PD-1 Inhibition in Congenital Pigment Synthesizing Metastatic Melanoma

Angela C. Weyand[1], Rajen J. Mody[1], Raja M. Rabah[2], and Valerie P. Opipari[1]

1, Division of Pediatric Hematology and Oncology, Department of Pediatrics and Communicable Diseases, University of Michigan Medical Center

2, Department of Pathology, University of Michigan Medical Center

Angela C. Weyand

1500 E. Medical Center Drive

Ann Arbor, MI 48109

(734)936-9814 (p)

(734)232-8740 (f)

acweyand@med.umich.edu

Abstract: 81 words

Main Text: 1335 words

Tables: none

Figures: 2

Supplemental files: none

Running title: PD-1 Inhibition in Congenital Metastatic Melanoma

Key words: metastatic melanoma, Pd-1 inhibition, pediatric, congenital, pigment-synthesizing

Key Abbreviations

MRI	Magnetic resonance imaging
PD-L1	Programmed death ligand 1
PET	Positron emission tomography
BRAF	v-Raf murine sarcoma viral oncogene homolog B
WES	Whole exome sequencing
RNA-seq	Ribonucleic acid sequencing
KMT2C	Lysine Methyltransferase 2C
MLL3	Mixed lineage leukemia protein 3
PD-1	Programmed death 1
CD8	Cluster of differentiation 8
ANC	Absolute neutrophil count
IgG4	Immunoglobulin G4
CD4	Cluster of differentiation 4

Abstract

An newborn female was born with a congenital pigment synthesizing melanoma of the scalp. Further workup revealed metastatic disease within the liver, lungs, and left tibia. Whole exome sequencing was performed on multiple samples which revealed one somatic mutation KMT2C at low allelic frequency but no B-RAF, NF-1 mutation. PD-L1 was moderately expressed. Treatment was initiated with the PD-1 inhibitor nivolumab. The patient tolerated this treatment well with minimal toxicity. She is now over a year out from initial diagnosis, continuing on nivolumab, with stable disease.

Case

A newborn female was noted to have a dark raised scalp lesion (Fig. 1). The lesion expanded and became more raised. The patient underwent excisional biopsy. Initial pathology was consistent with malignant blue nevus. A second review revealed pigment synthesizing melanoma (Fig. 2a), 7.3mm in depth with 2 mitoses/mm^2. There were positive margins but no ulceration or angiolymphatic invasion. Some areas were consistent with pre-existing sclerotic blue nevus and there were overlapping features with malignant blue nevus. A chest radiograph was negative and abdominal ultrasound demonstrated two suspicious lesions. Magnetic resonance imaging (MRI) revealed six hepatic lesions. She underwent biopsy of these and pathology was consistent with metastatic melanoma (Fig. 2b). Brain MRI and bone marrow examination were negative. Whole body positron emission tomography (PET) imaging revealed disease in her left posterior cervical chain (SUV 2.9) lung (SUV 2), liver (SUV 3.1), abdomen (SUV 9.24), and left tibia (SUV 10.4) with a pathologic fracture. Microarray analysis from the liver lesion identified a gain of chromosome 6 and chromosome 9. The patient's mother had remained healthy

throughout the pregnancy without any melanoma history or concerning skin lesions on full dermatologic examination.

The patient was enrolled on a prospective integrative clinical sequencing trial (PEDS-MIONCOSEQ) and underwent paired tumor/normal whole exome sequencing (WES) and tumor transcriptome sequencing (RNA-Seq). Specifics of the sequencing procedure and bioinformatics analysis have been described previously [1].. Only one somatic mutation, KMT2C (MLL3, p. N729D) was detected, at a low allele frequency (7%) in a 2nd sample while on treatment for 16 weeks. This somatic mutation was not seen in the original biopsy. No mutations were detected in BRAF, NRAS, TSC1/2 genes and no pathogenic variants were detected on germline sequencing. No remarkable gene copy number changes or driving fusions were detected. PD-L1 was moderately expressed.

Treatment was initiated with nivolumab, a programmed cell death protein 1 (PD-1) inhibitor dosed at 3mg/kg every two weeks. First dose was 50%, second dose 75%, and subsequent dosing at 100%. With the first dose, she developed Grade II diarrhea[2] which resolved quickly and did not recur. Following her first treatment, she developed a raised bony scalp lesion, concerning for progressive disease versus pseudo-progression. Following her third treatment, she developed left sided posterior cervical lymphadenopathy. With subsequent treatments, parents noted that the bony lesion and lymphadenopathy increased in size following treatments, and regressed prior to her next treatment. Reassessment of disease status was performed after six doses with whole body PET scan and abdominal MRI. The PET scan demonstrated significant lymphadenopathy, which clinically had not been present at initial PET scan, an increase in her pulmonary nodule, and otherwise stable disease. Abdominal MRI showed slight increase in hepatic lesion size. A lymph node biopsy was

performed following her eighth dose. Pathology revealed metastatic melanoma with large areas of coagulative necrosis (Fig. 2c). This could be consistent with treatment effect but necrosis has also been described within untreated lesions, although was not seen in initial pre-treatment biopsies in our patient[3]. 1-2% of tumor cells were weakly positive with PD-L1 by immunohistochemistry and a small number of CD8 positive infiltrating lymphocytes were noted nearby (Fig. 2d). Given evidence of treatment effect, clinical stability, and tolerance of therapy, the decision was made to continue with nivolumab. Disease evaluation was performed following 14 doses with whole body PET and abdominal MRI. PET scan demonstrated stable number and size of lesions, with global decreased avidity (SUV max of 3.8). MRI of her abdomen showed stable number of lesions with slight decrease in size. Following her 16th dose, she developed grade I anemia (hemoglobin 10.2 g/dL) and grade III neutropenia (ANC nadir 0.8K/uL) [2]. Repeat disease evaluation following 35 doses revealed stable disease.

Discussion

Childhood melanoma has been reported to account for 0.3-0.4% of all melanoma[4] and between 1-3% of pediatric malignancies. Congenital metastatic melanoma is an extremely rare condition that is defined as metastatic melanoma recognized at birth. Its incidence is difficult to determine given its rarity. Congenital melanoma is generally subdivided into three separate entities based on etiology: 1. Transmission from an affected mother by metastatic spread through the placenta. 2. Primary de novo cutaneous congenital melanoma arising in utero. 3. Primary melanoma arising within a giant congenital melanocytic nevus[5].

Pigment synthesizing melanoma, an uncommon variant, typically present as blue-black papules and its incidence is skewed toward the first or second decade of life. [6]

The most common location is the scalp. It is generally known as a low-grade malignancy[7]. It has the tendency to recur locally with satellite nodules and regional lymph node spread but distant metastasis is rare. In a review of 190 cases of pigment synthesizing melanoma, only 7 (3.7%) were congenital and only 2(1.1%) presented with distant metastases. One congenital case was found to have lymph node and liver disease multiple months after presentation[8]. Due to this rarity, optimal treatment is unknown. Additionally, treatment of metastatic melanoma has historically been unsuccessful. Biological basis of neonatal melanoma is unknown. They are usually not driven by BRAF. Our tumor had a low frequency MLL3 mutation which has been reported in other relapsed/ refractory solid tumors and lymphoma[9,10]. No pathogenic variants were reported in germline sequencing which was reassuring and consistent with otherwise negative family history of cancers.

Nivolumab, a fully human IgG4 human antibody to the PD-1 immune checkpoint, has been approved for the treatment of patients with metastatic or unresectable melanoma since 2014. By blocking PD-1, nivolumab restores anticancer immune responses through abrogation of T cell inhibition mediated through the PD-1 pathway. A large phase III study in previously untreated adults with metastatic melanoma with wild type BRAF demonstrated overall one year survival of 72.9%, median progression free survival of 5.1 months and an objective response rate of 40%[11], significant improvements over prior standard of care. Common adverse events seen included nausea (16.5%), fatigue (19.9%) and pruritus (17.0%). Additionally, checkpoint inhibitors have been associated with autoimmune toxicities ranging from colitis to endocrinopathies. In general, the PD-1 inhibitors seem to have less severe toxicities, with fewer severe adverse effects and decreased rates of discontinuation due to toxicity. This has been postulated to be due to the fact that

CTLA-4 inhibitors activate a wider range of T-cell subtypes[12]. Furthermore, autoimmunity secondary to PD-1 inhibition tends to occur later in the treatment course (at a median of 59-64 days) and is more likely to involve thyroid dysfunction rather than colitis or hypophysitis[13,14]. We have conducted regular screening assessments for endocrine dysfunction, as well as clinical monitoring, and plan to continue this throughout her treatment course.

Only one published study exists looking at checkpoint inhibition in pediatric populations[15]. Toxicities of this treatment were similar to that seen in adults but unfortunately, no objective responses were observed. There is no published data on checkpoint inhibition in infancy. The efficacy of this approach in infants with immature immune systems is unknown. However, data showing cord blood with increased expression of the PD-1 molecule on CD4+ T cells in comparison to adult peripheral blood upon activation raises the possibility of utility in this population. This case demonstrates the safe use of PD-1 checkpoint inhibition in a young infant. [16]. Our patient initially experienced some early Grade I-II diarrhea and later developed Grade II anemia and Grade III neutropenia, none of which required a change in therapy. She has continued to grow and develop normally. Following initiation of nivolumab, she developed left sided posterior cervical lymphadenopathy. Given later improvement, this likely represents pseudo-progression, a phenomenon that is seen with the new immunotherapies during which an initial increase in tumor size is seen prior to subsequent improvement. On follow-up radiologic and clinical evaluations, our patient continues to demonstrate stable disease. Pigment synthesizing melanoma tends to be an indolent disease so it is difficult to ascertain exactly what contribution the nivolumab has made to her clinical stability. However, given lack of significant toxicity, overall tolerability, and stable disease, we plan to

continue this treatment. If she develops progressive disease, consideration will be given to combination therapy with CTLA-4 inhibition.

Ethics Statement: Informed consent (from the patient's mother) was obtained and properly documented.

Conflict of Interest statement: The authors have no relevant disclosures.

1. Mody RJ, Wu YM, Lonigro RJ, et al. Integrative Clinical Sequencing in the Management of Refractory or Relapsed Cancer in Youth. *Jama.* 2015;314(9):913-925.
2. NCI. Common Terminology Criteria for Adverse Events v4.0 NCI, NIH, DHHS. NIH publication # 09-7473. 2009.
3. Bahrami A, Lee S, Wu G, et al. Pigment-Synthesizing Melanocytic Neoplasm With Protein Kinase C Alpha (PRKCA) Fusion. *JAMA dermatology.* 2016;152(3):318-322.
4. Hamm H, Hoger PH. Skin tumors in childhood. *Deutsches Arzteblatt international.* 2011;108(20):347-353.
5. Trozak DJ, Rowland WD, Hu F. Metastatic malignant melanoma in prepubertal children. *Pediatrics.* 1975;55(2):191-204.
6. Zembowicz A, Mihm MC. Dermal dendritic melanocytic proliferations: an update. *Histopathology.* 2004;45(5):433-451.
7. Antony FC, Sanclemente G, Shaikh H, Trelles AS, Calonje E. Pigment synthesizing melanoma (so-called animal type melanoma): a clinicopathological study of 14 cases of a poorly known distinctive variant of melanoma. *Histopathology.* 2006;48(6):754-762.
8. Vyas R, Keller JJ, Honda K, Cooper KD, Gerstenblith MR. A systematic review and meta-analysis of animal-type melanoma. *Journal of the American Academy of Dermatology.* 2015;73(6):1031-1039.
9. Parsons DW, Li M, Zhang X, et al. The genetic landscape of the childhood cancer medulloblastoma. *Science (New York, NY).* 2011;331(6016):435-439.
10. Morin RD, Assouline S, Alcaide M, et al. Genetic Landscapes of Relapsed and Refractory Diffuse Large B-Cell Lymphomas. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2016;22(9):2290-2300.
11. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *The New England journal of medicine.* 2015;372(4):320-330.
12. Ott PA, Hodi FS, Robert C. CTLA-4 and PD-1/PD-L1 blockade: new immunotherapeutic modalities with durable clinical benefit in melanoma patients. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2013;19(19):5300-5309.
13. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. *The New England journal of medicine.* 2015;372(26):2521-2532.
14. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *The New England journal of medicine.* 2015;373(1):23-34.
15. Merchant MS, Bernstein D, Amoako M, et al. Adjuvant Immunotherapy to Improve Outcome in High-Risk Pediatric Sarcomas. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2016;22(13):3182-3191.
16. Chiou VL, Burotto M. Pseudoprogression and Immune-Related Response in Solid Tumors. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2015;33(31):3541-3543.

Figure Legends:

Figure 1. Initial appearance of the scalp lesion prior to resection

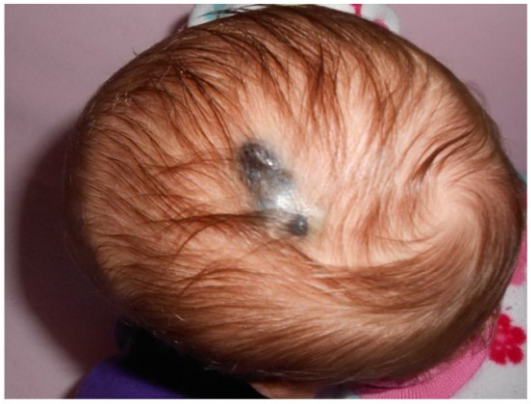

Figure 2. Skin biopsy with atypical spindled and epithelioid heavily pigmented melanocytic cells in the dermis (A). Similar atypical melanocytic cells noted in the liver (B) and lymph node (C). Few CD8 positive small lymphocytes (arrows) are noted adjacent to the melanoma cells (D).