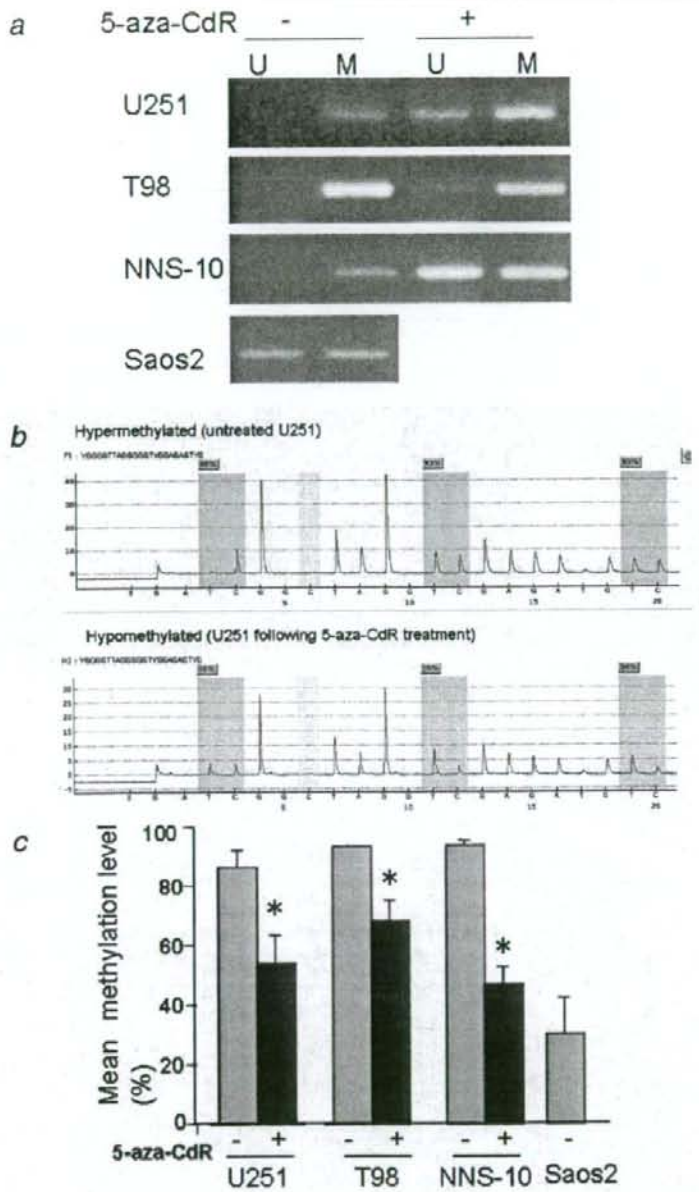


FIGURE 3 – *NY-ESO-1* methylation analyses. (*a*) DNA methylation of the *NY-ESO-1*-gene was measured by MSP. The *NY-ESO-1* is heavily methylated in glioma cells but not in SaOS2 cells before 5-aza-CdR treatment, and it is hypomethylated following 5-aza-CdR exposure. U and M, reactions for unmethylated and methylated sequences, respectively. (*b*) Representative pyrograms of hyper- and hypomethylation. The sequence in the upper part of each pyrogram represents the sequence under investigation. The sequence below the pyrogram indicates the sequentially added nucleotides. The gray regions indicate the analyzed C/T sites; the percentage values for the respective cytosine methylation are provided above them. Yellow regions indicate the positions where a cytosine was added to verify the complete conversion from unmethylated cytosine to thymine. (*c*) Quantitative analyses of NY-ESO-1 methylation by pyrosequencing. Mean methylation levels ± SD were 86.3% ± 5.5%, 93.3% ± 0.6% and 93.3% ± 1.5% for U251, T98 and NNS-10 glioma cells, respectively, while it was 30% ± 12% for SaOS2 cells constitutively expressing NY-ESO-1. Following 5-aza-CdR treatment (+), methylation levels were significantly decreased to 54% ± 9.1%, 68% ± 6.9% and 46.7% ± 5.7% for U251, T98 and NNS-10. * $p < 0.05$ compared to untreated (−) cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

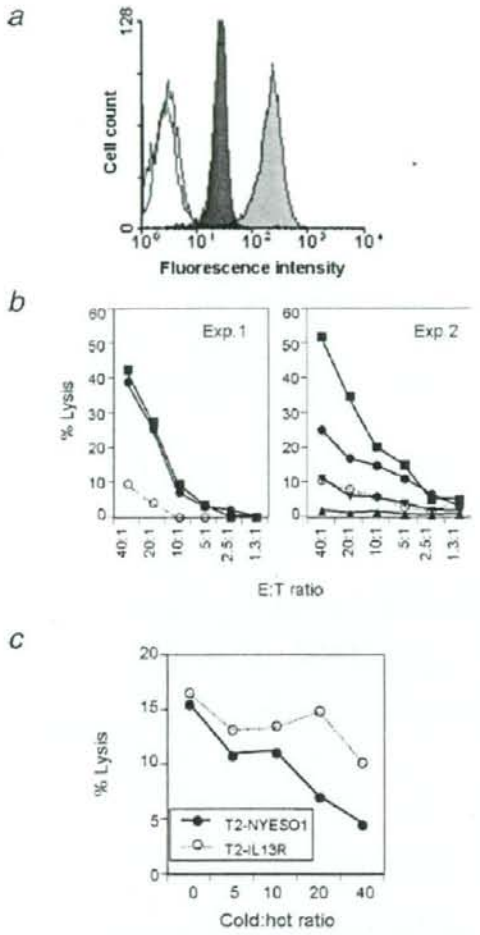


FIGURE 4 – Antigenic potentiation of glioma cells by 5-aza-CdR. (*a*) Upregulation of HLA class I. Untreated U251 glioma cells (black) and those treated with 5-aza-CdR (gray) were stained with anti-HLA class I mAbs (W6/32). Negative staining of untreated and treated U251 cells with isotype control mouse IgG2a is shown (white). (*b*) Cytolytic activity of 2 independently induced NY-ESO-1 CTL lines against U251 (HLA-A2$^+$) before (○) and after (●) 5-aza-CdR treatment; other NY-ESO-1$^-$ glioma cells, SK-MG-1 (A24, ▲); human osteosarcoma cells, SaOS-2 (A2, NY-ESO-1$^+$, ■); and human myeloid leukaemia cell line K562 (▼). Exp. 1, Experiment 1; Exp. 2, Experiment 2. (*c*) The CTL-mediated target cell (5-aza-CdR-treated U251) lysis was blocked using T2.A2 cells that had been loaded with the HLA-A2-binding NY-ESO-1 peptide p157-165 (SLLMWITQC) (●) but not with the control peptide, *i.e.*, the HLA-A2-binding IL-13Rα2 peptide p345-354 (WLPFGFILI) (○). The same CTL line as in the Exp. 2 of Figure 4*b* was used in the experiment (E:T ratio, 10:1).

glioma cells, administration of the agent did not induce CTA expression in these cells (Fig. 2*b* and Table II).

To assess whether the induction of CTA mRNA is followed by the production of the appropriate protein, western blotting for NY-ESO-1 was performed using untreated and 5-aza-CdR-treated glioma cell lines. The SaOS-2 osteosarcoma cell line, which constitutively expresses *NY-ESO-1* without 5-aza-CdR treatment, was used as a positive control. As shown in Figure 2*c*, 23-kDa bands corresponding to NY-ESO-1 were observed in all glioma cell lines treated with 5-aza-CdR but were absent in the untreated cells (all data not shown). This result suggested that the *NY-ESO-1* expression was induced after 5-aza-CdR treatment at the protein level as well as the mRNA level.

Quantitative CpG island mapping with Pyrosequencing™

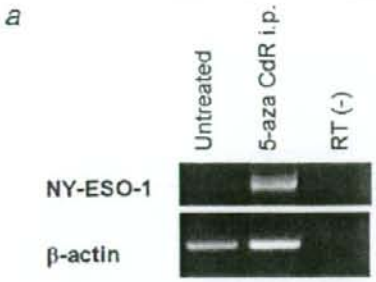
MSP experiments were performed to evaluate the methylation status of *NY-ESO-1* in cultured glioma cells. We observed that the *NY-ESO-1* is heavily methylated in glioma cells before 5-aza-CdR treatment and becomes hypomethylated following 5-aza-CdR exposure (Fig. 3*a*). To quantify the methylation of the CpG sites of *NY-ESO-1*, we employed a novel real-time DNA sequencing technology called Pyrosequencing™. This technology was originally developed for the analysis of single-base variations and enables the precise quantification of incorporated nucleotides at polymorphic positions. Treatment of the DNA with sodium bisulphite converts the epigenetic difference between methylated and unmethylated cytosine into a single-base variation of the C/T type. Therefore, Pyrosequencing is a very suitable tool for methylation analysis. Representative pyrograms for hypermethylated (untreated U251 cells) and hypomethylated (U251 cells following 5-aza-CdR exposure) are shown in Figure 3*b*. We identified the regions showing the largest differences in methylation and compared methylation levels of a small window (3 CpG sites) of *NY-ESO-1*. Mean methylation levels ± standard deviation (SD) were 86.3% ± 5.5%, 93.3% ± 0.6% and 93.3% ± 1.5%, for U251, T98 and NNS-10 glioma cells, respectively, while it was 30% ± 12% for SaOS2 cells constitutively expressing *NY-ESO-1*. Following 5-aza-CdR treatment, methylation levels were significantly decreased to 54% ± 9.1%, 68% ± 6.9% and 46.7% ± 5.7% for U251, T98 and NNS-10 ($p < 0.05$) (Fig. 3*c*). The MSP and Pyroseqencing data of other glioma cell lines and primary glioma cells were almost identical (data not shown). Taken together, this result is consistent with the hypothesis that 5-aza-CdR mediated NY-ESO-1 activation is a consequence of DNA demethylation.

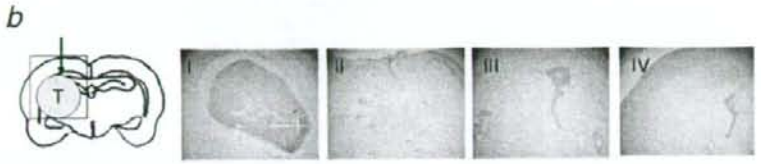
Upregulation of HLA class I in glioma cells

Our microarray data (supplemental data and Discussion) indicated that HLA class I molecules can be upregulated by ~3-fold (Table SII). Flow cytometric analysis confirmed that HLA class I expression was significantly increased in the 5-aza-CdR-treated glioma cells when compared to that in the untreated cells, indicating that 5-aza-CdR could affect the constitutive expression of HLA class I antigens in gliomas. The representative data of U251 cells are shown in Figure 4*a*. Combined with the analyses on the effect of 5-aza-CdR on *NY-ESO-1* expression, our study suggests that 5-aza-CdR may have potential therapeutic implications in *NY-ESO-1*-specific immunotherapy for human gliomas.

Antigenicity of forcibly expressed NY-ESO-1 in glioma cells by 5-aza-CdR

To evaluate the antigenicity of forcibly expressed *NY-ESO-1*, HLA-A2-restricted *NY-ESO-1*-specific CTL lines were generated, and their cytotoxicity against 5-aza-CdR-treated glioma cells was tested. Cytotoxic activity was observed only in SaOS-2 osteosarcoma cells (*NY-ESO-1*+ and HLA-A2) and 5-aza-CdR-treated U251 glioma cells, depending on the E:T ratios (Fig. 4*b*). In contrast, untreated U251 cells and other glioma cells (*NY-ESO-1*-negative and HLA-A2-negative) were resistant to lysis. K562 cells were included in order to assess the degree of the natural-killer activity of the CTL cultures; this activity was found to be negligible. Cold target inhibition assays demonstrated that cytotoxicity against 5-aza-CdR-treated U251 cells was specifically inhibited in the presence of T2.A2 cells that were prepulsed with the cognate but not those that were prepulsed with an irrelevant peptide (Fig. 4*c*). This indicated that CTL lines recognized the NY-ESO-1 pep-





Treatment				
i.p.	PBS	PBS	5-aza-CdR	5-aza-CdR
i.t.	PBS	CTL	PBS	CTL
Tumor volume (Mean±SD mm3)	32.2± 8.4	27.5 ± 8.9	12.2 ± 4.1	8.1 ± 2.5*

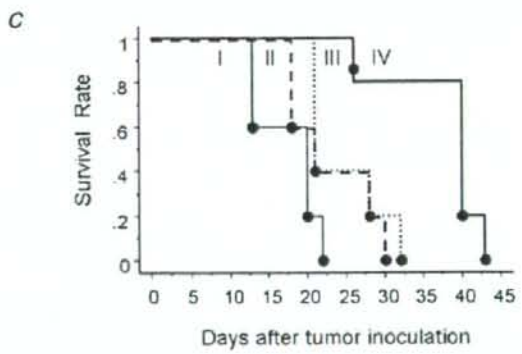


FIGURE 5 – Therapeutic effects of the adoptive transfer of NY-ESO-1-specific CTLs and 5-aza-CdR treatment in the human glioma orthotopic xenograft model. (*a*) RT-PCR for NY-ESO-1 in intracerebral glioma after i.p. injection of 5-aza-CdR. (*b*) Histopathological characteristics (haematoxylin and eosin, magnification: 40×) and tumor volumes of animals treated with PBS i.p. (8 pulses from the day after tumor inoculation) and PBS (2 μl) i.t. 6 days after tumor inoculation (Group I), PBS i.p. (8 pulses) and NY-ESO-1-specific bulk CTLs (1×10^6 cells/mouse in 2 μl) i.t. (Group II), 5-aza-CdR i.p. (0.2 mg/kg, 8 pulses) and PBS (2 μl) i.t. (Group III), or 5-aza-CdR i.p. (0.2 mg/kg, 8 pulses) and NY-ESO-1-specific bulk CTLs (1×10^6 cells/mouse in 2 μl) i.t. (Group IV). The mean tumor volume + SD in each group is shown in the table. The schema of brain coronal section shows the tumor (T), the injection site of CTL/PBS (arrow), and the magnified area (box). * $p < 0.05$ compared to other 3 treatment groups. (*c*) Kaplan–Meier survival curves of groups I–IV. The survival time of mice treated with 5-aza-CdR and NY-ESO-1-specific CTLs was significantly greater than that of mice in Groups I, II and III ($p = 0.0019$, 0.0126 and 0.0132, respectively).

tides that were processed and presented. These results together indicated that the amount of 5-aza-CdR-induced NY-ESO-1 in glioma cells was sufficient to render them sensitive to HLA-A2-restricted *NY-ESO-1*-specific CTLs *in vitro*.

Induction of NY-ESO-1 expression in intracerebral glioma by systemic administration of 5-aza-CdR

Previous evidence indicates that 5-aza-CdR can cross the BBB effectively, maintaining cytotoxic concentrations in the cerebrospinal fluid when administered *via* continuous intravenous infusion.[46] To evaluate whether *NY-ESO-1* expression could be induced *in vivo* after systemic delivery of 5-aza-CdR, we employed NOD/SCID mice transplanted intracerebrally with U251 glioma cells (NY-ESO-1 negative). After the mice were injected i.p. with a 0.2 mg/kg dose of 5-aza-CdR at 12 hr intervals for 4 days, the tumors were resected and subjected to RT-PCR analysis for *NY-ESO-1*. The results demonstrated that *NY-ESO-1* expression could be detected in intracerebral gliomas i.p. treated with 5-aza-CdR (Fig. 5*a*).

Growth suppression of glioblastoma xenografts after the adoptive transfer of anti-NY-ESO-1 CTLs

Next, we addressed whether adoptively transferred NY-ESO-1-specific CTLs posses cytotoxic activity against gliomas in which NY-ESO-1 was induced *in vivo* by 5-aza-CdR. In this model, U251 tumor cells were stereotactically implanted in the forebrain of NOD/SCID animals. An intraperitoneal injection of 5-aza-CdR (or PBS) was initiated from the next day for 4 consecutive days at 12-hr intervals. On day 6 after the tumor inoculation, the mice were treated by stereotactic delivery of either NY-ESO-1-specific bulk CTLs or PBS. On day 21 after the tumor inoculation, tumor volumes were evaluated in half of the animals. Tumor growth was significantly delayed in the animals that were administered both 5-aza-CdR (i.p) and CTLs (Group IV), whereas relatively larger tumors were observed in the other 3 groups (Groups I, II and III, $p < 0.05$) (Fig. 5*b*). In addition, we measured the survival of the remaining half of the animals. The Kaplan–Meier survival curves of 4 groups are shown in Figure 5*c*. The survival time of mice treated with 5-aza-CdR and NY-ESO-1-specific CTLs was significantly greater than that of mice in Groups I, II and III ($p = 0.0019$, 0.0126 and 0.0132, respectively). Interestingly, 5-aza-CdR injection alone (Group III) exerted a beneficial antitumor effect in terms of tumor volumes and survival ($p < 0.05$ *vs.* Group I). As discussed later, the formation of enzyme-DNA adducts mediated by *p53* induction may account for the efficacy and toxicity of 5-aza-CdR *in vivo*.

Discussion

The principal findings of this study are that in spite of relatively low frequency of CTA expression in gliomas, the DNA demethylating agent 5-aza-CdR remarkably induced the expression of CTAs, including NY-ESO-1-one of the most immunogenic CTAs-in glioma cells but not in normal human cells. The de novo expressed NY-ESO-1 was effectively recognized by the specific CTL lines both *in vitro* and in glioma orthotopic xenografts.

Expression of CTAs in human gliomas

A German group investigated the expression of 7 CTAs (SSX-1, SSX-2, SSX-4, SCP-1, TS85, NY-ESO-1 and MAGE-3) in 50 gliomas by RT-PCR. They demonstrated that SCP-1 was most frequently positive (40%), followed by SSX-4 (27%) and MAGE-3 (7%).[35] However, the expression frequency of CTAs in gliomas remains unclear, particularly when focusing on different ethnic groups. For example, NY-ESO-1 and LAGE-1 are expressed at much lower frequencies in lung cancer in the Japanese than in Caucasians; NY-ESO-1 is expressed in 2% of the Japanese *versus* 17 or 20% in Caucasians, and LAGE-1 is expressed in 9% of the Japanese *versus* 33% expression in Caucasians.[39] Liu *et al.*, have reported MAGE-1 expression in approximately 40% of glioblastoma primary cell lines and in established glioma cell lines, including U87MG, which is inconsistent with our results.[47] Although the reasons for the inconsistency remain unclear, the differences in ethnic groups and culture conditions (*e.g.*, passage and confluency) might be responsible. Nevertheless, overall, we found that gliomas have a very low frequency of CTA gene expression, such as NY-ESO-1, indicating that brain tumors are considerably unsuitable for CTA-based immunotherapy. To overcome this limitation, novel CTA induction strategies are required to evoke strong immune responses against gliomas.

CTA as targets of demethylation

A cascade of biochemical events for gene silencing is triggered by CpG island methylation that involves DNA methyltransferase activity, which in turn attracts histone deacetylases and histone methylases that eventually modify histones into a silenced chromatin state.[48] The agent 5-aza-CdR has been shown to interrupt this silencing cascade effectively by binding covalently to DNA methyltransferase and inhibiting its enzymatic activity. Numerous studies have shown the ability of 5-aza-CdR to reactivate the transcription of several tumor suppressor genes (*i.e. p16* and *MGMT*) in human tumors.[28,29] Previous evidence has clearly defined the regulatory role of DNA methylation in the constitutive expression of CTAs in melanomas and renal cell carcinomas and has demonstrated that *in vitro* treatment with 5-aza-CdR induces their expression in neoplastic cells. Therefore, CTAs are intriguing targets for demethylation. Here, we studied a large panel of CTAs and showed that 5-aza-CdR reactivated the expression of a variety of CTAs in human gliomas. This result is consistent with the report of Liu *et al.*, showing that 5-aza-CdR induced the mRNA expression of MAGE-1.[47] Then, we determined whether 5-aza-CdR-mediated NY-ESO-1 activation is a consequence of promoter DNA methylation by using MSP and quantitative Pyrosequencing. Although MSP is very sensitive and easy to use, it does not provide precise information about the methylation status of single CpG sites. Recently, Pyrosequencing technology was developed for the analysis of single-base variations. An indirect bioluminometric assay quantitatively measures the amount of pyrophosphate (Ppi) that is released from each incorporated dNTP. Through an enzymatic cascade, the release is converted into a light signal that is directly proportional to the amount of incorporated dNTP, appearing as peaks in a Pyrogram. Employing this technology, we first showed that only a small region of the *NY-ESO-1* CpG island provides relevant information for differential methylation analysis.

Expression profiling and gene ontology analysis after 5-aza-CdR treatment

To identify alterations in gene expression after 5-aza-CdR treatment, we conducted microarray experiments (supplementary data). Once the analysis was completed, we narrowed down the number of potential targets by selecting only those genes whose expression changed more than 2-fold in 2 independent RNA preparations. A total of 65 genes that fulfilled our criteria were upregulated, and 24 genes were downregulated following 5-aza-CdR treatment (Table SII). To identify the biological processes significantly involved in the drug effect, genes considered differentially expressed between the treated and control glioma cells were processed through the Gene Ontology (GO) program. The majority of the differentially expressed genes were related to biological process categories such as apoptosis (programmed cell death), cell proliferation, immune system process and tissue development (Table SIII). These categorized GO terms supported the fact that DNA methylation is associated with various biological epigenetic processes, including cell differentiation, development and oncogenic transformation. For the 11 upregulated genes underlined in Supplementary Table SII, we confirmed the microarray data using semiquantitative RT-PCR with the primers listed in Supplementary Table SIV on the same glioma cell line (U251) and 3 others (AO2, T98 and SKMG1). As shown in Supplementary Figure S1, we were able to show marked up-regulation of the tissue inhibitor of metalloprotease (TIMP) gene, which has been found to be silenced by aberrant promoter hypermethylation in other tumor types,[49] thus validating our screening procedures. Interestingly, p53, Gadd45, NF-κB and caspase 4 genes were activated by 5-aza-CdR, although the dose of 5-aza-CdR in this study (1 μM) did not affect the cell viability and morphology in glioma cells (data not shown). Recently, Kim *et al.*, attempted to identify genes silenced epigenetically in malignant gliomas by using a comprehensive and intense microarray technique coupled with the inhibition of DNA methylation and histone deacetylation.[50] Although they validated the reactivation of the MAGE genes in their micoarray screen, they mainly focused on novel targets harbouring the CpG island promoter. In addition to the role of 5-aza-CdR in the activation of epigenetically silenced genes, an important biological activity of this agent is the formation of enzyme-DNA adducts.[51] Karpf *et al.*, reported that the formation of covalent enzyme-DNA adducts and cellular toxicity resulted from the activation of *p53* as a cellular response to DNA damage.[52]

5-Aza-CdR as a potent immunostimulator

Our microarray data (Supplementary data) and the flow cytometric analysis showed that HLA class I expression was significantly increased in the 5-aza-CdR-treated glioma cells. In addition, the efficient recognition of 5-aza-CdR-treated U251 glioma cells by the HLA-A2 restricted NY-ESO-1 specific CTL lines demonstrated that de novo synthesized NY-ESO-1 antigen is functionally processed and presented. Thus, CTL-mediated lysis of glioma cells induced by 5-aza-CdR appears to represent a direct consequence of immunogenic peptides derived from *de novo* expressed NY-ESO-1 and loaded onto upregulated HLA class I molecules. Our *in vivo* study suggested that systemic administration of 5-aza-CdR may be useful in reverting the CTA-negative phenotype of gliomas through the Blood-Brain-Barrier. This evidence strongly identifies 5-aza-CdR as a potential pharmacological agent in designing and establishing new therapeutic strategies in combination with CTA-based immunotherapeutic approaches for glioma patients.

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Clinical Trial Note

A Multicenter Phase I Trial of Interferon-β and Temozolomide Combination Therapy for High-grade Gliomas (INTEGRA Study)

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A multicenter phase I clinical trial, namely, Integrated Japanese Multicenter Clinical Trial: A Phase I Study of Interferon-β and Temozolomide for Glioma in Combination with Radiotherapy (INTEGRA Study), is being conducted for patients with high-grade glioma in order to evaluate the safety, feasibility and preliminary clinical effectiveness of the combination of interferon-β and temozolomide. The primary endpoint is incidence of adverse events. The secondary endpoints are progression-free survival time and overall survival time. In addition, objective tumor response will be evaluated in a subpopulation of patients with the measurable disease. The reduction rate of tumor will be calculated according to Response Evaluation Criteria In Solid Tumors for measurable tumors as determined by magnetic resonance imaging. Subsequently, the overall response will be evaluated based on the results of measurable and non-measurable tumors. Ten newly diagnosed and 10 recurrent patients will be enrolled in this study.

Key words: chemo-phase I-II-III – clinical trials – CNS

INTRODUCTION

Gliomas account for ~40% of all brain tumors and are thus the most common primary tumors of the central nervous system. Primary brain tumors are classified according to their cell type and histological grade into categories defined by the World Health Organization (WHO) (1). High-grade (WHO grades III and IV) gliomas, which include anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), anaplastic oligoastrocytoma (AOA) and glioblastoma multiforme (GBM), are often resistant to treatment; GBM, the most common glioma in adults, kills patients within a median time span of a year after diagnosis despite treatment with aggressive surgical resection, nitrosourea-based chemotherapy and radiotherapy (2–4). A number of studies by large cooperative groups have shown the benefits of radiation therapy in doses up to 60 Gy after surgery for improving overall survival and time to progression (5). In Japan, nitrosourea agents such as 1-(4-amino-2-methyl-5-pyridiminyl)methyl-3-(2-chloroethyl)-3-nitrosourea and methyl-6-[3-(2-chloroethyl)-3-nitrosoureido]-6-deoxy-alpha-D-glucopyranoside have been used to treat malignant gliomas for a long time; however, this treatment offered few clinical benefits. Temozolomide (TMZ), an oral alkylating agent, has been demonstrated to possess antitumor activity against malignant gliomas, with minimal additional toxicity; furthermore, in a previous study of concomitant radiation therapy and chemotherapy with TMZ followed by adjuvant TMZ, survival duration substantially improved (6). In 2006, TMZ

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was certified as the treatment agent for malignant gliomas by the National Ministry of Health and Welfare of Japan, and a combination of radiotherapy and chemotherapy with TMZ is now used as the first-line therapy. However, its clinical outcomes depend on the *O*-(6)-methylguanine-DNA methyltransferase (MGMT) status, and MGMT modification is one of the key factors to obtain greater clinical benefits in the future.

Interferon-β (IFN-β) exhibits pleiotropic biological effects and has been widely used either alone or in combination with other antitumor agents in the treatment of malignant gliomas and melanomas (7). In the treatment of malignant gliomas, IFN-β can act as a drug sensitizer, enhancing toxicity against various neoplasms when administered in combination with nitrosourea. IFN-β and nitrosourea combination therapy has been particularly used for the treatment of gliomas in Japan (8). Previously, we demonstrated that IFN-β markedly enhanced chemosensitivity to TMZ in an *in vitro* study of human glioma cells (9); this finding suggested that one of the major mechanisms by which IFN-β enhances chemosensitivity is the downregulation of MGMT transcription via *p53* induction. This effect was also observed in an experimental animal model (10). These two studies suggested that chemotherapy with IFN-β and TMZ plus radiation might further improve the clinical outcome in malignant gliomas when compared with TMZ plus radiation therapy. Here, in order to evaluate the safety, feasibility and preliminary clinical effectiveness of the combination of IFN-β and TMZ, we are conducting a clinical study, namely, Integrated Japanese Multicenter Clinical Trial: A Phase I Study of Interferon-β and Temozolomide for Glioma in Combination with Radiotherapy (INTEGRA study). This study involves eight medical institutions, covering the entire regional population of Japan.

PROTOCOL DIGEST OF THE STUDY

Purpose

The main aim of this study is to evaluate the safety, feasibility and preliminary clinical effectiveness of IFN-β and TMZ for the treatment of malignant gliomas.

Study Setting and Protocol Review

This is a multicenter clinical trial involving eight neurosurgical institutions: Yamagata, Saitama Medical, Nippon Medical, Nagoya, Osaka, Kyoto, and Hiroshima Universities and Kitano Hospital. The protocol has been reviewed and approved by institutional review boards of each of these institutions.

Registration and Monitoring

Participating investigators are instructed to send an eligibility criteria report to the Data Center at Nagoya University, which is a third party different from the study director. Ten newly diagnosed and 10 recurrent patients are registered for a period of 6 months from December 2007. Data, including those of magnetic resonance imaging (MRI), blood tests, and pathology, will be collected at the data center. The quality of data will be checked and verified at the data center. If required, the data center would provide feedback to the institutions. The data center will send high-quality data to the study director. Committees of safety and efficacy (Dr Kazuo Tabuchi, Koyanagi Memorial Hospital, Saga), radiotherapy (Dr Shinji Naganawa, Department of Radiology, Nagoya University School of Medicine), pathological review (Dr Youichi Nagasato, Department of Pathology, Gunma University School of Medicine) and statistics (Dr Kunihiko Hayashi, Gunma University School of Health Science) will send their reports to the head office.

Endpoints

The primary endpoint is incidence of adverse events. The secondary endpoints are progression-free survival time and overall survival time. In addition, objective tumor response will be evaluated in a subpopulation of patients with measurable disease. The reduction rate of tumor will be calculated according to Response Evaluation Criteria In Solid Tumors for measurable tumors as determined by MRI. Non-measurable tumors are classified into four grades: complete remission, partial response, progression and not evaluable. Subsequently, the overall response will be evaluated based on the results of measurable and non-measurable tumors.

Eligibility Criteria

The eligibility criteria are as follows:

(i) Histologically confirmed diagnosis of newly diagnosed or recurrent high-grade glioma (AA, AO, AOA or GBM). More than 50% volume of tumor is located in the supratentorial region.
(ii) No tumor recognized in the optic nerve, olfactory nerve and pituitary gland on pretreatment MRI.
(iii) No dissemination detected by MRI. Age between 18 and 75 years at the time of registration.
(iv) Performance status is 0–2, 3 only due to neurological deficits.
(v) Sufficient organ function before chemotherapy according to the following laboratory data: WBC $\geq 3000/mm^3$ or neutrophils $\geq 1500/mm^3$, platelets $\geq 100\,000/mm^3$, hemoglobin ≥ 8.0 g/dl, bilirubin ≤ 1.5 mg/dl, serum glutamic oxaloacetic transaminase ≤ 100 IU, serum glutamic pyruvic transaminase ≤ 100 IU, creatinine ≤ 1.5 mg/dl, creatinine clearance ≥ 50 ml/min and electrocardiogram showing no serious arrhythmia and no serious ischemic heart disease.
(vi) No prior chemoradiotherapy for newly diagnosed patients.

(vii) The interval from the end of prior anti-tumor therapy (e.g. chemotherapy, radiotherapy, immunotherapy) must be at least 4 weeks for recurrent patients, regardless of the regimen.
(viii) Written informed consent.

Exclusion Criteria

The exclusion criteria are as follows:

(i) synchronous double cancer or metachronous double cancer in last 5 years; carcinoma *in situ* accepted;
(ii) meningitis or pneumonia;
(iii) pregnant, possibly pregnant, or nursing women;
(iv) mental disorder;
(v) uncontrolled diabetes mellitus (DM) or under treatment with insulin for DM;
(vi) myocardial infarction in last 3 months;
(vii) history of pulmonary fibrosis or interstitial pneumonia.

Treatment Methods

For newly diagnosed patients:

Radiotherapy	60 Gy/30 fr, 2 Gy ×5 days/week;
IFN-β	3 MIU/body, administered intravenously on alternate days during radiotherapy;
TMZ	75 mg/(m^2 day), daily from the first day to the last day of radiotherapy.

After completing this induction period, all patients will have 4 weeks of washout period, and they will be then shifted to adjuvant period.

IFN-β	3 MIU/body, administered on the first day morning every 4 weeks;
TMZ	150 mg/(m^2 day) (days 1–5: first cycle); 200 mg/(m^2 day) (days 1–5: second to sixth cycle).

In the absence of hematologic toxicity, the dose is increased to 200 mg/(m^2 day), beginning with the second cycle to the sixth cycle.

This cycle is repeated six times every 28 days in the absence of tumor progression, serious adverse events such as grade 4 hematological toxicity, refusal of therapy and deviation from the protocol.

For recurrent patients:

IFN-β	3 MIU/body, administered the first day morning every 4 weeks (day 1);
TMZ	150 mg/(m^2 day) (days 1–5: first cycle); 200 mg/(m^2 day) (days 1–5: second to sixth cycle).

In the absence of hematologic toxicity, the dose is increased to 200 mg/(m^2 day), beginning with the second cycle to the sixth cycle.

This cycle is repeated six times every 28 days.

This regimen has been considered to be the most promising based on previous clinical studies (8,11–14). Thus, dose-limiting toxicity was not evaluated in this study.

Follow-up and Statistical Methods

Disease progression and occurrence of new disease will be examined by MRI performed at baseline and at least after every 4–5 weeks during treatment. Blood tests and symptom checks will be carried out before treatment and at least after every 2 weeks during treatment. Follow-up will continue for 3 months from the end of treatment. In cases wherein therapy is discontinued due to toxicity, clinicians would follow-up patients until they recover from toxicity. In addition, overall survival, progression-free survival and treatment success curves are constructed as time-to-event plots by the Kaplan–Meier method.

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Conflict of interest statement

None declared.

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特集　知っておきたいがん放射線治療の知識

放射線治療の実際
肺　癌

Radiation therapy for lung cancer

角　美奈子
SUMI Minako

肺癌の治療過程における放射線治療は，三次元治療計画の浸透や定位放射線治療などの高精度放射線治療の普及により，より効果的かつ安全な治療として，大きな役割を果たしている．化学放射線治療は局所進行非小細胞肺癌および小細胞肺癌において，根治的治療としての役割を確立している．肺の有害事象対策としては，肺の線量に関する指標を利用して有害事象軽減のための工夫が行われている．

はじめに

治療装置や治療計画装置の進歩の結果としての三次元治療計画の浸透や定位放射線治療などの高精度放射線治療の普及により，肺癌領域は放射線治療の中でもとくに近年大きく変化した領域のひとつである．また，肺癌の治療戦略は放射線治療の進歩のみならず，positron emission tomography(PET)の普及による診断過程の変化や手術や化学療法と放射線治療の組み合わせによる集学的治療の evidence の蓄積により，診療過程そのものも変化している．

肺癌の放射線治療は，単独または集学的治療の一環として幅広く臨床応用されている．肺癌の罹患率の上昇とともに高齢者や合併症を有する症例も増加しており，放射線治療においては治療効果の向上とともに副作用の軽減にも工夫が必要であり，良好な QOL を得るための治療の確立が急がれている．

放射線化学療法(以下，cehmoradiation)および定位放射線治療については他項で詳述されており，本項では非小細胞肺癌(以下，NSCLC)については照射野と総線量についての最近の知見について，小細胞肺癌については予防的全脳照射の位置づけおよび限局型(以下，LDSCLC)における総線量および治療期間と治療成績に関して，さらに副作用として放射線肺臓炎と対策について最近の動向を示す．

I. NSCLC の放射線治療

肺癌の治療方針の決定に関する因子としては，組織型・病期・合併疾患も含めた全身状態がある．NSCLC ではすべての病期において根治的または緩和的な放射線治療の適応が考えられる．

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Key words : 放射線治療／非小細胞肺癌／小細胞肺癌／化学放射線療法

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Stage IおよびIIにおいては根治切除困難な症例もしくは手術拒否例において根治的放射線治療が考慮され，Stage IIIではBulky N2をはじめとする局所進行肺癌では化学療法を併用するchemoradiationが標準治療となっている．手術可能症例においても，術前または術後照射として放射線治療が実施される．Chemoradiationの詳細については他項に譲るが，NSCLCにおいても手術や化学療法を併用する集学的治療を考える場合は，年齢および合併疾患による臓器機能低下が治療の安全性を考える際に重要な検討課題となる．放射線治療にあたる医師と技師の間のみならず看護師や腫瘍内科医・外科医を含めた情報交換が必要であり，肺癌診療のシステムとチーム医療の確立を推進する必要がある

1．放射線治療計画と装置の進歩

肺癌に対する放射線治療の進歩としては，三次元放射線治療(Three-dimensional conformal radiotherapy：3DCRT)の応用がある(図1)．腫瘍の形状に則した照射野や線量分布の設定による周囲正常組織の線量の軽減は，治療成績の向上と有害事象の軽減をもたらしている．照射体積の決定において重要な役割を果たすのは画像診断であり，CTやMRI，PETなどの応用で腫瘍の浸潤・残存範囲や正常組織の機能を考慮した治療計画の可能性が実現されている．

非小細胞肺癌の放射線治療においては，

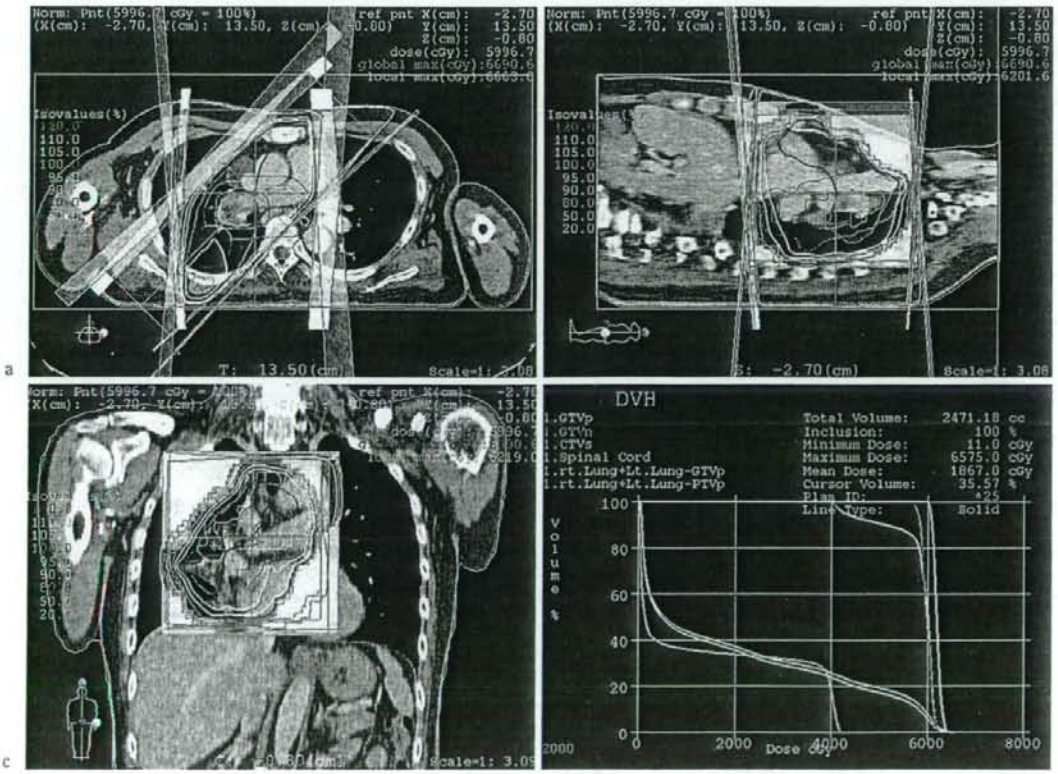

図1 右肺下葉の扁平上皮癌 T3N2M0 症例の三次元治療計画の例

a：アイソセンター面の横断像　b：アイソセンター面の矢状断像

c：アイソセンター面の冠状断像　d：Dose Volume Histgram

予防的縦隔照射体積を含む照射野に40 Gy照射後原発巣およびリンパ節転移にしぼり60 Gyまで照射予定腫瘍の縮小にしたがい，照射体積を変更する．

Gross Tumor Volume(GTV)=画像上の異常領域，腫瘍と考えられる領域

Clinical Tumor Volume(CTV)=GTV+顕微鏡的浸潤領域+肺門や縦隔リンパ節領域

Internal target volume(ITV)=CTV+IM(インターナルマージン：呼吸性移動)

Planning target volume(PTV)=ITV+SM(セットアップマージン)

という照射体積の設定が行われている．従来の二次元治療計画では70 Gy 以上の照射は正常組織の線量を考慮すると不適切とすされたが[1]，現在は 3D-CRT を応用した総線量増加が臨床試験により積極的に検討されている．

わが国においても放射線治療に関する実態調査の結果では，CT を用いた治療計画がすでに NSCLC の放射線治療においては過半数で施行されている[2]．3D-CRT の実施には三次元治療計画装置による照射体積や線量分布の検討および Multi-leaf Collimator による照射野形状の作成など，治療計画装置の利用のみならず治療装置のハード面での対応も必要である．肺癌の放射線治療ではエネルギーが低く半影の大きなコバルトによる治療は不適切とされ[3]，肺線量増加による有害事象のリスクを高くしないためには 6 MV 以上のエネルギーでの放射線治療が望ましいとされている．このように，Multi-leaf Collimator の搭載やより適切なエネルギー選択が可能な治療装置の使用など，使用される治療装置も対象となる疾患を考慮し選択されていく必要があり，治療装置の導入や更新に関し十分な配慮が必要である．

治療計画装置の進歩と治療計画の複雑化は，放射線治療の精度管理と質的保証を必要としている．治療にあたる放射線腫瘍医のみならず放射線治療技師についても認定制度の整備が進行中である．さらに，"放射線治療の品質管理に関わる作業を自ら責任を持って行うとともに，品質管理の観点からの病院全体の業務の監督，連絡・指示の伝達周知，管理部門への改善措置の提案等を行うとともに，それぞれの現場での自主的な品質改善活動(狭い意味での「品質管理」だけではなく，「放射線治療の質」自体の向上を目的とした幅広い活動)を行う" ことを任務とする放射線治療品質管理士制度が2004年に制定された．放射線治療では治療装置や治療計画装置の進歩とともに，線量測定・線量計算および装置の受入れ時や日常業務の中での定期点検など，品質管理の重要性を認識し適切な管理のもとに治療を実施する必要がますます高まっている．

2．NSCLC における放射線治療の標準的な照射体積と総線量

末梢型 I 期症例では原発巣のみの照射が行われ，N1 例に対する縦隔予防照射の意義は不明である．局所進行肺癌の根治照射においては，予防的リンパ節照射として同側肺門，気管分岐リンパ節，および上縦隔リンパ節までを CTV 含める照射体積が計画されてきた．ただし，対側肺門は CTV に含めない．また，Superior sulcus tumor では鎖骨上窩や椎体方向への浸潤傾向が強く，進行例にもかかわらず肺門リンパ節転移のない症例も少なからず存在するため，明らかなリンパ節腫大がみられない場合には肺尖部と鎖骨上窩を含めた限局した CTV が設定されてきた．症例ごとに呼吸性移動などによるインターナルマージンを確認ののち ITV を設定し，さらに0.5 cm 程度のセットアップマージンをつけ PTV を設定している．呼吸性移動は腫瘍の位置により異なる，一般的に上肺野より下肺野で大きくなる．

NSCLC において腫瘍制御に要する線量は，GTV には60 Gy/30回/ 6 週以上の線量が必要と考えられている．Hyperfractionation(過分割照射)は遅発性放射線反応の増加をさけつつ，総線量を増加することにより局所制御の向上をめざす方法として検討されてきた．RTOG 8108/8311の結果より 1 回1.2 Gy で 1 日 2 回照射において69.6 Gy が推奨線量とされたが[4]，過分割照射では急性期の有害事象が増加することが明らかとなりその後の chemoradiation を中心とする臨床試験では標準分割照射が実施されている．NSCLC の治療期間と治療成績の検討では，総治療期間の

増加により tumor control probability が低下することが示唆され[5][6]，RTOG による臨床試験の解析結果では unfavorable clinical feature (KPS < 90，weight loss > 5％，N3 stage など)がない症例において，局所制御と生存率の低下が頭頸部癌同様およそ1.5%/日となる可能性が指摘された[7][8]．臨床試験の立案や治療成績の比較においては，split の設定をはじめ総治療期間の延長の有無にも十分な配慮を払う必要がある．

3．局所進行 NSCLC に対する標準的治療とその成績

切除不能 III 期 NSCLC に対して実施されていた放射線療法単独の５年生存率は５％前後であり，再発形式は局所再発単独 43%，局所および遠隔再発 21%，遠隔再発単独 32%と[9]，局所および遠隔転移の制御ともに課題となっていた．その後の evidence の集積により化学療法を加えることにより生存期間が延長されることが示され，奏効率および生存期間が化学療法と放射線治療の継続併用法に比較し同時併用法の方が良好な結果であることが示された[10]-[12]．現在では化学療法と胸部放射線療法の同時併用療法が局所進行 NSCLC に対する標準治療であると考えられており，同時併用される化学療法としてはビノレルビン，パクリタキセル，ドセタキセルなどの新規抗癌剤とプラチナ製剤の併用療法が多く臨床応用されている[13]．切除不能 III 期 NSCLCの chemoradiation による生存期間中央値は16～22ヵ月，５年生存率は15～20%程度と向上したが[14]，化学療法と胸部放射線療法の同時併用療法における初回再発形式は，局所再発単独 17～29%，局所および遠隔再発４％～36%，遠隔再発単独 23～47%と報告されており[10][13]，現在においても局所制御の向上が局所肺癌治療戦略の重要課題の一つであることにかわりはない．

近年の放射線治療方法を検討した臨床試験において，予防的縦隔照射(Elective nodal irradiation：ENI)を省略することにより照射体積を縮小し総線量を増加することにより，局所制御の向上をはかる方法がある[15]．この背景には CT や PET によるリンパ節転移に関する診断能の向上および化学療法による occult micrometastases 制御への期待が存在している．しかしながら，現段階では臨床試験により検討中の治療方法であり今後ランダム化比較試験の結果により評価されるべき治療方法である．

II．手術の併用療法としての放射線治療

1．術後照射

1998年に発表された PORT meta-analysis では[16]，９つのランダム化比較試験における2,128例を解析し，術後照射施行例において予後が悪化することを報告した．術後照射は N0-1 症例においては有害事象が問題であり早期の完全切除例に対する術後照射は detrimental(有害)であると報告しているが，N2 症例に対してはその役割が明らかでなくさらなる検討が必要とされた．その後この検討については対象とされた報告の背景が時期的にも内容的にもさまざまであることなどによる反論も多く発表され，術後の化学療法に関する evidence の増加とともに切除症例に対する放射線治療の役割は，evidence を収集し前向きに評価していくことが必要と考えられている．現時点での術後照射の対象は，術後に腫瘍の残存の可能性のある症例と考えられている．

2．術前照射

術前治療としての集学的治療には，微小遠隔転移の制御や腫瘍の debulking による切除率の向上，外科切除時の腫瘍細胞の播種や散布防止などが目的とされるが，いまだ controversial である．縦隔鏡などで組織学的確診を得た N2 症例や Superior sulcus tumor など，十分な evidence の結果の蓄積が必要と考えられている．最近の Superior sulcus tumor に対する術前 chemoradiation に関する報告では[17][18]，完全切除率68～76%および５年生存率44～56%という成績も報告されており，集学的治療の有用性についてさらな

る検討がすすめられている．

III．LDSCLC における放射線治療

LDSCLC に分類される小細胞肺癌は一側胸郭に病変が限局するものであり，同側肺門・両側縦隔・両鎖骨上窩リンパ節転移を含む．悪性胸水（細胞診陽性あるいは細胞診陰性でも中等量以上の胸水）は除外されることが多い．LDSCLC に対する標準治療は，chemoradiation と考えられている．LDSCLC における化学療法と chemoradiation との比較試験のメタアナリシスでは，3年生存率が化学療法群8.9%に対し chemoradiation 群14.3%であり[19]，他のメタアナリシスでは chemoradiation により25.3%減少し，2年生存率が5.4%改善することが示されている[20]．放射線療法と化学療法のタイミングには早期併用と晩期併用がある．最近のメタアナリシスによればプラチナ製剤を含む化学療法と放射線治療の併用では，早期併用の方が5年生存率が良好であったことが示されている[21]．すなわち，化学療法もしくは放射線治療開始日より照射終了日までの期間が短いほど5年生存率は良好であり，1週間の延長で5年生存率が1.8%低下するとされた．

LDSCLC の照射体積は NSCLC と同様であるが，照射体積が大きくなる場合は導入化学療法後に照射することを検討する．LDSCLC に対する chemoradiation の場合，加速過分割照射法が用いられることが多い．米国で行われたランダム化比較試験において[22]，シスプラチンとエトポシドによる化学療法と当時併用する放射線治療の分割方法として加速過分割照射（45 Gy/30回/3週）と通常分割照射（45 Gy/25回/5週）が比較検討された．5年生存率の比較では通常分割照射群で16%であったのに対して，加速過分割照射群では26%と有意に向上した．この臨床試験では同じ45 Gy を照射期間を変えて比較しているため放射線生物学的には効果が劣ることが従来より指摘されており，近年通常分割照射では線量を増加した比較試験が実施されている．照射期間と生存期間をみたメタアナリシスによれば[23]，総線量を増加させるより全治療期間を延長させないことが治療成績に向上に寄与することが示されていることもあり，最適な総線量と分割方法および治療期間の選択についてはさらなる evidence の蓄積が重要と考えられる．

IV．EDSCLC における放射線治療の役割

局所療法としての放射線治療の有用性が確立している LDSCLC に対し，EDSCLC における放射線治療のあり方はいまだ十分な evidence がない．EDSCLC の標準治療は化学療法であるが，化学療法が奏功し遠隔転移が消失した場合には胸部照射が行われることがある．骨転移や脳転移などの転移巣に対する緩和的治療として放射線治療が行われることがある．

V．SCLC における予防的全脳照射

小細胞肺癌では予防的全脳照射（prophylactic cranial irradiation：PCI）が検討される．LDSCLC でも脳転移の発生率は50～60%とされ，7つの臨床試験を施行した987症例についてのメタアナリシスの結果[24]では，PCI は3年生存率を15.3%から20.7%へ5.4%上昇させていた（$P < 0.01$）．このメタアナリシスにおいて脳転移の累積発生率は PCI 未施行群で58.6%であったのが，8 Gy/1回より40 Gy/20回分割までのさまざまな線量での PCI 施行群では33.3%に減少していた．すなわち，脳転移発生率の PCI 施行による減少率は25.3%であった（95%信頼区間：19%～30%）と報告している．PCI の施行時期に関しては，厳密な比較試験はないものの，早期の PCI で脳転移抑制効果が高いことが示されている．PCI の最適な線量の検討について2008年の ASCO で発表された LDSCLC における比較試験の結果では[25]，25 Gy/10回分割と36 Gy/18回分割または36 Gy/24回分割（1日2回照射）の比較において，2年脳転移発生率は25 Gy 群が30%であるのに対

し36 Gy 群は24%であり(p=0.13)，2年生存率も25 Gy 群が42%であるのに対し36 Gy 群は37%であった(p=0.03)．この報告では25 Gy/10回分割が現時点でも PCI の標準治療としている．

EDSCLC における PCI に関しても，2007年の ASCO において化学療法で反応良好群に PCI を追加することによる生存率の向上が報告されており，LDSCLC 同様に PCI の有用性が指摘されている．

VI. 胸部放射線治療による有害事象

放射線治療による有害事象は急性と遅発性に分類されている．急性有害事象は治療中または治療直後に出現し治療終了後改善してくるが，遅発性有害事象は治療終了後月および年単位で出現してくる．肺癌の放射線治療で問題となる有害事象は急性では皮膚炎と食道炎であり，遅発性では肺の有害事象と脊髄症である．食道炎はとくに chemoradiation および総線量増加を目的とした臨床試験で，近年注目されるようになっている．

放射線治療後の肺の有害事象(RT-induced lung injury)は，肺癌の放射線治療症例において約20%で症状を有するとされる[26)27)]．急性期の肺臓炎の症状は息切れ，咳，発熱を主たる症状とし，放射線治療後1ないし6ヵ月で生じてくることが多いとされる．急性期の肺臓炎は典型的には steroid に反応するため predonisolone で40ないし60 mg/日を数週投与したのち，10 mg/日程度を週単位でゆっくり減量していく．遅発性の肺有害事象は照射後数ヵ月ないし数年単位で慢性の呼吸困難として発症し，照射された肺の繊維化を伴っている．繊維化の時期には steroid への反応性は低下していることが多く，対応としては必要に応じて酸素の補給を行う．

放射線治療後の肺の有害事象に関する因子としては，1回線量や総線量が検討されてきた．二次元治療計画と比較し三次元治療計画では，正常な肺の線量軽減を目的とした治療計画の比較検討が容易となっている．具体的な指標として，dose-volume histogram(DVH)の検討により(図2)，20 Gy や30 Gy が照射される正常肺の体積(V_{20}やV_{30})や肺の平均線量などが検討され，臨床的な肺の有害事象の発現と相関していることが報告されている[28)-32)]．

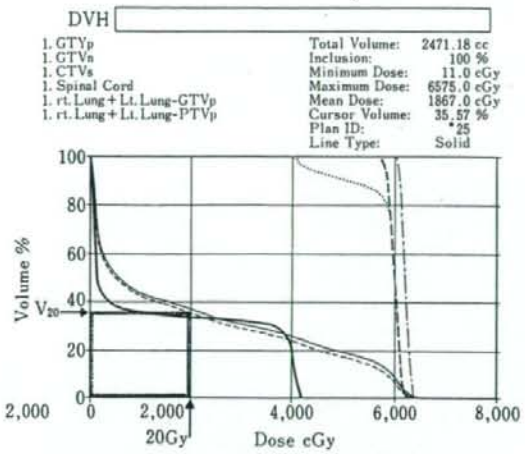

図2 Dose-Volume Histgram

V_{20}：左右肺の体積より PTV または GTV を引いた体積において20 Gy 以上照射される割合．

この症例では総線量60 Gy の計画で35%となる．

Graham らは V_{20}(the percent volume of the total lung exceeding 20 Gy)が，肺臓炎の良好な指標であると報告した[31)]．Grade 2 以上の肺臓炎発生は V_{20} とよく相関しており，V_{20} が22%未満の症例では発症はなく，Grade 2 の発生率は V_{20} が22～31%であった症例で7％ (Grade 3 は0％)であり，V_{20} が32～40%では13%(Grade 3 は5％)と増加し，V_{20} が40%を超えると36%(Grade 3 は23%)に達していた．RTOG 9311(Phase I/II)では，予防的リンパ節照射をしない 3D-CRT で総線量増加を行う多施設共同臨床試験が V20で層別化して実施され[33)]，V_{20}が25%未満の症例では83.8 Gy まで，V_{20}が25～36%であった症例では77.4 Gy まで安全な総線量増加が可能であったと報告された．

現時点では放射線治療後の肺の有害事象に関する指標間の優劣の評価は一定していないが，有害事象のリスクの検討や治療計画の比較にその有用

性を発揮している．体積のみでなく腫瘍の位置の重要性を指摘する報告もあり[31]，今後よりよい有害事象検討の指標の確立が望まれている．

おわりに

肺癌に対する標準的治療の確立には，局所制御率と生存期間の向上および有害事象の軽減を図るための工夫が必要である．新しい薬剤を含めた臨床試験が現在数多く施行されているが，放射線治療は対象に合わせた治療の工夫が可能である．高齢者やPSの低い症例への安全な治療の提供など，今後evidenceを収集し治療方法を確立していく必要がある．放射線治療の安全な実施に関しては，技術の高度化とともに治療の安全性や品質管理にさくべきman powerと時間の確保が急務であることを最後に指摘したい．

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Postoperative radiotherapy for non-small-cell lung cancer: Results of the 1999–2001 patterns of care study nationwide process survey in Japan

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Summary To investigate the practice process of postoperative radiation therapy for non-small-cell lung cancer (NSCLC) in Japan. Between April 2002 and March 2004, the Patterns of Care Study conducted an extramural audit survey for 76 of 556 institutions using a stratified two-stage cluster sampling. Data on treatment process of 627 patients with NSCLC who received radiation therapy were collected. Ninety-nine (16%) patients received postoperative radiation therapy between 1999 and 2001 (median age, 65 years). Pathological stage was stage I in 8%, II in 17%, IIIA in 44%, and IIIB in 20%. The median field size was 9 cm × 11 cm, and median total dose was 50 Gy. Photon energies of 6 MV or higher were used for 64 patients, whereas a cobalt-60 unit was used for five patients. Three-dimensional conformal treatment was used infrequently. Institutional stratification influenced several radiotherapy parameters such as photon energy and planning target volume. Smaller non-academic institutions provided worse quality of care. The study confirmed continuing variation in the practice of radiotherapy according to stratified institutions. Outdated equipment such as Cobalt-60 units was used, especially in non-academic institutions treating only a small number of patients per year.

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1. Introduction

Postoperative radiation therapy (PORT) decreases the risk of local–regional recurrence in patients with resected non-small-cell lung cancer (NSCLC) [1–3]. However, reduction in the frequency of local recurrence has not translated into a survival benefit in most studies. In 1998, the impact of PORT for NSCLC was analyzed in a meta-analysis of phase III trials [4]. After publication of the PORT meta-analysis, which emphasized deleterious effects in patients receiving PORT for completely resected N0-1 cases, much of the clinical focus on adjuvant therapy shifted to chemotherapy [5,6]. Thus, the role of PORT for patients at high risk for locoregional failure such as those with N2 disease remains unclear. Adjuvant chemotherapy trials have often permitted use of PORT as an option for patients with N2 disease [5,7]. One clinical study reported promising results for combined PORT and chemotherapy for patients with pathologic stage II or IIIA disease [8]. The results of these trials imply that PORT delivered using modern radiotherapy techniques may potentially provide a survival advantage for selected high-risk patients.

The Patterns of Care Study (PCS) is a retrospective study designed to investigate the national practice for cancer patients during a specific period [9,10]. In April 2002, the PCS started a nationwide survey for patients with NSCLC treated with radiation therapy in Japan. In the present report, we provide results of analyses focused on patients who received PORT for NSCLC during the study period. The objectives of this study were to reveal clinical practice patterns regarding PORT after publication of the PORT meta-analysis and to assess variation in clinical practice according to stratified institutions.

2. Materials and methods

Between April 2002 and March 2004, the PCS conducted a national survey of radiation therapy for patients with lung cancer in Japan. The Japanese PCS developed an original data format and performed an extramural audit survey for 76 of 556 institutions using a stratified two-stage cluster sampling. Data collection consisted of two steps of random sampling. Prior to random sampling, all institutions were classified into one of four groups. Criteria for stratification have been described elsewhere [10]. Briefly, the PCS stratified Japanese institutions as follows: A1, academic institutions such as university hospitals or national/regional cancer center hospitals treating $\geqq$ 430 patients per year; A2, academic institutions treating <430 patients; B1, non-academic institutions treating $\geqq$130 patients per year; and B2, <130 patients. The cut-off values in number of patients treated per year between A1 and A2 institutions and B1 and B2 institutions, respectively, were increased from those used in the previous PCS study because of the increase in the number of patients treated by radiation therapy in Japan [10]. Eligible patients had 1997 International Union Against Cancer (UICC) stage I–III NSCLC that was treated with PORT between 1999 and 2001, a Karnofsky Performance Status (KPS) >50 prior to start of treatment, and no evidence of other malignancies within 5 years. The current PCS collected specific information on 627 patients (A1:157, A2:117, B1:214, B2:139) who were treated with radiation therapy between 1999 and 2001. Of those, 99 (16%) patients (A1:15, A2:17, B1:45, B2:22) who received PORT constitute the subjects of the present analysis. The practice of PORT was investigated by reviewing items in each medical chart such as demographics, symptoms, history, work-up examinations, pathology, clinical stage, treatment course including radiation therapy, surgery and chemotherapy, and radiotherapy parameters. In addition, simulation films and linacgraphy of each patient were also reviewed by surveyors.

The PCS surveyors consisted of 20 board-certified radiation oncologists. For each institution, one radiation oncologist visited and surveyed data by reviewing patient charts. In order to validate the quality of collected data, the PCS utilized an internet mailing-list among all surveyors. In situ real-time check and adjustment of data input were available between each surveyor and the PCS committee. In tables, "missing" indicates that the item in the data format was left empty, whereas "unknown" means that the item in the format was completed with data "unknown". We combined "missing" and "unknown" in tables because their meanings were the same in most cases; no valid data were obtained in the given resources. Cases with missing or unknown values were included when both the percentage and significance value were calculated. Statistical significance was tested by the χ^2 test. A p-value less than 0.05 was considered statistically significant. Overall survival was assessed from the day of surgery and was estimated by the Kaplan–Meier product limit method using the Statistical Analysis System, Version 6.12.

3. Results

3.1. Patient and tumor characteristics

Patient and clinical tumor characteristics are shown in Table 1. Of the 99 patients who received PORT, 32 were treated at academic institutions and 67 at non-academic institutions. The proportion of patients with NSCLC who received PORT was significantly higher in non-academic institutions than in academic institutions (19% versus 12%, $p = 0.013$). Overall, median age was 65 years (range, 39–82), and the male to female ratio was 4:1. Ninety-three percent of patients had a KPS greater than or equal to 80%. Preoperative examinations included chest computed tomography (CT) in 97% of patients, bronchoscopy in 87%, brain CT or magnetic resonance imaging (MRI) in 75%, abdominal CT in 75%, bone scintigraphy in 83%, and mediastinoscopy in 4%. The primary tumor site was the upper lobe in 62 patients, middle lobe in 7, and lower lobe in 27. The remaining 2 patients had a primary tumor near the border of the upper and middle lobes that involved both lobes, and they were allocated to "others". Peripheral tumors were twice as common as central tumors. When tumors were analyzed by laterality, the ratio of right to left side primary site was 1.5. Clinical T- and N-classifications were T1 in 28 patients, T2 in 35, T3 in 24, T4 in 11, and N0 in 33, N1 in 19, N2 in 40, and N3 in 6, resulting in clinical stage I in 27 patients, II in 14, IIIA in 41, and IIIB in 16. The numbers less than 99 are due to missing or unknown data.