

## Potential of Germline *CDKN2A* I49T as a Targetable Driver Mutation: Prolonged Control of Refractory Osteosarcoma with CDK4/6 Inhibitor in a Familial Cancer

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### Abstract

**Objective:** Identifying the potential of germline *CDKN2A* I49T (also known as *p.149T: ATC>ACC*) in familial cancer and its potential as a targetable driver mutation in carcinogenesis.

**Method:** Germline mutational analysis was done using commercially available next generation sequencing (NGS) in kindred affected by cancers of lung, throat, gastrointestinal stromal tumor (GIST) and osteosarcoma using. Treatment of chemo-refractory osteosarcoma was done with CDK4/6 inhibitor palbociclib. Monitoring of response was done by serial computed tomography (CT) imaging.

**Results:** Two affected members in the kindred, one with GIST and one with osteosarcoma were tested and proven positive for germline *CDKN2A* I49T alteration. The patient with osteosarcoma experienced progression of the disease despite multiple surgical resections and combination chemotherapy. Patient had a sustainable response to CDK4/6 inhibitor palbociclib, with disease controlled for more than a year.

**Conclusion:** These findings suggested a familial cancer syndrome associated with germline *CDKN2A* I49T and showed its potential as a targetable driver mutation.

**Keywords:** Germline mutational analysis; Next generation sequencing; Osteosarcoma

**Abbreviations:** FAMMM: Familial Atypical Multiple Mole Melanoma; PARP: Poly (ADP-ribose) Polymerase; IHC: Immune-Histo-Chemistry; GIST: Gastrointestinal Stromal Tumor

### Introduction

*CDKN2A* is frequently altered in a variety of cancers. It occurs as somatic and germline mutations. *CDKN2A* mutations have been reported in rare osteosarcoma cases [1]. Germline *CDKN2A* mutations are reported in Familial Atypical Multiple Mole Melanoma (FAMMM). FAMMM patients frequently have multiple hyper pigmented nevi and higher frequency of melanoma and pancreatic cancer [2]. Brain tumors, head/neck and skin cancers have also been reported in mutation carriers [3-5].

At the cellular level, *CDKN2A* gene encodes a variety of proteins including p14 and p16. Clinical significance of *CDKN2A* I49T, which in turn affects p16 and p14, is not clear [6,7]. In reported families with I49T alteration, the altered gene did not happen to segregate with melanoma [8]. Here we report a family with germline *CDKN2A* I49T alteration and multiple family members affected with cancer. In at least two members, the germline mutation was confirmed. We also show the potential of germline *CDKN2A* I49T alteration as a targetable driver mutation, as the refractory osteosarcoma went into a prolonged remission after treatment with CDK4/6 inhibitor.

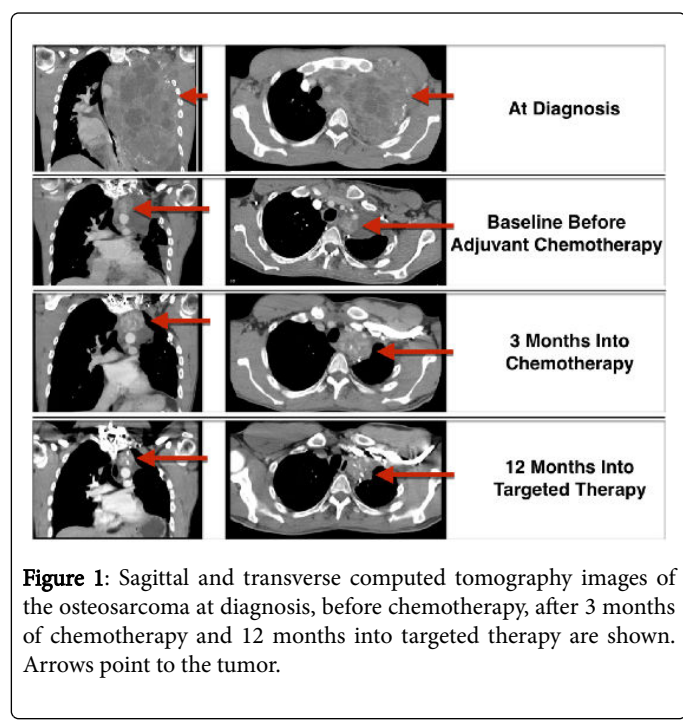
Pathology was reviewed and confirmed at Community Regional Medical Center, Fresno, California after reviewing of all samples with microscopic histology and immune-histo-chemistry (IHC) evaluation for vimentin, pancytokeratin, CD34, S100, desmin, SMA, EMA, CK7, CK20, STAT6, myogenin, ERG, SALL4, Beta-Catenin and MUC4. The pathology samples were additionally reviewed separately at University of California, San Francisco (UCSF) as well as in University of Southern California (USF). Tumor mutational analysis was done by commercially available FoundationOne™ test (Foundation Medicine, Cambridge, Massachusetts) on the paraffin block of the formaldehyde fixed samples of GIST tumor and osteosarcoma. Germline mutation tests were done on the peripheral blood by commercially available test CancerNext (Ambry Genetics, Aliso Viejo, California). Response to the treatment was monitored by serial computed tomography (CT) imaging.

### Case Presentation

A 28 years old male with no prior significant medical or surgical history presented to ER with progressive shortness of breath, bipedal swelling. His symptoms were worsening over course of a month. Chest X-ray and computed tomograms (CT scans) of the chest showed a large chest mass emanating from the left side rib, causing severe extrinsic compression on the mediastinum and invading through thoracic spinal column. Patient underwent surgical stabilization of thoracic vertebrae, during which, tissue samples were obtained from the hypervascular lesion invading into the vertebral bodies. Positron

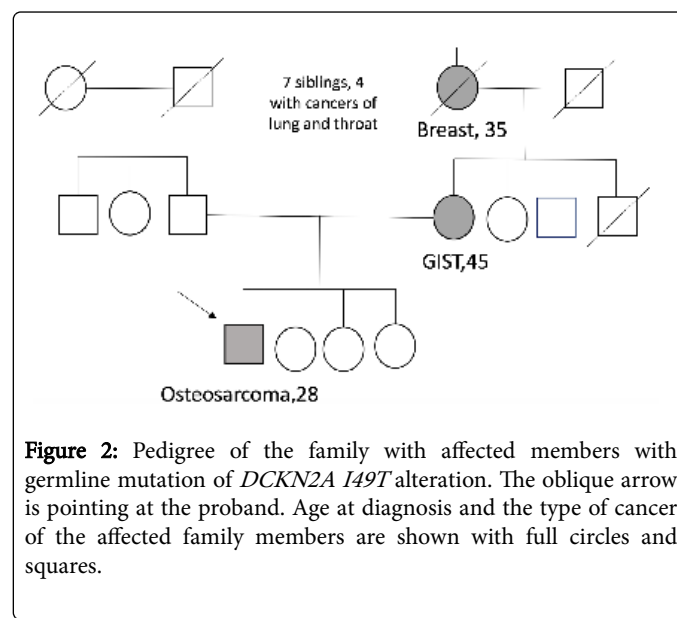
emission and computed tomography (PET/CT) obtained after this operation showed a low activity tumor with higher activity in certain areas of the tumor, which was attributed to the biopsied spots. Patient underwent a second surgical operation with sternotomy, left thoracotomy, resection of the chest wall mass with en-bloc tumor resection and partial excision of the left second rib. Surgical and pathology report showed gross residual disease. Post resection, patient recovered well. Pathology confirmed low grade osteosarcoma with spindle shape cells, only positive for vimentin, and negative for pancytokeratin, CD34, S100, desmin, SMA, EMA, CK7, CK20, STAT6, myogenin, ERG, SALL4, Beta-Catenin and MUC4. Final tissue diagnosis was periosteal osteosarcoma of the rib with secondary aneurismal bone cyst-like degeneration and possible focal de-differentiation into a high-grade osteosarcoma. Post-operation CT scan showed remaining lesions in the mediastinum as well as paraspinal regions. To identify whether these are metastatic foci, biopsy was obtained through endobronchial ultrasound (EBUS). Pathology of these biopsies was compatible with the original tumor histology, confirming metastasis. Based on these findings, case was discussed in multidisciplinary tumor board. Due to the distribution of the residual disease in mediastinum, ribs and spine, and also the low-grade nature of the bulk of the disease, adjuvant radiation was not recommended. Therefore, sample was sent for somatic genetic mutational analysis and in parallel, patient was treated with 4 cycles of systemic chemotherapy with gemcitabine 900 mg/m<sup>2</sup> (day 1 and 8) and docetaxel 100 mg/m<sup>2</sup> (day 8) on a 21-day cycle. Re-imaging after 3 months of chemotherapy showed progressive disease. Case was discussed again in the multidisciplinary tumor board and surgical resection was not recommended. Patient was also not interested in pursuing clinical trials at this time.

Next generation sequencing of osteosarcoma showed gene alterations in the following genes: *CDKN2A*, *TSC1*, *CDK4*, *KDM5A*, *PBRM1*, *KRAS*, *PAG1*, *NUP98*, *NOD1*, *HNF1A*, *DTX1*, *HDAC7*. Allele frequency showed that *CDKN2A* mutation has frequency of 47%.



Due to the progressive disease with *CDKN2A* alteration, patient was started on CDK4/6 inhibitor palbociclib 125 mg daily for 21 consecutive days and 7 days off treatment in a 28-day cycle. Follow up images after 3 months showed regression of the disease in the mediastinum, and eventually calcification formed in the mediastinal lesion on imaging 12 months on treatment (Figure 1). Patient has tolerated the treatment without complications and with normal blood tests. Absolute neutrophil counts remained within normal limits over the course of the next 12 months. Patient remained asymptomatic and fully functional.

Family history of the patient was positive for patient's mother with gastrointestinal stromal tumor (GIST) of small intestine, diagnosed at age 45. She was treated successfully with surgical resection and adjuvant imatinib. Besides patient's mother, there was significant family history of cancer. Maternal grandmother was also diagnosed with breast cancer at age 35, died at age 40. Four of grandmother's seven siblings reportedly had cancer; most of them developed cancer prior to the age of 45. Two of the affected members had lung cancer and one had throat cancer (Figure 2).



Notably none of them had history of smoking. Mother's GIST tumor was also analyzed with next generation sequencing and showed mutation in the following genes: *CDKN2A*, *c-KIT*, *RBI*, *SPTA1*, *ERBB2*, *LRP1B*, *EPHA5*, *POLE*. Notably, allele frequency was close to 50% in several genes: *CDKN2A* (47%), *RBI* (56%), *SPTA1* (50%), *EPHA5* (48%), and *POLE* (52%) (Table 1). Since tumor samples of patient and his mother showed *CDKN2A* I49T alteration, patient and his mother's were tested for germline mutation and both were found to be positive for germline *CDKN2A* I49T mutation.

Patient			Mother		
Gene	Protein effect	Allele Frequency	Gene	Protein effect	Allele Frequency
<i>CDKN2A</i>	I49T	47%	<i>CDKN2A</i>	I49T	47%
<i>TSC1</i>	D24FS*4	7%	<i>KIT</i>	W557S	37%
<i>CDK4</i>	Amplified x7		<i>RBI</i>	L809fs*17	56%

<i>KDM5A</i>	Amplified x7		<i>SPTA1</i>	R891*	50%
<i>PBRM1</i>	Y1577D	51%	<i>ERBB2</i>	Q1148L	7%
<i>KRAS</i>	M189L	77%	<i>LRP1B</i>	I844M	48%%
<i>PAG1</i>	E281K	50%	<i>EPHA5</i>	S124F	10%
<i>NUP98</i>	D1757V	50%	<i>POLE</i>	G380S	52%
<i>NOD1</i>	V268M	52%			
<i>HNF1A</i>	Amplified x6				
<i>DTX1</i>	Amplified x6				
<i>HDAC7</i>	Amplified x7				

**Table 1:** Altered genes, type of genetic alteration and allele frequencies reported in the osteosarcoma and gastrointestinal stromal tumor (GIST). Coding alterations are reported with the amino acid alterations or the copy number as amplifications.

## Discussion

Pathogenicity and association of *CDKN2A I49T* with familial cancer has been a matter of controversy. As noticed in FAMMM, *CDKN2A I49T* did not segregate with melanoma [8]. Additionally, while somatic *CDKN2A* deletion is frequently reported in human osteosarcoma, germline *CDKN2A* mutation is common in canine osteosarcoma [1,9]. In the present study, none of the family members had melanoma, dysplastic nevi, glioblastoma or pancreatic cancer, common features of FAMMM. Instead, there was a range of early age breast cancer, lung cancer, head and neck cancer, GIST and osteosarcoma. It is possible that families affected by germline *CDKN2A I49T* mutation carry a different risk of malignancies compared to those with FAMMM.

Osteosarcoma is usually divided into high grade and low-grade subtypes. Most of the non-metastatic low-grade osteosarcomas are treated by surgical resection. Treating low grade osteosarcoma with islands of high grade disease has been a matter of controversy. In a study in 2015 it was suggested that if high grade is less than 50% of the tumor, complete surgical resection is the only necessary treatment [10]. The current case had slow growing osteosarcoma with small islands of high grade cancer and despite several attempts, total resection was not possible. Imaging showed that despite combination chemotherapy, tumor was growing and forming new lesions. The main challenge in this case, was to find a targetable driver mutation.

It is known that *CDKN2A I49T* alteration affects p16 [6,7]. Cellular and molecular studies in *CDKN2A I49T* mutant cells have shown a different intracellular distribution of p16 [11]. Among products of *CDKN2A*, p16, is a natural inhibitor of CDK4/6 [12]. Therefore in the osteosarcoma case with germline *CDKN2A* mutation, a CDK4/6 inhibitor could be a potential candidate to replace the dysfunctional protein. Treatment with palbociclib, a CDK4/6 inhibitor, resulted in prolonged control of the disease, supporting the potential of germline mutation in *CDK4/6 I49T* for targeted tumor therapy. Other targetable germline mutations include *BRCA1*, *BRCA2* and *ATM* in breast and ovarian cancers. Such cancers are candidates for treatment with ADP-ribose polymerase (PARP) inhibitors [13]. Further studies are needed to explore and confirm the potential of CDK4/6 inhibitors in treating patients with germline *CDK4/6* mutations.

## Conclusion

Germline *CDKN2A I49T* mutation is a potentially targetable driver mutation and can be associated with familial cancer.

## Declarations

Ethics approval and consent to participate was waived, obtained by UCSF Fresno institutional review board.

Consent for publication was obtained from the patient.

## Availability of Data and Materials

Please contact author for data requests.

## Competing Interests

The authors declare that they have no competing interests.

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