(Patient name)

(Date)

Dear (Name/Parent name),

Thank you for partnering with Count Me In and participating in the Osteosarcoma Project.

Thank you for sharing a saliva sample with us and giving us permission to obtain some leftover tumor tissue from the hospital(s) where you have received your care. With your help, we are continuing to learn more about osteosarcoma.

We want to share with you some of the genetic information that we found within your tumor sample. Genetic changes found in your tumor can help us to learn more about how osteosarcoma develops and grows. These results are shared with you to provide you with information about how your participation in Count Me In is helping researchers, in accordance with your request to receive these results. These results are not meant to replace a clinical genetic test of your tumor. These results may not provide all of the information your doctor needs to make recommendations for your treatment. The information provided here was obtained from sequencing performed in a clinical laboratory and so if you choose to share these results with your doctor, your doctor can place these in your medical record. If you or your doctor thinks having your tumor sequenced or profiled will help with your care, then we recommend that standard testing for clinical purposes be ordered by your doctor.

We obtained leftover tumor tissue from your biopsy or surgery from (procedure date), identified as (Institution & SPID/Accession Number). A clinical report from the laboratory is provided at the end of this letter.

The following cancer genetic alterations were found in your tumor. This information helps us understand how osteosarcoma develops and grows. Each alteration is described with the gene name, the alteration, and what we understand about how it may be important in osteosarcoma cells. References are provided at the end of this report:

[The genes described below serve as examples of how results will be described to participants.]

- ***MYC* amplification** : The *MYC* gene (also known as *c-MYC*) is a member of a family of genes that makes proteins involved in many cell functions, including cell growth, cell maturation, and cell death. Genetic changes in the DNA sequence of the MYC gene have been found in many types of cancer, including osteosarcoma.[1-3] Amplification means that there are many extra copies of this gene within the tumor cells, at least a 3 -5 fold increase.[4] This change may cause osteosarcoma cells to grow.[5,6]
- ***CDKN2A* deletion:** The *CDKN2A* gene makes two major proteins involved in controlling cell growth, cell division, and a type of cell death called apoptosis. Variants (changes) in

the *CDKN2A* gene may cause cells to grow and divide too quickly or in an uncontrolled way or may keep cells fr om undergoing apoptosis. This may cause abnormal cells, including cancer cells, to grow. *CDKN2A* gene mutations have been found in melanoma and in many other types of cancer. The *CDKN2A* gene is a type of tumor suppressor gene.[7] A deletion of *CDKN2A* allows osteosarcoma cells to divide and grow.[8]

- ***TP53* c.818G>A, p.R273H (NM_000546.4)**: The *TP53* gene (also known as p53) makes a protein that is found inside the nucleus of cells and plays a key role in controlling cell division and cell death. Variants (changes) i n the *TP53* gene may cause cancer cells to grow and spread in the body. These changes have been found in many types of cancer. The *TP53* gene is a type of tumor suppressor gene.[9] The variant (or mutation) described as p.R273H changes the way that the p53 protein interacts with other proteins in the cell, including how DNA replicates.[10]

The NCI Dictionary of Cancer Terms may be a useful resource for definitions of genomic and clinical terms used above.

With your participation, and the help of others in the osteosarcoma community, we look forward to learning more about how to get better at caring for pa tients with osteosarcoma.

Yours,
Count Me In Team

References for genetic alterations:
[The references below serve as examples based on the genetic alteration examples]

1. https://www.cancer.gov/publications/dictionaries/cancer -terms/def/myc -gene-family
2. https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=4609
3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3092215/
4. https://pubmed.ncbi.nlm.nih.gov/3113893 1/
5. https://pubmed.ncbi.nlm.nih.gov/23461061/
6. https://www.nature.com/articles/s41413 -018-0009-8
7. https://www.cancer.gov /publications/dictionaries/cancer -terms/def/cdkn2a -gene
8. https://www.sciencedirect.com/science/article/pii/S0304383520300343
9. https://www.cancer.gov/publications/dictionaries/cancer -terms/def/tp53 -gene
10. https://cancerres.aacrjournals.org/content/80/3/394

Boston Children's Hospital
Until every child is well

Department of Pathology
Boston Children's Hospital BCH3027
300 Longwood Avenue
Boston, MA 02115
Phone: (617) 355-7431

BCH MRN:
Patient Name:
DOB/Age/Sex:
Patient Location:
Patient Type:
Admit Date:
Discharge Date:
Ordering Provider:
Accession:

BROAD INSTITUTE | CLINICAL RESEARCH SEQUENCING PLATFORM

Final Diagnosis

Test: [Analysis of Whole Exome Sequencing]
Specimen received: [Originating institution] [SPID/Accession Number] [Block ID]
Tissue: [Tumor site], [histology/ diagnosis]
Original specimen collection date: [Procedure DATE]
Estimated percentage of neoplastic cells in submitted specimen: [%]

RESULT:

MYC amplification

CDKN2A deletion

TP53 c.818G>A, p.R273H

INTERPRETATION:

SUMMARY OF TECHNICAL REPORT

[Data from the Broad CRSP lab]]

CHURCH MD, ALANNA
(Electronically signed by)
Verified: [DATE]
AC /EB
Department of Anatomic Pathology
300 Longwood Avenue
Boston,MA
(617) 355-7431

Sample Received
slides labeled with the patient's name and [SPID/Accession Number]

Sample Description
slides labeled with the patient's name and [SPID/Accession Number]

Test Description

This test represents a collaboration between the Broad Clinical Research Sequencing Platform (CRSP) and the Boston Children's Hospital Laboratory for Molecular Pediatric Pathology (LaMPP). Tumor samples are sequenced at the Broad laboratory, with data transfer and analysis at Boston Children's Hospital.

The Broad Institute's clinical research lab provides Whole Exome Sequencing with somatic variant calling across Tumor/Normal pairs including SNV and Indel calls. Briefly, the assay leverages the Broad-developed solution-phase hybridization assay with TWIST biosciences exome enrichment (35Mb of exome content) and Illumina short read sequencing technology.

Data are transferred to the Boston Children's Hospital Laboratory for Molecular Pathology (LaMPP) for data review and interpretation. A subset of the sequenced genes are interpreted:

[GENE LIST INCLUDING TRANSCRIPT IDs]

Genetic alterations are classified into four tiers based on their level of clinical significance in cancer diagnosis, prognosis and/or therapeutics, according to the standards and guidelines recommended by the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists.

Tier I: Biomarkers which are diagnostic for a specific type of tumor, based on professional guidelines or well-powered studies with consensus from experts; biomarkers which are prognostic for a specific type of tumor based on professional guidelines or well-powered studies with consensus from experts; biomarkers that predict response or resistance to therapies for a specific type of tumor based on professional guidelines or well-powered studies with consensus from experts.

Tier II: Biomarkers of diagnostic significance for a specific type of tumor based on the results of small studies, or that may assist disease diagnosis themselves or along with other biomarkers based on small studies or a few case reports; biomarkers of prognostic significance based on the results of multiple small studies or that may assist disease prognosis along with other biomarkers based on small studies or a few case reports; biomarkers that predict response or resistance to therapies for a different type of tumor, or that serve as inclusion criteria for clinical trials, or that show plausible therapeutic significance based on preclinical studies.

Tier III: Variants of unknown clinical significance.

Tier IV: Benign or likely benign variant (these variants will not be reported).

References:

1. Gnirke et al. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. Nature Biotechnology 27, 182 - 189 (2009).
2. Fisher et al. A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries. Genome Biology 12:R1 (2011).
3. Cibulskis et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nature Biotechnology 31, 213–219 (2013).
4. Allen et al. Whole-exome sequencing and clinical interpretation of FFPE tumor samples to guide precision cancer medicine Nature Medicine 20. 682-8. (2014).
5. Li MM, Datto, M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, Tsimneridou AM, Vnencak-Jones CL, Wolff DJ, Younes A, Nikiforova MN. Standards and Guidelines

for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diag, 2017 Jan;19(1):4-23.

Laboratory Information

The Broad Clinical Research Sequencing Platform (CRSP), under direction of Heidi Rhem, is a clinical laboratory accredited by the College of American Pathologists (CAP), licensed by the State of Massachusetts and registered with the Centers for Medicare and Medicaid Services to provide testing under the CLIA regulations. CLIA # 22D2055652; MA License # 5347; CAP # 8707596

The analysis and reporting of the data are performed by the Laboratory for Molecular Pediatric Pathology (LaMPP) in the Department of Pathology at Boston Children's Hospital under the supervision of Marian H. Harris, MD, PhD, LaMPP Director (CLIA # 22D0001844). This laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 as qualified to perform high-complexity clinical testing. This test has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.