A case of metastatic adenocarcinoma of unknown primary in a pediatric patient: opportunities for precision medicine

John Prensner[1], Juan Putra[2], Sara Vargas[2], Alanna Church[2], Katherine Janeway[1], Nadine McCleary[3], and Steven DuBois[1]

[1]Dana-Farber/Boston Children's Cancer and Blood Disorders Center
[2]Boston Children's Hospital
[3]Dana-Farber Cancer Institute

August 25, 2020

Abstract

Cancer of unknown primary (CUP) is a common diagnosis in adult oncology, but is extremely rare in pediatrics, with few published reports and none in the era of molecular profiling. Here, we describe a 13-year old boy with metastatic adenocarcinoma of unknown primary that was refractory to conventional chemotherapy. Molecular profiling revealed an activating ERBB2 D769Y variant. He subsequently achieved disease control on a lapatinib-containing regimen, and upon progression, he transiently responded to neratinib prior to dying from disease. Our case reports a rare example of pediatric CUP and highlights the utility of molecular profiling in these cases.

Word count: 98/100

Introduction

Cancer of unknown primary (CUP) remains one of the most lethal malignancies in adults, representing 3% of all cancer diagnoses and the fourth most common cause of cancer death worldwide [1]. However, nearly all cases occur in older adults, with a median age of ˜65-70 years, and are presumed to have spread from occult primary sites of the common adult carcinomas, such as lung, gastrointestinal or pancreatobiliary carcinomas. Data on young adults and children are sparse, and when CUP occurs, it is almost never carcinoma. Single-institution case series have defined only rare pediatric patients with CUP, and these cases reflect sarcomas, melanomas, and embryonal tumors [2-6]. Reports of carcinoma of unknown primary in children exist only as single case reports [7]. Irrespective of histology, outcomes in both adults and children with CUP are abysmal. Median survival time for adults treated with combination chemotherapy is ˜9-10 months [8, 9], and similar numbers have been reported in the young adult population [6].

Paradigms for the pathological workup of carcinoma of unknown primary are based on findings in adult carcinoma [10]. Likewise, there have been numerous efforts to use tumor gene expression to classify a tissue of origin for adults with CUP [11, 12]. Despite these efforts, a randomized clinical trial in which patient treatments were stratified by putative tissue of origin showed only a modest improvement in overall survival compared to historical controls (median 12.5 months versus 9.1 months) [13]. Adult CUP frequently harbors *TP53* , *KRAS* ,*CDKN2A* , or *ARID1A* alterations, consistent with suspicions that most tumors arise from gastroesophageal, biliary tract, or pancreatic tumors [14]. A small analysis of 17 patients treated with targeted therapy matched to observed molecular alterations reported only 2 patients with stable disease and the remainder with progressive disease [15].

Here, we present a case of metastatic adenocarcinoma of unknown primary in a 13-year old boy. We employed

molecular profiling of his tumor to uncover a targetable *ERBB2* kinase domain mutation. Our patient subsequently had a clinical response with a combination of lapatinib as a targeted agent and chemotherapy, with excellent quality of life during this time prior to eventually dying from disease.

Case Description

Our patient was a 13-year old male who presented to our emergency room for shortness of breath, hypoxia and lower back pain. One week prior, he presented to an outside hospital for shortness of breath, where a chest x-ray showed bilateral infiltrates and was interpreted as atypical pneumonia, for which the patient completed a 5-day course of azithromycin. He had a history of type 1 diabetes, diagnosed 1 year previously. Family history was negative for cancer onset under age 60 in any family members.

Initial chest x-ray at our center showed bilateral lung opacities concerning for malignancy. Laboratory work-up demonstrated high LDH, uric acid, alkaline phosphatase, and inflammatory markers. Staging CT scans of the chest, abdomen, and pelvis, and an MRI of the brain, spine and orbit demonstrated widespread bone lesions, no intraparenchymal brain metastases, and no obvious primary tumor (**Figure 1A**). Bone lesions were FDG-avid by FDG-PET scan. A scrotal ultrasound demonstrated bilateral edema with a question of an infiltrative process. Elevated tumor markers included CEA (228.7 ng/mL), alkaline phosphatase (1491 unit/L), and CA19-9 (71 unit/L). His β-human chorionic gonadotropin (β-HCG) and α-fetoprotein (AFP) tumor markers were within normal limits.

Needle biopsy of a right iliac crest lesion showed malignant epithelial cells with interspersed mucogenic cells and poorly formed glands, consistent with metastatic adenocarcinoma. A concurrent pleural fluid sample showed identical cells. By immunohistochemistry, tumor cells were positive for CAM 5.2, MNF116, CK20 (strong), CK7 (focal), CD99, CEA, and p63, and negative for chromogranin, synaptophysin, TTF-1, NUT, and desmin (**Figure 1B, Supplemental Figure 1**). These stains failed to establish a definitive primary site of origin, but supported the possibility of a gastrointestinal or pancreatobiliary site [16]. Next generation sequencing of 447 cancer-related genes, with a previously validated OncoPanel assay [17], revealed inactivating variants in *ARID1A* (in-frame internal deletion of exon 20, 10% of 372 reads) and *CTCF* p.Q198* (26% of 304 reads), and a variant in the *ERBB2* kinase domain, p.D769Y (28% of 228 reads) (**Figure 1C, Supplemental Table 1)** . There were copy number variants of 1q, 7p, 7q, 8p, 8q, and 11 (**Supplemental Figure 2**). Mutational burden was 4.5 mutations/megabase and mismatch repair proficiency. Germline genetic testing of 48 cancer predisposition genes at a reference laboratory revealed a variant of unknown significance in*APC* (p.A2795T), which was also detected in his tumor sequencing.

While tumor profiling was pending, the patient began empiric treatment with carboplatin and paclitaxel [18], but after one cycle he experienced worsening thrombocytopenia, increased bony pain and respiratory distress requiring positive pressure ventilation. A chest CT scan demonstrated tumor progression which was corroborated by increased tumor markers.

The activating *ERBB2* D769Y variant had previously been shown to exhibit sensitivity to several inhibitors in model systems [19]. Our patient was therefore started on lapatinib (1250mg daily), an oral kinase inhibitor targeting the ERBB2 and EGFR kinases with known safety data in pediatric patients [20, 21], as well as capecitabine (850mg/m^2 twice daily), according to adult clinical trial data in *ERBB2* -altered gastroesophageal adenocarcinoma [22]. Concurrent oxaliplatin (130mg/m^2, day 1) was initially held due to the patient's poor performance status but was added in subsequent cycles. Within 10 days of starting treatment with lapatinib and capecitabine, his symptoms improved and his CEA decreased by more than 50%.

The patient remained on lapatinib with combination chemotherapy for a total of 5 cycles, with a CEA nadir of 25.4 ng/mL, representing a 93% reduction compared to his pre-lapatinib CEA. His alkaline phosphatase decreased from 1795 unit/L to 356 at its nadir (80% decrease) (**Figure 2A**). Radiographically, he had widespread disease regression and dramatically reduced PET-avidity of tumor sites (**Figure 2B**). He had good quality of life at home during these treatment cycles, and his clinical course was complicated only by bilateral pulmonary emboli, treated with enoxaparin as an outpatient.

After 5 cycles, the patient exhibited worsening pain and thrombocytopenia, and PET/CT imaging demonstrated diffusely worsening disease. His CEA rose to 434 ng/mL. He initiated neratinib (240mg daily) for more potent ERBB2 inhibition [19] and vinorelbine (25mg/m^2, days 1 & 8) [23]. His tumor exhibited a transient response, with a reduction of his CEA to 119 ng/mL (72.5% reduction); but this rapidly rose within several weeks, along with worsening pain and respiratory status. Since his performance status was too poor for cross-sectional imaging, X-ray images of his chest and pelvis showed increased disease burden.

He was then consented to pembrolizumab, although his diagnostic biopsy was negative for PD-L1 staining. He received one dose of pembrolizumab (2mg/kg). He died twenty-four hours later from cardiopulmonary arrest.

A limited autopsy of the chest, abdomen and pelvis revealed extensive pulmonary intralymphatic carcinomatosis, mediastinal lymph node metastases, and epicardial lymphatic involvement by tumor. No clear primary site was identified.

Discussion

Here, we present a challenging case of metastatic adenocarcinoma of unknown primary in a pediatric patient. This rare pediatric diagnosis represents <1% of solid tumors in childhood, with few published reports in the literature. Indeed, prior reports of cancer of unknown primary in children almost exclusively reflect non-carcinoma histology and date to an era before advanced immunohistochemical, cytogenetic, and molecular characterization of tumors, and before PET scans [2, 4, 5]. Whether these cases would be considered to be, or treated as, CUP today is unclear.

Our patient died from disease approximately 6 months after diagnosis, in keeping with a median survival of ˜9-10 months in the adult literature [9]. Moreover, we were unable to locate a primary tumor on autopsy, which in adult series may occur in up to 50% of cases [24].

Our case also demonstrates the potential for precision medicine in this patient population. Indeed, up to 10% of CUP in adults may harbor an*ERBB2* alteration, as well as smaller percentages with alterations in genes encoding other targetable kinases [19]. While our patient developed therapy-resistant disease, he had excellent quality of life at home for most of his disease course. We conclude that pediatric oncology patients diagnosed with CUP may benefit from a comprehensive molecular evaluation to determine whether a targetable event is present and clinically actionable.

Word count: 1361/1200

Acknowledgements

We are deeply indebted to the patient's family for enabling us to share his case.

Ethics Statement

Radiological images of the patient are used with the consent of his family.

Conflict of Interests:

John R. Prensner: none to declare

Juan Putra: none to declare

Sara O. Vargas: Occasional consulting for various medicolegal cases.

Alanna J. Church: Prior consulting fee from Bayer Oncology, Samba Scientific, and Jackson Laboratories. Prior travel expenses from Bayer Oncology.

Katherine A. Janeway: Consulting fees from Bayer and Ipsen. Travel expenses from Loxo Oncology.

Nadine J. McCleary: None to declare

Steven G. DuBois: Prior consulting fee from Bayer and Loxo Oncology. Prior travel expenses from Loxo Oncology, Roche, and Salarius Pharmaceuticals.

References

1. Pavlidis N and Pentheroudakis G. Cancer of unknown primary site. Lancet 2012:379(9824):1428-1435.

2. Didolkar MS, Fanous N, Elias EG and Moore RH. Metastatic carcinomas from occult primary tumors. A study of 254 patients. Ann Surg 1977:186(5):625-630.

3. Pappo AS, Kuttesch JF, Kaste SC, Parham DM, Rao BN and Pratt CB. Malignant melanocytic lesions of unknown primary site in children and adolescents. Med Pediatr Oncol 1995:24(5):315-320.

4. Kuttesch JF, Jr., Parham DM, Kaste SC, Rao BN, Douglass EC and Pratt CB. Embryonal malignancies of unknown primary origin in children. Cancer 1995:75(1):115-121.

5. Etcubanas E, Peiper S, Stass S and Green A. Rhabdomyosarcoma, presenting as disseminated malignancy from an unknown primary site: a retrospective study of ten pediatric cases. Med Pediatr Oncol 1989:17(1):39-44.

6. Raghav K, Mhadgut H, McQuade JL, Lei X, Ross A, Matamoros A, Wang H, Overman MJ and Varadhachary GR. Cancer of Unknown Primary in Adolescents and Young Adults: Clinicopathological Features, Prognostic Factors and Survival Outcomes. PLoS One 2016:11(5):e0154985.

7. Cheng JC, Esparza SD, Knez VM, Sakamoto KM and Moore TB. Severe lactic acidosis in a 14-year-old female with metastatic undifferentiated carcinoma of unknown primary. J Pediatr Hematol Oncol 2004:26(11):780-782.

8. Greco FA, Burris HA, 3rd, Litchy S, Barton JH, Bradof JE, Richards P, Scullin DC, Jr., Erland JB, Morrissey LH and Hainsworth JD. Gemcitabine, carboplatin, and paclitaxel for patients with carcinoma of unknown primary site: a Minnie Pearl Cancer Research Network study. J Clin Oncol 2002:20(6):1651-1656.

9. Pentheroudakis G, Greco FA and Pavlidis N. Molecular assignment of tissue of origin in cancer of unknown primary may not predict response to therapy or outcome: a systematic literature review. Cancer Treat Rev 2009:35(3):221-227.

10. Chu PG, Chung L, Weiss LM and Lau SK. Determining the site of origin of mucinous adenocarcinoma: an immunohistochemical study of 175 cases. Am J Surg Pathol 2011:35(12):1830-1836.

11. Tothill RW, Kowalczyk A, Rischin D, Bousioutas A, Haviv I, van Laar RK, Waring PM, Zalcberg J, Ward R, Biankin AV, Sutherland RL, Henshall SM, Fong K, Pollack JR, Bowtell DD and Holloway AJ. An expression-based site of origin diagnostic method designed for clinical application to cancer of unknown origin. Cancer Res 2005:65(10):4031-4040.

12. Varadhachary GR, Talantov D, Raber MN, Meng C, Hess KR, Jatkoe T, Lenzi R, Spigel DR, Wang Y, Greco FA, Abbruzzese JL and Hainsworth JD. Molecular profiling of carcinoma of unknown primary and correlation with clinical evaluation. J Clin Oncol 2008:26(27):4442-4448.

13. Hainsworth JD, Rubin MS, Spigel DR, Boccia RV, Raby S, Quinn R and Greco FA. Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients with carcinoma of unknown primary site: a prospective trial of the Sarah Cannon research institute. J Clin Oncol 2013:31(2):217-223.

14. Ross JS, Wang K, Gay L, Otto GA, White E, Iwanik K, Palmer G, Yelensky R, Lipson DM, Chmielecki J, Erlich RL, Rankin AN, Ali SM, Elvin JA, Morosini D, Miller VA and Stephens PJ. Comprehensive Genomic Profiling of Carcinoma of Unknown Primary Site: New Routes to Targeted Therapies. JAMA Oncol 2015:1(1):40-49.

15. Subbiah IM, Tsimberidou A, Subbiah V, Janku F, Roy-Chowdhuri S and Hong DS. Next generation sequencing of carcinoma of unknown primary reveals novel combinatorial strategies in a heterogeneous mutational landscape. Oncoscience 2017:4(5-6):47-56.

16. Stella GM, Senetta R, Cassenti A, Ronco M and Cassoni P. Cancers of unknown primary origin: current perspectives and future therapeutic strategies. J Transl Med 2012:10:12.

17. Garcia EP, Minkovsky A, Jia Y, Ducar MD, Shivdasani P, Gong X, Ligon AH, Sholl LM, Kuo FC, MacConaill LE, Lindeman NI and Dong F. Validation of OncoPanel: A Targeted Next-Generation Sequencing Assay for the Detection of Somatic Variants in Cancer. Arch Pathol Lab Med 2017:141(6):751-758.

18. Briasoulis E, Kalofonos H, Bafaloukos D, Samantas E, Fountzilas G, Xiros N, Skarlos D, Christodoulou C, Kosmidis P and Pavlidis N. Carboplatin plus paclitaxel in unknown primary carcinoma: a phase II Hellenic Cooperative Oncology Group Study. J Clin Oncol 2000:18(17):3101-3107.

19. Bose R, Kavuri SM, Searleman AC, Shen W, Shen D, Koboldt DC, Monsey J, Goel N, Aronson AB, Li S, Ma CX, Ding L, Mardis ER and Ellis MJ. Activating HER2 mutations in HER2 gene amplification negative breast cancer. Cancer Discov 2013:3(2):224-237.

20. Fouladi M, Stewart CF, Olson J, Wagner LM, Onar-Thomas A, Kocak M, Packer RJ, Goldman S, Gururangan S, Gajjar A, Demuth T, Kun LE, Boyett JM and Gilbertson RJ. Phase I trial of MK-0752 in children with refractory CNS malignancies: a pediatric brain tumor consortium study. J Clin Oncol 2011:29(26):3529-3534.

21. Fouladi M, Stewart CF, Blaney SM, Onar-Thomas A, Schaiquevich P, Packer RJ, Goldman S, Geyer JR, Gajjar A, Kun LE, Boyett JM and Gilbertson RJ. A molecular biology and phase II trial of lapatinib in children with refractory CNS malignancies: a pediatric brain tumor consortium study. J Neurooncol 2013:114(2):173-179.

22. Hecht JR, Bang YJ, Qin SK, Chung HC, Xu JM, Park JO, Jeziorski K, Shparyk Y, Hoff PM, Sobrero A, Salman P, Li J, Protsenko SA, Wainberg ZA, Buyse M, Afenjar K, Houe V, Garcia A, Kaneko T, Huang Y, et al. Lapatinib in Combination With Capecitabine Plus Oxaliplatin in Human Epidermal Growth Factor Receptor 2-Positive Advanced or Metastatic Gastric, Esophageal, or Gastroesophageal Adenocarcinoma: TRIO-013/LOGiC–A Randomized Phase III Trial. J Clin Oncol 2016:34(5):443-451.

23. Awada A, Dirix L, Manso Sanchez L, Xu B, Luu T, Dieras V, Hershman DL, Agrapart V, Ananthakrishnan R and Staroslawska E. Safety and efficacy of neratinib (HKI-272) plus vinorelbine in the treatment of patients with ErbB2-positive metastatic breast cancer pretreated with anti-HER2 therapy. Ann Oncol 2013:24(1):109-116.

24. Blaszyk H, Hartmann A and Bjornsson J. Cancer of unknown primary: clinicopathologic correlations. APMIS 2003:111(12):1089-1094.

25. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C and Schultz N. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012:2(5):401-404.

26. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C and Schultz N. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013:6(269):pl1.

Figure legends:

Figure 1: Diagnosis of metastatic cancer of unknown primary.A) *Left* , A diagnostic sagittal T1-weighted MRI image showing extensive vertebral disease. *Right* , A post-contrast chest CT image showing extensive nodular opacities disease burden. **B)**Histological images from the patient's right iliac crest biopsy showing: (**B1**) malignant epithelial cells with glandular differentiation and mucin production (hematoxylin and eosin; original magnification, 10x). Neoplastic cells were immunoreactive for (**B2**) CK7 (focal and

weak; original magnification, 10x), (**B3**) CK20 (focal and strong; original magnification, 10x), and (**B4**) CDX2 (focal and weak; original magnification, 20x). **C)** A lollipop diagram of observed *ERBB2* variants in the MSKCC-IMPACT study dataset, courtesy of cBioPortal.org [25, 26]. The patient's D769Y variant (in red) lies within the kinase domain, within a cluster of known activating variants (recurrent variants are highlighted).

Figure 2: Response and resistance to lapatinib therapy.A) A line plot showing the change over time for the CEA and alkaline phosphatase biomarkers for the patient during different treatment regiments. **B)** Sagittal, axial and coronal FDG-PET scan images for the patient at diagnosis, during clinical response to lapatinib, and at conclusion of lapatinib therapy following disease progression. Several sentinel sites of disease are indicated with arrows for ease of visualization.

Hosted file

`JRP_PBC_Figure1_v08.20.20.pdf` available at https://authorea.com/users/353116/articles/477176-a-case-of-metastatic-adenocarcinoma-of-unknown-primary-in-a-pediatric-patient-opportunities-for-precision-medicine

Hosted file

`JRP_PBC_Figure2_v08.20.20.pdf` available at https://authorea.com/users/353116/articles/477176-a-case-of-metastatic-adenocarcinoma-of-unknown-primary-in-a-pediatric-patient-opportunities-for-precision-medicine