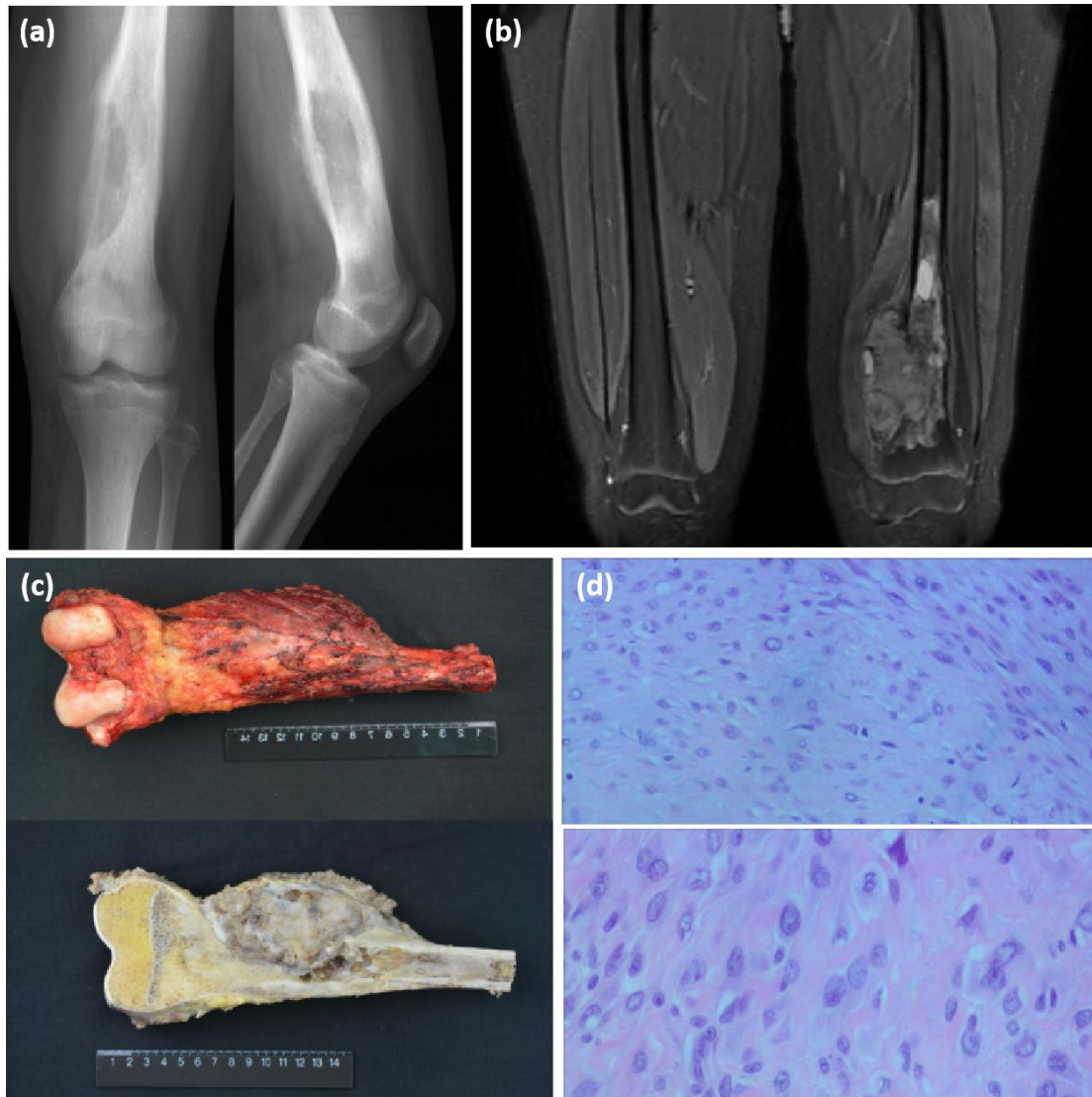


Medical Materials of three patients

Patient no. 1



Supplementary Figure 1 Medical materials of patient no.1. (a) X-ray showed a localized mass on the left distal femur. (b) MRI show a localized mass on the left distal femurs. (c) Gross specimen of the resected primary tumor. (d) Hematoxylin and eosin (H&E) – stained slide of the primary tumor showed osteosarcoma.

Patient no.1 was an 18-year-old adolescent who was referred to our institution with chief complaint of progressive pain on left distal thigh for 4 months.

Radiograph demonstrated a mass on the left distal femur and a core biopsy proved an osteosarcoma. He received neo-adjuvant chemotherapy including adriamycin (A), cisplatin (P), methotrexate (M) and ifosfamide (I) (APMI) before limb-salvage surgery. Pathology of the resection specimen verified a fibroblastic osteosarcoma and the necrosis rate was 64.2%. He completed the postoperative chemotherapy with 4 cycles of APMI for 28 weeks. A nodule (3mm) on the right upper lobe was found by routine follow-up 6 months after the completion of adjuvant chemotherapy. The nodule was watched cautiously and found to be stable until one year after primary treatment. A PET-CT scan showed hypermetabolic lesions (7mm) of the right upper lobe and a local recurrence at primary surgical site (SUV_{max}=9.5). Salvaged chemotherapy of ifosfamide and etoposide (IE) was given, being followed by surgical resection of recurrent tumor and lung metastasectomy. Pathology diagnosis of both lesions were osteosarcoma. Nine months after the lobectomy, another metastasis to the left lower lobe was detected on CT scan. Second metastasectomy was performed. The pathology of P1_M2 showed an undifferentiated sarcoma. A second local recurrence and a third lung metastasis at right lower lobe was found at 5 months later. Combined of IE anti-PD-1 strategy was introduced 12 weeks before surgical removal. Both local recurrent tumor and lung metastasis were resected. Pleura dissemination was observed during the video-associated thoracic surgery (VATS). The patient received palliative chemotherapy of IE combined with anti-PD-1 strategy till now.

Patient no.2

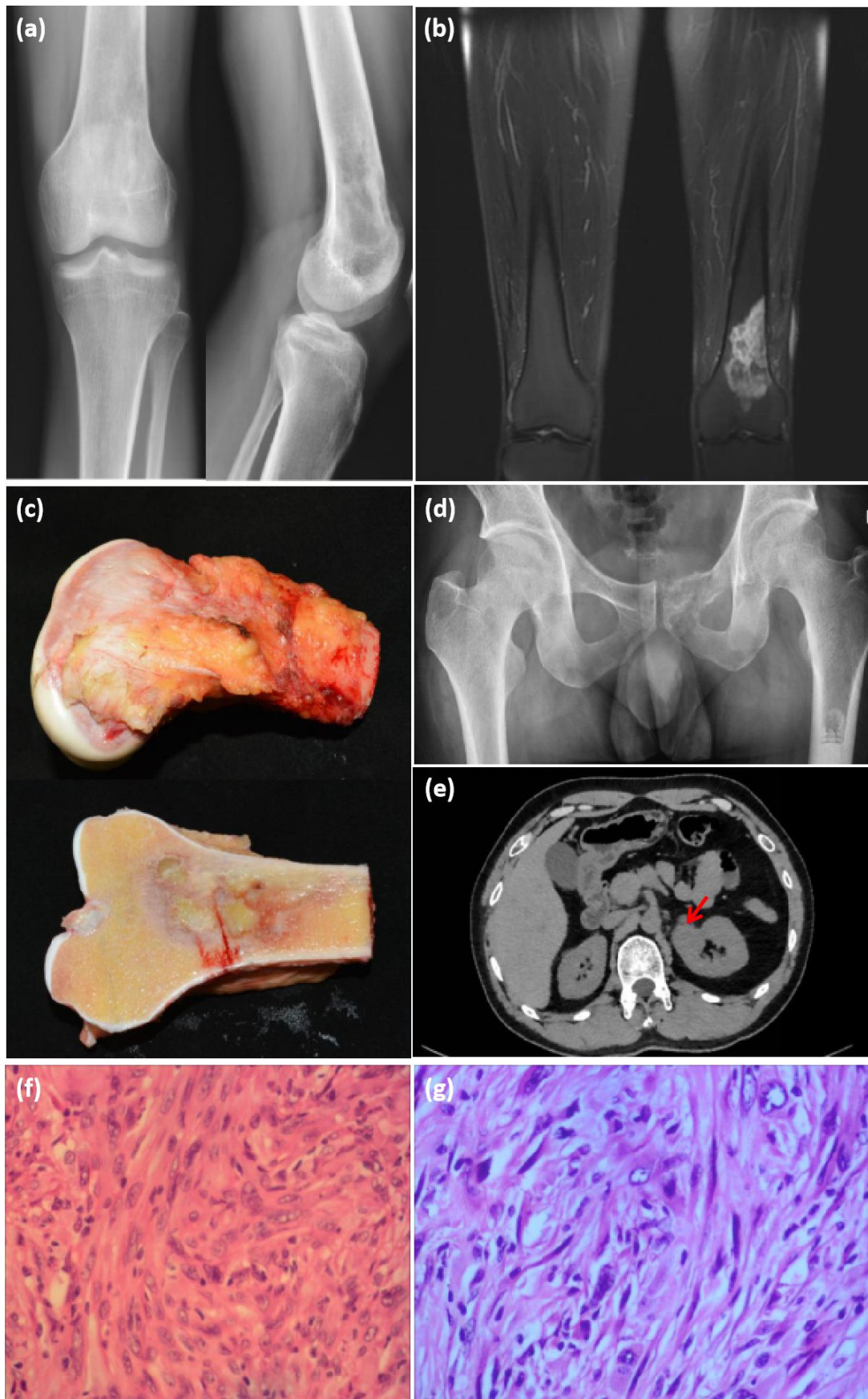


Figure 2 Medical materials of patient no.2. (a) X-ray showed a localized mass on the left distal femur. (b) MRI show a localized mass on the left distal femurs. (c) Gross specimen of the receted primary tumor. (d) X-ray of the pelvis showed osteolytic lesion on left pubis. (e) CT showed solitary mass on the left kidney (red arrow). (f) Hematoxylin and eosin (H&E)–stained slide of the primary lesion showed typical osteosarcoma. (g) H&E-stained slide of the renal metastasis showed undifferentiated sarcoma.

Patient no.2 was a 27-year-old male who presented with 1 month of pain in left distal thigh. Radiograph demonstrated an osteolytic lesion at distal femur. Osteosarcoma was proved by core needle biopsy. Neo-adjuvant chemotherapy of APMI was given before limb-salvage surgery. Postoperative pathology reported a fibroblastic osteosarcoma and necrosis rate of 92.8%. Postoperative chemotherapy was uneventful. Pubis metastasis was found at 4 years after completion of primary treatment. Resection of pubis was performed and the pathology diagnosis was undifferentiated sarcoma. Additional two cycles of API protocol were given after surgery. Kidney metastasis was found at nine months after completion of API chemotherapy. Left nephrectomy was carried out. Solitary soft tissue metastasis in right iliac fossa was found 3 months after nephrectomy. The metastasis was removed. Both lesions were proved to be undifferentiated sarcoma. Rapid systematic progression developed half year afterwards. PET-CT showed distal metastases including pancreas, lung, mesentery, muscles and lymph nodes. The patient was on palliative chemotherapy of IE combined with tyrosine kinase inhibitor (TKI), anlotinib.

Patient no. 3

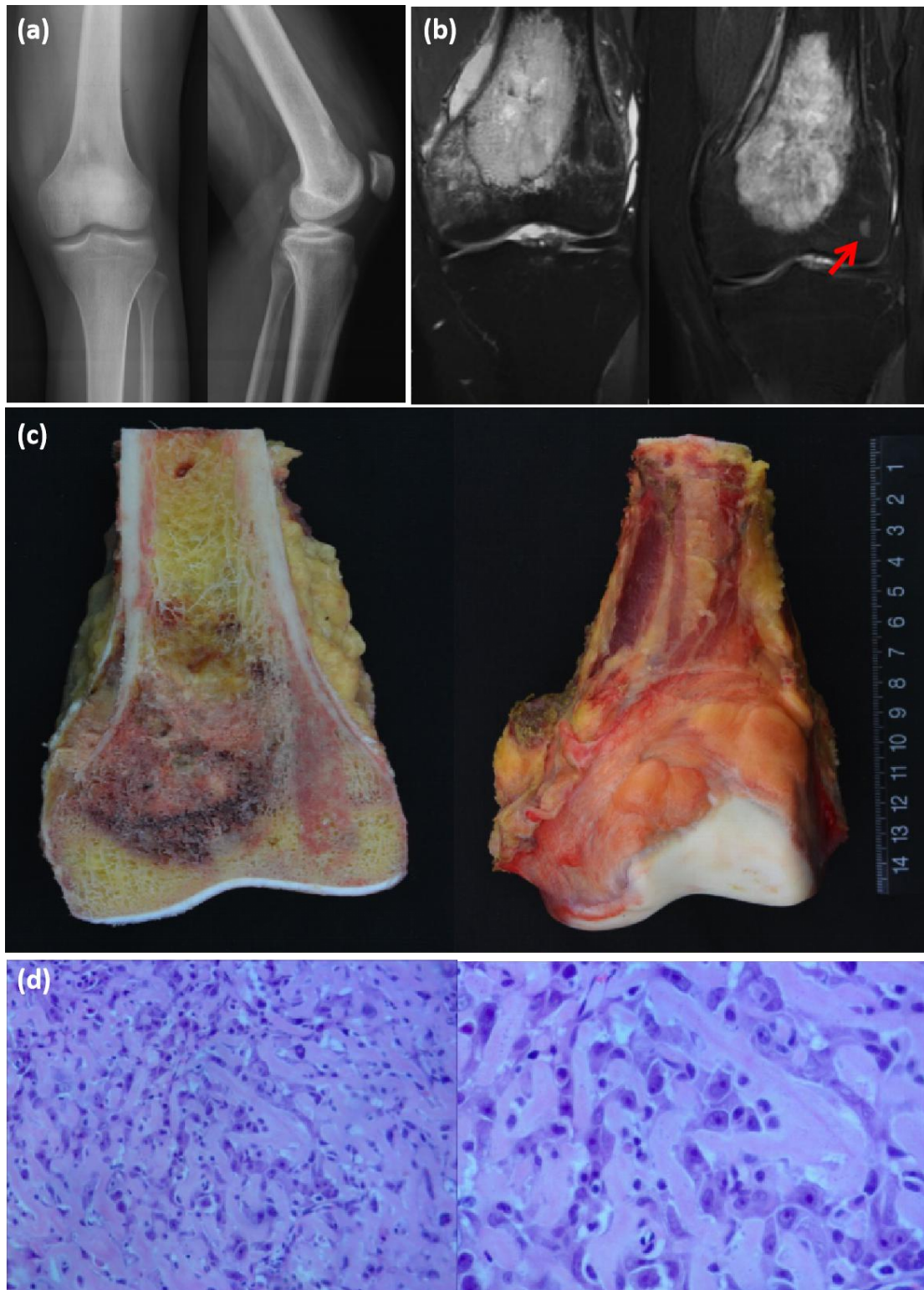


Figure 3 Medical materials of patient no.3. (a) X-ray showed a localized mass on the left distal femur. (b) MRI show a localized mass and a nearby skip lesion (red arrow)

on the left distal femurs. (c) Gross specimen of the resected primary tumor. (d) Hematoxylin and eosin (H&E) - stained slide of the primary tumor showed osteosarcoma.

Patient no.3 was a 13-year-old boy diagnosed of osteosarcoma at left distal femur. A skip lesion was observed on preoperative MRI. Routine preoperative of APMI was given, followed by distal femur replacement. The tumor showed good response to preoperative chemotherapy. The resection specimen proved to be an osteosarcoma and was analyzed for a necrosis rate of 85.3%. Postoperative chemotherapy of APMI was uneventful. Proximal sartorius metastasis was found at 5 months after completion of postoperative chemotherapy. Then the regional metastasis was resected. Rapid systematic progression was found even the patient was on IE and apatinib.

YuanSu Panel Gene List

ABCB1
ABL1
ABL2
ACVR1B
ACVR2A
ADAM29
ADGRA2
AKT1
AKT2
AKT3
ALK
ALOX12B
AMER1
ANTXR2
APC
APEX1
APOBEC3B
AQP3
AR
ARAF
ARAP3
ARFRP1
ARHGAP4
ARHGAP6
ARHGDIA
ARHGEF10
ARHGEF17
ARHGEF25
ARHGEF3
ARID1A
ARID1B
ARID2
ASXL1
ATF1
ATM
ATR
ATRX
AURKA
AURKB
AXIN1
AXIN2
AXL

B2M
BAP1
BARD1
BCL2
BCL2L1
BCL2L11(BIM)
BCL2L2
BCL6
BCL7A
BCOR
BCORL1
BCR
BIRC3
BIRC5
BLK
BLM
BMPR1A
BMX
BRAF
BRCA1
BRCA2
BRD4
BRIP1
BTG1
BTG2
BTK
BUB1
CALR
CAMTA1
CARD11
CASP8
CBFB
CBL
CCN6
CCNB3
CCND1
CCND2
CCND3
CCNE1
CD1A
CD1B
CD1C
CD1D
CD1E

CD22
CD274(PD-L1)
CD36
CD70
CD74
CD79A
CD79B
CDC42
CDC73
CDH1
CDK12
CDK2
CDK4
CDK6
CDK8
CDKN1A
CDKN1B
CDKN2A
CDKN2B
CDKN2C
CEACAM3
CEBPA
CFTR
CHD2
CHD4
CHEK1
CHEK2
CIC
CLDN18
COL1A1
COL2A1
CRBN
CREB3L1
CREB3L2
CREBBP
CRKL
CRLF2
CSF1
CSF1R
CSF3R
CSK
CSNK1A1
CTCF
CTLA4

CTNNA1
CTNNA2
CTNNA3
CTNNB1
CUL3
CUL4A
CXCR4
CYLD
CYP17A1
CYP2D6
DAXX
DCTN1
DDR1
DDR2
DEF6
DICER1
DIS3
DLC1
DNMT3A
DNMT3B
DOT1L
DPYD
DYNLL1
E2F3
ECT2
EED
EGF
EGFR
EMSY
EP300
EPAS1
EPCAM
EPHA2
EPHA3
EPHA5
EPHA6
EPHA7
EPHA8
EPHB1
EPHB4
ERBB2(HER2)
ERBB3
ERBB4
ERCC1

ERCC2
ERCC3
ERCC4
ERCC5
ERG
ERRFI1
ESR1(ER)
ETV1
ETV4
ETV5
ETV6
EWSR1(EWS)
EZH2
EZR
FAM135B
FANCA
FANCC
FANCD2
FANCE
FANCF
FANCG
FANCI
FANCL
FANCM
FARP1
FAS
FAT1
FAT3
FAT4
FBXO31
FBXW7
FEN1
FEV
FGF1
FGF10
FGF12
FGF14
FGF18
FGF19
FGF2
FGF21
FGF23
FGF3
FGF4

FGF5
FGF6
FGF7
FGF9
FGFR1
FGFR2
FGFR3
FGFR4
FGR
FH
FLCN
FLI1
FLT1
FLT3
FLT4
FNDC3B
FOS
FOXL2
FOXO1
FOXP1
FPR1
FRS2
FUBP1
FUS
FYN
GABRA6
GALNT12
GATA1
GATA2
GATA3
GATA4
GATA6
GID4
GLI1
GLI2
GLI3
GNA11
GNA13
GNAQ
GNAS
GREM1
GRIN2A
GRM3
GSK3B

H2AX
H3-3A
H3-3B
H3C2
HCK
HDAC1
HDAC2
HDAC9
HGF
HMGA1
HMGA2
HNF1A
HOXB13
HRAS
HSD3B1
HSP90AA1
HTATIP2
ID3
IDH1
IDH2
IDO1
IGF1R
IGF2
IKBKE
IKZF1
IL7R
INHBA
INPP4B
IRF1
IRF2
IRF4
IRS2
ITK
JAK1
JAK2
JAK3
JAZF1
JUN
KAT6A
KDM5A
KDM5B
KDM5C
KDM6A
KDR

KEAP1
KEL
KIT
KLF5
KLHL6
KMT2A
KMT2C
KMT2D
KNSTRN
KRAS
LCK
LIMK1
LMO1
LRIG1
LRP1
LRP1B
LRP2
LTK
LYN
LZTR1
MACC1
MAF
MAGI2
MALAT1
MAML2
MAP2K1(MEK1)
MAP2K2(MEK2)
MAP2K4
MAP3K1
MAP3K13
MAP4K5
MAPK1
MAX
MCF2L
MCL1
MDM2
MDM4
MECOM
MED12
MEF2B
MEN1
MERTK
MET
MGMT

MITF
MKNK1
MLH1
MLLT3
MPL
MR1
MRE11
MS4A1
MSH2
MSH3
MSH6
MST1R
MTAP
MTG1
MTOR
MUC16
MUTYH
MYB
MYBL1
MYC
MYCL
MYCN
MYD88
MYH11
MYOD1
NAB2
NBN
NCOA2
NCOR1
NECTIN4
NEK11
NET1
NF1
NF2
NFE2L2
NFIB
NFKBIA
NKX2-1
NOTCH1
NOTCH2
NOTCH3
NOTCH4
NPAT
NPM1

NR4A3
NRAS
NRG1
NRG3
NSD1
NSD2
NSD3
NT5C2
NTHL1
NTRK1
NTRK2
NTRK3
NUP88
NUP93
NUTM1
OBSCN
P2RY8
PAK1
PAK3
PALB2
PARP1
PARP2
PARP3
PARP4
PAX3
PAX5
PAX7
PBRM1
PBX1
PCA3
PDCD1(PD-1)
PDCD1LG2
PDCD1LG2(PD-L2)
PDGFB
PDGFRA
PDGFRB
PDK1
PHF6
PHOX2B
PIK3C2B
PIK3C2G
PIK3C3
PIK3CA
PIK3CB

PIK3CD
PIK3CG
PIK3R1
PIK3R2
PIM1
PKD2
PKN1
PLA2G1B
PLCG2
PML
PMS2
POLB
POLD1
POLE
POT1
PPARG
PPP2R1A
PPP2R2A
PRDM1
PREX2
PRF1
PRKACA
PRKACB
PRKAR1A
PRKCI
PRKDC
PRKN
PRPF38B
PRSS1
PRSS8
PTCH1
PTEN
PTK2
PTK6
PTPN11
PTPRO
PTPRT
QKI
RAC1
RAD17
RAD21
RAD50
RAD51
RAD51B

RAD51C
RAD51D
RAD52
RAD54B
RAD54L
RAF1
RANBP2
RARA
RASA1
RB1
RBM10
RECQL
RECQL4
REL
RELA
RELB
RET
REV3L
RGS7
RHBDF2
RHEB
RHOA
RICTOR
RIT1
RNASEL
RNF43
ROCK1
ROCK2
ROS1
RPTOR
RSPO2
RSPO3
RUNX1
RUNX1T1
RXRA
SDC4
SDHA
SDHAF2
SDHB
SDHC
SDHD
SERPINB3
SERPINB4
SETBP1

SETD2
SF3B1
SGK1
SHOC2
SHQ1
SIK1
SKP2
SLC1A2
SLC34A2
SLC6A2
SLIT2
SLX4
SMAD2
SMAD3
SMAD4
SMARCA2
SMARCA4
SMARCB1
SMARCD1
SMARCE1
SMO
SNCAIP
SND1
SOCS1
SOX10
SOX2
SOX9
SPEN
SPINK1
SPOP
SPTA1
SRC
SRGAP1
SRMS
SRSF2
SS18
SSX1
STAG2
STAT3
STAT4
STAT6
STK11
STK24
SUFU

SUZ12
SYK
TACSTD2
TAF1
TBX3
TCF3
TCF7L2
TEK
TENT5C
TERC
TERT
TET1
TET2
TET3
TFE3
TFEB
TGFB1
TGFB2
TIE1
TIPARP
TLX1
TMEM127
TMPRSS2
TNFAIP3
TNFRSF14
TNFRSF19
TNFSF11
TNFSF13B
TNK2
TOP1
TOP2A
TP53
TP63
TPMT
TRAF7
TRIO
TSC1
TSC2
TSHR
TSPAN1
TSPAN31
TYK2
TYRO3
U2AF1

UGT1A1
USP6
VEGFA
VGLL3
VHL
WEE1
WEE2
WNK1
WRN
WT1
XIAP
XPO1
XRCC2
XRCC3
YAP1
YES1
YWHAE
ZBTB2
ZFHX3
ZNF217
ZNF703
ZNF750
ZRSR2

Osteosarcoma Metastasis-associated Mutations

ANGPTL4
CCDC80
CCL5
COX-2
CSF-1
CTGF
CXCL8
CXCR4
CXCR4/CXCL12 pathway
DAB2
DEPDC6
DLX4
EHF
endothelin-1
EREG
ezrin
FGFR
FHOD3
FOXQ1
gp78
ID1
IL6
interleukin-11
LOX
LOX
MET
MMP-1
MMP-9
mTOR
NEDD9
Notch pathway
OSMR
PAX3
PCBD1
PHLDA1
PKC
RANKL
RHoC
SERPINE2
SLC1A3
TGFB2
TGF β

TP53 deletion

TPM1

TWIST1

VEGF

WNT/ β -catenin pathway

Δ Np63 pathway