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Genitourinary Cancer

Treatment of CD30-Expressing Germ Cell Tumors and Sex Cord Stromal Tumors with Brentuximab Vedotin: Identification and Report of Seven Cases

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Immunoconjugates • CD30 antigen • Germ cell tumor • Sex cord stromal tumor • Clinical trial

Abstract

Background. Cytotoxic therapy for relapsed and refractory germ cell tumors or metastatic sex cord stromal tumors is rarely effective and is often accompanied by high adverse event rates. Expression of CD30 has been observed in testicular cancers, and patients with CD30-expressing embryonal carcinomas have worse progression-free survival and overall survival than those with CD30-negative tumors. The objective of this study (NCT01461538) was to characterize the antitumor activity of brentuximab vedotin in patients with CD30-expressing nonlymphomatous malignancies. Enrolled patients included seven patients with relapsed or refractory germ cell tumors or metastatic sex cord stromal tumors described in this case series.

Materials and Methods. Forty patients with relapsed or refractory germ cell tumors, metastatic sex cord stromal tumors, or testicular tumors were screened for CD30 expression; 14 patients had tumors that expressed CD30. Seven patients with CD30-expressing testicular cancer were enrolled in the treatment study: five patients with germ cell tumors, one patient with a Leydig cell tumor, and one patient with a Sertoli cell tumor. Patients were treated with brentuximab vedotin at initial doses of 1.8 or 2.4 mg/kg every 3 weeks. Response assessments were performed at cycles 2 and 4 and every 4 cycles thereafter while the patient was receiving treatment.

Results. Two of seven patients achieved an objective response, including one durable complete response and one partial response at a single time point. Both responding patients had germ cell tumors. Treatment with brentuximab vedotin was generally well tolerated.

Conclusion. Treatment of relapsed or refractory germ cell tumors with brentuximab vedotin can induce durable responses with a manageable toxicity profile. ***The Oncologist*** 2018;23:316–323

Implications for Practice: This case series of seven patients with relapsed or refractory CD30-expressing germ cell tumors (GCTs) or sex cord stromal tumors demonstrates that brentuximab vedotin has activity against GCTs and is well tolerated in heavily pretreated patients with these aggressive tumor types. One patient achieved a complete response that has been durable for almost 4 years since the discontinuation of treatment with brentuximab vedotin. Therefore, brentuximab vedotin may be a valuable option for physicians who care for this difficult-to-treat patient population.

Introduction

Testicular cancer is the most common solid tumor among men aged 15–44 years [1]. Testicular cancers are classified as germ cell tumors (GCTs) or non-germ cell tumors, with sex cord stromal tumors (SCSTs) comprising the majority of the latter category. Rarely, GCTs are present solely in extragonadal sites, such as the anterior mediastinum or retroperitoneum. The histology of GCTs is divided into seminomas and nonseminomatous GCTs (NSGCTs), and NSGCTs can be further subdivided into embryonal carcinomas (ECs), teratomas, yolk sac tumors, and choriocarcinomas. Germ cell tumors are extremely sensitive to cisplatin-based chemotherapy compared with other solid tumors, and more than 70% of patients with GCTs are cured with initial cisplatin-based chemotherapy with or without adjunctive surgery [2–4]. Among patients with GCTs in whom initial treatment is not successful, salvage treatments, including chemotherapy (conventional or high-dose) or additional surgeries, have increased the survival rate [5, 6]. Independent risk factors that indicate a worse prognosis in patients treated

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with high-dose salvage chemotherapy include platinum-refractory disease, persistent disease following two or more lines of prior chemotherapy, and a high-risk International Germ Cell Cancer Collaborative Group (IGCCCG) stage at the time of initial treatment [7]. Individuals who relapse following treatment with high-dose salvage chemotherapy (HDCT) have an especially dire prognosis, with a long-term survival rate of $<$5% [8].

Available treatment options for patients with multiply relapsed disease include gemcitabine, oxaliplatin, oral etoposide, paclitaxel, or additional salvage surgeries [2, 5]. However, these treatments rarely lead to durable disease remissions and are often accompanied by high adverse event rates [7, 9]. A recent retrospective analysis of patients with relapsed or refractory GCT treated with a range of therapies across several phase II trials demonstrated a low objective response rate (ORR; 1%) and a median overall progression-free survival of only 1 month [5].

Sex cord stromal tumors (Sertoli and Leydig cell tumors) are usually benign. However, approximately 10% of tumors in adults are malignant and can metastasize and ultimately lead to death [10]. Patients with metastatic Sertoli and Leydig cell tumors also currently face limited treatment options. Radiation and chemotherapy are usually not effective in the presence of metastatic disease, and surgical resection is therefore the recommended treatment option. Orchiectomy may be followed by retroperitoneal lymph node dissection if there is evidence of regional lymph node metastasis or if high-risk features are identified within the primary tumor [10, 11].

Expression of CD30 has been observed in a number of nonlymphomatous malignancies, including testicular cancer. Specifically, EC, which is present in approximately 90% of NSGCTs [12] either alone or, more commonly, in conjunction with other histologies as part of a mixed cell GCT, has been shown to express high levels of CD30, whereas other GCTs do not express CD30 [13]. Additionally, CD30 is not expressed in normal adult, neonatal, or fetal testicular tissue, suggesting that therapies targeting CD30 may be useful in the treatment of certain subtypes of testicular cancer. A recent examination of CD30 expression as a prognostic indicator in patients with relapsed or refractory EC demonstrated that patients with CD30-expressing tumors had worse progression-free survival and overall survival than patients with CD30-negative tumors. Moreover, serial biopsy results demonstrated that CD30 expression was persistent in tumors after relapse, further highlighting its potential usefulness as a therapeutic target [14].

Brentuximab vedotin (Adcetris, Seattle Genetics, Inc., Bothell, WA) is an anti-CD30 antibody-drug conjugate (ADC) consisting of the chimeric anti-CD30 monoclonal IgG1 antibody (cAC10), specific to human CD30, covalently attached to the microtubule-disrupting agent monomethyl auristatin E (MMAE) by a protease-cleavable linker. The ADC binds to CD30-expressing cells, leading to internalization of the ADC-CD30 complex and the release of MMAE via proteolytic cleavage within the cell. Binding of MMAE to tubulin disrupts the microtubule network within the cell, inducing cell cycle arrest and apoptotic death of the cell [15]. Targeted delivery of MMAE to CD30-expressing cells is the primary mechanism of action of brentuximab vedotin [16]; however, additional proposed mechanisms of tumor cell killing that may contribute to the clinical activity of brentuximab vedotin include antibody-dependent cellular phagocytosis, immunogenic cell death, and the bystander effect [17–23].

Two pivotal phase II studies have demonstrated the efficacy and safety of brentuximab vedotin administered every 3 weeks (Q3W) as a single agent for treating relapsed or refractory Hodgkin lymphoma (HL) and systemic anaplastic large cell lymphoma (ALCL), both of which express high levels of CD30. The ORR for patients with HL was 75%, with approximately one third of patients achieving a complete remission (CR) [24]. An ORR of 86% was observed in patients with systemic ALCL, with over 50% of patients achieving a CR [25]. In both studies, brentuximab vedotin was generally well tolerated with manageable side effects.

A phase II, open-label study to evaluate the antitumor activity, safety, pharmacokinetics, and immunogenicity of brentuximab vedotin in patients with CD30-expressing nonlymphomatous malignancies (NCT01461538) was sponsored by Seattle Genetics, Inc. Seven patients with CD30-expressing GCTs and SCSTs (four patients with testicular GCTs, one patient with an anterior mediastinal GCT, one patient with a Sertoli cell tumor, and one patient with a Leydig cell tumor) were enrolled in the treatment study and were administered brentuximab vedotin at starting doses of either 1.8 mg/kg or 2.4 mg/kg Q3W. The outcomes of these seven patients are presented in this case series.

Materials and Methods

Patients

Patients with CD30-expressing nonlymphomatous tumors were identified through a companion screening protocol that employed immunohistochemical staining (IHC) of tissue biopsy samples using an anti-BerH2 antibody [26]. Patients with histologically confirmed CD30-expressing tumors ($\geq$10% CD30 expression) must have also failed, refused, or have been deemed ineligible for standard therapy. Patients must have had measurable disease, defined for solid tumors as at least one nonresectable lesion $\geq$10 mm in the longest diameter. Patients were required to be at least 12 years of age (or $\geq$6 years of age with permission from the sponsor) and have an Eastern Cooperative Oncology Group Performance Status score of 0 or 1 or a Karnofsky or Lansky Performance Status score $\geq$70. Screening and treatment studies were performed at investigational sites following approval by an investigational review board (IRB), and all patients provided informed consent prior to administration of any study treatment. Institutional review board oversight for the seven patients described in this study report was performed by the Indiana University IRB (IRB study #1111007468) and the U.S. Oncology IRB (Protocol #10328).

Study Design and Treatment

During the screening study, CD30 expression was assessed in eligible patients with GCTs, SCSTs, unclassified sex cord tumors, and unclassified testicular tumors. Seven patients with CD30-expressing disease were subsequently enrolled in the treatment study from May 2012 to February 2014 and were treated with brentuximab vedotin administered via intravenous infusion over a period of 30 minutes on day 1 of each 21-day cycle. Patients enrolled under the original protocol received an initial dose of 1.8 mg/kg Q3W (Cohort 1), the recommended single-

agent dose for the treatment of relapsed or refractory HL and systemic ALCL. However, the original phase I dose escalation study of brentuximab vedotin in hematological malignancies (NCT00430846) demonstrated acceptable toxicity up to a dose level of 2.7 mg/kg Q3W. Therefore, after completion of enrollment of Cohort 1 in this study, the protocol was amended to evaluate a second cohort of patients dosed with 2.4 mg/kg brentuximab vedotin Q3W. Following enactment of the amendment, patients who were initially treated with 1.8 mg/kg brentuximab vedotin Q3W (Cohort 1) and were still on-study could also have their dose increased to 2.4 mg/kg Q3W at the discretion of the investigator. Dose reductions for tolerability were allowed during the course of the study. Response assessments were performed at cycles 2 and 4 and every four cycles thereafter while the patient was receiving treatment. Efficacy assessments included radiographic tumor imaging of the chest, abdomen, and pelvis graded using Response Evaluation Criteria in Solid Tumors version 1.1 [27]. Patients with stable disease or better were eligible to continue treatment with brentuximab vedotin until disease progression, unacceptable toxicity, or study closure. In addition, physical examination, serum chemistry panels, coagulation panels, complete blood counts with differential, performance status, and select vital sign measurements were performed on day 1 of each cycle. Patients were monitored for unacceptable toxicities. Adverse events were recorded for the safety reporting period from day 1 predose through the end of treatment visit or 30 days after the last study treatment, whichever was later.

Chart Review

This study was not specifically designed to evaluate patients with GCTs or SCSTs; rather, it was designed to evaluate patients with a broad range of nonlymphomatous lesions. Therefore, chart reviews were conducted by the investigators to collect relevant clinical testicular cancer information that may not have been collected as part of the study. The results of this chart review in concert with data collected during the original study are presented for each of the seven patients with CD30-expressing GCTs and SCSTs who were treated with brentuximab vedotin.

Results

A total of 40 patients with GCTs, SCSTs, unclassified sex cord tumors, and unclassified testicular tumors were screened for CD30 expression by IHC. Of these, 14 patients were considered to have CD30-expressing tumors, using a cutoff for positivity of $\geq$10% staining by IHC. Seven patients (five patients with GCTs, one patient with a Leydig cell tumor, and one patient with a Sertoli cell tumor) with CD30-expressing disease were subsequently enrolled in the treatment study. In total, two patients achieved an objective response, including one complete response and one partial response at a single time point, the former of which is ongoing. Additionally, three patients achieved a best response of stable disease (Fig. 1).

Case 1

A 24-year-old male patient initially presented with a poor-risk, metastatic, mixed testicular GCT (60% embryonal cell and 40% choriocarcinoma). He had lung, liver, and brain metastases; retroperitoneal disease; and a human chorionic gonadotropin

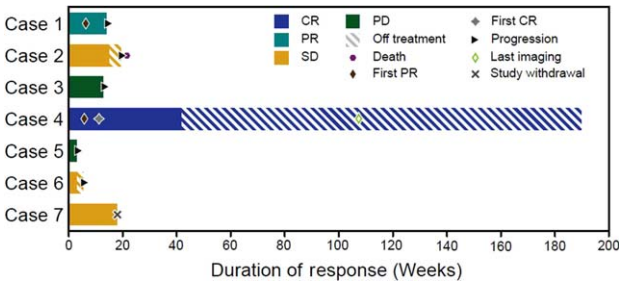

Figure 1. Best responses in patients with testicular cancer treated with brentuximab vedotin. Swimmer's plot showing the best response and duration of response in all patients with testicular cancer treated with brentuximab vedotin. Solid bars represent time on treatment, and hatched bars represent time off treatment. Time of progression is noted by a black arrow, onset of partial responses by a brown diamond, onset of complete responses by a gray diamond, and time of death by a purple hexagon. The patient in Case 1 achieved a partial response at a single time point only.

Abbreviations: CR, complete remission; PD, progressive disease; PR, partial response; SD, stable disease.

(HCG) level >180,000 mIU/mL. The patient underwent a left radical orchiectomy, a left suboccipital craniotomy, and gamma knife radiation treatment to the cerebellum. Frontline chemotherapy included three cycles of bleomycin, etoposide, and cisplatin (BEP) and one cycle of etoposide and cisplatin (EP). He subsequently relapsed with pulmonary disease and was treated with one cycle of paclitaxel and gemcitabine, followed by a HDCT regimen consisting of two cycles of carboplatin plus etoposide with autologous stem cell rescue and radiation therapy to the right hilum. After relapsing again, he received additional salvage therapy with four cycles of gemcitabine, oxaliplatin, and paclitaxel. After achieving a best response of stable disease, his disease progressed 5 months later. At that point his archived orchiectomy tumor specimen was evaluated for CD30 expression, which was found to be present in 90% of tumor cells, and he was enrolled in the treatment study.

At baseline staging, the patient's HCG was 5,571 mIU/mL, and he had target lesions on the spleen, periaortic lymph node, right lobe of the lung, and right iliacus muscle (Table 1). The patient began treatment with brentuximab vedotin 1.8 mg/kg Q3W. His HCG decreased to 1,924 mIU/mL after the first cycle of treatment and further decreased to 45 mIU/mL after the second treatment cycle. Cycle 2 response assessments indicated a partial response to therapy. After two additional cycles of brentuximab vedotin, he experienced an increase in HCG to 502 mIU/mL and a further increase to 4,076 mIU/mL after the fourth cycle. At that point a computed tomography (CT) scan also showed evidence of disease progression at multiple sites. No additional therapy was given, and the patient subsequently died of progressive disease approximately 8 months after coming off the study.

Case 2

A 33-year-old male patient presented with a poor-risk, metastatic, mixed testicular GCT consisting of EC, choriocarcinoma, and teratoma. He initially presented with an enlarged left supraclavicular lymph node, large bilateral pulmonary metastases, retroperitoneal disease, and a tumor in his right testis. The patient's HCG was 133,161 mIU/mL. Frontline treatment included four cycles of etoposide, ifosfamide, and cisplatin

Table 1. Clinical features of patients with germ cell tumors or sex cord stromal tumors treated with brentuximab vedotin

Patient	Tumor type	Sites of metastasis at enrollment	Best response	CD30 expression	H-score	CD30 cellular localization	HCG: mIU/mL	AFP: ng/mL
1	Mixed GCT: EC, choriocarcinoma	Spleen, lung, iliacus muscle, periaortic lymph nodes	Partial response[a]	90%	190	Membrane, cytoplasm	BL 5,571.0, min 45.3 (C3D1)	N
2	Mixed GCT: EC, choriocarcinoma, teratoma	Lung, supraclavicular lymph nodes, retroperitoneal lymph nodes	Stable disease	90%	195	Membrane, cytoplasm	BL 1,0401.0, min 52.7 (C4D1)	BL 3.1, min 2.5 (C5D1)
3	EC	Liver, mesentery/ small intestine, retroperitoneum	Progressive disease	100%	265	Membrane, cytoplasm, Golgi	BL 1.3	BL 2.9
4	Mixed GCT[b]: EC, seminoma, teratoma	Lung; mediastinal, hilar, and supraclavicular lymph nodes	Complete response	100%	270	Membrane, cytoplasm, Golgi	N	N
5	Primary mediastinal GCT	Mediastinum, carina, aortic arch, lung, lytic bone lesions	Progressive disease	80%	230	Membrane, cytoplasm	N	BL 151.5, min 151.5 (BL)
6	Sertoli cell	Lung; supraclavicular, subcarinal, and paratracheal lymph nodes	Stable disease	98%	206	Membrane, cytoplasm	N	N
7	Leydig cell	Lung; supraclavicular, subcarinal, and retrocrural lymph nodes; pelvis	Stable disease	100%	225	Membrane, cytoplasm	N	N

H-score (range 0–300) was calculated as the product of the intensity of CD30 staining (0–3) multiplied by the percentage of positively staining cells (0–100).
[a]This patient achieved a partial response at a single time point only.
[b]Metastatic disease displayed pure EC histology.
Abbreviations: AFP, alpha-fetoprotein; BL, baseline value; C, cycle; D, day; EC, embryonal carcinoma; GCT, germ cell tumor; H-score, histo score; HCG, human chorionic gonadotropin; min, minimum on-study value; N, normal or not performed.

(VIP). Prior to the start of therapy, a biopsy of his left supraclavicular lymph node revealed 75% EC, 20% choriocarcinoma, and 5% mature teratoma. After completing four cycles of VIP, he underwent a delayed orchiectomy that revealed only mature teratoma. He then relapsed with his HCG increased, and he was found to have a new brain metastasis that was treated with stereotactic radiosurgery, with complete disappearance of his tumor. He was then treated with an HDCT regimen of carboplatin and etoposide, followed by an autologous stem cell transplant. Stable disease was achieved for 1 month, after which the patient's disease progressed. At that point, his left supraclavicular lymph node sample was evaluated for CD30 expression and was found to have 90% of malignant cells stain positive for CD30 by IHC. He was then enrolled in the treatment study.

At the baseline visit, the patient's HCG was 10,401 mIU/mL. Lesions were present in a left supraclavicular lymph node, right and left upper lungs, and a left retroperitoneal lymph node (Table 1). The patient began receiving brentuximab vedotin at a dose of 1.8 mg/kg Q3W. His HCG decreased to 832 mIU/mL following the first treatment cycle, decreased further to 111 mIU/mL following the second cycle, and reached a nadir of 53 mIU/mL after the third treatment cycle. Tumor assessments following cycle 2 indicated stable disease with no progression of target lesions and no new nontarget lesions. Following the fourth treatment cycle, the patient's HCG increased to 439 mIU/mL, but radiologically he continued to have stable disease. After cycle 5 of brentuximab vedotin, the patient's HCG had increased to 1,186 mIU/mL, and his left lung lesion had increased in size. New lesions were also present in the liver and lung. The patient was considered to have progressive disease and discontinued treatment with brentuximab vedotin. The patient subsequently died of progressive disease less than 2 months after coming off the study.

Case 3

A 58-year-old male patient was diagnosed with a metastatic pure EC with good risk per the IGCCCG criteria. He underwent a left radical orchiectomy followed by four cycles of BEP at an outside institution and achieved stable disease. He relapsed with metastatic disease in the retroperitoneal lymph nodes and was treated with a HDCT regimen of two cycles of carboplatin and etoposide and tandem autologous stem cell transplant. He achieved stable disease but relapsed after 7 months with liver and mesenteric metastases, at which time he received oral etoposide. A duodenal mass sample was evaluated for CD30 expression and was determined to be 100% CD30-positive. He then enrolled in the treatment study.

At baseline, the patient had two lesions on the liver, a mesenteric mass on the small intestine (Table 1), and HCG and alpha-fetoprotein (AFP) within the normal range. The patient

received one cycle of brentuximab vedotin at a dose of 1.8 mg/kg but experienced multiple adverse events and subsequently discontinued treatment. At the end of treatment visit, the patient's imaging studies showed unequivocal progression of nontarget lesions in the liver. The patient subsequently had rapid deterioration and died of progressive disease 2 months after coming off the study.

Case 4

A 26-year-old male patient initially presented with a right testis mass and underwent a right radical orchiectomy. Tumor pathology demonstrated a mixed GCT composed of 90% EC and smaller components of seminoma and teratoma. On staging, he was found to have metastatic disease involving the lungs and retroperitoneal, mediastinal, and left supraclavicular lymph nodes. Tumor markers were normal. For good risk, stage III-A, mixed NSGCT, he received four cycles of EP and achieved a partial response with normalization of his tumor markers but had residual retroperitoneal nodes and small remaining lung nodules. Although a retroperitoneal lymph node dissection demonstrated no evidence of viable disease, shortly thereafter he was found to have enlarging mediastinal lymph nodes and lung nodules without elevated tumor markers. Viable EC was demonstrated on a thoracoscopic biopsy specimen. The patient was treated with HDCT using a regimen of paclitaxel and ifosfamide followed by carboplatin and etoposide, including three cycles of high-dose carboplatin and etoposide, each followed by stem cell reinfusion. He achieved a near complete response and was again placed on surveillance. However, approximately 5 months later, he was noted to have enlarging right lower lobe lung nodules and underwent a right lower lobectomy and regional lymph node dissection, with pathology demonstrating viable EC in both right lower lobe lung nodules as well as intralobular lymph nodes. On active surveillance, the patient developed progression in the lungs and mediastinal lymph nodes approximately 2 months later. He was enrolled in a phase II clinical trial of 5-fluorouracil, oxaliplatin, and leucovorin plus alvocidib (flavopiridol, Tolero Pharmaceuticals, Inc.) but progressed after two cycles. A lung tumor sample from the patient's thoracotomy was evaluated for CD30 expression by IHC and was found to be 100% CD30-positive. He was subsequently enrolled in the treatment study.

At baseline, the patient had lesions on the medial, lower right lobe of the lung and on the inferior hilum of the right lung (Table 1). Nontarget lesions were also present in supraclavicular and mediastinal lymph nodes. The patient began receiving brentuximab vedotin at a dose of 1.8 mg/kg Q3W. He achieved a partial response after two cycles, with the right lung lesion completely resolving and the right hilar lesion decreasing in size, and converted to a complete response after four cycles (Fig. 2). The patient continued treatment with brentuximab vedotin 1.8 mg/kg Q3W, and a complete response to treatment was maintained through the cycle 12 response assessment (Fig. 2). After 14 cycles of therapy, he began experiencing progressive lower extremity weakness, with difficulty climbing stairs and pain in his posterior calves. On examination, he was noted to have lower extremity weakness, including decreased strength in bilateral ankle plantar and dorsiflexion and hyporeflexia of the knees and ankles. Strength in the wrist extensors bilaterally was more subtly decreased. Peripheral sensory and motor neuropathy events, including those observed in this patient, are known side effects of treatment with brentuximab vedotin [28]. Given these symptoms and findings, cycle 15 was not administered and the patient was subsequently placed on close monitoring. As of his last follow-up, complete response has been maintained without evidence of disease at more than 46 months from his last dose of brentuximab vedotin and over 55 months from study enrollment. He has had near complete resolution of weakness in his hands with significant improvement in strength in his lower extremities.

Case 5

A 28-year-old male patient was diagnosed with an anterior mediastinal GCT composed of 95% EC and 5% seminoma/yolk sac tumor. He received frontline therapy of four cycles of BEP and achieved a partial response, after which his disease relapsed in the anterior mediastinum and subcarinal lymph nodes. He then received one cycle of paclitaxel, cisplatin, and ifosfamide. The patient's mediastinal tumor was assessed for CD30 expression and was found to be 80% CD30-positive by IHC. The patient subsequently enrolled in the treatment study.

At baseline, he had disease in his mediastinum, carina, and aortic arch (Table 1), and his AFP was elevated at 152 ng/mL. The patient received one cycle of 2.4 mg/kg brentuximab vedotin but began experiencing symptomatic deterioration 1 week after the first dose and subsequently discontinued treatment. The patient died of progressive disease approximately 5 months after discontinuing the study drug.

Case 6

A 32-year-old male patient presented with a diagnosis of stage I seminoma. The patient underwent a radical right orchiectomy with adjuvant radiation to the right iliac and periaortic lymph nodes, after which he remained disease-free for approximately 2.5 years. He then relapsed radiographically in an interaortocaval lymph node and was treated with three cycles of BEP, followed by retroperitoneal and right iliac lymph node dissections, spermatic cord excision, and retroperitoneal mass excision. Pathology revealed residual GCT with a question of EC and seminoma elements. Subsequently, he had no evidence of disease for approximately 19 months, at which point a chest x-ray demonstrated new bilateral pulmonary metastases. The patient received one cycle of VIP and sought further medical attention for consideration of a tandem transplant. At that time, the previous pathology specimens were re-examined and the patient's tumor was determined to be a Sertoli cell tumor and not a seminoma as originally diagnosed. He underwent two cycles of paclitaxel and gemcitabine and a thoracotomy with wedge resection of pulmonary metastases, but his disease progressed. The patient was subsequently enrolled in a clinical trial of the gamma secretase (Notch) inhibitor MK-0752, which he discontinued because of an adverse event. The patient's thoracotomy sample was evaluated for CD30 expression and was found to be 98% CD30-positive by IHC. He was then enrolled in the treatment study.

At baseline, the patient had target lesions on the subcarinal lymph node, the paratracheal lymph node, the lower lobes of the right and left lungs, and the left supraclavicular lymph node (Table 1). The patient initiated treatment with 1.8 mg/kg brentuximab vedotin Q3W and received four cycles of treatment. He achieved stable disease after two cycles of treatment, but

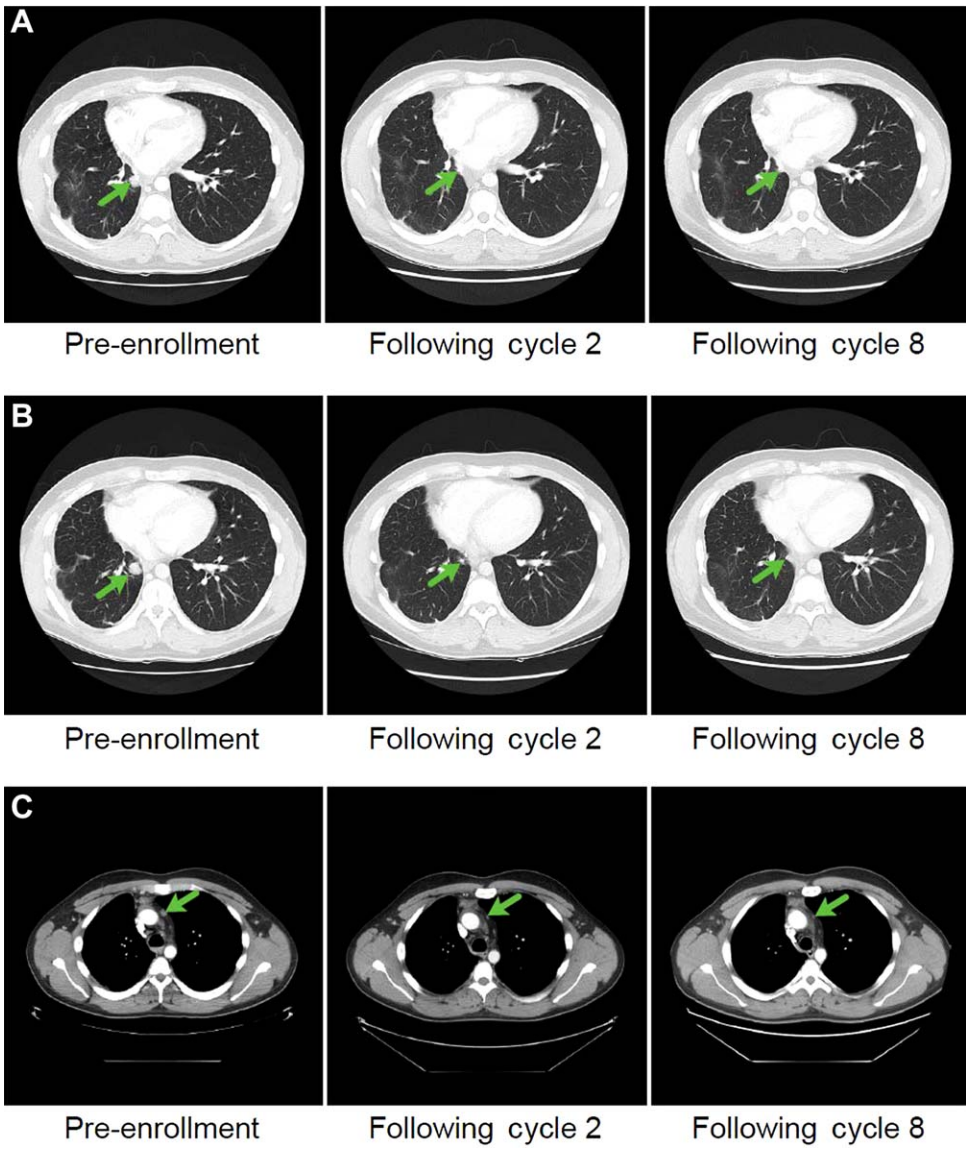

Figure 2. Treatment with brentuximab vedotin induced a complete response in a patient (Case 4) with a germ cell tumor. Computed tomography scans over time of a right lung nodule **(A)**, second right lung nodule **(B)**, and mediastinal lymph node **(C)** in the patient in Case 4 who was treated with 1.8 mg/kg brentuximab vedotin. After cycle 2, the patient achieved a partial response that converted into a durable complete response after cycle 4.

all lesions showed evidence of progression at the cycle 4 assessment visit. Treatment with brentuximab vedotin was then discontinued, and the patient died 9 months later from progressive disease.

Case 7

A 61-year-old male patient was diagnosed with a stage I, 3.8-cm, metastatic Leydig cell tumor that was treated with a left orchiectomy. The patient remained disease-free for approximately 2 years following surgery, at which time an abdominal CT scan revealed extensive retroperitoneal adenopathy and several small bilateral pulmonary metastases. The patient underwent a retroperitoneal lymph node dissection, left nephrectomy, and partial thoracotomy, which revealed 21 lymph nodes involved with a metastatic Leydig cell tumor. Several lung nodules were also removed, with pathology demonstrating involvement by the Leydig cell tumor. Immunohistochemistry revealed 100% expression of CD30 in the tumor in his kidney and lymph node samples. The patient had stable disease for approximately 2 months, after which his disease progressed and he was enrolled into the treatment study.

At baseline, lesions were present in a subcarinal lymph node, a supraclavicular lymph node, upper and lower lobes of the right lung, and the pelvis (Table 1). The patient initiated treatment with brentuximab vedotin at a dose of 1.8 mg/kg Q3W. After two cycles of treatment, the patient achieved stable disease, which was maintained at the cycle 4 assessment. The patient's cycle 5 and cycle 6 doses of brentuximab vedotin were increased to 2.4 mg/kg Q3W as a result of the approval of a protocol amendment to test the safety and efficacy of this higher dose level. His stable disease was maintained through six cycles of treatment, at which point he experienced progression of a cough and fatigue and the onset of shortness of breath. The patient opted to withdraw from the study because symptomatic deterioration and subsequently died of progressive disease 1 month after coming off the trial.

Discussion

In this case series, seven patients with relapsed or refractory CD30-expressing GCTs or SCSTs were treated with brentuximab vedotin at starting doses of 1.8 mg/kg or 2.4 mg/kg. One patient (Case 4) achieved a complete response after four cycles of treatment with brentuximab vedotin that has been maintained for more than 46 months since discontinuation of therapy following cycle 14. One patient (Case 1) achieved a partial response to treatment at a single time point following two cycles of treatment with brentuximab vedotin but discontinued after four cycles of treatment because of progressive disease. Three patients (Cases 2, 6, and 7) also achieved stable disease that was maintained for up to six cycles of treatment, whereas two patients (Cases 3 and 5) had a best response of progressive disease.

Germ cell tumors that are refractory to multiple courses of chemotherapy, including high-dose treatment with stem cell rescue, historically have a low rate of response to many single and combination chemotherapeutic regimens [2, 9, 29]. In a retrospective study examining the outcomes of 90 patients with relapsed or refractory GCTs enrolled in seven single-agent, phase II trials, no patients achieved a complete response, 1 patient (1%) achieved a partial response, and 15 patients (17%) achieved stable disease [5]. In an additional trial, the single-agent mesenchymal-epithelial transition (c-MET) inhibitor tivantinib did not demonstrate any activity in patients with relapsed or refractory GCTs, with no patients achieving complete or partial response [30]. Therefore, the objective responses seen in two of the five patients with GCTs in this study indicate that brentuximab vedotin may have significant clinical activity in this difficult-to-treat patient population.

Brentuximab vedotin is an ADC that targets CD30-expressing cells. Patients with a variety of non-Hodgkin lymphomas exhibiting CD30 expression levels $\geq$10% have responded to treatment with brentuximab vedotin in clinical trials [17, 31–33]. Preclinical evidence suggests that even in mixed histology tumors, cells lacking CD30 are susceptible to treatment if they are in close proximity to CD30-expressing cells [22]. Collectively, these data suggest that tumor CD30 expression levels may vary widely while still responding to treatment with brentuximab vedotin and support the rationale for selection of a $\geq$10% CD30 expression level for inclusion in the current study.

The most widely recognized extralymphoid expression of CD30 is in EC, and the presence of CD30 has been correlated with the presence of EC in mixed GCTs [14]. Additionally, CD30 expression has been shown to be retained in patients with metastatic GCTs even after multiple chemotherapeutic regimens [14], and has been identified as a prognostic factor for metastatic disease, suggesting that CD30 is a promising target for therapy in relapsed or refractory GCTs. In this subset of refractory GCTs, the patient in Case 4 who achieved a durable complete response had a mixed GCT in the testis with metastatic disease consisting entirely of EC. Thoracic metastases removed after HDCT demonstrated very strong 3+ IHC staining for CD30 (80%), which was observed at the cell membrane as well as diffusely in the cytoplasm and in the Golgi. However, high levels of CD30 expression did not appear to correlate with response to treatment in all patients (Table 1), and a patient with a pure EC (Case 3) did not achieve a response. The lack of response in many patients with high levels of CD30 in this study may be due to acquired or intrinsic resistance to brentuximab vedotin. The cellular mechanisms of resistance to brentuximab vedotin are not well characterized, but elucidating these mechanisms is an active area of research. Data from studies in brentuximab vedotin-resistant cell lines have offered insight into global cellular changes following treatment, with potential mechanisms of resistance including loss of CD30 expression and overexpression of the multi-drug resistance 1 (MDR1) drug exporter [34]. The relative significance of these in vitro mechanisms as they relate to clinical isolates is still being evaluated.

As this study was exploratory in nature, only a limited number of patients with GCTs or SCSTs were enrolled. Although two of five patients with GCTs achieved objective responses, additional studies in larger patient cohorts will be required to determine the clinical activity of brentuximab vedotin in relapsed or refractory GCT. Additionally, studies examining the use of brentuximab vedotin in both CD30-expressing and CD30-negative tumors in patients with relapsed or refractory GCTs and SCSTs will help to further elucidate the role of CD30 in these diseases. A phase II, open-label efficacy study further examining the use of brentuximab vedotin in relapsed or refractory GCTs, sponsored by Seattle Genetics, Inc. (NCT02689219), is currently underway. Further studies are also required to determine whether brentuximab vedotin may be useful in combination therapy with other active agents that do not share an overlapping toxicology profile.

Conclusion

This case series demonstrates the activity of brentuximab vedotin in a small cohort of seven patients with relapsed or refractory CD30-expressing GCTs or SCSTs, including one patient who achieved a durable complete response that remains ongoing more than 46 months after discontinuation of treatment. Therefore, brentuximab vedotin may be a treatment option in this particularly aggressive disease.

Acknowledgments

We thank Chantelle Rein-Smith of Whitsell Innovations, Inc., for aid in manuscript preparation (funded by Seattle Genetics, Inc.). T.B. is currently affiliated with Willamette Valley Cancer Institute, Eugene, OR.

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Disclosures

Costantine Albany: Seattle Genetics, Inc. (SAB, RF); **Neil Josephson**: Seattle Genetics, Inc. (E, OI); **Darren R. Feldman**: Novartis, Seattle Genetics, Inc. (RF). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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