation of PRT with 65 CGE/26 fractions. However, 2 of 11 patients who received this treatment developed gastrointestinal toxicity grade of ≥2. Moreover, these 11 patients showed significantly worse LPF rates than those who received 76 CGE/20 fractions of PRT. Three patients who received 60 CGE/10 fractions of PRT were controlled locally. Although our current data are based on a limited number of patients, precluding definitive conclusions, they suggest a low α/β ratio (22) of HCC, and this assumption should be examined further in clinical trials. Based on currently available data, efforts to exclude the gastrointestinal loop from the PTV by using, for example, surgical manipulations, seem to be positively considered in order to expand the role of PRT for HCC.

CONCLUSIONS

In conclusion, PRT achieved excellent local progression-free rates when aggressive, high-dose/fractionation was administered. Child-Pugh class A patients with ICG R15 of less than 40% tolerated PRT of a large irradiated volume well, despite development of transient PHI. However, in Child-Pugh class B patients, it seems reasonable to minimize the irradiated volume to prevent detrimental liver damage induced by PRT and underlying liver diseases. A V30 of less than 25% in the noncancerous portion of the liver is considered an indicator of the safety of PRT in patients who have pre-PRT ICG R15 of 20% to 50%. We believe that there are extremely few indications for PRT in patients who have ICG R15 of 50% or higher. Gastrointestinal toxicity is a major drawback of PRT for tumors adjacent to the gastrointestinal tract, and surgical manipulation to exclude the intestinal loop from the PTV should be positively considered as indicated. If these issues are carefully considered, with special attention to the patterns of tumor spread, when determining the

CTV, aggressive high-dose PRT could become a legitimate treatment for a certain population of patients with unresectable HCC for whom there is no standard treatment available other than TACE or liver transplantation.

REFERENCES

1. Krishnan S, Dawson LA, Seong J, *et al.* Radiotherapy for hepatocellular carcinoma: An overview. *Ann Surg Oncol* 2008;15: 1015–1024.
2. Bush DA, Hillebrand DJ, Slater JM, *et al.* High-dose proton beam radiotherapy of hepatocellular carcinoma: Preliminary results of a phase II trial. *Gastroenterology* 2004;127:S189–S193.
3. Kawashima M, Furuse J, Nishio T, *et al.* Phase II trial of radiotherapy employing proton beam for hepatocellular carcinoma. *J Clin Oncol* 2005;23:1839–1846.
4. Chiba T, Tokuuye K, Matsuzaki Y, *et al.* Proton beam therapy for hepatocellular carcinoma: A retrospective review of 162 patients. *Clin Cancer Res* 2005;11:3799–3805.
5. Kosuge T, Makuuchi M, Takayama T, *et al.* Long term results after resection of hepatocellular carcinoma: experience of 480 cases. *Hepato-Gastroenterol* 1993;40:328–332.
6. The Liver Cancer Study Group of Japan. Predictive factors for long term prognosis after partial hepatectomy for patients with hepatocellular carcinoma in Japan. *Cancer* 1994;74:2772–2780.
7. Makuuchi M, Sano K. The surgical approach to HCC: Our progress and results in Japan. *Liver Transpl* 2004;10:S46–S52.
8. Tsai TJ, Chau GY, Lui WY, *et al.* Clinical significance of microscopic tumor venous invasion in patients with resectable hepatocellular carcinoma. *Surgery* 2000;127:603–608.
9. Vauthey JN, Lauwers GY, Esnaola NF, *et al.* Simplified staging for hepatocellular carcinoma. *J Clin Oncol* 2002;20:1527–1536.
10. Imamura H, Sano K, Sugawara Y, Kokudo N, Makuuchi M. Assessment of hepatic reserve for indication of hepatic resection: Decision tree incorporating indocyanine green test. *J Hepatobiliary Pancreat Surg* 2005;12:16–22.
11. Esnaola NF, Lauwers GY, Mirza NQ, *et al.* Predictors of microvascular invasion in patients with hepatocellular carcinoma who are candidates for orthotopic liver transplantation. *J Gastrointest Surg* 2002;6:224–232.
12. Cammà C, Schepis F, Orlando A, *et al.* Transarterial chemoembolization for unresectable hepatocellular carcinoma: Meta-analysis of randomized controlled trials. *Radiology* 2002;224: 47–54.
13. Takayasu K, Arii S, Ikai I, *et al.* Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. *Gastroenterology* 2006;131: 461–469.
14. Lawrence TS, Tesser RJ, Ten Haken RK. An application of dose volume histograms to the treatment of intrahepatic malignancies with radiation therapy. *Int J Radiat Oncol Biol Phys* 1990;19:1041–1047.
15. Tsujii H, Tsuji H, Inada T, *et al.* Clinical results of fractionated proton therapy. *Int J Radiat Oncol Biol Phys* 1993;25:49–60.
16. Ando K, Furusawa Y, Suzuki M, *et al.* Relative biological effectiveness of the 235 MeV proton beams at the National Cancer Center Hospital East. *J Radiat Res* 2001;42:79–89.
17. Schwartz JD, Schwartz M, Mandeli J, Sung M. Neoadjuvant and adjuvant therapy for resectable hepatocellular carcinoma: review of the randomised clinical trials. *Lancet Oncol* 2002;3: 593–603.
18. Méndez Romero A, Wunderink W, Hussain SM, *et al.* Stereotactic body radiation therapy for primary and metastatic liver tumors: A single institution phase I-II study. *Acta Oncol* 2006;45: 831–837.
19. Choi BO, Jang HS, Kang KM, *et al.* Fractionated stereotactic body radiotherapy in patients with primary hepatocellular carcinoma. *Jpn J Clin Oncol* 2006;36:154–158.
20. Liang SX, Zhu XD, Lu HJ, *et al.* Hypofractionated three-dimensional conformal radiation therapy for primary liver carcinoma. *Cancer* 2005;103:2181–2188.
21. Avila MA, Berasain C, Sangro B, Prieto J. New therapies for hepatocellular carcinoma. *Oncogene* 2006;25:3866–3884.
22. Thames HD, Withers HR, Peters LJ, *et al.* Changes in early and late radiation responses with altered dose fractionation: Implications for dose-survival relationships. *Int J Radiat Oncol Biol Phys* 1982;8:219–226.

第7回DIA日本年会
グローバル開発における日本の役割・日本の貢献
大学・研究機関・企業での臨床試験の実際
国立がん研究センター東病院での
がんペプチドワクチン療法臨床試験の取り組み

独立行政法人国立がん研究センター東病院　がん治療開発部機能再生室長　中面　哲也

我々は，国立がん研究センターで医師主導の臨床研究として「がんペプチドワクチン（peptide vaccine）療法」を行っていますが，このような免疫療法はまだエビデンス（evidence）が確立されておらず，我々が臨床研究をどんどんやっていかないといけない分野だと思っています。その中から良いものがあれば製薬会社にぜひ拾っていただいて，開発していただきたいと思っております。全部最初から治験でやるというのは無理がありますので，我々のような者が医師主導の臨床研究を行って，いろいろな抗原をどんどんみつけて良いものを製薬会社の人に拾っていただき，治験として開発していただくというのが良いと思っております。なお，本日（2010.10.29）は，この後，癌治療学会ともブッキングしておりまして，パネルディスカッションは残念ながら出られないのですが，ご容赦ください。

先日の朝日新聞の記事にありましたように，がんワクチンがちょっと叩かれています。明日（2010.10.30）もがん免疫学会主催で緊急のシンポジウムが東京大学医科学研究所で開催されます。私はそちらでも話しますけれども，今日は20分の持ち時間なので，いろいろ話したいことはあるのですが，限られた時間内で，できるだけのことを話したいと思います。詳細は「臨床医薬」にも掲載されますし，西條長宏先生が監修された「Mebio」という雑誌の2010年12月号に特集が組まれますので，そちらもご覧いただければと思います。

私はもともと外科医で肝胆膵外科のレジデント（resident）をやっていたということもあり，国立がん研究センターに呼ばれて丸5年になります。江角先生に呼んでいただいたのですが，最初はこのGlypican-3（GPC3）のペプチドワクチンのフェーズⅠ（phase Ⅰ）を立ち上げるということで呼ばれました。国立がんセンターというところは化学療法が専門の先生が多いため，免疫療法を信用していない先生が多くて，かなり立ち上げに苦労しました。この臨床試験も，倫理審査委員会に出してから承認までに1年間かかりました。最近では西條先生はじめ諸先生方もペプチドワクチンをある程度評価してくださっていることから，がんセンターの中でも免疫療法の地位は確立できたと考えております。ただ，まだまだエビデンスが足りませんので，我々は一生懸命頑張っていきたいと思っています。

今日は主にこのGPC3についての発表になります。

GPC3というのは，もともと当時東京大学医科学研究所の中村祐輔先生のラボ（labo）のcDNAマイクロアレイ（microarray）のデータからみつけたものです。肝細胞がんの8割で高発現している遺伝子で，正常の臓器にはほとんど発現していない遺伝子としてみつけました。胎盤と胎生期の肝臓で発現している遺伝子で，胎盤と胎生期の肝臓は免疫学的に守られていますので，これがもし有望な抗原であれば，副作用が起きない理想的な抗原になるということでみつけました。蛋白レベルで免疫染色をしても肝細胞がんでは染まりますが胆管細胞がんでは

全く染まらず，きれいな特異性があり，胎盤と胎生期の肝臓ではヒトでもマウスでも同じように発現が認められます。

GPC3は，肝細胞がんでは8割の人が陽性なのですが，陰性の2割の人は予後が良くて，陽性の8割の人は予後が悪いという結果が得られています。

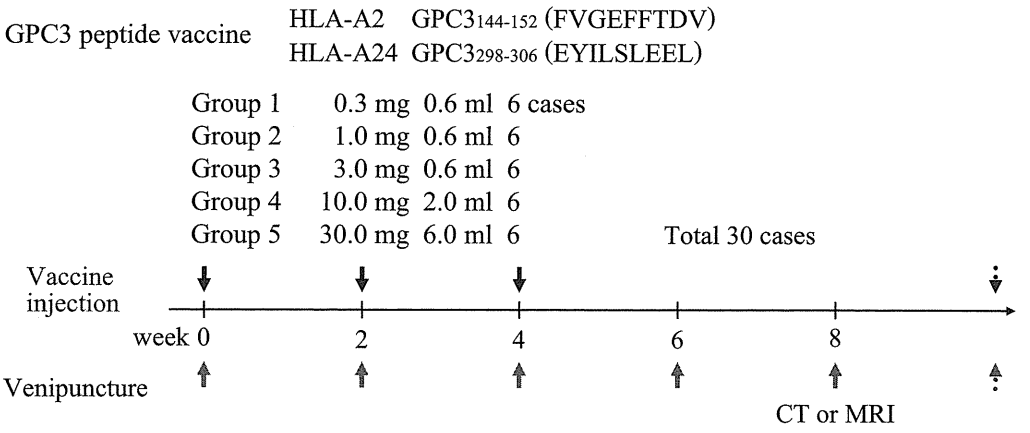
我々は熊本大学時代からこのGPC3におけるHLA-A24とHLA-A2，つまり，日本人に多いタイプのHLAに対するペプチドを同定して参りました。HLA-A24が日本人の6割で，HLA-A2は欧米白人においてメジャーなものですが，日本人の4割が陽性です。2つのペプチドを合わせると，日本人の85%に対応可能です。マウスの実験もいろいろとやり，ペプチドが有望で副作用を起こさない抗原であるということを証明してきました（**表1**）。

苦労した末に，2007年2月にスタートした臨床第1相試験は，進行肝細胞がんを対象としたスタディ（study）です。当初は再発予防のアジュバント・セッティング（aduvant setting）でやろうとしたのですが，「進行がんでやりなさい」ということで，進行がんでスタートしました。使っているのはこの2種類のペプチドで，あまり免疫療法には投与量は関係ないといわれていたのですが，抗がん剤に倣う形で，0.3mg，1 mg，3 mgとドース・エスカレーション（dose escalation）していく設定にしました。

3 mgが一番有望な結果が出ましたので，10mg，30mgを追加することになりましたが，**図1**の

表1　GPC3 is an ideal tumor antigen for immunotherapy in mouse models

We identified
HLA-A24 (*A*2402*)-restricted GPC3$_{298\text{-}306}$ (EYILSLEEL),
HLA-A2 (*A*0201*)-restricted GPC3$_{144\text{-}152}$ (FVGEFFTDV),
Nakatsura T. *Clin. Cancer Res.* 10 : 8630-8640 2004.
Komori H. *Clin. Cancer Res.* 12 : 2689-2697 2006.
IFA is one of indispensable adjuvants for peptide-based immunotherapy, and the immunological effect of peptide vaccines depends on the dose of peptide injected.
Motomura Y. *Int. J. Oncol.* 32 : 985-990, 2008.



- The principal endopoints : toxicity and immunological responses
- The secondary endopoint : clinical responses.
  Clinical responses at 2 months after 1st vaccination (RECIST criteria)
  Monitoring the level of serum tumor markers

図1　Phase I clinical study of GPC3 derived peptide vaccine

量をみると分かるように，3 mg まではペプチドが溶けるのですが 30mg となるとペプチドは溶けないので，混ぜる IFA（Incomplete Freund's adjuvant：不完全フロイントアジュバント）の量も 3 倍，10 倍になりました。6 cc の中には 3 cc の IFA が入っていますので，30mg の場合は IFA の副作用が 10 倍高いということになります。進行がんでフェーズⅠですので，限られた設定で 2 週間に 1 回，3 回ワクチンして，2 ヵ月間で評価するという設定をとらざるを得ませんでしたが，患者さんの QOL（Quality Of Life）がかなり良かったものですから，「もっと打ってくれ」という患者さんの要望が強くなりました。そこで，最初のころの前半の患者さんは継続投与ができなかったのですが，あとで倫理審査委員会に承認していただいて，後半の 12 人は継続投与が可能になっています。フェーズⅠのエンドポイント（end point）は，安全性と免疫学的な有効性，そして二次的にクリニカル（clinical）なレスポンス（response）をみるということです。

ペプチドワクチンは「何で効くのか」というメカニズム（mechanism）なのですが，まず肝細胞がんの HLA（Human Leucocyte Antigens：ヒトリンパ球抗原）class 1 には――すべての細胞に HLA class 1 が出ています――**図 2** で示す GPC3 のペプチドが出ています。通常はキラーＴ細胞が GPC3 のペプチドを見分けて殺しているはずなのですけれども，その機構が破綻しているのが，がん患者さんにあらわれているがんの塊です。正常細胞にはこの GPC3 のペプチドは出ていません。このペプチドは 9 アミノ酸が繋がったものですから人工的につくることができ，GMP（Good Manufacturing Practice：優良医薬品製造基準）グレードのものをつくってもらい使っております。それを IFA と混ぜて白い液を患者さんの脇の下の皮膚，皮内に注射しています。皮内にはランゲルハンス（Langerhans）細胞というプロフェッショナル（professional）抗原提示細胞がたくさんおり，ふだんはこのランゲルハンス細胞は自分の細胞の中でできたペプチドを出しているのですが，大量に GPC3 のペプチドを打ちますと，これが置きかわって，GPC3 ペプチドを乗せたランゲルハンス細胞がたくさんでき上がります。それが脇の下のリンパ節に移動して，GPC3 のペプチドを認識する T-cell receptor（T 細胞抗原受容体）を持つ CD8 陽性のキラーＴ細胞（Killer T cell）を活性化して増やすというメカニズムになっており，増えたキラーＴ細胞は GPC3 のペプチドを出している肝臓がんの細胞だ

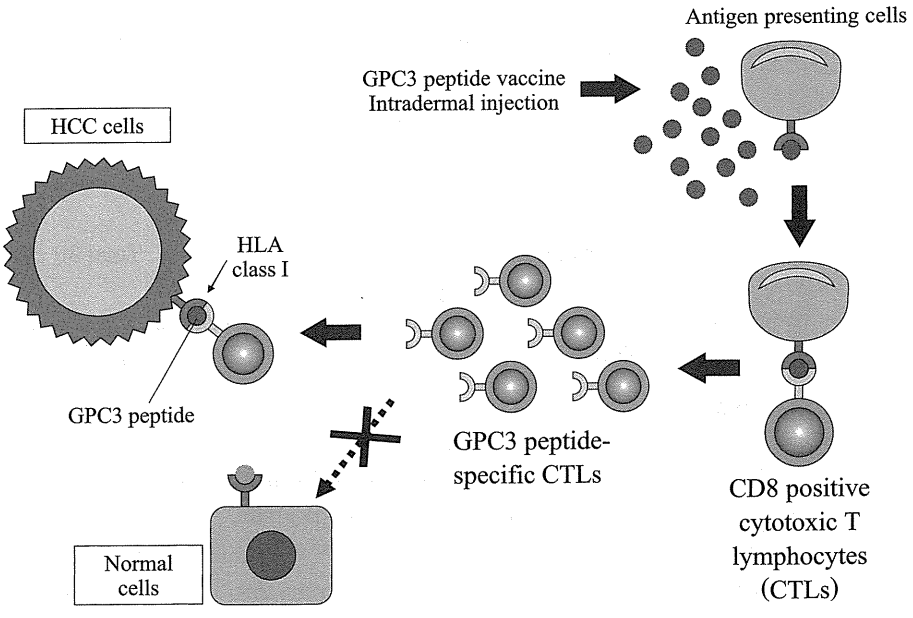


図 2　The mechanism of inducing tumorspecific CTLs by peptide vaccine

けを殺して，正常は殺さないという仕組みです。

今回のフェーズⅠは，33人がエンロール（enroll：登録）して，3人は3回ワクチンの2ヵ月が完遂できませんでしたので，今回は完遂できた30人での解析結果を示します（**図3**）。

対象は，比較的肝機能の良い人が入っているのですけれども，半分はステージ4（Stage 4）の遠隔転移のある患者さんで，半分は肝臓のがんが大きくて治療できない，有効な治療がないという患者さんが入っております。HLA-A24とA2は半分ぐらいが入っていまして，前治療は様々で，いろいろな治療が抵抗性になった患者さんが30人入っています（**表2**）。

GPC3の免疫染色とHLA class1の免疫染色における30人の内訳ですが，8割以上の人がGPC3も発現していて，HLA class1も発現しているという患者さんたちです（**表3**）。

まず安全性ですが，DLT（Dose-Limiting Toxicity：用量規制毒性）は一例も出現しませんでした。グレード2までの副作用でおさまっています。全員に起こるのが，投与局所の発赤，

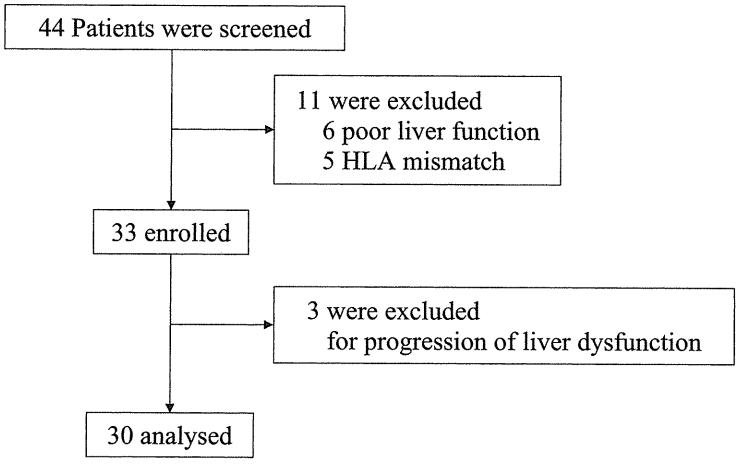


図3 Patient's Enrollment

表2 Patient characteristics

Age : 42-77 (mean 64.83) Sex M 25 F 5
PS 0 : 29 1 : 1 Child-Pugh A : 25 B : 5
Stage Ⅱ : 5, ⅢA : 6, ⅢC : 4, Ⅳ: 15
HCV : 14, HBV : 7, NBNC ; 9
HLA-A24 :16, HLA-A2 :14 (0201 : 11, 0206 : 3, 0207 : 2)
Prior therapy
TACE 8, TAI 6, Chemotherapy 7, RT 5, ope 2, RFA 1, none 1

表3 Immunohistochemical analysis of GPC3 and HLA class I

• Glypican-3 (GPC3)	• HLA class I
• ++ : 5 (19.2%)	• ++ : 3 (11.5%)
• + : 16 (61.5%)	• + : 20 (76.9%)
• − : 5 (19.2%)	• − : 3 (11.5%)
• NT : 4	• NT : 4
• Positive rate 80.7%	• Positive rate 88.4%

硬結で，これはグレード1で起こります。打ったときの一過性の発赤ですが，これは7割の人に認められ異所性の発疹が2人に認められました。37度5分以上の熱は一過性の熱なのですが，30人中6人に認められ38度以上は3人でした。いずれも解熱鎮痛剤を使用しないで，一過性に自然解熱しており，かゆみが5例あり，この5人には抗ヒスタミン剤の塗り薬を処方しています（**表4**）。

次に免疫学的な有効性について述べます。ペプチドを打つと本当にペプチド特異的キラーT細胞はできるのかというところがこの試験の大きなポイントで，エクスビボ（ex vivo：生体外）のIFN-γ（Interferon-gamma：インターフェロンガンマ）のエリスポット・アッセイ（ELISPOT assay：測定法）とデキストラマー（Dextramer）を使ったフローサイトメーター（flow cytometer）の解析を行いました。HIV（Human Immunodeficiency Virus）のエイズウイルスのペプチドに対するキラーT細胞は我々の体にはいませんので，これがネガティブコントロール（negative control）となります。打ったGPC3のペプチドに対するキラーT細胞はどれぐらいいるかというアッセイ（assay）ですが，この30mgを3回打った患者さんでは，投与前は血液中の50万個のリンパ球の中には1個もペプチド特異的キラーT細胞はいないのですが，2回ワクチンを打った後に50万個中441個，0.09%のペプチド特異的なキラーT細胞が出現しました。フローサイトメーターでも同じで，この0.1%というのはどのくらいインパクトを持つかといいますと，全身で1兆個のリンパ球がいますけれども，10億個のペプチド特異的なキラーT細胞が，わずか2回か3回のワクチンで誘導されたことになります。

全員でこの解析をやり，ほとんどの患者さんでワクチン前に比べてワクチン後にペプチド特異的なキラーT細胞の数が増えています。しかも，その投与量の依存性が確認されていて，投与量が増えれば増えるほど，最高の数が**図4**のように増えていっているということが分かります。

平均と中央値をとってみても，0.3mgのワクチンよりも3 mgのワクチンが良いということは明らかで，「30mgは意味があるか」というのは議論があるところでした（**図5**）。

もう1つ，今回のエビデンスとしては生検はしづらいのですが，7人の患者さんからワクチン後に腫瘍の生検をさせていただきました。そうしますと，7人中5人でワクチン前のがんの中には茶色に染まったキラーT細胞はほとんどいないのですが，ワクチン後にがんの中にキラーT細胞がたくさん入っている像が確認できました。

臨床効果ですけれども，ネクローシス（necrosis）が起こった患者さんの例では，この患者さんでは一番大きい腫瘍に真っ黒い壊死が起こり，こちらの患者さんの場合たくさんの肝細胞がんの中に壊死が起こりました。

次に縮小した症例ですけれども，肺門のリンパ節が縮小した症例と，胸部のリンパ節が縮小した症例があります。

表4 Toxicity

Adverse Event (CTCAE v3.0)	Grade 1	Grade 2
Allergic reaction (Flushing or Rash) 24 (80%)	transient flushing 22 (73.3%)	ectopic rash or flushing 2 (6.7%)
Allergic reaction (Drug fever) 6 (20%)	drug fever of ＜38°C 3 (10%)	drug fever of ≧38°C 3 (10%)
Injection site reaction 30 (100%)	erythema ; induration 30 (100%)	pain or swelling with inflamation 0
Itching 5 (16.7%)	mild or focal itcing 5 (16.7%)	severe or wide itcing 0

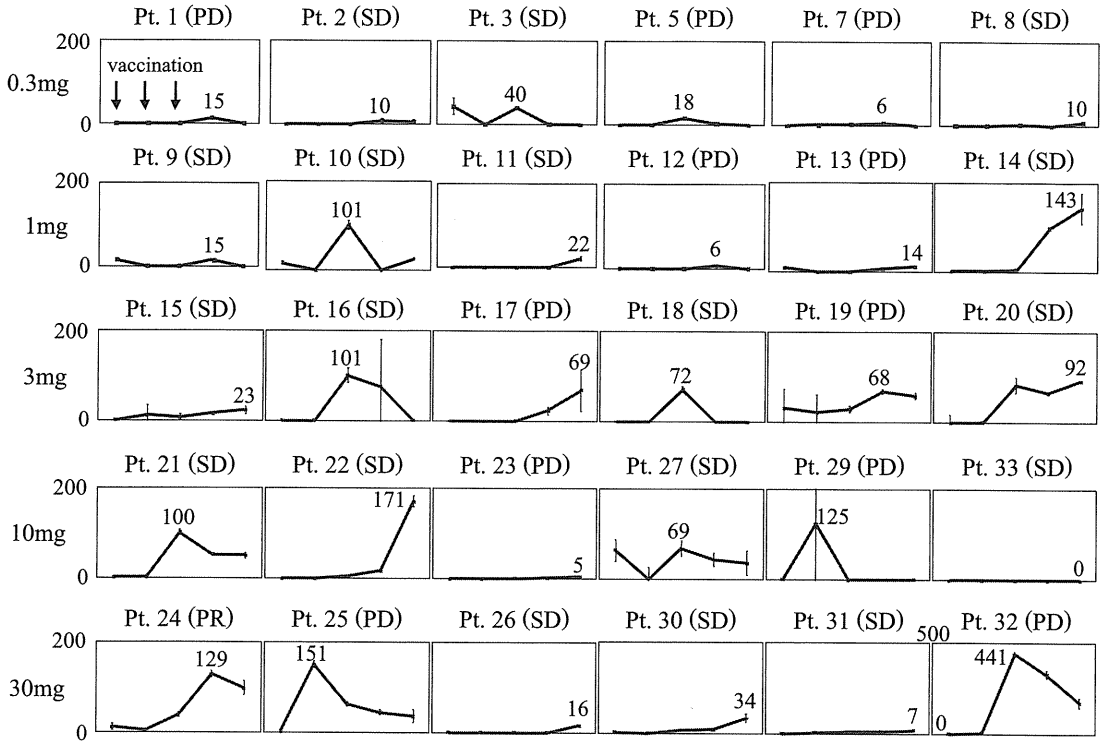


図4　Immunological responses (1)

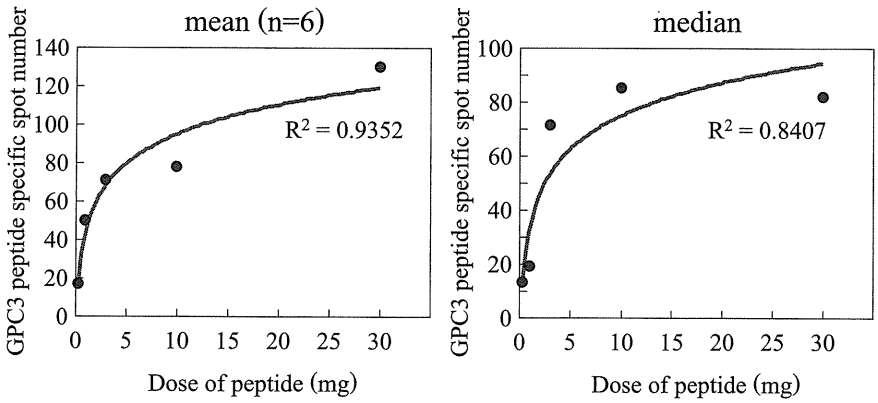


図5　Immunological responses (2)

次に一番レスポンスがあったPR（Partial Response：部分寛解）の患者さんの結果を示します。ワクチン前の状態で，多発の肝細胞がん，骨転移，肺転移，リンパ節転移の患者さんが30mgを3回打った結果，5.5cmの骨転移が大幅に縮小して，別の骨転移も真っ黒く壊死になってしまいました。1.4cmの肝臓内の腫瘍2個は完全に消えて，エコー（echo：超音波検査）でもみえなくなりました。1.4cmの腫瘍が，CT（Computed Tomography）上は同じ大きさで残っているようにみえるのですが，ここを生検させてもらうと，何とがんの中の半分はキラーT細胞という状態で，がんの量としては半分になっていることが分かりました。

表5が臨床効果のまとめです。RECIST criteria（Response Evaluation Criteria In Solid Tumorsによるガイドライン）で1例のPRと，2ヵ月間で12例のSD（Stable Disease：不変）ということになっています。ネクローシスか，サイズの縮小がみられた患者さんが30例中5

表5　Clinical responses at 2 months after 1st vaccination (RECIST criteria)

peptide dosage	Clinical response				necrosis or size reduction of tumors	decrease tumor markers in the sera
	PR	SD	PR+SD	PD		
0.3mg	0	3	3 (50.0%)	3	1	1/6 (16.7%)
1.0mg	0	4	4 (66.7%)	2	0	4/6 (66.7%)
3.0mg	0	4	4 (66.7%)	2	3	5/5 (100%)
10mg	0	4	4 (66.7%)	2	0	6/6 (100%)
30mg	1	3	4 (66.7%)	2	1	6/6 (100%)
total	1	18	19 (63.3%)	11	5 (16.7%)	22/29 (75.9%)

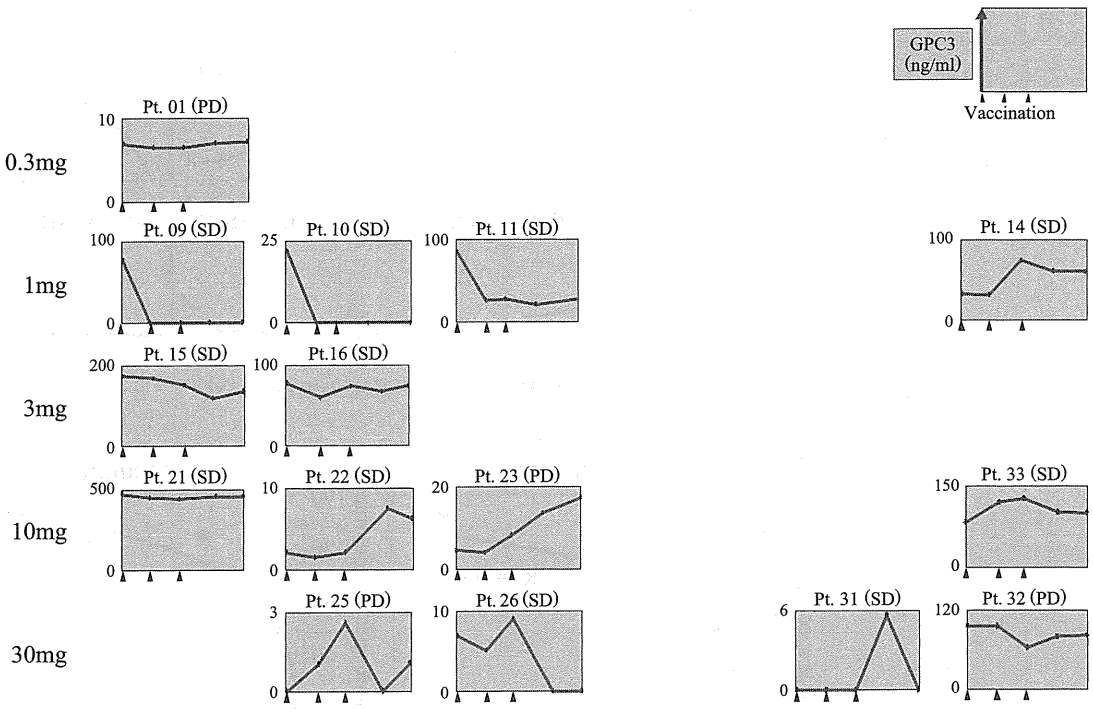


図6　Decrease of GPC3 in the sera 100% 15/15 SD rate：73% 11/15

例で，AFP（Alpha Fetoproteins：癌胎児性蛋白），PIVKA-Ⅱ（Protein Induced by Vitamin K absence or Antagonist-Ⅱ：ビタミンK依存性凝固因子前駆体Ⅱ），GPC3の腫瘍マーカー（tumor marker）が2ヵ月の間に1回でも下がった患者さんは76%という結果です。

GPC3は腫瘍マーカーにもなるのですが，投与前に陽性だった15例全員がワクチン期間中に1回はGPC3が下がり，その下がった人のSD率は73%ですので，全体のSD率63%より高いということで，バイオマーカー（Biomarker：生物指標化合物）にもなり得ると考えています（図6）。

PIVKA-Ⅱも同じで，6割の人がワクチン投与によって下がったのですが，下がった人は75%がSDになっています（図7）。

今回投与量は0.3mgから30mgまで，3回しか打っていない人が18人含まれていますので，全く意味がないのですが，overall survival（OS：全生存期間　9ヵ月）はソラフェニブ（sorafenib：複数のキナーゼ阻害薬）のフェーズⅡの結果と比べると，遜色ない結果が得られています。本当に進行がんの方の予後を延ばせるのかというのは，今後のフェーズⅡとしての

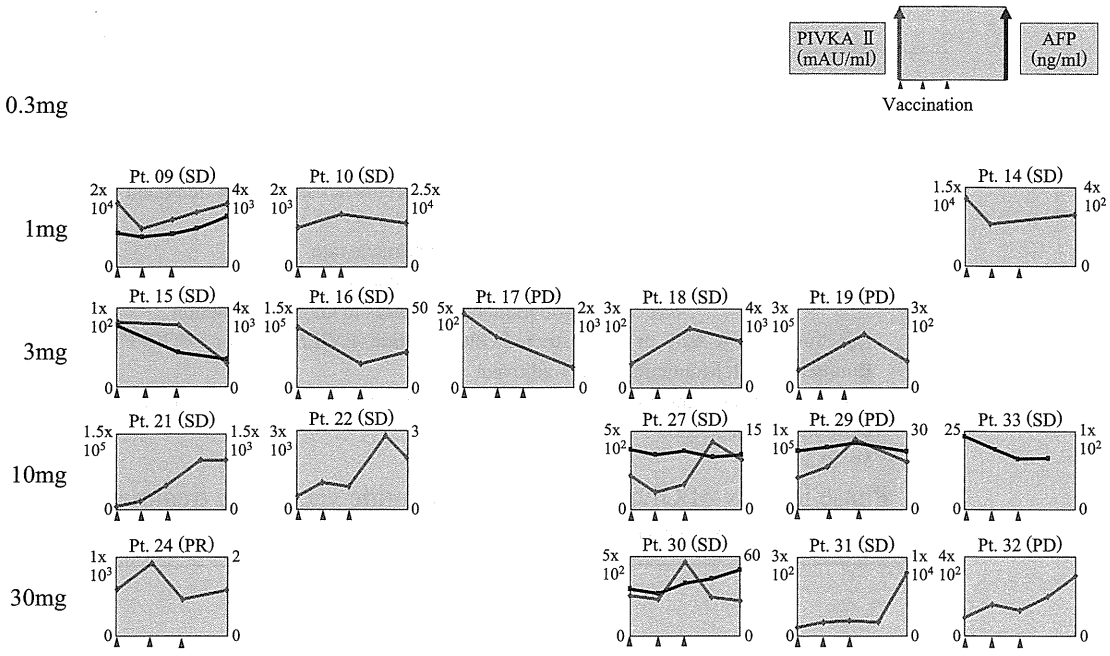


図 7　Decrease of PIVKA Ⅱ in the sera 59% 16/27 SD rate : 75% 12/16

表 6　Result of the Phase I study

- Toxicity consisted of mainly local erythema at the GPC3 vaccine injection sites in all patients.
- As for immunological response, we found an increase of the GPC3 peptide-specific CTLs in 5×10^5 Peripheral Blood Mononuclear Cells (PBMCs) of almost all patients by ex vivo IFN-γ Enzyme-Linked ImmunoSpot (ELISPOT) assay.
- Furthermore, about 60% of cases showed stable disease (SD) at 2 months after 1st vaccination. Some cases showed necrosis or reduction of tumor after vaccination.
- Serum tumor marker levels decreased in many cases after vaccination.
- One of 3 cases received 30mg, 3 times vaccination showed partial response (PR), including disappearance of some tumors.

課題になっていきます。

表 6 はフェーズⅠの結果のまとめですが，安全性に問題がなく免疫学的に有効性が確認され，臨床的にもそこそこいけたのではないかと考えております。では，進行がんの人に本当にQOL を保ちながら OS（overall survival）を延ばせるのか――QOL を保つのは間違いないと思っているのですが，抗がん剤と同じくらいの OS が期待できれば，QOL がいい分，十分，薬になると考えております。

現在進行中の臨床試験ですが，そのフェーズⅠの結果を踏まえて 30mg で PR が出たのですが，3 mg でも十分有効そうだということと投与局所の副作用，あるいはグレード 2 の副作用が 30mg では多いということで，推奨投与量は 3 mg に設定しています。これは切除後，ラジオ波後の初回治療の患者さんですけれども，我々の施設でも 1 年再発率 4 割，2 年再発率 6 割以上という結果になっていて，それをどれくらいの確率に落とせるかという臨床試験で，1 アーム（arm）なのですが，40 例で現在実施していて，24 例登録したところです。このよう

表7　A Phase Ⅱ study is on going

A Phase Ⅱ study of Glypican-3 (GPC3) peptide vaccine as adjuvant treatment for Hepatocellular carcinoma (HCC) after Surgical resection or Radiofrequency ablation (RFA)

To evaluate efficacy and safety of GPC3 peptide vaccine in the adjuvant treatment of HCC after potentially curative surgical resection or RFA
Primary endpoint : The one-year and two-year recurrence rate
Secondary endpoint : Adverse effects of GPC3 vaccination
GPC3-specific immune-responses to GPC3 vaccination
Injection of HLA-A24- or -A2-restricted GPC3 peptide (EYILSLEEL or FVGEFFTDV)
Emulsified with Montanide ISA51 adjuvant
3 mg intradermally injection, every 2 weeks, 6 times, and every 2 months, 4 times, total 10 times in a year, until disease recurrence
N=40 cases

Phase Ⅱ study of Glypican-3 (GPC3) peptide vaccine as treatment for clear cell adenocarcinoma of ovary

表8　Next plan

- We are planning the randomized Phase Ⅱ study of Sorafenib +/− GPC3 peptide vaccine for advanced HCC patients.
- GPC3 is also expressed on the hepatoblastoma, Wilmus tumor, squamous cell carcinoma of the lung, and so on. We are also planning Phase Ⅰ or Ⅱ studies on these cancers.

なワクチン療法は再発予防とか予防に生かすべきだと思っていて，もちろん進行がんの患者さんのQOLを保ったまま予後を延ばせるかという方も薬にしたいのですが，再発予防，あとは予防です。B型肝炎やC型肝炎の患者さんは日本には350万人といわれていますから，その患者さんたちの肝細胞がんの発症を抑えるということを考えながらやっていきたいと考えています（表7）。

また，卵巣の明細胞腺がんでも名古屋大学でフェーズⅡがスタートしており，そのほか，ウィルムス・テューマー（Wilms tumor：ウィルムス腫瘍）やヘパトブラストーマ（hepatoblastoma：肝芽腫），肺の扁平上皮がんでもGPC3が出ていることが確認されていますので，これらの臨床試験も計画中です。また進行肝細胞がんに関しては，ソラフェニブが標準治療になっていますので，ソラフェニブとGPC3併用あり・なしのランダム化比較試験（randomized controlled trial：無作為化対照試験）を計画して，近いうちに，倫理審査委員会に出すところです。また，ペプチドワクチン単独での免疫学的な腫瘍内の有効性をみるという臨床試験も倫理委員会に申請中です。ということで終わりなのですが，いろいろ言いたいことはあるのですが，このようながんワクチンは本当に有効なのかということは，我々が臨床研究でどんどん臨床試験をやっていくしかないと考えておりまして，それでいいものが出てきたら製薬会社の人にぜひ拾っていただいて，治験をやって確認していただいて，ここから薬が出ていくことを期待しております。がん患者さんのために何とか我々も頑張って，製薬業界の方にも手伝っていただき，どんどん薬が出ればと考えております（表8）。

以上です。