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Primary Bone Tumours – Guiding Treatment Using Histology and Molecular Genetics
Diagnosing Bone Tumours – A challenge?

- Rare
- Specialist Centres
- Improved imaging
- Biomarkers – prognostic
  - Ewing sarcoma
- Stratification
Classification Primary Bone Tumours

- Round cell tumours
- Bone-forming
- Cartilage-forming
- Osteoclast-rich
- Fibro-osseous
- Pleomorphic

- Is it a tumour? - exclude reactive, metabolic..
- Benign vs malignant; primary vs metastatic
Recurring errors – uncommon but should no longer happen

- Osteosarcoma called lymphoma
- Lymphoma called osteosarcoma
- Undifferentiated neoplasm is called primary malignant bone tumour but is metastatic carcinoma…
- Clinical information and critical immuno
CD99 – NOT SPECIFIC
CK MNF116:
MYOEPITHELIAL TUMOUR? MELANOMA? SYN SARC?
t(11;22)(q24;q12), resulting in a EWSR1–FLI1 gene fusion
EWSR1 – NFATc2 amplification
Ewing sarcoma – tibia 25 year old
Negative for EWSR1 fusion
CD99 Membranous  TdT expression
Dx: Acute lymphoblastic lymphoma
EWSR1 structural alteration before making a diagnosis of Ewing sarcoma — exclude (varies with age)

- Lymphoma (CD45, CD20)
- Plasma cell tumour (CD138)
- Myeloid leukemia
- LCH (S100, CD68, CD1a, **BRAF/NRAS**)
- Carcinoma (metastatic) — problematic with age; Ewing sarcoma occurs in 60 year olds
- Melanoma (S100, Melan A, HMB45)
- Synovial sarcoma (SYT/SSX1)
Round cell tumour in bone: 
? Ewings but negative for EWSR1 fusion gene 
~10% of such tumours having excluded lymphoma, melanoma etc
More spindled and myxoid 2/3\textsuperscript{rd} of EWSR1-fusion neg RCTs have CIC-DUX4/4L fusions
Round cell tumour: Cic-Dux4
lobular, extrnsive necrosis, mitotic figures
Round cell tumour: Cic-Dux4

Treatment?
CIC FISH on Small Round Cell Tumor

Normal metaphase slide

Negative, 2 copies

Positive, 07 0588

Negative, 3 copies 08 2013

Normal metaphase slide

Positive, 05 2347
Ewing sarcoma-like tumour
BCOR (Xp11.4) and CCNB3 (Xp11.22)
CCNB3 overexpression – immuno: Treatment?

- CD99 negative
Myxoid liposarcoma FUS-CREB
- metastasises to bone - vertebra
Ewing Family of Tumours

EWSR1 partners with ets family members
- 85% EWSR1 – FLi1 fusions
- EWSR1-ERG, ETV1, ETV4, FEV

Non-ets partners
- EWSR1-NFATc2, SMARCA5, PATZ, SP3

Ewing-like tumours
- BCOR-CCNB3
- CIC-DFX4* - mainly soft tissue
- Fli immuno – not specific; WT1, not specific
- FISH vs RT-PCR or NGS?
Rarely occurring in bone – *EWSR1*

- Clear cell sarcoma *EWSR1CREB1*
- Mixed tumour /myoepithelioma
  
  *EWSR1-POU5F1*  *EWSR1-PBX1*
  
  - ***Could be confused with Cic-Dux tumour***
- Angiomatoid fibrous histiocytoma
  
  *EWSR1-ATF1/ EWSR1-CREB1*
- Desmoplastic Small Round Cell Tumour
  
  *EWSR1-WT1*
- Extraskeletal mesenchymal chondrosarcoma
  
  *EWSR1-NR4A3*

- *NEXT GENERATION SEQUENCING*
Bone-Forming Neoplasms
High grade osteosarcomas – neoadjuvant therapy
- Highly variable histology
- Immunohistochemistry does not help other than haematological biomarkers (CD45, CD20)
- Copy number +++
OSTEOBLASTIC OSTEOSARCOMA
CHONDROBLASTIC OSTEOSARCOMA
FIBROBLASTIC OSTEOSARCOMA
TELANGIECTATIC
OSTEOSARCOMAS

ROUND CELL

? ABC

? Lymphoma, LCH, Ewings, Melanoma, Carcinoma
Bone tumour: ? osteosarcoma
CD20        CD45
B cell lymphoma

Round cell tumours – always exclude lymphoma/leukaemia
Easy to do - good immuno, cheap, fast: good treatment options
Could this be a carcinoma, melanoma? Imaging? Immuno not very helpful
Cartilaginous tumours
- anatomical classification
High grade Tumours

IDH1 – 50%
TP53
CDKN2A

Excludes OS
CDKN2A never loss or gain in CS G1
GI – always disomic
Bland spindle cells: ? Parosteal osteosarcoma

MDM2:CEN12
Sensitivity
Specificity

80% of cases
Low grade central osteosarcoma
MDM2 amplification -
Low Grade Central OS

MDM2 amplification
Differential Diagnosis: Fibrous dysplasia vs Low Grade Central Osteosarcoma

Fibrous dysplasia

Most common benign fibro-osseous lesion (5-7% of all benign bone tumours)

Fibrous dysplasia and parosteal osteosarcoma share histological features.

GNAS1 mutation

Parosteal osteosarcoma

Malignant surface bone tumour (4% of all osteosarcomas)

MDM2 amplification
GNAS1 mutation

Rapid growth
Mayo study: Carter et al. 2014. AJSP
Parosteal osteosarcoma have GNAS mutations – 5/9 cases

GNAS mutations are not detected in parosteal and low-grade central osteosarcomas – Modern Pathology in press

- 97 FFPE low-grade OS from
- 90 patients: 62 parosteal OS, MDM2 amplification in 79%
- Quality of DNA checked with ddPCR
The value of some basic understanding of research – how to interpret data; review MS
100,000 Genomes Project
DNA extraction from FFPE - quality

Time for change…
- Fixation in FFPE within 2 hours
- Buffered formalin
- Small fragment
- Not more than 24 hours
- Vacuum pack and kept cold
Aneurysmal bone cyst
Pain at back of neck. Relatively acute onset about 8 weeks earlier and with increasing swelling. No history of trauma or significant other history.
USP6 break apart probe present in 75% of ABC and > 90% of nodular fasciitis
Giant cell tumour of bone & Chondroblastoma
H3F3A (H3.3) mutations

- GCT H3F3A, Glycine to tryptophan (W) (p.G34W) – 96%
- Chondroblastoma H3F3 A/B – Lysine to methionine Lys36Met (p.K36M) 95%

- Mutually exclusive with USP6, IDH1, GNAS1 alterations
- Present in small bone of hands and feet
- Not present in 70+ non-ossifying fibroma,
- Not present in giant cell reparative tumours of the jaw
- Not present in chondromyxoid fibroma

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Ephyseal tumour
12 years old

Other osteoclast-rich tumours

Chondroblastoma
Giant cell tumour of bone

- Giant cell tumours of the small bones of hand and feet, and jaw
Giant cell tumour of bone & Chondroblastoma
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Malignant Giant Cell Tumour

98% of GCT harbour G34W mutation in H3F3A gene
Wild type for H3.3 mutations...Malignant GCT of Left Talus
Dx; osteosarcoma vs malignant GCT - treatment
Osteoclast lesions of the jaw
H3.3 mutations - ? useful

Still need morphology to make dx of GCT but in the absence of H3.3 mutation be cautious about making such a dx.

Mutually exclusive with ABC
Chordoma – brachyury positive
nothing useful for stratification for therapies
Summary

- Round cell tumours – exclude lymphoma, CA, melanoma
- Only Ewing dx with genetic alteration
- Think about CIC-DUX, BCOR- fusion in Ewing-like tumour
- MDM2 amplification in LGOS (central and parosteal)
- GNAS in Fibrous Dysplasia but not LGOS
- IDH1 mutations in 60% of CS; p16 loss in high grade tumor
- IDH1 mutations in dediff CS…excludes dx of OS
- H3,3 mutation in 96% of chondroblastoma and GCT – not in OC-rich lesions of jaw
- Occasionally present in malignant GCT /OS
- USP6 rearrangement in ABC
The end

Thank you for your attention