Bone Biopsy Protocol for Advanced Prostate Cancer in the Era of Precision Medicine

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Supplementary Information

Supplementary Table 1. Recommendations for handling of bone biopsies

Interventional Radiology	Place bone/bloodclot immediately on a Telfa non-stick pad and put on wet ice
	Transport to surgical pathology department
Surgical pathology department	Measure specimen, label cryomolds, cork disk and cassettes
	Split bone biopsy in compact and marrow part
	Place bone marrow and bloodclots on a thin layer of OCT in cryomolds
	Freeze cryomolds in methylbuthan and dry ice
	Fill up cryomold with OCT, cover with labeled cork disk and freeze
	If material was kept for organoids, prepare cytology smear
	If smear is positive and estimated tumor cell count is more than 10%, place material in cell culture media and process
	If smear is negative, freeze material in cryomold
	Wrap compact bone part in tissue paper, place in formalin/decalcifying agent and submit for clinical diagnosis
	Cut cryomolds once clinical diagnosis is established
Slide review	Review frozen H&E slides for tumor cell content and quality
	Scan selected slide with representative histology
	Annotate areas with highest tumor cell content
	Select slide for DNA and RNA
	Sent annotated slides and cryomolds to wet lab for DNA/RNA extraction and NGS testing

Englander Insitute for Precision Medicine Program at Weill Cornell Medicine

STANDARD OPERATING PROCEDURES (SOP)

PRECISION MEDICINE (PM) TISSUE PROCESSING

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A. Introduction

Purpose

This manual of procedures (MOP) is to standardize the method for collecting and handling biospecimens collected from participants in the “Precision Medicine” program. The biospecimens include needle biopsies of tumors, bone marrow biopsies, excisional biopsies of tumors for the extraction of DNA and RNA.

It is critical that all samples have to be collected, processed and stored in a uniform and consistent manner. Deviations from these procedures should be annotated in the meta-data that accompanies the samples.

Scope

These procedures apply to all personnel involved in the collection and handling of tumor biopsies.

Precaution

Universal precautions for handling potentially infectious biospecimens should be followed at all times (e.g. gloves; safety needles; etc). All acquisition materials should be disposed of in appropriate biohazard containers.

B. Material and Equipment required

MATERIAL	NUMBER/AMT	VENDOR	CAT #
Tissue-Tek® Cryomold®, Standard-size molds	1 to 5 per sample	Fisher	NC9511236
Tissue Tek® O.C.T. Compound, Sakura®Finetek	1 BOTTLE	Fisher	14-373-65
Cork Disk 22mm	1 per crymold		
Insulated NalGene Container	1		
Dry ice	1 container		
Wet ice	1 container		
Methylbutane	As needed		
100% Alcohol - 200 Proof Pure Ethanol	As needed		
95% Alcohol – 190 Proof Pure Ethanol			
Blue Pads and Markers	As needed	Fisher	507105 NC9319816
1.7 ml Posi-Click tube (Denville)	8	Fisher	C2170
Gloves (non-sterile)	1 box		
Ruler	1		
Camera	1		
Superfrost plus slides	10 slides		
Scalpel	3		
Tweezers	1		
Microscope	1		
Hematoxylin			
Eosin			
Bluing Reagent			
Large container for water			
Xylene			
Manual Hand Staining Unit			
Thermo Scientific Cytoseal- XYL			
Microscope Cover Glass 24x50mm			

-21 Cryostat	1		
Large Forceps - 12”	1		
Paper towel			
Insulated NalGene Container	1		
Small Zip lock bag - 3x6	1 per case		
Metal Chucks	1 per block		
Leica Slide Etcher			
24 Count Slide Rack	1		
Spec-Tec Resistant Disposable Cut Gloves	1		
Small Pencil Thin Brush	1		
High-profile disposable Blades #818 (10x50)	1		
Biohazard Sharps Disposal Container	1		
-80°C Freezer	1		

C. Preparing for tissue collection

1. Contact info

- Contact all the team members once the date of the procedure is known. A good communication between the research staff and the surgery team is required to ensure that the sample is collected according to the SOP.

2. Preparation and PM specimen Worksheet

- Arrive at the collection site at least 15 min ahead of the scheduled time to allow sufficient time to set up laboratory supplies and ensure rapid transport of specimens to the laboratory after collection.

- Prepare and bring the following lab supplies:
  - 1 Tweezer
  - Wet ice 1 container
  - Blue Pads and Markers
  - 8 Pre-chilled 1.7mL Posi-click tubes
  - 2 scalpels
  - 10 slides of which 5 prelabeled

- Bring a PM worksheet with the corresponding PM identifier to the collection site.

3. Pre-collection Labeling

- Label 2 to 5 Posi-click tubes 1.7 ml
- Label 2 to 5 tubes 5ml
- Label with the following information:
  - Date of procedure
  - Specimen PMID

D. Tissue Sample Collection

1. While the tissue sample collection takes place:

- Record the following information on a PM worksheet:
  - Specimen PMID
  - Date of procedure
  - Type of specimen
  - Site
  - Number of specimen
  - Time of collection
  - Research team members

2. Once tissue sample is collected:

- DO NOT PLACE IN FORMALIN
- Transfer freshly collected tissue specimen(s) with tweezers into the pre-chilled tubes.
- Place tubes in wet ice.
- Dispose the needles or tweezers in the appropriate biohazard waste container(s).
- Bring the tissue specimen(s) to the Pathology Department as soon as possible.

E. Pathology evaluation

1. Labeling

- Label the Tissue-Tek®Cryomolds®. Preparing more (2-4) cryomolds helps ensure that the team is prepared if additional cryomolds are needed.
- Label the cork to be placed atop of the Tissue-Tek®Cryomolds® before freezing.
- Label 1 Superfrost plus slide per cryomold.
- Label with the following information:
  - Specimen PMID
  - 1-4 (this will help to identify each piece of tissue individually)

2. Gross examination

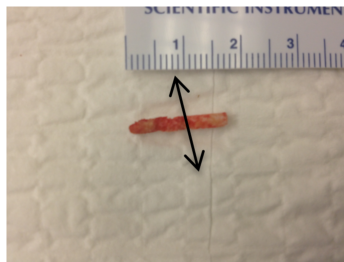
***NOTE: Gross examination will be done by a pathologist.**

- Take a picture of the specimen. Place small tissue cores in PBS buffer to prevent adhesion of tissue to glass slide. See image below.

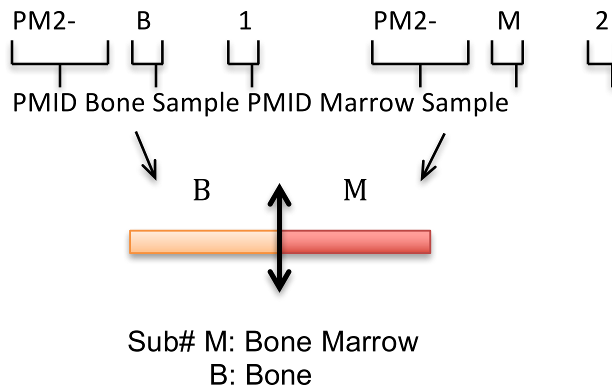
- Report the gross examination:
  - Tissue type
  - Number of specimens
  - Size, weight of each specimen
  - Gross description
- Using sterile forceps, place the specimen in a cryomold prefilled with a drop of Tissue-Tek®O.C.T. Compound, Sakura®Finetek, and pre labeled with the specimen PM identifier (PMID). In case multiple biopsies are received, use only cryomold per core.
- Fill the cryomold with O.C.T. medium ensuring no air bubbles are present.
- Place a labeled cork on top of the O.C.T. and place the cryomold into the methylbutane/dry ice combination for no less than 60 seconds.
- Using the large Forceps remove the frozen tissue blocks from the methylbutane /dry ice combination and place in the -21 Cryostat.

- In case of a Bone Biopsy

- Each bone biopsy has to be cut in two parts: cortical bone (hard, whitish or pale in color) and bone marrow (soft, reddish in color). See photo and schematic figure below.



- Place cortical bone in formalin for standard calcification (clinical processing), and hand in with clinical requisition form to the Pathologist Assistant.
- Place bone marrow in a cryomold prefilled with a drop of Tissue-Tek®O.C.T. Compound, pre labeled with the specimen PMID.



- Fill the cryomold with O.C.T. medium ensuring no air bubbles are present.
- Place a PMID-labeled cork on top of the O.C.T. and place the cryomold into the methylbutane /dry ice combination for no less than 60 seconds.
- Using the large forceps remove the frozen tissue blocks from the methylbutane /dry ice combination and place in the -80^{0}C freezer.

- In case blood clots are received with a Bone Biopsy

- Prepare a Diff-Quik stained smear from blood clots
- Follow the steps above for bone marrow part of the biopsy

3. Procedure for tissue cutting

***NOTE: For safety purposes the Spec-Tec Cut Resistant Disposable Cut Gloves should be worn under the non-sterile glove for additional protection.**

- Using the Leica Slide Etcher. Print corresponding slides (PM1, #1-4) on the Superfrost Plus Slides for each tissue block that has been frozen.

***NOTE: All slides must remain at room temperature in order to ensure that the frozen tissue will adhere to the slide**.

- Place metal chucks in the -21 cryostat (1 chuck per tissue block).
- Cover the entire top of the chuck with the Tissue Tek OCT compound.
- Remove the frozen block from the Tissue Tek standard size cryomold and place the cork directly on to the OCT covered chuck.
- Allow 1 to 2 minutes for the OCT compound to fully freeze on the chuck before cutting.
- For H&E staining, the cryostat needs to have a thickness setting of 5 microns.
- Place a High Profile Disposable Blade in the blade holder and lock.
- Place metal chuck in block holder and adjust for cutting.
- Slowly level the frozen block with the blade and begin cutting.
- Cut block slowly until the tissue is fully faced.

- Cut the fully faced section and pull on to the cryostat's frozen block using a small pencil thin brush.
- Pick up tissue using the labeled Superfrost Plus Slide and place in the 24 count slide rack.
- Continue until all frozen blocks have been cut.
- Once all blocks have been cut, place 24 count slide rack in 100% alcohol – 200 Proof Pure Ethanol and prep slides for staining.

4. Procedure for slide staining

***NOTE: Slide Staining should always occur under a fume hood.**

- Stain 1 H&E slide per specimen.
- Fill the large container or bucket with water and place on the side of the Manual Hand staining Unit.
- Leave slide rack in 100% alcohol for 2/3 minutes.
- Remove 24 count slide rack from 100% alcohol and place in 95% alcohol for 2/3 minutes.
- Remove 24 count slide rack from 95% alcohol and rinse thoroughly in the large container or bucket filled with water.
- Place 24 count slide rack in hematoxylin for 45 seconds – Agitate gently.
- Remove from hematoxylin and place 24 count slide rack in water and rinse thoroughly to remove excess hematoxylin.
- Discard dirty water and refill the container or bucket with clean water.
- Place 24 count slide rack in Bluing Reagent (Lithium Carbonate) for 10 seconds – Agitate gently.
- Remove 24 count slide rack from Bluing Reagent (Lithium Carbonate) and rinse in water.
- Place 24 count slide rack in 95% alcohol for 5-10 seconds
- Remove from 95% alcohol and place 24 count slide rack in Eosin for 7-10 seconds.
- Remove 24 count slide rack from Eosin and blot on paper towel lightly once or twice to remove excess Eosin.
- Place 24 count slide rack in 95% alcohol for 5 seconds.
- After 5 seconds, remove from 95% alcohol and place in 100% alcohol for 5-10 seconds.
- Remove from 100% alcohol and place into a 2nd container filled with 100% alcohol for 5-10 seconds.
- After 5-10 seconds, remove the 24 count slide rack and place it into a 3rd container filled with 100% alcohol for 5-10 seconds.
- Remove the 24 count slide rack from the 3rd container filled with 100% alcohol and blot on a paper towel to drain excess alcohol.

- After draining excess alcohol onto the paper towel, place the 24 count slide rack into xylene for 10 seconds.
- After 10 seconds, remove from xylene and place in a 2nd container filled with xylene for 10 seconds.
- After 10 seconds in the 2nd container of xylene, the staining process is complete.
- To cover slip the slides, leave the 24 count slide rack in xylene and remove slides individually.
- Place 1 to 2 drops of Thermo Scientific Cytoceal – XYL on the tissue that is on the slide.
- Take 1 microscope cover glass and gently place it over the tissue that is on the slide.
- Blot the slide and cover glass on paper towel to remove any excess Cytoceal that may be around the edges of the slide.
- Place slide in a 20 count slide holder booklet and let dry.
- Continue this until all slides are cover slipped.

5. Pathology evaluation

- After slides are dried, a pathologist reviews the H&E slides. At this point, if any additional slides need to be cut, instruction will be given to do so.

  - Evaluate size of tissue, confirm the presence of tumor and evaluate tumor content (percentage of tumor involvement) for each block.
  - Note presence of necrosis and/or normal tissue if present.
  - Document above information in pathology report.
  - If multiple blocks/passes have been analyzed, document best blocks that should be used for analysis in pathology report- these are blocks with the highest tumor content.
  - Representative images are taken from H&E slides.

6. Procedure to convert frozen tissue into formalin-fixed paraffin-embedded (FFPE) tissue

- After pathology evaluation of frozen material, the pathologist determines which cryomold is converted to FFPE if clinically indicated, *e.g.* no diagnostic tissue seen on clinical slide(s). FFPE tissue is used for clinical documentation (when applicable) and to perform subsequent assays (e.g. IHC, FISH).

  - Let cryomold thaw for approx. 2 minutes until OCT starts to melt.
  - Separate excess OCT from tissue.
  - Wrap tissue in lens paper and place it plastic cassette.
  - Place cassette in formalin.

7. Scanning slides

- Send H&E stained slides for scanning (Aperio AT2 by Leica Biosystems Inc.)

F. Specimen Storage

1. Specimen Storage

- After all slides have been reviewed

  - Take all metal chucks out of the cryostat and place under the hood or on the bench.
  - Remove frozen blocks from metal chuck using forceps.
  - Place frozen blocks in the plastic zip lock bag that was labeled with the PM Identifier and the date.
  - Transfer cryopreserved labeled specimen(s) to an -80°C freezer at the designated floor.

2. Information uploaded into the LIMS

- Record the following information into the LIMS system in the Tissue Processing file:

  - PM number
  - Date of procedure
  - Number of specimens
  - Site
  - Time of collection
  - Time of freezing
  - Diagnosis
  - Representative H&E images of each specimen.
  - Date/time of specimen(s) placed at -80°C.
  - Location of the specimen.

Precision Medicine Program at WCM

STANDARD OPERATING PROCEDURES (SOP)

Tissue Specimen Collection

PM#....

General information

Date of procedure .../.../...

Specimen PMID PM..........

Type of specimen ....

Research team ....

Pathologist ....

Technician ....

	Sample 1	Sample 2	Sample 3	Sample 4
Time of collection				
Type of specimen				
Site				
Size (mm)				
Time of freezing				
Diagnosis				

Supplementary Figure 1. Example of a WES report

NewYork-Presbyterian
Weill Cornell Medical Center

Institute for Precision Medicine Report - Preliminary

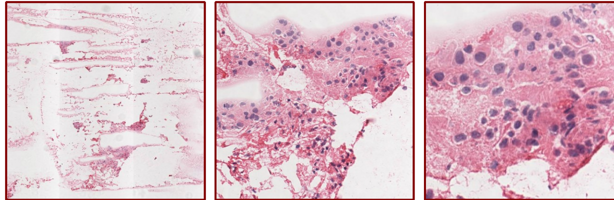
Patient ID: **Diagnosis:** Metastatic prostatic adenocarcinoma **Report date:** Nov. 18, 2014

CLINICAL INFORMATION

Patient ID:
Physician:
Diagnosis: Metastatic prostatic adenocarcinoma
Site: Bone Biopsy
Specimen IDs (case/control)

Sample type (case/control): Frozen Tissue / Blood
Sample collected (case/control): (8/25/2014) / (9/11/2014)
Sample received (case/control): (9/23/2014) / (9/23/2014)
Neoplastic content: 46.4%

CASE IMAGES



RESULTS

GENOMIC ALTERATIONS: Summary

Somatic alterations in clinically relevant genes

A set of 49 clinically relevant genes was investigated. 1 alteration was found in these genes (listed below).

Somatic alterations of unknown significance in known cancer genes

A set of 509 known cancer genes was investigated. 7 alterations in these cancer associated genes were found (listed below).

Somatic alterations of unknown significance

11 gene(s) with point mutations or indels and 11 copy number alteration(s) were found (listed below).

Clinically relevant genomic alterations

These alterations occur in genes that are deemed clinically relevant because: they are targets of drugs, they confer resistance or susceptibility to treatment, or for other clinically relevant reasons (see Appendix).

Gene name	FDA approved drugs with indication (if any)	Interpretation
CDKN2A focal loss	none	CDKN2A loss may be associated with increased sensitivity to CDK4/6 inhibitors. However these inhibitors are currently undergoing clinical trials and their efficacy and/or lack of toxicity has not yet been demonstrated.

VAF: variant allele frequency

Institute for Precision Medicine Report - Preliminary

Notes

The status of alterations in gene(s) **BRAF:** is indeterminate because the coverage was below the optimal levels of this method (<10 reads). Hence, analysis of the alteration(s) with an independent methodology will be performed.

Genomic alterations of unknown significance in cancer genes

These alterations occur in genes that are cancer associated, but their impact on the disease is unknown (see Appendix).

Copy number alterations

Gene name	Description	Classification of alteration	Altered region
IRF4	interferon regulatory factor 4	FOCAL DELETION	chr6:203,540-572,580
NDRG1	N-myc downstream regulated 1	LARGE SCALE AMPLIFICATION	chr8:81,733,717-146,279,471
RECQL4	RecQ protein-like 4	LARGE SCALE AMPLIFICATION	chr8:81,733,717-146,279,471
COX6C	cytochrome c oxidase subunit VIc	LARGE SCALE AMPLIFICATION	chr8:81,733,717-146,279,471
EXT1	multiple exostoses type 1 gene	LARGE SCALE AMPLIFICATION	chr8:81,733,717-146,279,471
MLLT3	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 3 (AF9)	FOCAL DELETION	chr9:20,620,805-23,693,463

Genomic coordinates are based on human reference GRC37/hg19. Large scale alterations involve at least 50 genes.

Somatic mutations and indels

Gene name	Gene description	Classification	Reference Allele	Tumor Allele 1	Tumor Allele 2	AA change	Tumor (Normal) read depth	Tumor VAF
TP53 chr17:7577580	tumor protein p53	missense	A	A	G	p.Y234C	154 (207)	24.03%

AA: amino-acid; VAF: variant allele frequency; Genomic coordinates are based on human reference GRC37/hg19 and are 1-based.

Genomic alterations of unknown significance

These alterations are not known to have any effect on the disease, but are here reported in the event that in the future progress in scientific knowledge could determine their role (see Appendix).

Somatic mutations and indels

Gene name	Classification	Reference Allele	Tumor Allele 1	Tumor Allele 2		
IRF5 chr7:128587351	inframe deletion	ACTCTGCAGCCGCCCACTCTGCGGCCGCCT	-	ACTCTGCAGCCGCCCACTCTGCGGCCGCC	p.P168_nofs	50 (1
GRAMD3 chr5:125802022	missense	A	A	G	p.D74G	56 (6
RPL13AP20 chr12:13029069	frameshift insertion	-	+A	-	p.Q221_fs	19 (1
KRTAP5-5 chr11:1651585	inframe deletion	CTGCTGCCAGTCCAGCTGCTGTAAGCCTTA	-	CTGCTGCCAGTCCAGCTGCTGTAAGCCTT	p.P172_nofs	92 (4

ATXN7 chr3:63981669	missense	C	C	T	p.S724F	110 (9

Gene name	Classification	Reference Allele	Tumor Allele 1	Tumor Allele 2	AA change	Tumor (Normal) read depth	Tumor VAF
EOMES chr3:27763427	inframe insertion	-	+CGGCGC	-	p.A120_nofs	12 (13)	66.7%
PRIM2 chr6:57398186	frameshift insertion	-	+A	-	p.E297_fs	188 (214)	35.1%
GRM3 chr7:86415634	missense	G	G	A	p.A176T	135 (94)	33.3%
KLHL29 chr2:23865523	missense	G	G	A	p.G248E	42 (36)	57.1%
MESP2 chr15:90320134	inframe deletion	GGGCAGGGGCAG	-	-	p.Q183_nofs	20 (17)	90.0%
EP400 chr12:132547068	inframe insertion	-	+GCA	-	p.R2719_nofs	70 (76)	38.6%

AA: amino-acid; VAF: variant allele frequency; Genomic coordinates are based on human reference GRC37/hg19 and are 1-based.

Copy number alterations

Location (Chr:Start-End)	Type	Number of genes	Gene names (if less than 6)
chr1:1,670,404-1,670,486	FOCAL DELETION	1	SLC35E2
chr1:8,022,888-11,159,831	FOCAL DELETION	36	too many to show
chr16:71,482,196-71,605,533	FOCAL DELETION	4	CHST4; ZNF23; TAT; ZNF19
chr19:5,786,806-5,787,125	FOCAL DELETION	1	DUS3L
chr4:435,669-437,310	FOCAL AMPLIFICATION	2	ZNF721; ABCA11P
chr6:203,540-572,580	FOCAL DELETION	2	DUSP22; EXOC2
chr7:38,301,793-38,357,068	FOCAL AMPLIFICATION	1	TARP
chr8:81,733,717-146,279,471	LARGE SCALE AMPLIFICATION	357	too many to show
chr9:20,620,805-23,693,463	FOCAL DELETION	30	too many to show
chrY:14,954,276-23,763,771	FOCAL AMPLIFICATION	35	too many to show
chrY:2,655,335-14,898,592	FOCAL AMPLIFICATION	40	too many to show

Genomic coordinates are based on human reference GRC37/hg19. Large scale alterations involve at least 50 genes.

Method

Genomic DNA was extracted from macrodissected formalin-fixed paraffin-embedded (FFPE) tumor, or cored frozen, OCT-embedded tumor and peripheral blood lymphocytes of the patient's specimens using the Promega Maxwell 16 MDx. Estimation of tumor content is based on analysis of the sequencing data using CLONET version 1.0 [1]. Sequencing was performed using Illumina HiSeq 2500 (2x100bp). A total of 21,522 genes were analyzed with an average coverage of 80x (85x) using Agilent HaloPlex. 65,474,425 (68,348,586) short reads were aligned to GRC37/hg19 reference using BWA [2] and processed accordingly to Whole Exome Sequencing Test for Cancer ExaCT1 - pipeline v0.9. The capture efficiency is 82.90% (83.95%). **NB:** numbers in parentheses refer to the control sample.

1. Baca, S, Prandi D. et al. Punctuated evolution of prostate cancer genomes. Cell 2013 Apr 25;153(3):666-77. doi: 10.1016/j.cell.2013.03.021.
2. Li, Heng, and Durbin Richard. Fast and Accurate Long-read Alignment with Burrows-Wheeler Transform. Bioinformatics 2010;26(5)(March

1):589–595. doi:10.1093/bioinformatics/btp698

Appendix

Clinically relevant genes: These genes are deemed clinically relevant because: they are targets of drugs, they confer resistance or susceptibility to treatment, or for other clinically relevant reasons. As the scientific knowledge increases, this list will be updated accordingly. A total of 94 alterations in 49 genes are considered in this report.

Somatic alterations of unknown significance in cancer genes or in other genes: These genes may not be related to the disease. Current scientific knowledge cannot determine the impact of these alterations on the disease. These genes are included herein in the event they become clinically relevant as our knowledge increases. Specifically, this report considers a total of 509 cancer genes that are listed in the section 'Genomic alterations of unknown significance in cancer genes' if alterations are found.

Alterations are not listed in ranked order: The order of the alterations reported as clinically relevant or of unknown significance is **not** associated with predicted effect on tumor development, progression, or resistance to treatment.

Treatement decisions: The treating physician is responsible to select the most appropriate course of treatment. Decision making about therapy should not be based solely on the information contained in this report.

List of clinically relevant and known cancer genes:
ABL1, ABL2, AKT1, AKT2, AKT3, ALK, AR, AURKA, BCL2, BRAF, BRCA1, BRCA2, CD79B, CDK4, CDK6, CDKN2A, CEBPA, CRKL, DNMT3A, EGFR, ERBB2, ERBB3, ERBB4, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2, KIT, KRAS, MAP2K1, MAP2K2, MCL1, MET, NRAS, PDGFRA, PIK3CA, PTCH1, PTEN, SMO, TSC1, ACSL3, AF15Q14, AF1Q, AF3p21, AF5q31, AKAP9, ALDH2, ALO17, APC, ARHGEF12, ARHH, ARID1A, ARID2, ARNT, ASPSCR1, ASXL1, ATF1, ATIC, ATM, ATRX, AXIN1, BAP1, BCL10, BCL11A, BCL11B, BCL3, BCL5, BCL6, BCL7A, BCL9, BCOR, BCR, BHD, BIRC3, BLM, BMPR1A, BRD3, BRD4, BRIP1, BTG1, BUB1B, C12orf9, C15orf21, C15orf55, C16orf75, C2orf44, CAMTA1, CANT1, CARD11, CARS, CBFA2T1, CBFA2T3, CBFB, CBL, CBLB, CBLC, CCDC6, CCNB1IP1, CCND1, CCND2, CCND3, CCNE1, CD273, CD274, CD74, CD79A, CDH1, CDH11, CDK12, CDKN2a(p14), CDKN2C, CDX2, CEP1, CHCHD7, CHEK2, CHIC2, CHN1, CIC, CIITA, CLTC, CLTCL1, CMKOR1, CNOT3, COL1A1, COPEB, COX6C, CREB1, CREB3L1, CREB3L2, CREBBP, CRLF2, CRTC3, CTNNB1, CYLD, D10S170, DAXX, DDB2, DDIT3, DDX10, DDX5, DDX6, DEK, DICER1, DNM2, DUX4, EBF1, ECT2L, EIF4A2, ELF4, ELK4, ELKS, ELL, ELN, EML4, EP300, EPS15, ERCC2, ERCC3, ERCC4, ERCC5, ERG, ETV1, ETV4, ETV5, ETV6, EVI1, EWSR1, EXT1, EXT2, EZH2, EZR, FACL6, FAM22A, FAM22B, FAM46C, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FBXO11, FBXW7, FCGR2B, FEV, FGFR1OP, FH, FHIT, FIP1L1, FLI1, FLJ27352, FNBP1, FOXL2, FOXO1A, FOXO3A, FOXP1, FSTL3, FUBP1, FUS, FVT1, GAS7, GATA1, GATA2, GATA3, GMPS, GOLGA5, GOPC, GPC3, GPHN, GRAF, H3F3A, HCMOGT-1, HEAB, HERPUD1, HEY1, HIP1, HIST1H3B, HIST1H4I, HLF, HLXB9, HMGA1, HMGA2, HNRNPA2B1, HOOK3, HOXA11, HOXA13, HOXA9, HOXC11, HOXC13, HOXD11, HOXD13, HRPT2, HSPCA, HSPCB, IGH@, IGK@, IGL@, IL2, IL21R, IL6ST, IL7R, IRF4, IRTA1, ITK, JAK1, JAK3, JAZF1, JUN, KCNJ5, KDM5A, KDM5C, KDM6A, KDR, KIAA1549, KIF5B, KLF4, KLK2, KTN1, LAF4, LASP1, LCK, LCP1, LCX, LHFP, LIFR, LMO1, LMO2, LPP, LRIG3, LYL1, MADH4, MAF, MAFB, MALT1, MAML2, MAP2K4, MAX, MDM2, MDM4, MDS1, MDS2, MECT1, MED12, MEN1, MITF, MKL1, MLF1, MLH1, MLL, MLL2, KMT2D, MLL3, MLLT1, MLLT10, MLLT2, MLLT3, MLLT4, MLLT6, MLLT7, MN1, MPL, MSF, MSH2, MSH6, MSI2, MSN, MTCP1, MUC1, MUTYH, MYB, MYC, MYCL1, MYCN, MYD88, MYH11, MYH9, MYST4, NACA, NBS1, NCOA1, NCOA2, NCOA4, NDRG1, NF1, NF2, NFE2L2, NFIB, NFKB2, NIN, NKX2-1, NONO, NOTCH1, NOTCH2, NPM1, NR4A3, NSD1, NT5C2, NTRK1, NTRK3, NUMA1, NUP214, NUP98, OLIG2, OMD, P2RY8, PAFAH1B2, PALB2, PAX3, PAX5, PAX7, PAX8, PBRM1, PBX1, PCM1, PCSK7, PDE4DIP, PDGFB, PDGFRB, PER1, PHF6, PHOX2B, PICALM, PIK3R1, PIM1, PLAG1, PML, PMS1, PMS2, PMX1, PNUTL1, POT1, POU2AF1, POU5F1, PPARG, PPP2R1A, PRCC, PRDM1, PRDM16, PRF1, PRKAR1A, PRO1073, PSIP2, PTCH, PTPN11, RAB5EP, RAC1, RAD51L1, RAF1, RALGDS, RANBP17, RAP1GDS1, RARA, RB1, RBM15, RECQL4, REL, RET, RNF43, ROS1, RPL10, RPL22, RPL5, RPN1, RUNDC2A, RUNX1, RUNXBP2, SBDS, SDC4, SDH5, SDHB, SDHC, SDHD, SEPT6, SET, SETBP1, SETD2, SF3B1, SFPQ, SFRS3, SH2B3, SH3GL1, SIL, SLC34A2, SLC45A3, SMARCA4, SMARCB1, SMARCE1, SOCS1, SOX2, SRGAP3, SRSF2, SS18, SS18L1, SSH3BP1, SSX1, SSX2, SSX4, STAT3, STK11, STL, SUFU, SUZ12, SYK, TAF15, TAL1, TAL2, TCEA1, TCF1, TCF12, TCF3, TCF7L2, TCL1A, TCL6, TERT, TET2, TFE3, TFEB, TFG, TFPT, TFRC, THRAP3, TIF1, TLX1, TLX3, TMPRSS2, TNFAIP3, TNFRSF14, TNFRSF17, TNFRSF6, TOP1, TP53, TPM3, TPM4, TPR, TRA@, TRAF7, TRB@, TRD@, TRIM27, TRIM33, TRIP11, TSC2, TSHR, TTL, U2AF1, USP6, VHL, VTI1A, WAS, WHSC1, WHSC1L1, WIF1, WRN, WT1, WTX, WWTR1, XPA, XPC, XPO1, YWHAE, ZNF145, ZNF198, ZNF278, ZNF331, ZNF384, ZNF521, ZNF9, ZRSR2.

Disclaimer

The information here provided is for investigational use only. We do not exclude the possibility of other genomic alterations present that could not have been identified for biological or technical reasons.

Institute for Precision Medicine Report - Preliminary

Gene variants present in less than 20% of cells may not be detected by this test.
Some regions of genes cannot be fully evaluated for mutations or indels because of lack of sufficient coverage, including: BRAF(chr7:140433812-140624564)

This method has not been cleared by the FDA. The analytical performance characteristics have been determined by the Institute for Precision Medicine/New York Hospital Laboratories.

The report was generated at 23:36:41 EST - Nov 18, 2014; based on version v1.1-1-g0b26ce0 of software IPM-reportGenerator.

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Supplementary Figure 2. Example of a RNAseq report

CLINICAL INFORMATION

Patient ID:
Physician:.
Specimen ID:

SUMMARY

- Druggable Outliers found in the case.
- List of fusions (known, oncogenes) detected.
- Pathways enriched.

OUTLIERS

Outliers, by definition mean highly expressed genes in a sample, when compared to cohort . The outliers are only calculated for 74 druggable cancer genes (using Sanger database and Drugbank) using z-scores across a cohort. See appendix for figures.

Method of Detection	Druggable Outliers
1.) Across IPM	PIM1, CCND3

FUSIONS

The top fusions with maximum support of spanning pairs have been listed. The ones that are known fusions or oncogenes was also the another criteria. No fusions were found .

Gene_1	Gene_2	Spanning pairs	Junction Reads

PATHWAYS ENRICHMENT

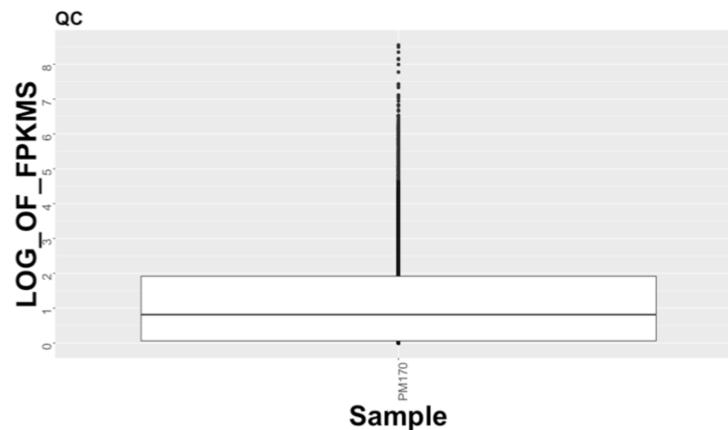
Pathways enrichment was done by Gene Set Enrichment Analysis, and using GTEX Normals as Controls. The significant ones are mentioned below. See appendix for figures.

PATHWAY	Enrichment Status
AKT1	No
ERBB2	No
AR	No

APPENDIX

1.) QC control

The QC of this sample seems to be fine as per the boxplot of FPKMS.



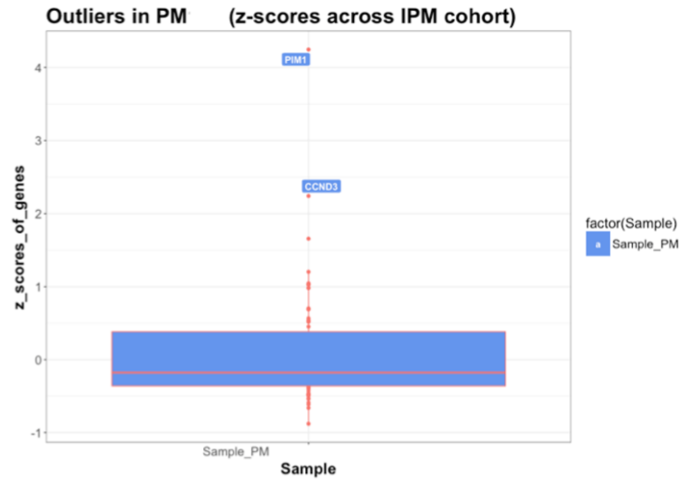
2.) OUTLIERS

PIM1, CCND3 are outliers across IPM cohort

Method: Z-scores across IPM

List of Druggable Cancer genes used for Outlier Detection

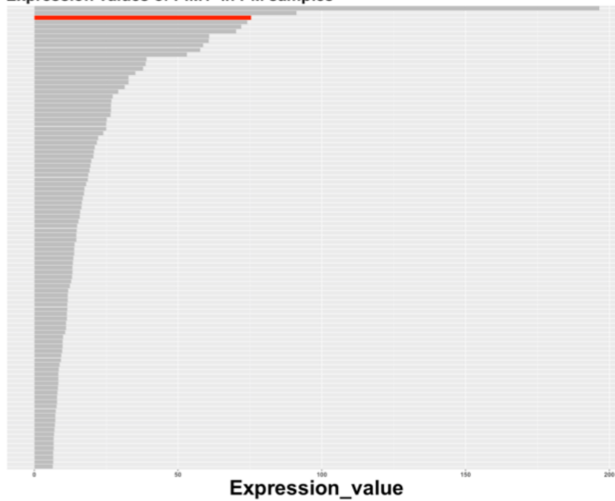
ABL1,ABL2,ACSL3,ACVR1,AKT1,ALDH2,ALK,ATIC,ATP1A1,BCL2,BCR,BRAF,CACNA1D,CALR,CARS,CCND1,CDK12,CDK4,CDK6,CDKN1B,CDKN2C,CREB1,DNMT3A,EGFR,ELN,ERBB2,FCGR2B,FGFR1,FGFR2,FGFR3,FLT3,GNAS,GPHN,GRIN2A,IKBKB,ITK,JAK1,JAK2,JUN,KCNJ5,KDR,KIT,KLK2,LCK,MAP2K1,MAP2K2,MDM2,MET,MPL,NCOA1,NCOA2,NFKB2,NT5C2,NTRK1,PDGFRA,PDGFRB,PIK3CA,PIK3R1,PIM1,POLE,PPARG,PRKAR1A,PTPN11,RAF1,RARA,RET,SDHB,SDHC,SDHD,SMO,STAT5B,SYK,TOP1,TPR


Outliers in PM (z-scores across IPM cohort)
PIM1
CCND3
z_scores_of_genes
4
3
2
1
0
-1
Sample_PM
Sample
factor(Sample)
Sample_PM

3.) PATHWAYS ENRICHMENT

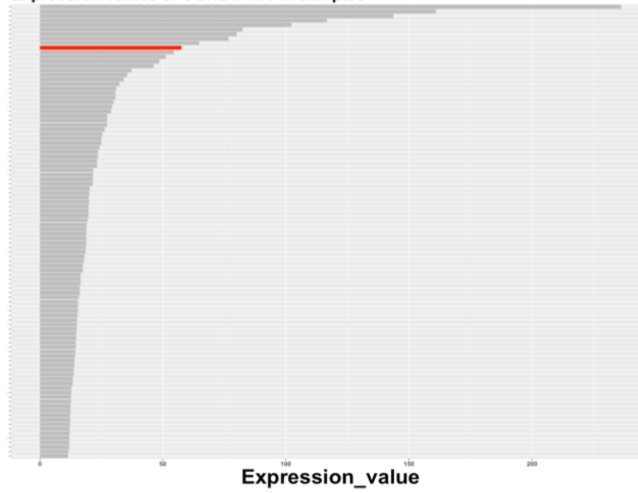
a.) PIM1 Over expression

Expression values of PIM1 in PM samples

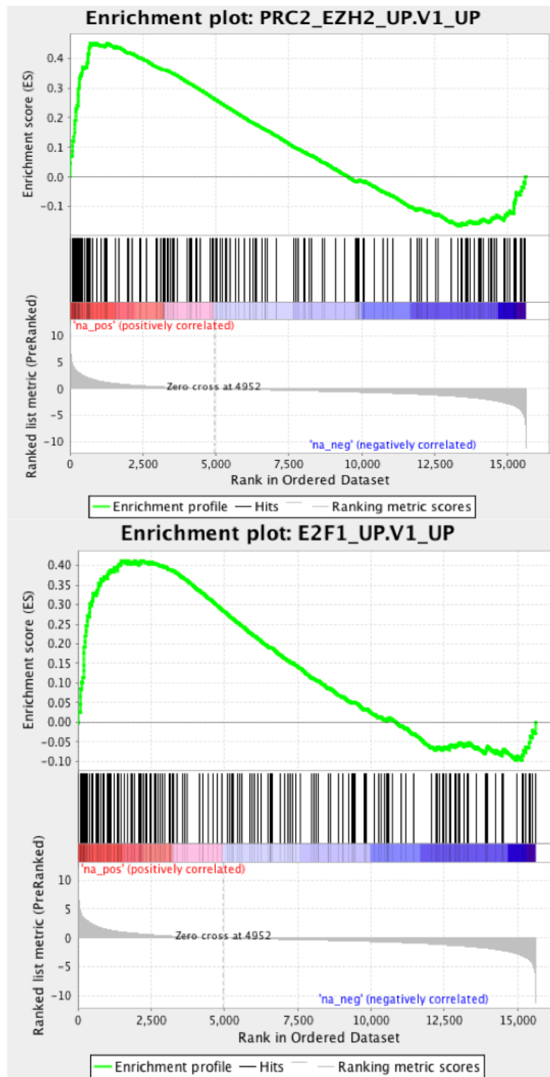


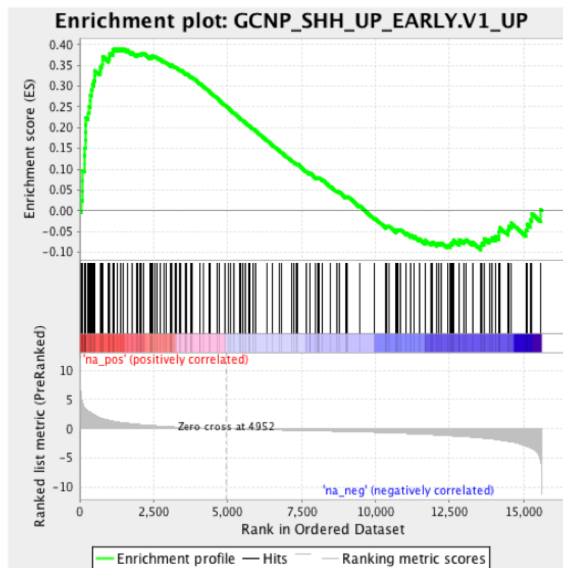
b.) CCND3 over expression

Expression values of CCND3 in PM samples



4) Other Pathways enrichment (GSEA)





5.) METHODS

i) **Outliers:** The outliers are only calculated for 74 druggable cancer genes (using Sanger database and Drugbank) using z-scores across a cohort.

ii) **Fusions:** For fusions, Fusion catcher and STAR-fusion are used. The fusions that are known, oncogenes, or with maximum support of junction reads and spanning pairs are reported.

iii) **Pathways Enrichment:** GSEA pre-ranked is used for this. The matched tissue normals are selected from GTEX. **Log2fold change** of the tumor vs. the mean of matched normal is calculated for all genes, which becomes the input to GSEA. The genesets used are **Oncogenic Signatures** available in **GSEA** and 90 curated genesets of druggable cancer genes. The significant pathways that have p-value < .05 and FDR < 0.25 are reported.