Nonmyeloablative Allogeneic Stem Cell Transplantation for Nasopharyngeal Carcinoma

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Key Words. Nonmyeloablative allotransplant • Nasopharyngeal cancer • Graft-versus-tumor

Disclosures: Whay K. Chia: None; Who W. Wang: None; Wan T. Lim: None; Wai M. Tai: None; Li Sun: None; Choon H. Thng: None; Yatanar Soe: None; Swee P. Yap: None; Eng H. Tan: None; Han C. Toh: None.

The article reports on the use of bone marrow transplant for nasopharyngeal carcinoma, which is an investigational treatment.

The content of this article has been reviewed by independent peer reviewers to ensure that it is balanced, objective, and free from commercial bias. No financial relationships relevant to the content of this article have been disclosed by the authors or independent peer reviewers.

ABSTRACT

We present a case of a patient with metastatic nasopharyngeal carcinoma who failed two lines of palliative combination chemotherapy and was treated with allogeneic nonmyeloablative stem cell transplantation (NST). This patient achieved a durable tumor response, dramatic relief of his symptoms, and elimination of tumor in his bone marrow—an effect likely achieved via a graft-versus-tu-

mor response. Although NST has been explored previously in solid tumors, such as renal cell carcinoma and breast cancer, it has not been widely explored in nasopharyngeal carcinoma. We also present data from a flow cytometric immune analysis and cytokine enzyme-linked immunosorbent assay analysis in the pre- and post-NST period. *The Oncologist* 2010;15:1192–1197

BACKGROUND

Nasopharyngeal carcinoma (NPC) is an Epstein–Barr virus (EBV)-driven malignancy [1, 2] that is common in south China, Taiwan, Hong Kong, and Singapore [3]. Although the disease is chemosensitive in its early stages, resistance invariably develops, and the prognosis for metastatic patients who have progressed on two or more lines of chemotherapy is poor [4]. Allogeneic nonmyeloablative stem cell transplant (NST) has been shown to be effective in hematological malignancies.

More recently, it has been explored in several solid tumors, including renal cell carcinoma, breast cancer, ovarian cancer, and malignant melanoma, with mixed results [5–7]. Here, we report the successful treatment of a patient with advanced NPC who was treated with a human leukocyte antigen (HLA)-identical sibling NST. Despite the presence of rapidly progressing bulky disease and bone marrow involvement, the tumor regressed substantially and the patient remained progression free for >1.5 years.

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Figure 1. CT scan images demonstrating a reduction in size of the tumor masses (indicated by the arrowheads) after NST. Abbreviation: NST, nonmyeloablative stem cell transplantation.

CASE REPORT

A 48-year-old Chinese gentleman presented in March 2003 with EBV-derived World Health Organization type 3, T2bN2M0 NPC and completed treatment with radical radiotherapy. His disease recurred a year later in March 2004, when he presented with persistent cough and severe weight loss, and computed tomography (CT) scan showed multiple new lung metastases, enlarged mediastinal lymph nodes, and a large left pleural effusion. He was treated successfully with six cycles of paclitaxel and carboplatin chemotherapy until the end of 2004, with a good partial response, and chemotherapy was stopped. He achieved only a brief recess, and in May 2005 his disease progressed and chemotherapy was reinitiated, and he received another four cycles of paclitaxel and carboplatin chemotherapy with stable disease as his best response. In September 2005, he became highly symptomatic with cough, shortness of breath with wheezing, and severe bony pain that was not well controlled with opioids. CT scans showed new metastases in the spine, an increase in the size of the lung metastases, and mediastinal lymphadenopathy. A bone marrow biopsy was performed and this showed metastatic carcinoma. He then consented and enrolled in an NST protocol that was approved by the ethics board of the National Cancer Centre.

On a protocol that was adapted from Spitzer et al. [8], he received a conditioning regimen of i.v. cyclophosphamide

(50 mg/kg per day) on day -5 to day -3 and a single fraction of 7 Gy thymic irradiation, before receiving an unmanipulated, HLA-identical, peripheral stem cell graft from his EBV seropositive brother. In vivo T cell depletion was achieved through the administration of rabbit antithymocyte globuline on days -1 , $+1$, $+2$, and $+3$. He tolerated the transplant well and was discharged home well on day $+17$. Prophylactic cyclosporine was discontinued on day 28. Post-NST, however, the donor chimerism level in his peripheral blood mononuclear cells (an indicator of stem cell engraftment) continued to remain low, at 12%–25%. Because of persistently low donor chimerism levels, the patient received four cycles of donor lymphocyte infusions (DLIs) on days $+63$, $+90$, $+104$, and $+135$.

Although a transient chemotherapy effect was observed on his day $+30$ CT scan, with a decrease in lung metastasis, the day $+60$ CT scan showed only stable disease. However, a CT scan performed after DLIs on day $+130$ showed 34% tumor shrinkage in the mediastinal lymph nodes and lung metastasis that was coincident with the onset of limited cutaneous graft-versus-host disease (GVHD). The latter was manifest by an erythematous fine macular rash on the forearms that was confirmed on skin biopsy. He was treated with cyclosporine (300 mg) and prednisolone (60 mg) daily from day $+154$, and the rash largely resolved by day $+160$. Steroids were stopped on day $+205$ and cyclosporine was stopped on day $+247$,

Figure 2. (A) Patient's rising donor chimerism was temporally related to tumor response; (B) CD3⁺, CD4⁺, and CD8⁺ T-cell recovery after NST; (C) CD4⁺CD25^{high} Treg showed a reduction post NST.

Abbreviation: Treg, T regulatory cell.

3 months after restarting it. He never resumed immunosuppressive drugs after that because there was no recurrence of GVHD. On day +160, when his donor chimerism level reached 100%, a repeat CT scan showed further tumor shrinkage. The patient remained well 1 year after NST, and a CT scan on day +360 continued to demonstrate further tumor shrinkage (Fig. 1). Furthermore, a bone marrow biopsy, which

had been positive pretransplant and at day +100, finally showed no detectable NPC on day +360. His symptoms of cough, shortness of breath, and severe bone pain resolved completely, and his Eastern Cooperative Oncology Group performance status score improved from 2 pre-NST to 0 one year after the NST.

The temporal relationship among rising donor chimer-

Figure 3. (A, B): Patient's CD8⁺CCR7⁺CD45RA⁻ effector memory cells and CD3⁺CD8⁺CD56⁺ (NK)T-like cells showed a rise after day +100; (C) plasma IL-21 levels showed a significant rise post NST that correlated with tumor response.

Abbreviation: IL, interleukin; NK, natural killer.

ism, onset of GVHD, and tumor response (Fig. 2A) is highly suggestive of a graft-versus-tumor effect, indicating that the alloimmune response is capable of controlling NPC. In addition, his plasma EBV DNA titer, which was >44,000 copies/ml pre-NST, was significantly reduced to 940 copies/ml on day +392 post-NST. In all, this patient

achieved a response duration of 396 days and a progression-free survival time of 525 days—an outcome that is both meaningful and difficult to achieve with conventional chemotherapy in the salvage setting. In later months, the patient experienced disease progression in the mediastinal lymph nodes and he developed hemoptysis for which pal-

liative radiotherapy was commenced. Unfortunately, he passed away shortly after radiotherapy was completed on day +774, approximately 2 years after the NST.

In order to characterize systemic immune reconstitution post-transplantation, we performed cytokine arrays and a four-color flow cytometry analysis on the patient's peripheral blood mononuclear cells. This showed that the CD3⁺, CD4⁺, and CD8⁺ T-cell frequencies recovered to normal levels by 1 year post-NST (Fig. 2B). We were also interested in analyzing the levels of T regulatory cells (Tregs) (defined as CD3⁺CD4⁺CD25^{high} cells) because these immunosuppressive cells play a crucial role in maintaining immune tolerance [9]. Levels of peripheral Tregs were found to be highly elevated at baseline, representing 14% of all CD3⁺CD4⁺ cells. In the immediate post-NST period, his Tregs showed a steep fall to 0% at day +30—a phenomenon that may be explained by the use of cyclophosphamide in the pre-NST conditioning regimen [10]. The levels of Tregs continued to remain low until day +392 (Fig. 2C). Conversely, levels of CD8⁺CCR7[−]CD45RA[−] effector memory cells and CD3⁺CD8⁺CD56⁺ natural killer (NK)T-like cells showed a rise after day +100, indicating relative expansion in the number of activated antitumor immune cells (Fig. 3A, 3B). The increases in these specific immune cell populations correlated well with tumor responses in this patient.

We performed a plasma protein array analysis on the patient's pre-NST, day +30, day +60, and day +100 thawed plasma samples, and this showed a significant rise in interleukin (IL)-21 post-NST. These results were validated using sandwich enzyme-linked immunosorbent assay to quantitate the IL-21 level in plasma. This showed that his plasma IL-21 levels increased by a factor of 42× post-NST and remained persistently high, in a pattern that appeared to mirror the tumor response (Fig. 3C). IL-21 is a cytokine with structural homology to IL-2, IL-4, and IL-15; it is produced by activated CD4⁺ T cells and NKT cells and exerts a wide variety of actions, including activation, differentiation, expansion, and maturation of NK, B, CD8, CD4, and dendritic cells, resulting in antitumor activity [11]. IL-21 has been shown to suppress FoxP3 and enhance generation of CD8⁺ antitumor cytotoxic T lymphocytes, whereas

IL-21 blockade has been shown to reduce GVHD mortality through inducible Treg generation [12–13]. In our analysis, the increase in IL-21 levels appeared to correlate with an increase in NKT-like cells and a reduction in Tregs in this patient.

In an analysis of 11 patients with solid tumors who have undergone NST at our center, IL-21 has emerged as a significant factor predicting tumor response (unpublished data). This raises interesting questions regarding whether IL-21 can be used as an adjuvant to improve outcomes for patients post-NST. Indeed phase II trials using recombinant IL-21 monotherapy in metastatic melanoma and renal cell carcinoma patients have reported encouraging clinical responses [14].

CONCLUSION

We report a novel approach in the treatment of metastatic NPC. Although this patient had a poor prognosis because of symptomatic bulky disease, bone marrow involvement, and rapid progression after two lines of combination chemotherapy, he achieved a good response with allogeneic NST. The temporal relationship among increasing donor chimerism, DLIs, and response (as measured by the Response Evaluation Criteria in Solid Tumors) strongly suggests a graft-versus-tumor effect. Our immune analysis suggests a correlation among clinical response, plasma IL-21 increase, Treg level decrease, and an increase in effector memory and NKT-like immune cells. Although the results from this single-patient report demonstrate compelling NST activity in metastatic NPC, this needs further characterization in prospective clinical trials before it can be routinely applied in the clinical setting.

ACKNOWLEDGMENT

This protocol was funded by the Biomedical Research Council of Singapore.

AUTHOR CONTRIBUTIONS

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