Advantages and Limitations in the Use of Combination Therapies with Charged Particle Radiation Therapy

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Abstract

Purpose: Studies are currently underway to help provide basic and clinical evidence for combination particle beam radiation therapy, on which there are few published reports. The purpose of this article is to summarize the current status in the use of particle beams combined with other modalities.

Results: Following from experiences in x-ray radiation therapy, combination therapy with proton beams (PBT) has been attempted, and several clinical studies have reported improved survival rates for patients with non–small cell lung cancer, pancreatic cancers, esophageal cancers, and glioblastomas. Recently, basic studies combining PBT with PARP inhibitors and histone deacetylase inhibitors have also reported promising results. In the area of carbon ion therapy (CIT), there are few clinical reports on combination therapy; however, the number of basic research reports exceeds that for PBT. So far, the combined use of cytotoxic drugs with CIT yields independent additive effects. In addition, it is notable that combination therapy with CIT is effective against radioresistant cancer stem-like cells. Recent evidence also suggests that local radiation therapy can induce an effective antitumor immune response. There has been an increased use of combination immune-modulating agents and cytokines with particle beams, especially CIT. The field of radiation therapy is evolving from a strong reliance on local-regional treatment to a growing reliance on systemic immunotherapy.

Conclusions: The combined use of anticancer agents with particle radiation therapy has a considerable potential effect. Future research in molecular targeting therapy and immunotherapy may help identify the most efficacious approach for combination therapy with protons and carbon ions.

Keywords: particle therapy; proton therapy; carbon-ion therapy; chemotherapy; immunotherapy; molecular targeting therapy

Introduction

Particle radiation therapy for solid cancers should offer biological advantages that are yet to be understood as physical advantages. One of the reasons is that uncertainty in biological issues is much greater than that in physical issues. From a clinical point of view, it is strongly desirable to clarify not only the biological effects of particles themselves but also advantages or limitations of conjunctive use of particle beams with other biological modalities. The expected advantages of the combining radiation therapy are (1) tumor tissue radiosensitization, ideally tumor specific; (2) normal tissue protection; and (3) induction of bystander or abscopal effect in distant regions.
For proton beam radiation therapy (PBT), combination therapy has been attempted from experiences in x-ray treatments, wherein the combination of radiation with cytotoxic chemotherapy has become a standard treatment option for most locally advanced cancers. In contrast, the same does not hold true for carbon ions (C-ions), because their physical and biological characteristics are very different from photons. Robust scientific background is required to translate combination therapies with C-ions into clinical practice.

In this mini review, I summarize the status of the combined use of particle beams with other modalities from a biological point of view, focusing on the advantages and limitations of these methods.

Combination Therapy with Protons

Clinical Research on Combination Therapy with Protons

The number of PBT facilities has been increasing worldwide. Currently, there are 69 facilities in operation, 42 under construction, and 22 at the planning stage [1]. With the increased use of PBT, many clinical trials involving the conjunctive use of anticancer drugs with PBT have been attempted, based on experiences in photon therapy, as the generic relative biological effectiveness (RBE) of the proton to the photon is 1.1.

Review of the literature, that is, main publications on non–small cell lung cancers (NSCLCs), pancreatic cancers, esophageal cancers, and glioblastoma are listed in Table 1. Among these, improvements in survival rates, compared to previous data on conventional x-ray therapy, were demonstrated in stage II to III NSCLC [2–6]. Hoppe et al [4] mention that the improved survival rate, as compared to other reports of patients with stage III NSCLC, could be attributed to the safe delivery of a relatively high radiation dose of 74 GyE with protons, even to large bulky inoperable tumors, and other reports agree with this point. However, recently Liao et al [7] reported that although passive beam proton therapy (PSPT) significantly reduced heart exposure in terms of both radiation dose and heart volume, there was no benefit in either grade ≥ 3 radiation pneumonitis or local failure as compared to intensity-modulated (photon) radiation therapy (IMRT). The median overall survival times were 29.5 months for patients in the IMRT group and 26.1 months for patients in the PSPT group [7]. A phase III randomized trial comparing overall survival after photon versus proton chemoradiotherapy for inoperable stage II-IIIB NSCLC (RTOG1308) has been undergoing since 2013. The efficacy of proton chemoradiotherapy compared to photon chemoradiotherapy should be clarified with a high level of evidence.

Similar levels of positive results with combination therapies have been reported in pancreatic cancers [8–10]. Although 1 patient showed grade-5 gastrointestinal toxicities with a high-dose PBT of 70.2 GyE concurrent with gemcitabine, progression-free and overall survival rates were more favorable than those in conventional chemoradiotherapy [8]. In combination with capecitabine, neoadjuvant short-course proton-based chemoradiation (25 GyE) has been reported to be associated with favorable local control and low incidence of toxicity in resectable pancreatic cancers [9]. Also, a definitive therapy combining proton therapy (59.4 GyE) with concomitant capecitabine was well tolerated, without serious gastrointestinal toxicity, and showed encouraging results in unresectable nonmetastatic pancreatic cancers [10].

The major benefit of PBT for esophageal cancers is that the dose to the spinal cord, heart, and lungs can be significantly reduced. Lin et al [11] reported results of definitive therapy or neoadjuvant plus surgery using PBT and “various types” of chemotherapy, and they concluded that acute treatment-related toxicities and perioperative morbidities are relatively low, whereas tumor response and disease-related outcomes are encouraging. Also, Ishikawa et al [12] summarized the results of 40 cases of stage I-III esophageal cancers treated by PBT with concurrent chemotherapy consisting of cisplatin and 5-fluorouracil. They concluded that this can be a promising concurrent chemoradiotherapy for patients with esophageal cancer, especially with regard to late cardiopulmonary toxicity.

In malignant gliomas, we previously reported the safety and efficacy of postoperative hyperfractionated concomitant boost proton radiation therapy with nimustine hydrochloride (ACNU) for supratentorial glioblastoma multiforme (GBM) [13]. We also reported that although localized radiation necrosis was inevitable, this high-dose proton beam therapy with the concurrent use of ACNU was able to control GBM pathogenesis, based on the analysis of long-term survivors [14]. In addition, the concurrent use of temozolomide (TMZ) has a tendency for better survival as well as a lower incidence of hematologic toxicity than ACNU [15].

The potential advantages of PBT combined with chemotherapy for these solid tumors is that one can minimize toxicity to the surrounding vital organs while delivering high doses to the sensitized tumors. These potential advantages of PBT are applicable to other locally advanced solid tumors. As PBT becomes more accessible and indications become wider, other
| Tumor type | Author (year) | Ref. No. | No. of patients | Study type | Treatment method | Efficacy | Incidence of G ≥ 4 Toxicity |
|------------|--------------|----------|----------------|------------|------------------|---------|---------------------------|
| NSCLC      | Sejpal S (2011) | 2 62    | A comparative study among proton, 3D-CRT, and IMRT | 74GyE/2GyE, paclicaxel and carboplatine | Median OSs were 17.7 months for the 3D-CRT group, 17.6 months for the IMRT group, and 24.4 months for PBT group | Three grade 4 fatigue in the proton therapy group |
|            | Oshiro Y (2014) | 3 15    | Phase II study | 74GyE/2GyE, cisplatin and vinoreline | The mean survival time was 26.7 months | Four grade 4 neutropenia |
|            | Hoppe BS (2015) | 4 14    | Phase II study | 74-80 GyE/2GyE, paclicaxel and carboplatine | Two-year OS rate was 57%. | None |
|            | Nguyen QN (2015) | 5 134 (II: 21, III:113) | prospective nonrandomized case-only observational study. | 60–69.6 GyE, 70–72 GyE, 74 GyE/2GyE, paclicaxel and carboplatine | At a median follow-up of 4.7 years, median OSs were 40.4 months in stage II group and 30.4 months in stage III group. | One grade 4 esophagitis in 74GyE group |
|            | Chang JY (2017) | 6 64    | Phase II study | 74GyE/2GyE, paclicaxel and carboplatine | Median OS was 26.5 months. | One grade 4 bronchial fistula |
|            | Liao Z (2018) | 7 57    | Randomized trial compared PSPT versus IMRT | 74GyE/2GyE, standard platinum- and taxane-based chemotherapy | Median OSs were 29.5 months for IMRT group and 26.1 months for PSPT group. | None in the PSPT group |
| Pancreatic Cancer | Terashima K (2012) | 8 50    | Phase I/II study for unresectable pancreatic cancers | 50GyE/2GyE, 67.5GyE/2.7GyE, 70.2GyE/2.7GyE, gemcitabine | One-year OS rate was 76.8%. | Three acute grade 4 hematologic toxicity |
|            | Hong TS (2014) | 9 50    | Phase I/II short course neoadjuvant study for resectable pancreatic cancers | 25GyE/5GyE, capectabine | At a median follow-up of 38 months, the median PFS for the entire group was 10 months, and OS was 17 months. | None |
|            | Sachsman S (2014) | 10 11   | Phase I/II study for unresectable pancreatic cancers | 59.4GyE/1.8GyE, capectabine | One- and two-year OS rates were 61% and 31% with a median OS of 18.4 months. | None |
| Esophageal Cancer | Lin SH (2012) | 11 62   | Retrospective analysis of a prospective study | 50.4 (36-57.6) GyE/1.8GyE, various types of chemotherapies | Three-year OS rate was 51.7% | One grade 5 ventricular tachycardia |
|            | Ishikawa H (2015) | 12 40   | Retrospective analysis of a prospective study | 60GyE/2GyE, cisplatinum and 5-fluorouracil | Two- and three-year OS rates for the whole patient were 75.1% and 70.4%, respectively | Tow grade 4 hematomal toxicity |
| Glioblastoma multiforme | Mizumoto M (2010) | 13 20   | Phase I/II study | X-ray; 50.4GyE, 1.8GyE, proton; 46.2GyE/1.4GyE, nimustine-hydrochloride | One- and two-year OS rates were 71.1% and 45.3%, respectively. The median OS was 21.6 months. | Six grade 4 hematologic toxicity |
|            | Mizumoto M (2016) | 15 46   | Retrospective study comparing nimustine hydrochloride and temozolomide with protons | X-ray: 50.4GyE/1.8GyE, proton; 46.2GyE/1.4GyE, nimustine-hydrochloride or temozolomide | One- and two-year OS rates were 82.6 and 47.6 %, respectively. Median OS was 21.1 months with no significant difference between the nimustine hydrochloride and temozolomide groups. | Fourteen grade 4 hematologic toxicity with nimustine hydrochloride, Three grade 4 hematologic toxicity with temozolomide |

**Abbreviations:** Ref, reference; G, grade; GyE, Gray equivalent; 3D-CRT, 3 dimensional radiotherapy; IMRT, intensity modulated radiotherapy; OS, overall survival; PFS, progression free survival; PSPT, passive scattering proton therapy.
protocols will be designed to sensitize tumor tissues and protect normal tissues, for establishing the next-generation standard chemo-PBT.

Prospective randomized comparisons with modern technology of x-ray IMRT and protons of broad or scanning beams is strongly desired to obtain scientific results with high-evidence levels.

Preclinical Analysis in Combination Therapy with Protons

Combination with new drugs has been researched, particularly on molecular targeting agents in vitro and in vivo. Most of these approaches target DNA damage-repair–associated molecules. Hirai et al [16] reported that the PARP inhibitor enhances radiosensitivity to the proton beam irradiation by inhibiting DNA repair, possibly by increasing the conversion of non–double-strand break lesions to lethal DNA damage. They reported that the combination effect was more significant in the Bragg peak region than in the entrance region. With high conformity and dose concentration property of proton beams, PARP inhibitor may be useful as a radiosensitizer when combined with proton beam irradiation.

Further, Yu et al [17] reported that the histone deacetylase inhibitor (HDACi) valproic acid sensitizes hepatocellular carcinoma cells to proton beams. They found that valproic acid sensitized more hepatocellular carcinoma cells to proton than to photon irradiation, inducing prolonged proton-induced DNA damage and augmented proton-induced apoptosis. In addition, we recently reported that another HDACi, suberoylanilide hydroxamic acid (SAHA), successfully sensitized cancer cells to radiation, even with high LET radiation [18]. Importantly, we proved that SAHA selectively sensitized cancer cells to photon and particle radiation (Figure 1). SAHA is an epigenetic agent with a variety of actions. Although the total picture of SAHA’s
unique radiosensitization mechanism has not been elucidated yet, it has been reported that SAHA suppresses homologous recombination repair only in cancer cells [19, 20]. Based on the radiation dose control, enhancement ratio, and potential lower side effects for normal cells, it is assumed that the SAHA treatment may be the most effective when combined with protons.

The conjunctive use of novel molecular targeting drugs with PBT has a considerable potential to improve the outcome for patients; however, the scientific biological basis of the combination therapy needs to be clarified both in vitro and in vivo. Further development of this research area is expected.

**Combination Therapy with C-ions**

**Clinical Research on Combination Therapy with C-ions**

Currently, there are 11 facilities in operation, 4 under construction, and 1 in the planning stage [1]. Compared to that for protons, the number of clinical reports for combination therapy involving cytotoxic drugs and C-ions therapy is very limited, and the summaries of clinical studies are listed in Table 2.

For GBM, Mizoe et al [21] reported a phase I/II clinical trial for patients with malignant gliomas (16 anaplastic astrocytomas and 32 GBMs), treated with combined x-ray radiation therapy, ACNU chemotherapy, and C-ion radiation therapy [21]. The results showed the potential efficacy of chemoradiotherapy for malignant gliomas in terms of the improved survival rate for those patients who received higher C-ion doses.

Jingu et al [22] reported a prospective definitive treatment study for patients with malignant mucosal melanoma, treated with C-ions (57.6 GyE in 16 fractions) and combined use of dacarbazine, ACNU, and vincristine. Their results are favorable as compared to previously reported x-ray chemoradiotherapy, and they found that minimum apparent diffusion coefficient value in magnetic resonance images can be a predictive factor of survival rates [22].

In pancreatic cancers, Shinoto et al reported a phase I preoperative, short-course, dose-escalation study on resectable pancreatic cancers [23], and a prospective study of definitive treatment with C-ions combined with full-dose gemcitabine on unresectable locally advanced pancreatic cancers [24]. They demonstrated that C-ion radiation therapy concurrent with chemotherapy was well tolerated and was effective in both preoperative and definitive therapy in those patients.

As multiple clinical studies on the combination therapy of anticancer drugs with C-ion radiation therapy are undergoing [25], the efficacy of combination therapy should be shown in the near future. Prospective randomized comparisons of C-ions, at least with modern technology of x-ray IMRT in common cancers, is desired to obtain scientific results with high-evidence levels.

**Preclinical Analysis in the Combination Therapy with C-ions**

There have been many basic radiobiological studies on the adjunctive use of anticancer drugs and C-ions. It has been known that C-ion beams induce cluster DNA damage resulting in a higher proton RBE of 2.5, compared to that for photons. From their high linear energy transfer (LET), one can expect that the combination of cytotoxic drugs with C-ions may induce a “synergistic” cytotoxic effect in cancer cells. However, Combs et al [26] demonstrated that the combination of TMZ and C-ions leads to an additive effect comparable to that of photons. In addition, they showed that combinations of camptothecin, gemcitabine, paclitaxel, and cisplatinum with C-ions also yielded additive results. This is in good agreement with the findings of the other group that reported TMZ causes additive cytotoxicity when combined with radiation, regardless of the radiation quality [27]. Dehne et al [28] investigated the putative cytotoxic effects in 4 different hepatocellular carcinoma cell lines after irradiation with photons or carbon ions in combination with new targeted molecular agents. Their result demonstrated that the combination of C-ions with either of those agents exhibited independent toxicities in all cell lines. They assumed that combining C-ions with systemic substances only has independent effects because heavy ions cause direct damage owing to their high-LET character resulting in clustered DNA double strand breaks (DSBs). Although one can reduce the C-ion dose when combined with other anticancer drugs, these results indicate that effects of the combinations of anticancer drugs with C-ions are expected to be additive.

However, it is worth noting that C-ions are effective against radioresistant cancer stem-like cells (CSCs). Sai et al [29] reported that combining C-ions with cisplatin is an effective method to target triple-negative breast CSCs. Further, they reported that a C-ion beam combined with gemcitabine has superior potential to kill pancreatic CSCs [30]. They mention that these combinations induce irreparable clustered DNA DSBs, apoptosis, autophagy, and subsequent cell death at relatively low doses, compared to a C-ion beam alone or x-rays combined with GEM [28]. As CSCs are considered to play central roles for
### Table 2. Conjunctive use of C-ions ans Chemotherapies

| Author (year) | Ref. No. | Types of malignancy       | No. of patients | Study type                  | C-ion irradiation          | Combined Chemotherapy     | Efficacy                      | Toxicity                           |
|--------------|----------|---------------------------|----------------|----------------------------|----------------------------|----------------------------|-------------------------------|-----------------------------------|
| Mizoe JE (2007) | 21       | Glioblastoma multiforme   | 32             | Phase I/II, Definitive     | X-ray: 50 Gy/25 fractions C-ion: dose was increased from 16.8 to 24.8 GyE in 10% incremental steps. | nimustine-hydrochloride       | Median OSs were 7 months for the low-dose group, 19 months for the middle-dose group, and 26 months for the high-dose group. | No grade 3 or higher acute reaction. Four cases of grade 2 brain morbidity. Four cases of grade 2 brain reaction. |
| Jingu K (2010) | 22       | Malignant mucosal melanoma | 37             | Perspective, Definitive    | 57.6 GyE/16 fractions dacarbazine nimustine-hydrochloride vincristine | Three-year OS rate was 65.3% with a median follow-up period of 19.0 months. | No serious complication       |
| Shinoto M (2013) | 23       | Resectable pancreatic cancers | 26             | Phase I preoperative, short-course, dose-escalation study | Dose was increased by 5% increments from 30 GyE to 36.8 GyE. | not specified             | Five-year OS rates for all 26 patients and for those who underwent surgery were 42% and 52%, respectively. | No dose-limiting toxicity. One patient developed acute grade 3 toxicity (liver abscess). One patient developed late grade 4 toxicity (portal vein stenosis). |
| Shinoto M (2016) | 24       | Locally advanced pancreatic cancer | 66             | Prospective, Definitive    | Dose was escalated from 43.2 to 55.2 GyE at 12 fractions. | gemcitabine               | Two-year OS rates in all patients and in the high-dose group with stage III (45.6 GyE) were 35% and 48%, respectively. | Dose-limiting toxicity was observed in 3 patients. Grade 3 infection in 1 patient and grade 4 neutropenia in 2 patients. Grade 3 gastric ulcer and bleeding in one patient. |

**Abbreviations:** Ref, reference; GyE, Gray equivalent; OS, overall survival.
therapy resistance and tumor recurrence, targeting CSCs by using the combination of C-ions and cytotoxic drugs is an ideal strategy for the treatment of refractory solid cancers.

As for recent reports on the conjunctive use of molecular targeting agents and C-ions, the combined use of Hsp90 inhibitor–sensitized cancer cells with C-ions, as well as x-rays, provides effective tumor growth delay without affecting noncancerous cells. It has been assumed that the underlying mechanism for this radiosensitizing effect is the inhibition of 2 major DNA DSB repair pathways by the Hsp90 inhibitors [31, 32]. In addition, similar to protons, PARP-1 depletion radiosensitized cancer cells to C-ions [33]. They showed that PARP-1 depletion along with C-ion exposure synergistically increases apoptosis and decreases metastatic properties in HeLa cells. Thus, PARP inhibitors inhibit not only DNA repair but also the metastatic property of cancer cells [31, 33].

Compared to cytotoxic drugs in which the conjunctive effect was additive or independent, molecular targeting agents may be worth further research to identify an approach for an effective combination therapy with C-ions.

**Immuno-particle Beam Oncology**

The concept of the conjunctive use of immunotherapy with particle therapy is totally different from combination with cytotoxic drugs. Recent reports show that localized irradiation of tumors can act as an immune adjuvant, which can induce a systemic tumor immune response by killing tumor cells in situ [34–36]. As shown in Figure 2, the underlying mechanism is the induction of immunogenic cell death in the tumor microenvironment and the sequential activation of systemic cellular immunity [37, 38] (Figure 2). Danger signals and the release of tumor-specific antigens after exposure to ionizing radiation can convert an irradiated tumor into an in situ vaccine. We previously reported that the preventive abscopal effect mediated by CD8+ T-cells was observed in the brain when tumors inoculated in the thigh were cured by x-ray irradiation [39]. However, to induce the abscopal effect correctly, localized radiation alone is not usually sufficient because spontaneous tumors are usually poorly immunogenic [36, 37]. Several studies on mice models have reported positive results for using a combination of radiation therapy and immunotherapy [40–42]; however, most of the studies focused on immune response with x-ray radiation. Recently, Girdhani et al [43] reported that critical proangiogenic and immune-inhibitory factors, including vascular endothelial growth factor, interleukin-6 and-8, and hypoxia-inducible factor-1 alpha, were significantly downregulated after high-energy proton irradiation [43]. Also, Gameiro et al [44] demonstrated that proton irradiation mediated calreticulin cell-surface expression on tumor cells, increasing the...
sensitivity to cytotoxic T-lymphocyte killing. These findings suggest that proton radiation could gain expanded therapeutic use owing to its ability to suppress neovascularization and immune inhibitory mechanism [45]. In addition to the physical benefits, these unique biological effects of particle irradiation may prove superior in the systemic effect as compared to x-rays [46, 47].

In clinical trials with PBT, we performed a Phase I study of a combination of an intratumoral injection of hydroxyapatite as an immune adjuvant following PBT to prevent local or distant recurrence by activation of immune systems in patients with locally advanced or recurrent hepatocellular carcinoma. We reported that this combination therapy was feasible and safe, and four of the nine patients were progression free for more than one year [48]. As for C-ions, in combination with dendritic cells injection, the C-ion beam correlated with a greater amount of immune activation and prevention of metastases in mouse models [49, 50, 51]. Further, in clinical case reports, abscopal responses have been reported in patients treated with local C-ion therapy [52, 53]. Further investigation is warranted to exploit this strategy to eliminate cancer cells spreading to non-irradiated field. At this point, the combination of particle therapy with immune system modulators such as immune checkpoint inhibitors and cytokines may be promising approaches. In addition, tumor-specific immune response may be obtained by converting tumors into effective in situ vaccines by using particle radiation therapy. At the same time, investigation on the underlying immune mechanisms leading to an efficient immune response is essential to translate into more effective novel therapeutic approaches.

**Conclusive Remarks**

The conjunctive use of other anticancer agents with particle radiation therapy has a considerable potential to improve the outcome for patients. The scientific biological basis of the combination therapy needs to be clarified both in vitro and in vivo for clinical translation. Molecular targeting agents may be worth further research to identify an approach for an effective combination therapy with protons and C-ions. In addition, recent reports show that localized irradiation of tumors can act as an immune adjuvant. As compared to x-rays, particle irradiation may be proved to be superior in the systemic immune effect.

**ADDITIONAL INFORMATION AND DECLARATIONS**

**Conflicts of Interest:** The authors have no conflicts to disclose.

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