Complete Response Induced by Concurrent Chemoradiotherapy in a Patient with NUT Carcinoma: A Case Report

Joji Muramatsu¹, Kohichi Takada¹, Shintaro Sugita², Takaaki Tsuchiya³, Keisuke Yamamoto⁴, Masaru Takagi⁵, Kazuyuki Murase¹, Saki Ameda¹, Yohei Arihara¹, Koji Miyanishi¹, Koh-Ichi Sakata¹ and Junji Kato¹

Abstract:
An 18-year-old man presented with sudden vision loss in his left eye. Magnetic resonance imaging revealed a tumor that had invaded the left optic nerve, originating from the left posterior ethmoid sinus. Immunohistochemical analyses identified positive staining for NUT protein in the nuclei of tumor cells. We diagnosed locally advanced NUT carcinoma (NC) and initiated concurrent chemoradiotherapy (CCRT), consisting of chemotherapy with vincristine, doxorubicin, and cyclophosphamide, alternating with ifosphamide and etoposide, plus radiation therapy. The patient achieved a complete response. CCRT can be a useful treatment option for adolescent and young-adult patients with locally advanced unresectable NC.

Key words: NUT carcinoma, concurrent chemoradiotherapy, VDC-IE regimen

(Intern Med Advance Publication) (DOI: 10.2169/internalmedicine.7741-21)

Introduction
NUT carcinoma (NC), which usually involves midline body parts, such as the head, neck, and thorax, is a rare and highly aggressive carcinoma that occurs in young individuals. NUT carcinoma consists mainly of sheets of undifferentiated cells with focal abrupt dyskeratosis and squamous differentiation. This tumor entity is defined by an acquired chromosomal rearrangement of the NUT gene at the 15q14 locus. The diagnosis requires identifying the chromosomal rearrangement of NUT using fluorescence in situ hybridization (FISH), reverse transcription polymerase chain reaction, or next-generation sequencing (1). Furthermore, a specific monoclonal antibody against NUT has been frequently used for the diagnosis of NC, showing a specificity of 100% and sensitivity of 87% (2). Although NC has a dismal prognosis, recommended therapies have not been established, especially in patients who are ineligible for surgical intervention.

We herein report the successful treatment of an adolescent and young-adult (AYA) patient with unresectable NC using concurrent chemoradiotherapy (CCRT).

Case Report
An 18-year-old man presented with sudden vision loss in his left eye. He was initially diagnosed with left retrobulbar neuritis and treated with steroid-pulse therapy for three days by an ophthalmologist. However, his left vision did not improve. Magnetic resonance imaging (MRI) revealed a tumor in the left posterior ethmoid sinus that had directly invaded the left optic nerve and epidural space (Fig. 1A). To manage the tumor, the patient was referred to the Department of Otolaryngology and Medical Oncology at our hospital.
Laboratory examinations showed a slightly elevated level of squamous cell carcinoma antigen [1.8 ng/mL (normal...
The malignant cells showed nuclear staining for INI-1 and stained positive for NUT in nuclei (Fig. 3B), CK5/6 (mild), p40 (focal), and vimentin but stained negative for CD3, CD4, CD8, CD20, CD56, S100, synaptophysin, and Epstein-Barr Virus-encoded small RNA in situ hybridization (EBER-ISH). A FISH analysis did not reveal a NUTM1-BRD4 rearrangement, but an analysis of NUTM1 showed a NUTM1 split signal in 90% of the tumor (Fig. 4). Taken together, these results led to a final diagnosis of locally advanced unresectable NC.

We initiated CCRT, consisting of chemotherapy with VDC (vincristine at a dose of 1.5 mg/sqm on day 1, doxorubicin at a dose of 75 mg/sqm on days 1-2, and cyclophosphamide at a dose of 1,200 mg/sqm on day 1), alternating with IE (ifosfamide at a dose of 1.8 g/sqm on days 1-5 and etoposide at a dose of 100 mg/sqm on days 1-5), repeated every 14 days, according to a regimen for Ewing sarcoma (7), plus radiation therapy (70 Gy/35 Fr) (Fig. 5). To avoid cardiotoxicity of doxorubicin, we administered the VDC regimen for up to 5 cycles, subsequently replacing this with a VAC [consisting of vincristine, actinomycin D (at a dose of 1.25 mg/sqm on day 1), and cyclophosphamide]-IE regimen, which was continued for 4 cycles. Conventional radiation therapy was applied to the tumor simultaneously starting two weeks before the first VDC treatment. The patient was treated with 2.0 Gy once daily, with a total number of 20 fractions delivering a cumulative dose of 40 Gy to the tumor. Thereafter, intensity modulated radiotherapy was continued with 2.0 Gy once daily up to a cumulative dose of 30 Gy to control the tumor completely. Except for Grade 3 febrile neutropenia, no other serious adverse events were observed.

After completion of the CCRT, we conducted MRI and PET-CT (Fig. 6) and biopsied the tumor area. Images and a pathological examination revealed no evidence of residual tumor, so we determined the patient to have achieved a complete response (CR). The patient maintained a CR for seven months after the CCRT was completed.

However, follow-up MRI revealed a relapsed tumor in the left ethmoid sinus that had invaded directly into the skull base. We treated the relapsed tumor in the patient with pro-
Figure 3. Microscopic findings of the resected tumor. Hematoxylin and Eosin staining (A, x200). NUT staining (B, x200).

Table 1. Typical Immunohistochemical Staining Patterns of Various Small Round-cell Tumors in the Ethmoid Sinus.

| Histology                                      | CK5/6 | p40 | cluster of differentiation | S100 | synaptophysin | INI-1 | EBER-ISH | NUT |
|-----------------------------------------------|-------|-----|---------------------------|------|---------------|-------|----------|-----|
| NC1                                           | 5/6+  | +   | -                         | -    | +             | -     | -        | +   |
| SNUC1                                          | 5/6-  | -   | -                         | -    | +             | -     | -        | -   |
| Lymphoepithelial carcinoma1                    | 5/6+  | +   | -                         | -    | +             | +     | -        | +   |
| Malignant lymphoma4                            | 5/6-  | -   | +                         | -    | -             | +     | **       | -   |
| Olfactory neuroblastoma5                      | 5/6-  | -   | +                         | +    | +             | +     | -        | -   |
| SMARCB1(INI-1)-deficient sinonasal carcinoma4  | 5+    | +   | -                         | -    | (focal)       | -     | -        | -   |
| ESFT (PNET)6                                   | 5/6-  | -   | +                         | +    | +             | +     | -        | -   |
| Our case                                       | 5/6+(mild) | (focal) | - | - | + | - | - | + |

CK5/6: cytokeratin5/6, EBER-ISH: Epstein–Barr Virus-encoded small RNA in situ hybridization, ESFT (PNET): Ewing sarcoma family of tumors (peripheral primitive neuroectodermal tumor), INI-1: integrase interactor 1, NC: NUT carcinoma, SMARCB1: SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1, SNUC: sinonasal undifferentiated carcinoma

*CD3, 4, 8, 20, 56. **generally negative, except for NK/T cell lymphoma (100% positive), Hodgkin lymphoma (40% positive), or Burkitt lymphoma (10% positive).

Figure 4. Results of a fluorescence in situ hybridization (FISH) analysis of NUTM1 showing a NUTM1 split signal (arrows) and a fused signal (arrowhead) in the tumor.

A recent retrospective study demonstrated that the median overall survival (OS) was 6.5 months in patients with NC, with around a 70% chance of death within a year (8), highlighting the need to develop effective treatment strategies for this disease. Currently, most patients with NC receive multimodal treatment composed of a combination of surgery, chemotherapy, and radiation. In patients with NC of the head and neck, aggressive surgery with or without post-operative chemo-radiotherapy or radiation is recommended, as the treatment is associated with good clinical outcomes (9). Of note, upfront complete resection prolonged the OS significantly. However, in our case, we conducted CCRT since the patient was ineligible for complete resection, as the tumor had directly invaded the epidural space; the patient also wanted to retain his physical appearance.

We selected CCRT consisting of a VDC-IE regime as the first-line treatment because several reports have demon-
Clinical course

| VDC : VCR 1.5 mg/sqm (max 2mg) (day 1) |
|--------------------------------------|
| DXR 75 mg/sqm/48h (day 1-2)          |
| CPA 1,200 mg/sqm (day 1)             |
| IE : IFO 1.8 g/sqm/day (day 1-5)    |
| ETP 100 mg/sqm/day (day 1-5)        |
| VAC : VCR 1.5 mg/sqm (max 2mg) (day 1) |
| Actinomycin 1.25 mg/sqm (day 1)     |
| CPA 1,200 mg/sqm (day 1)            |
| PBR (70Gy/35Fr)                     |

Figure 5. Clinical course. CPA: cyclophosphamide, CR: complete response, DXR: doxorubicin, ETP: etoposide, IE: ifosfamide, and etoposide, IFO: ifosfamide, m: months, PBR: proton beam radiotherapy, RT: radiotherapy, VAC: vincristine, actinomycin D, and cyclophosphamide, VCR: vincristine, VDC: vincristine, doxorubicin, and cyclophosphamide, X: initiation of therapy

| RT (70Gy/35Fr)       | Local recurrence | CR |
|----------------------|------------------|----|
| V D I C E V D I C E V D I C E V D I C E V A C E V A C E V A C E V A C E | X + 9m | CR |
| V D I C E V D I C E V D I C E V D I C E V A C E V A C E V A C E V A C E | X + 16m | CR |
| V D I C E V D I C E V D I C E V D I C E V A C E V A C E V A C E V A C E | X + 18m | CR |

Figure 6. Magnetic resonance imaging (MRI) (A) and positron emission tomography (PET)-computed tomography (CT) (B) after treatment. MRI and PET-CT did not reveal any abnormalities with signs of relapse.

...strated that such a regimen was effective for Ewing sarcoma; combined with radiotherapy and surgery, this regimen was also effective for patients with NC (Table 2). To maximize the dose intensity, we treated the patient every two weeks (15). According to a previous report and our case, regimens for Ewing sarcoma are adequate and useful for not only pediatric but also AYA patients with NC (13). Unfortunately, other chemotherapeutic regimens have not been successful clinically (9, 16). Therefore, at present, regimens for Ewing sarcoma are the best strategies for patients with advanced-stage NC or in a CCRT or adjuvant chemotherapy setting.

Recently, several bromodomain and extra-terminal (BET) protein inhibitors have induced clinical responses in patients with NC (17, 18). Consequently, BET inhibitors will continue to be used for patients with NC and to improve the prognosis in the near future.

After definitive CCRT, the patient presented with local recurrence within the irradiated field. Re-irradiation of the relapsed tumor ran the risk of inducing bilateral blindness because the optic chiasm and normal right optic nerve had already been irradiated. Consequently, we selected PBR as radical treatment for the relapsed tumor. The patient achieved a CR without any severe adverse events, such as blindness. To our knowledge, no reports have demonstrated the efficacy of PBR for NC. This case suggests that PBR may be an effective therapeutic option for NC originating in the head and neck region.

The factors of primary tumor site, lymph nodes/distant metastases, and the type of fusion gene present have been identified as prognostic factors for NC (8). Harboring an \textit{NUTM1-BRD4} fusion gene was significantly associated with...
Table 2. Efficacies of Regimens for Ewing Sarcoma in Patients with NUT Carcinoma.

| Case | Age (y) Sex | Primary Metastasis | NUT fusion type | 1st line therapy | 2nd line Therapy | Efficacy | OS |
|------|-------------|--------------------|----------------|------------------|------------------|----------|----|
| 1    | 10/M        | ilium -            | BRD4           | VAI-PAI-VAI x4 RT(60 Gy/40 Fr) | -             | CR       | 13 y (alive) |
| 2    | 9/M         | sublingual gland cervical L/N | NA | Surgery VAI-PAI-VAI x4 RT (54 Gy/32 Fr) | - | CR | 6 y (alive) |
| 3    | 9/M         | parotid gland cervical L/N | NA | Surgery VAI-PAI-VAI x4 RT (59.4 Gy/33 Fr) | - | CR | 15 M (alive) |
| 4    | 49/M        | nasal sinuses ilium | NA | VDCx6 CDDP RT (75 Gy/35 Fr) Surgery | - | PD | 9 M |
| 5    | 15/F        | nasal sinuses -    | BRD3           | VAI-PAI-VAI x4 RT (68.4 Gy/38 Fr) | - | CR | 34 M (alive) |
| 6    | 12/F        | nasal sinuses vertebral body, right sacrum, femur, tibia | NA | VDC-IE x14 RT (55.8 Gy) Surgery | - | CR | 40 M (alive) |
| Our case | 18/M       | ethmoid sinus - | NA | VDC-IE x17 RT (70 Gy/35 Fr) PBR (70 Gy [RBE]/35 Fr) | CR | 26 M (alive) |

Cases 1–3, and 5 were treated with a vincristine, doxorubicin, ifosfamide (VAI)–cisplatin, doxorubicin, ifosfamide (PAI) regimen. Cases 4 and 6 and our case were treated with a vincristine, doxorubicin, and cyclophosphamide (VDC) or VDC–ifosfamide and etoposide (IE) regimen. CDDP: cisplatin, CR: complete response, L/N: lymph node, NA: not applicable, OS: overall survival, PD: progressive disease, PBR: proton beam radiotherapy, RBE: relative biological effectiveness, RT: radiotherapy

a poor OS (8, 19). In our case, an NUTM1-BRD4 fusion gene was not detected with a FISH analysis, and we speculate that this may be one of the reasons why the patient showed a such good clinical outcome.

Our findings suggest that CCRT, comprising a VDC-IE regimen, can be a useful treatment option for AYA patients with NC, even when the cancer is at a locally advanced unresectable stage.

The authors state that they have no Conflict of Interest (COI).

Acknowledgements

The authors would like to thank the patient and his family for allowing the publication of this case study.

References

1. Mao N, Liao Z, Wu J, et al. Diagnosis of NUT carcinoma of lung origin by next-generation sequencing: case report and review of the literature. Cancer Biology & Therapy 20: 150-156, 2019.
2. Haack H, Johnson LA, Fly CJ, et al. Diagnosis of NUT midline carcinoma using a NUT-specific monoclonal antibody. Am J Surg Pathol 33: 984-991, 2009.
3. Franchi A. Pathology of Sinonasal Tumors and Tumor-Like Lesions. 1st ed. Springer, Germany, 2019: 110-113.
4. Ayee R, Ofori MEO, Wright E, Quaye O. Epstein Barr Virus Associated Lymphomas and Epithelia Cancers in Humans. Journal of Cancer 17: 1737-1750, 2020.
5. Wooff JC, Weinreb I, Perez-Ordonez B, Magee JF, Bullock MJ. Calretinin staining facilitates differentiation of olfactory neuroblastosoma from other small round blue cell tumors in the sinonasal tract. Am J Surg Pathol 35: 1786-1793, 2011.
6. Shanfeld RL, Edelman J, Willis IE, et al. Immunohistochemical analysis of neural markers in peripheral primitive neuroectodermal tumors (pPNET) without light microscopic evidence of neural differentiation. Applied Immunohistochemistry & Molecular Morphology 5: 78-86, 1997.
7. Chin M, Yokoyama R, Sumi M, et al. Multimodal treatment including standard chemotherapy with vincristine, doxorubicin, cyclophosphamide, ifosfamide, and etoposide for the Ewing sarcoma family of tumors in Japan: results of the Japan Ewing Sarcoma Study 04. Pediatric Blood & Cancer 67: e28194, 2020.
8. Chau NG, Ma C, Danga K, et al. An Anatomical Site and Genetic-Based Prognostic Model for Patients With Nuclear Protein in Testis (NUT) Midline Carcinoma: Analysis of 124 Patients. JNCI Cancer Spectrum 4: pk094, 2019.
9. Chau NG, Hurwitz S, Mitchell CM, et al. Intensive treatment and survival outcomes in NUT midline carcinoma of the head and neck. Cancer 122: 3632-3640, 2016.
10. Mertens F, Wiebe T, Adlercreutz C, Mandahl N, French CA. Successful treatment of a child with t(15;19) - positive tumor. Pediatric Blood & Cancer 49: 1015-1017, 2007.
11. Storck S, Kennedy AL, Marcus KJ, et al. Pediatric NUT-midline carcinoma: Therapeutic success employing a sarcoma based multimodal approach. Pediatr Hematol Oncol 34: 231-237, 2017.
12. Arimizu K, Hirano G, Makiyama C, Matsuo M, Sasaguri T, Makiyama A. NUT carcinoma of the nasal cavity that responded to a chemotherapy regimen for Ewing’s sarcoma family of tumors in Japan: a report of two cases. J Clin Oncol 20: 4148-4154, 2012.
13. Leeman R, Pinkey K, Bradley JA, et al. NUT Carcinoma Without Upfront Surgical Resection: A Case Report. J Pediatr Hematol Oncol 2020.
14. Sopie J, Greffe B, Trece AL. Metastatic NUT Midline Carcinoma Treated With Aggressive Neoadjuvant Chemotherapy, Radiation, and Resection: A Case Report and Review of the Literature. J Pediatr Hematol Oncol 43: e73-e75, 2021.
15. Wonner RB, West DC, Krailo MD, et al. Randomized Controlled Trial of Interval-Compressed Chemotherapy for the Treatment of Localized Ewing Sarcoma: A Report From the Children’s Oncology Group. J Clin Oncol 30: 4148-4154, 2012.
16. Prasad M, Baheti A, Ramadwar M, Chinnaswamy G, Vora T, Qureshi S. Pediatric NUT Carcinoma Is a Rare and Challenging Tumor: Single Center Experience of Five Children. Oncologist 24: e1232-e1235, 2019.
17. Shapiro GI, LoRusso P, Dowlati A, et al. A Phase 1 study of RO 6870810, a novel bromodomain and extra-terminal protein inhibitor, in patients with NUT carcinoma, other solid tumours, or diffuse large B-cell lymphoma. Br J Cancer 124: 744-753, 2020.
18. Lewin J, Soria JC, Stathis A, et al. Phase Ib Trial With Birabresib, a Small-Molecule Inhibitor of Bromodomain and Extraterminal Proteins, in Patients With Selected Advanced Solid Tumors. J Clin Oncol 36: 3007-3014, 2018.
19. French CA, Kutok JL, Faquin WC, et al. Midline carcinoma of children and young adults with NUT rearrangement. J Clin Oncol 15: 4135-4139, 2004.