Diagnostic Value of Next-Generation Sequencing in an Unusual Sphenoid Tumor

FARZAD JAMSHIDI,^a ERIN PLEASANCE,^b YVONNE LI,^b YAOQING SHEN,^b KATAYOON KASAIAN,^b RICHARD CORBETT,^b PETER EIREW,^c AMY LUM,^a PAWAN PANDOH,^b YONGJUN ZHAO,^b JACQUELINE E. SCHEIN,^b RICHARD A. MOORE,^b ROD RASSEKH,^d DAVID G. HUNTSMAN,^{a,e} MEG KNOWLING,^f HOWARD LIM,^f DANIEL J. RENOUF,^f STEVEN J.M. JONES,^b MARCO A. MARRA,^{b,g} TORSTEN O. NIELSEN,^{a,e} JANESEA LASKIN,^f STEPHEN YIP^{a,e}

^aCentre for Translational and Applied Genomics, British Columbia Cancer Agency, Vancouver, British Columbia, Canada; ^bCanada's Michael Smith Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, British Columbia, Canada; ^cBritish Columbia Cancer Research Centre, Vancouver, British Columbia, Canada; ^dDivision of Oncology/Hematology/BMT, Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada; ^ePathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada; ^fDepartment of Medical Oncology, British Columbia Cancer Agency, Vancouver, British Columbia, Canada; ^gDepartment of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada

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

Key Words. Sphenoid • Rhabdoid • Atypical teratoid/rhabdoid tumor • Next-generation sequencing • *SMARCB1*

ABSTRACT

Extraordinary advancements in sequencing technology have made what was once a decade-long multi-institutional endeavor into a methodology with the potential for practical use in a clinical setting. We therefore set out to examine the clinical value of next-generation sequencing by enrolling patients with incurable or ambiguous tumors into the Personalized Onco-Genomics initiative at the British Columbia Cancer Agency whereby whole genome and transcriptome analyses of tumor/normal tissue pairs are completed with the ultimate goal of directing therapeutics. First, we established that the sequencing, analysis, and communication with oncologists could be completed in less than 5 weeks. Second, we found that cancer

diagnostics is an area that can greatly benefit from the comprehensiveness of a whole genome analysis. Here, we present a scenario in which a metastasized sphenoid mass, which was initially thought of as an undifferentiated squamous cell carcinoma, was rediagnosed as an *SMARCB1*-negative rhabdoid tumor based on the newly acquired finding of homozygous *SMARCB1* deletion. The new diagnosis led to a change in chemotherapy and a complete nodal response in the patient. This study also provides additional insight into the mutational landscape of an adult *SMARCB1*-negative tumor that has not been explored at a whole genome and transcriptome level. *The Oncologist* 2014;19:623–630

Implications for Practice: We show that use of next-generation sequencing in clinical settings is practical and can benefit patients because of the ability to define tumors genetically.

INTRODUCTION

Rhabdoid tumors are aggressive pediatric tumors, most commonly occurring in brain, kidney, and soft tissues [1]. Although rare, they have also been reported in adults [2]. Loss of expression of *SMARCB1*, a member of the SWI/SNF chromatin-remodeling complex, is a key molecular characteristic of these tumors [3]. Exome sequencing and copy number array data indicate that, other than biallelic inactivation of *SMARCB1*, the landscapes of rhabdoid tumors are unusually stable, with mutation rates that are among the lowest observed in any cancer [4]. Other cancers shown to have *SMARCB1* loss include epithelioid sarcomas, renal medullary carcinomas, epithelioid malignant peripheral nerve sheath tumors, extraskeletal myxoid chondrosarcoma, myoepithelial carcinoma, variants of chordoma, and hepatoblastoma [5], as

well as cribriform neuroepithelial tumors [6]. Consequently, examination of *SMARCB1* status can serve as an excellent tool to narrow possibilities when the aforementioned tumors are in the differential diagnosis.

Here, we report the first whole genome and transcriptome analysis of an *SMARCB1*-negative tumor resembling a rhabdoid tumor. It was not until the whole genome sequencing and comprehensive genomic analysis was completed that the tumor was accurately diagnosed, which led to changes in treatment protocol with therapeutic response. This scenario also points out the value of whole genome sequencing and genomic analysis in management of rare/ambiguous tumors.

A previously healthy 32-year-old man presented to the emergency department with a sudden loss of vision of the

Correspondence: Stephen Yip, M.D., Ph.D., Centre for Translational and Applied Genomics, British Columbia Cancer Agency, 675 West 10th Avenue, Vancouver, British Columbia V5Z 4E6, Canada. Telephone: 604-877-6000, ext. 3105; E-Mail: syip@bccancer.bc.ca Received October 9, 2013; accepted for publication March 3, 2014; first published online in *The Oncologist Express* on May 7, 2014. ©AlphaMed Press 1083-7159/2014/\$20.00/0 <http://dx.doi.org/10.1634/theoncologist.2013-0390>

Figure 1. Imaging. **(A):** From left to right, sagittal and coronal and sections from the patient's magnetic resonance imaging at initial presentation. There is a destructive process (arrow) in the central base of the skull that is centered in the sphenoid and extends to the posterior ethmoid. There is extension in the posterior orbit (more prominent on the right) with destruction of the medial wall at the orbital apex. **(B):** Fat-saturated T1-weighted magnetic resonance imaging representations from postsurgery. **(C):** The recurrence with the white arrow pointing to the main tumor mass. The recurrent tumor extended into the ethmoid sinus.

Abbreviations: A, anterior; L, left; P, posterior; R, right.

lower visual field in the left eye. This was preceded by a 1-month history of nasal congestion, headaches, and epistaxis. Computed tomography revealed a destructive process of the skull base. Magnetic resonance imaging (MRI) showed a mass lesion centered within the sphenoid sinus and extending to the posterior ethmoid sinus (Fig. 1A). The extradural mass exerted compressive pressure on the optic nerves. The patient underwent bilateral sphenoidotomy and debulking of the lesion (Fig. 1B). The biopsied tumor cells displayed a “small blue round cell” appearance (Fig. 2 left); after extensive workup, the tumor was categorized as a poorly differentiated squamous cell carcinoma based on strong immunohistochemical reaction to p63 and cytokeratin 5 (supplemental online Fig. 1) [7, 8]. A list of immunohistochemistry results can be found in the supplemental online Appendix 1. Postsurgery, the patient underwent neoadjuvant chemotherapy with two cycles of cisplatin/fluorouracil/docetaxel and thereafter high-dose radiotherapy with concurrent weekly cisplatin. Thirteen months after completion of chemoradiation, MRI (Fig. 1C) and positron emission tomography (PET) scans revealed local tumor recurrence with metastases to the right mediastinal, hilar, and upper retroperitoneal lymph nodes. Cisplatin-docetaxel was initiated, but local tumor progression was noted after only three cycles. The recurrent tumor (Fig. 2, right) showed histology similar to the original (Fig. 2, left). At this point, the patient was

enrolled in the Personalized OncoGenomics (POG) initiative at the British Columbia Cancer Agency to provide therapeutically actionable information based on detailed profiling of the tumor. POG aims to use comprehensive sequencing and bioinformatics approaches to promote understanding of treatment-resistant cancers. The patient underwent a biopsy of the recurrent ethmoidal tumor for whole genome/transcriptome and focused deep amplicon sequencing.

MATERIALS AND METHODS

Ethics and Data Privacy

An informed consent was acquired from the patient. All procedures were approved by the University of British Columbia Research Ethics Committee (REB# H12-00137). Patient anonymity was maintained within the research team with an external identification code assigned to the case that was used to communicate clinically relevant information to physicians. The patient consented to potential publication of findings. Raw data generated from sequencing and downstream analytics were maintained within a secure computing environment.

Nucleic Acid Extraction and Sequencing

Biopsy samples were taken from the recurrent mass, embedded in a small amount of optimal cutting temperature compound and sectioned for RNA and DNA extractions.

Figure 2. Hematoxylin- and eosin-stained section from the 2010 primary tumor on the left and the 2012 recurrence on the right. Magnification, $\times 40$.

Genome and transcriptome libraries were constructed using sections with 70% tumor content, and a matching transcriptome library with 0% tumor content was prepared for comparison with the tumor transcriptome data. In addition, RNA and DNA were isolated from blood samples and the paraffin embedded material from the original tumor for sequencing library construction.

Sequencing and Bioinformatic Analysis

Paired-end reads from all libraries were generated on an Illumina HiSeq2000 sequencer (Illumina Inc., San Diego, CA, <http://www.illumina.com>; supplemental online Appendix 1). Focused deep amplicon sequencing of both tumor specimens and blood was performed using the AmpSeq Cancer panel (version 2.0) on the Ion Torrent PGM platform (Life Technologies, Burlington, ON, Canada, <http://www.lifetechnologies.com>). [9]. A combination of established analytical pipeline and custom bioinformatic tools was used for identifying somatic nucleotide and copy number aberrations. These are described in detail in supplemental online Appendix 2.

Immunohistochemistry

Standard 4- μ m paraffin block tissue sections from the 2010 primary and 2012 recurrence formalin-fixed paraffin embedded (FFPE) blocks were performed on a semiautomated Ventana Discovery XT (Ventana Medical Systems, Tucson, AZ, <http://www.ventana.com>). Standard CC1 antigen retrieval with a 2-hour primary incubation was done. The complete list of primary antibodies is provided in supplemental online Appendix 2. For *SMARCB1*, the primary mouse BAF47 antibody from Becton Dickinson and Company (Franklin Lakes, NJ, <http://www.bd.com>) was used in a 1:50 dilution followed by a 16-minute incubation with prediluted horseradish peroxidase-conjugated anti-mouse secondary antibody.

Fluorescent In Situ Hybridization

Fluorescent in situ hybridization (FISH) was performed on 4- μ m thick full sections from the 2012 biopsy FFPE block. The slides were baked overnight at 70°C, deparaffinized in xylene, dehydrated with ethanol washes, incubated for 1 hour in 10 mM citric acid at 80°C, and treated with pepsin for 20 minutes at 37°C. After washing and dehydration, nick-translated fluorescent probes were applied, followed by hybridization at 37°C for 16 hours. The probes used were red fluorescence-labeled RP11-71G19 to detect *SMARCB1* and green fluorescence-labeled RP11-262A13 to mark chromosome 22. DAPI II was applied to mark the nuclei before viewing under fluorescent microscopy.

RESULTS

Copy Number

Analysis of whole genome sequence data revealed that both the primary and the recurrence samples had a highly stable genome with infrequent copy number variations (Fig. 3). There was, however, homozygous loss of a region in chromosome 22 involving 15 genes including *SMARCB1* (Fig. 4A). No other known tumor suppressor genes exhibited homozygous loss. Homozygous loss of the *SMARCB1* locus and protein expression was confirmed with FISH and immunohistochemistry (Fig. 4). Based on these data, the patient was switched to an ifosfamide-based chemotherapy regimen [10].

The recurrence sample had heterozygous deletions on chromosomes 8, 9, 11, and 12. The deletions on chromosomes 11 and 12 were also seen in the original 2010 sample, but the ones on chromosomes 8 and 9 seemed to be newly acquired in recurrence (supplemental online Fig. 2). The newly acquired deletions on chromosome 8 seem to be linked to an internal chromosomal rearrangement also detected in the tumor transcriptome (Fig. 3). Interestingly, one of the recurrence-specific deletions involved metastasis suppressor protein 1 (*MTSS1*), encoding an actin cytoskeleton interacting protein, which has been shown to be lost in metastases [11]. The recurrence of the tumor was associated with pulmonary nodal metastasis. There were no areas of copy number gain in the recurrence sample, and sporadic small areas of amplification were seen in the 2010 FFPE samples throughout the genome (Fig. 4A, bottom). However, our experience has shown that these are frequently noise resulting from the poor quality of DNA purified from FFPE materials. A complete list of somatic deletions is provided in supplemental online Appendix 1.

Single Nucleotide Variants and Insertion/Deletions

Panel-based sequencing of tumors did not reveal any somatic single nucleotide variants (SNVs) or insertion/deletions (indels). Whole genome sequencing of the recurrent tumor revealed a total of 4 million candidate single nucleotide changes. After filtering this list against the matched germline sequence obtained from the blood sample, 4,206 candidate SNVs and 583 somatic indels were detected, of which 39 SNVs and 2 indels were found in protein coding regions and were nonsynonymous (supplemental online Appendix 1). Among the genes with predicted mutations were *KIAA1468* and *SRPK3* with splice site mutations, *PIP5K1C* with loss of a stop codon, *HELZ* with a nonsense mutation at amino acid position 1735,

Figure 3. Circos plot showing the genomic landscape. From outer circle: chromosomes, somatic single nucleotide variations, CNV, LOH, and structural variations. Single nucleotide variations are indicated by blue dots. CNV gains are shown in red and losses in green. LOH is shown in blue bars. Structural variations, fusions, and inversions are shown as lines connecting breakpoints, with orange lines indicating transcriptome and blue lines indicating genome data.

Abbreviations: CNV, copy number variations; LOH, loss of heterozygosity.

and *EPHX2* with a nonsense mutation at amino acid position 3. Mutations affecting *EPHX2*, an epoxide hydrolysis enzyme, could potentially influence the efficacy of epoxide-based therapies such as etoglucid. The *HELZ* and *SRPK3* mutations were present in both the recurrence and the primary tumor, but the other mutations were specific to the recurrence sample. *HELZ* is a member of the superfamily I of RNA helicases [12] and has been suggested to have a tumor suppressive role in a variety of cancers [13]. *SRPK3* is a serine/threonine kinase required for normal muscular differentiation [14]. Furthermore, there were two regions of small insertion/deletions, both of which caused frame shifts (involving two genes, *SUCNR1* and *PLEKHH1*); both of these were found only in the recurrence. *SUCNR1* is a receptor for succinate [15] and *PLEKHH1* is a pleckstrin homology domain-containing protein whose function is not characterized. Neither of these genes has been directly linked to cancer previously and the relevance of mutations in them is unclear.

Rearrangements

We used the whole genome and RNA sequencing data generated from the recurrent tumor sample to search for rearrangements. A total of 42 rearrangements in the genome and 5 rearrangements in the transcriptome were detected (with all of the latter also detected in the genome). Of the

5 rearrangements detected at both DNA and RNA levels, 1 involved already-known fusion-associated genes. The myeloid/lymphoid or mixed-lineage leukemia translocated to 6 (*MLLT6*) is a previously described cancer-associated fusion partner [16, 17] that was fused to *SEZ6L* in this case. The fusion breakpoints are in the first intron of *SEZ6L* and the 5' UTR of *MLLT6*, and this is not a previously described pattern of *MLLT6* fusions. *MLLT6* is fused to *MLL* in a subset of acute myeloid leukemias, where it is the 3' partner in a variety of transcripts [17]. *SEZ6L* has been reported to be interrupted in a subset of lung cancers [18]. Both genes had reduced expression.

Expression

The expression pattern was complex but corresponded with the expected homozygous deletion of *SMARCB1*, with decreased expression of this gene, decreased expression of *CDKN1A* and *CDKN1C*, and increased expression of *CDK1*, *CDK2*, *CDK4*, *CDK6*, *CCNE1*, *CCNA1*, and *GLI1* consistent with the proliferative nature in the tumor (supplemental online Fig. 3). The direct impact of loss of *SMARCB1* on *GLI1* and *CCNA1* expression has been described previously [19, 20]. Surprisingly, *CDKN2A* expression was increased relative to compendium. In addition, some well-known oncogenes (*IGF1R*, *EGFR*, *HER2*, *FGFR*, *MYC*, *ABL1*, *BCL2*) displayed increased expression (supplemental online Appendix 1). *TP53*

Figure 4. Loss of *SMARCB1*. **(A):** Copy number status shown across chromosome 22 from the 2010 primary (bottom) and the 2012 recurrent (top) tumors. The single homozygously deleted region found in both samples includes 15 genes, of which the only established tumor suppressor was *SMARCB1* (supplemental online Appendix 1). The focal amplifications seen in the 2010 specimen are artifact created because of poorer quality and presequencing amplification step of formalin-fixed paraffin embedded samples. **(B):** Immunohistochemistry done using BAF47 antibody targeting *SMARCB1* protein. All tumor cells from both the 2010 primary (**Bi**) and 2012 recurrence (**Bii**) were negative. Endothelial cells staining served as an internal positive control. Fluorescent in situ hybridization confirmed homozygous deletion of *SMARCB1* (**Biii**). The red probe, RP11-71G19, hybridizes to a region spanning *SMARCB1* and the green probe, RP11-262A13, hybridizes to the telomeric region as a marker for chromosome 22. A normal cell with intact copies of chromosome 22 can be seen in the top left. The surrounding tumor cells have lost both red signals corresponding to the homozygous *SMARCB1* deletion, whereas they still maintain copies of chromosome 22 shown by the green fluorescent signals.

expression was similarly increased and *EZH2* had slight increase in expression. Interestingly, *EZH2* has been suggested to be upregulated leading to a global increase in H3K27me3 when *SMARCB1* is lost [21]. Findings of potential therapeutic utility included overexpression of both *EGFR* and *HER2*, which made this patient a candidate for lapatinib-based therapy. Lapatinib has been previously described to be highly cytotoxic on central nervous system (CNS) rhabdoid tumor cell lines in vitro [22].

Therapeutic Impact

With the change in diagnosis, the patient was started on a rhabdoid tumor-oriented ifosfamide and etoposide chemotherapy [10] regimen; after 2 cycles, we noted a complete response of the thoracic nodal metastases (Fig. 5). In addition, the patient was considered for clinical trials of histone deacetylase inhibitors [23] and lapatinib [22]. Furthermore, the main sphenoid mass stabilized with the change in chemotherapy, with minimal growth compared with the rapid progression during the previous 5 months. However, due to the location of the tumor being close to the optic nerves and its size at this point, the patient lost his vision 2 months after

the change in therapy. There was an unfortunate bacterial meningitis, likely secondary to the myelosuppression of the chemotherapy and the damage to the blood-brain barrier by the tumor. From that point onward, the patient did not fully recover and decided not to continue therapy. Once off treatment, he continued to deteriorate and unfortunately died 6 months after the initial change in chemotherapy.

DISCUSSION

Realignment of Diagnosis

This case illustrates a scenario in which whole genome sequencing enabled improvement of health care delivery. The tumor in this case was originally a diagnostic challenge; however, the critical finding of homozygous deletion of *SMARCB1* after whole genome sequencing narrowed the list of possibilities to a select group of conditions. Most of the possibilities other than rhabdoid tumors could be ruled out based on additional next-generation sequencing information, such as lack of T-brachyury overexpression for a subset of chordomas, and/or additional immunohistochemical information. The genomic stability of the tumor was also a unique

Figure 5. Patient response. **(A):** Positron emission tomography (PET) showing recurrence of the tumor as well as nodal metastases to the right mediastinum, right hilum, and upper retroperitoneum (shown by white arrows). The hypodense lesion in the lateral right lobe of the liver had been stable in size since previous PET computerized tomography scans and likely represents a benign lesion. **(B):** Complete nodal response after next-generation sequencing-based diagnosis of rhabdoid tumor with *SMARCB1* loss and change in treatment.

feature, described previously for rhabdoid tumors [4], that shed light on the identity of the tumor. Epithelioid sarcomas, for example, are aneuploid in the majority of cases [24] and can have a complex karyotype [25].

This led to benefit to the patient at three levels: First, with the new diagnosis, the treatment strategy changed to one featuring application of a rhabdoid tumor-oriented chemotherapeutic regimen. Second, new possibilities of targeted therapies and clinical trials focusing on *SMARCB1* inactivation including histone deacetylase inhibitors and lapatinib [22, 23] could be considered. Third, disease-specific prognostic information became available for the patient and his family.

This case represents a highly unusual scenario, as rhabdoid tumors are generally pediatric tumors and there have been only 32 cases of adult CNS rhabdoid tumors, known as atypical teratoid/rhabdoid tumors (AT/RT), reported in the literature [2]. Although the tumor was in the vicinity of the CNS, thus making adult AT/RT a possible diagnosis, the brain parenchyma was not involved and the tumor seemed to be extra-axial,

Figure 6. An overview of the timeline from initial presentation to realignment of diagnosis after deep sequencing results. The sequencing and analysis were completed in only 5 weeks, which led to a new therapeutic approach. This is a relatively short period compared with the overall course of treatment.

primarily involving the sphenoid sinus. Thus, we believe that the tumor could be best labeled as a rhabdoid malignancy with *SMARCB1* loss. The genomic stability, together with biallelic loss of *SMARCB1* and the histologic and immunohistochemical features, made rhabdoid tumor the most appropriate diagnosis.

In this case, whole genome and transcriptome sequencing was completed in 9 days, and the data were analyzed and interpreted in an additional month from the date of the biopsy (Fig. 6). We have shown that whole genome sequencing can be used in a clinically practical time frame and can be of clinical and diagnostic value.

Although one could argue in hindsight that use of an *SMARCB1* immunohistochemical test early on could have improved the treatment and avoided the efforts and costs that went into the next-generation sequencing study, the diagnosis of a rhabdoid tumor was not considered in the initial differential diagnosis. This was because of the extremely unusual presentation of the *SMARCB1*-negative tumor in this case. We are not suggesting that next-generation sequencing should replace simple cost-effective immunohistochemical tests; however, the comprehensiveness of this approach has great benefit when one is dealing with unusual presentations of rare tumors such as this case, which are diagnostic challenges and can be missed in typical clinical settings. Moreover, data from next-generation sequencing facilitate recalibration of the initial pathological diagnosis and can serve an important role as a quality assurance/control tool.

A Look Into Genomics of Rhabdoid Tumors and Comparison With Literature

Looking at the overall landscape, the genome of the tumor was stable (Fig. 3). This was also found by Lee et al., who looked at a larger cohort of cases but whose study was based on exome sequencing [4]. As part of a comprehensive whole genome analysis, we examined fusions and expression patterns, discovering features that have not been reported in the literature for *SMARCB1*-negative tumors. We found several fusions and noted a cluster of fusion breakpoints close to the *SMARCB1* locus. This could indicate susceptibility of this region to chromosomal rearrangements and could explain a contributing factor to the mechanism of homozygous deletion of *SMARCB1*. Furthermore, the heterozygous deletion of *MTSS1*,

a gene linked to tumor metastasis, was seen in the recurrence case, which was accompanied by distant nodal metastasis.

The expression pattern was generally concordant with loss of *SMARCB1* as predicted from in vitro cell line models [19, 20, 26]; however, there were deviations. For instance, with loss of *SMARCB1*, *GLI1* [19] and *CCNA1* [20] are predicted to increase in abundance; indeed, we saw similar effects (supplemental online Appendix 1). *GLI1* is a strong interactor of *SMARCB1* and is known to be upregulated in rhabdoid tumors [19]. However, one of the key targets of *SMARCB1* in regulation of the cell cycle as seen in cell line models [16], namely *CDKN2A*, was not downregulated relative to the normal compendium. *SMARCB1* has been shown to have a direct effect on *CDKN2A* transcription levels in cell lines [20, 21], with loss of *SMARCB1* leading to downregulation of *CDKN2A*. On the other hand, *EZH2*, predicted to be upregulated with loss of *SMARCB1* [21], was only slightly overexpressed compared with normal compendium.

It should be noted that the absence of an exact matched normal tissue, due to unknown origin of this tumor, and use of a compendium of 16 normal tissue types for the expression analysis could have confounded the results. However, the ready observation of dysregulation of other genes such as *GLI1*, *CCNA1*, *EGFR*, and *HER2* using the same methodology signifies the importance of pathways other than *EZH2* and *CDKN2A* in this case. Interestingly, in terms of the SWI/SNF complex itself, the majority of known members were expressed but there seemed to be a ninefold overexpression of *ACTL6A* relative to *ACTL6B*. This could indicate an abundance of a neural progenitor form of the SWI/SNF complex as opposed to the SWI/SNF found in differentiated neurons [27]. *ACTL6A* overexpression has been linked to an undifferentiated state in tumors such as rhabdomyosarcoma [28]. Overall, the most significantly affected pathways were cyclins and cyclin-dependent kinases, growth factor receptors such as *EGFR* and *FGFR*, and chromatin regulators such as *HDACs* and *SMARCB1*.

Practical Application of Next-Generation Sequencing

In this study, we confirm the paucity of somatic mutations in rhabdoid tumors, which highlights dramatic discrepancy between aggressive clinical behavior and the stable genomic landscape. This emphasizes the importance of abnormalities in the SWI/SNF complex in oncogenesis. In this patient, whole genome sequencing was instrumental in assisting in the correct diagnosis of the tumor based on the finding of homozygous *SMARCB1* loss. This resulted in a switch in chemotherapy protocol, leading to diminution of the distant metastases. Unfortunately, the sphenoid sinus mass did not show a prominent response with the new protocol. Tumor volume and difficulty in achieving effective intratumoral drug delivery could partly explain the suboptimal response at the primary site.

One could argue that the patient would have benefited therapeutically if genome and transcriptome profiling were to have occurred earlier during the course of his disease. The genomic information allowed for recalibration of the original pathological diagnosis, which illustrates the most powerful

aspect of close integration of next-generation sequencing technology with diagnostic pathology in the clinical realm. The identification of molecular targets with therapeutic implications is an obvious goal that has not achieved practical universal benefits in oncology. Currently, there exist only a handful of pathway-specific targeting agents in clinical oncology, whereas a large number of aberrant pathways are identified in cancer.

Interestingly, a case of “molecular rediagnosis” from an ovarian teratoma to a rhabdoid tumor based on exome sequencing has also been reported recently [29]. However, in this study, we have also highlighted the practicality of next-generation sequencing in a clinical setting and time frame.

ACKNOWLEDGMENTS

We acknowledge the generous financial support of the British Columbia Cancer Foundation. The Terry Fox Research Institute supported Farzad Jamshidi directly and Torsten O. Nielsen, David G. Huntsman, and Stephen Yip indirectly. Farzad Jamshidi is supported by a Vancouver Coastal Health-Canadian Institutes of Health-University of British Columbia/Ph.D. Studentship Award. Marco A. Marra acknowledges the support of Genome BC, Genome Canada, and the Canada Research Chairs program. We thank Dr. Don Wilson and Dr. Monty Martin for the PET scan and MRI images, respectively, as well as Dr. Arif Janjua for providing clinical information. We are grateful to Peggy Tsang, Julie Lorette, Alexandra Fok, and Robyn Roscoe for organizing project sessions, material transfer, and histology. We also thank Simon Haile Merhu, Helen McDonald, Heather Kirk, Katty Cruz, Tina Thorne, and Michelle Moksa, who helped with the sequencing and library construction. Last, we are greatly indebted to the patient for his participation in this study.

We acknowledge the generous financial support of the British Columbia Cancer Foundation.

AUTHOR CONTRIBUTIONS

Conception/design: David G. Huntsman, Howard Lim, Daniel J. Renouf, Steven J. M. Jones, Marco A. Marra, Torsten O. Nielsen, Janessa Laskin, Stephen Yip

Provision of study material or patients: Meg Knowling, Steven J.M. Jones, Marco A. Marra, Janessa Laskin, Stephen Yip

Collection and/or assembly of data: Farzad Jamshidi, Erin Pleasance, Yvonne Li, Yaoqing Shen, Katayoon Kasaian, Peter Eirew, Amy Lum, Pawan Pandoh, Yongjun Zhao, Jacqueline E. Schein, Richard A. Moore, Torsten O. Nielsen, Janessa Laskin, Stephen Yip

Data analysis and interpretation: Farzad Jamshidi, Erin Pleasance, Yvonne Li, Yaoqing Shen, Katayoon Kasaian, Richard Corbett, Peter Eirew, Rod Rassekh, David G. Huntsman, Meg Knowling, Howard Lim, Daniel J. Renouf, Steven J.M. Jones, Marco A. Marra, Torsten O. Nielsen, Janessa Laskin, Stephen Yip

Manuscript writing: Farzad Jamshidi, Stephen Yip

Final approval of manuscript: Farzad Jamshidi, Erin Pleasance, Yvonne Li, Yaoqing Shen, Katayoon Kasaian, Richard Corbett, Peter Eirew, Amy Lum, Pawan Pandoh, Yongjun Zhao, Jacqueline E. Schein, Richard A. Moore, Rod Rassekh, David G. Huntsman, Meg Knowling, Howard Lim, Daniel J. Renouf, Steven J.M. Jones, Marco A. Marra, Torsten O. Nielsen, Janessa Laskin, Stephen Yip

DISCLOSURES

Stephen Yip: Gerson Lehrman Group Research (C/A); **Meg Knowling:** GlaxoSmithKline, Bayer (H), Pfizer (RF); **Howard Lim:** Roche, Bayer (C/A). 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

REFERENCES

1. Roberts CW, Biegel JA. The role of SMARCB1/INI1 in development of rhabdoid tumor. *Cancer Biol Ther* 2009;8:412–416.
2. Shonka NA, Armstrong TS, Prabhu SS et al. Atypical teratoid/rhabdoid tumors in adults: A case report and treatment-focused review. *J Clin Med Res* 2011;3:85–92.
3. Bikowska B, Grajkowska W, Jóźwiak J. Atypical teratoid/rhabdoid tumor: Short clinical description and insight into possible mechanism of the disease. *Eur J Neurol* 2011;18:813–818.
4. Lee RS, Stewart C, Carter SL et al. A remarkably simple genome underlies highly malignant pediatric rhabdoid cancers. *J Clin Invest* 2012;122:2983–2988.
5. Hollmann TJ, Hornick JL. INI1-deficient tumors: Diagnostic features and molecular genetics. *Am J Surg Pathol* 2011;35:e47–e63.
6. Park JY, Kim E, Kim DW et al. Cribriform neuroepithelial tumor in the third ventricle: A case report and literature review. *Neuropathology* 2012;32:570–576.
7. Bourne TD, Bellizzi AM, Stelow EB et al. p63 Expression in olfactory neuroblastoma and other small cell tumors of the sinonasal tract. *Am J Clin Pathol* 2008;130:213–218.
8. Franchi A, Moroni M, Massi D et al. Sinonasal undifferentiated carcinoma, nasopharyngeal-type undifferentiated carcinoma, and keratinizing and nonkeratinizing squamous cell carcinoma express different cytokeratin patterns. *Am J Surg Pathol* 2002;26:1597–1604.
9. Bashashati A, Ha G, Tone A et al. Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling. *J Pathol* 2013;231:21–34.
10. Gururangan S, Bowman LC, Parham DM et al. Primary extracranial rhabdoid tumors. Clinicopathologic features and response to ifosfamide. *Cancer* 1993;71:2653–2659.
11. Xie F, Ye L, Ta M et al. MTSS1: A multifunctional protein and its role in cancer invasion and metastasis. *Front Biosci (Schol Ed)* 2011;3:621–631.
12. Wagner DS, Gan L, Klein WH. Identification of a differentially expressed RNA helicase by gene trapping. *Biochem Biophys Res Commun* 1999;262:677–684.
13. Nagai H, Yabe A, Mine N et al. Down-regulation in human cancers of *DRHC*, a novel helicase-like gene from 17q25.1 that inhibits cell growth. *Cancer Lett* 2003;193:41–47.
14. Nakagawa O, Arnold M, Nakagawa M et al. Centronuclear myopathy in mice lacking a novel muscle-specific protein kinase transcriptionally regulated by MEF2. *Genes Dev* 2005;19:2066–2077.
15. He W, Miao FJ, Lin DC et al. Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* 2004;429:188–193.
16. Moore SD, Strehl S, Dal Cin P. Acute myelocytic leukemia with t(11;17)(q23;q12-q21) involves a fusion of *MLL* and *AF17*. *Cancer Genet Cytogenet* 2005;157:87–89.
17. Strehl S, König M, Meyer C et al. Molecular dissection of t(11;17) in acute myeloid leukemia reveals a variety of gene fusions with heterogeneous fusion transcripts and multiple splice variants. *Genes Chromosomes Cancer* 2006;45:1041–1049.
18. Nishioka M, Kohno T, Takahashi M et al. Identification of a 428-kb homozygously deleted region disrupting the *SEZ6L* gene at 22q12.1 in a lung cancer cell line. *Oncogene* 2000;19:6251–6260.
19. Jagani Z, Mora-Blanco EL, Sansam CG et al. Loss of the tumor suppressor *Snf5* leads to aberrant activation of the Hedgehog-Gli pathway. *Nat Med* 2010;16:1429–1433.
20. Oruetxebarria I, Venturini F, Kekarainen T et al. p16^{INK4a} is required for hSNF5 chromatin remodeler-induced cellular senescence in malignant rhabdoid tumor cells. *J Biol Chem* 2004;279:3807–3816.
21. Wilson BG, Wang X, Shen X et al. Epigenetic antagonism between polycomb and SWI/SNF complexes during oncogenic transformation. *Cancer Cell* 2010;18:316–328.
22. Singh A, Lun X, Jayanthan A et al. Profiling pathway-specific novel therapeutics in preclinical assessment for central nervous system atypical teratoid rhabdoid tumors (CNS ATRT): Favorable activity of targeting EGFR- ErbB2 signaling with lapatinib. *Mol Oncol* 2013;7:497–512.
23. Knipstein JA, Birks DK, Donson AM et al. Histone deacetylase inhibition decreases proliferation and potentiates the effect of ionizing radiation in atypical teratoid/rhabdoid tumor cells. *Neuro Oncol* 2012;14:175–183.
24. Pastel-Levy C, Bell DA, Rosenberg AE et al. DNA flow cytometry of epithelioid sarcoma. *Cancer* 1992;70:2823–2826.
25. Lualdi E, Modena P, Debiec-Rychter M et al. Molecular cytogenetic characterization of proximal-type epithelioid sarcoma. *Genes Chromosomes Cancer* 2004;41:283–290.
26. Versteeg I, Medjane S, Rouillard D et al. A key role of the hSNF5/INI1 tumour suppressor in the control of the G1-S transition of the cell cycle. *Oncogene* 2002;21:6403–6412.
27. Staahl BT, Tang J, Wu W et al. Kinetic analysis of npBAF to nBAF switching reveals exchange of SS18 with CREST and integration with neural developmental pathways. *J Neurosci* 2013;33:10348–10361.
28. Taulli R, Foglizzo V, Morena D et al. Failure to downregulate the BAF53a subunit of the SWI/SNF chromatin remodeling complex contributes to the differentiation block in rhabdomyosarcoma. *Oncogene* 2013 [Epub ahead of print].
29. Witkowski L, Lalonde E, Zhang J et al. Familial rhabdoid tumour “*avant la lettre*”—From pathology review to exome sequencing and back again. *J Pathol* 2013;231:35–43.

See <http://www.TheOncologist.com> for supplemental material available online.