Genomic markers in Sarcomas in daily practice
From molecular diagnosis to therapeutic target

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31st ECP - Nice - September 10th 2019 -
Molecular Exams in routine

่า A molecular exam is defined by the association of 4 kind of actors

1 or a set of molecular Markers to assess

1 type technology

1 patient sample

1 Analysis depending of the context

= 1 molecular exam

่า For each of them, there’s many specifications, constraints, limitations ...
For each molecular marker a proper tool
Focus on current daily practice (Curie Experience)

Classical Technics

- RT QPCR
- Expression Profiles

NGS

- RNAseq
- Circos
- CNV/LOH
- Mutations

Low Cov Exome

Exome

Sanger sequencing

- Aneuploid
- Amplification (double minutes)

Fusion

Point Mutation

NGS Panel
Exploring more than one locus: aCGH

- DNA microarrays

- Improving sensibility, specificity, resolution, design
- Cost effective (1 aCGH < 2 FISH)
Copy Number Alteration & Genomic Index

- **Flat profile**: QC OK, No CNA, Genomic Index: GI = 0

- **Rearranged profile**: Gains, Losses, Amplifications, HomoZ Deletions...

  | Nbre Bkpts | 0 | 0 | 0 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 5 |
  | Nb Alts    | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 2 | 2 | 3 | 3 |

- Genomic Index = \((\text{Alterations})^2 / \text{Nb rearranged chromosomes}\)

  - GI = \((4^2)/4 = 4\)
SARCGYN : Uterine Leiomyoma vs Leiomyosarcoma on microbiopsy before surgery

- **Case 1** 43 yo women
  - Pain
  - Radiology leiomyoma vs leiomyosarcoma?
  - Pathologic review (Stanford criteria):
    - Atypia: None
    - Necrosis: 0
    - Differentiation: 1
    - Mitosis: 3

  ➔ Leiomyoma

- **CGHa analysis**

  ![CGHa analysis graph](image)

  2 breakpoints
  1 focal loss / chr7

  ➔ Genomic Index = \((1^2)/1 = 1\)
Case 2 64 yo women
Metrorrhagia
Radiology leiomyoma vs leiomyosarcoma?
Pathologic review (Stanford criteria):
  Atypia: yes
  Necrosis: 1
  Differentiation: 3
  Mitosis: 21

Leiomyosarcoma

CGHa analysis

39 breakpoints
41 aberrations among the 22 chromosomes
  gains, wide or thight losses
  Homozygous deletions: PTEN (ex 1 & 2) et RB1 (ex 1 à 17)

Genomic Index = (41^2)/22 = 76
SARCGYN: aCGH a useful tool

25 CGHa: Bkpts and GI evaluation

S. Croce et al. Modern Pathology 2015

Uterine smooth muscle tumor analysis by comparative genomic hybridization: a useful diagnostic tool in challenging lesions

25 cases complex genomic profiles:
  Bkpts [28;50] GI [42;144]

17 cases simple genomic profiles:
  Bkpts [0;8] GI [0;9]

Leiomyoma 88%
  15 leiomyomas & 2 STUMPs

Leiomyosarcoma 100%

Very specific test with high sensitivity
SARCGYN : 2 more cases

✦ Case 25
15 breakpoints & GI = 28 ✦ grey zone ?

Breakpoint chr2 / ALK, validation by FISH, RNAseq : TNS1-ALK
✦ Inflammatory Myofibroblastic Tumor

✦ Case 26
30 yo ; Histo : Diff 1, w/o atypia & necrosis
12 breakpoints & GI = 12,8 (threshold/tool)

✦ Staff review : Leiomyoma
Copy Number Alteration & Genomic Index

SYNOBIO in Pediatric Synovial Sarcomas

A. Flat profile

B. GI=1

C. GI=33

Rearranged profiles
RNAseq & chromosomal translocation identification

- **Known recurrent translocations** (ex EWSR1-FLI1)

  - 11
  - 22

- **New Fusion explosion** (partners and alternative imbalance)
RNAseq in routine use: fusions – expression – mutations

- Fusions identification
  Supervised analysis
  Bed custom V2: 1241 genes 1107 fusions

  Exploratory analysis by 5 distinct algorithms
  (DeFuse, TopHat Fusion, FusionMap, StarFusion, Fusion Catcher)

- Expression pattern
  Molecular markers validation
  Clinics vs Pathological review
  Outliers identification
  Clustering & Boxplot

- Expressed mutation
  Tumoral first (RNA then DNA)
  At Germline level
  Predisposition gene (TP53, DICER1, Ras...)
  Inform consent
Daily practice examples

- For ‘classical’ well known fusions

M 60 yo
Soft part sarcoma
Synovial Sarcoma vs LipoSarcoma?

- Fusion SYT-SSX2

M 60 yo
Soft part sarcoma
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Circos Plot

Clustering
Within Syno+

CINSARC F. Chibon
signature assessment

Boxplot en Log2 FPKM
MDM2 overexpression vs other Syno+

RNAseq for Diagnosis, Prognosis and Theranostic
Daily practice exemples

- RhabdomyoSarcomas: Fused or not fused? and much more ...
  - Fusion detection

- Expression Profiling

- IHC Correlation

- Expressed Mutation detection: therapeutic targets and markers follow up (cfDNA)

RMS epithelioïd
MYOD1 p.L122R 98%

RMSe
FGFR4 p.V550L 99%

RMSe
NRAS p.Q61K 36%

RMSe
PIK3CA p.Q546K 37%

RMSe
DICER1 (Som & Germline) p.(D1810A) & p.(Leu345TyrF5Ter8)
Daily practice examples ✨ For atypical, new, or unexpected fusions

💡 **Case 1**
Young Baby Boy  
Malignant round cells sarcoma

INI1+, Muscular, epithelial, lymphoid markers neg  
IHC ALK, ROS1, BRAF antiPDL1 neg

Pathological review: YWHAE/FAM22 fusion? -> RNAseq

➡️ RNAseq: no fusion but Specific BCOR-Fused Cluster ➡️ BCOR-ITD validation

💡 **Case 2**
Young boy, Buttock lesion, Metastatic

Small round cell tumor  
INI1+, Ki67 30/40%, IHC not conclusive

➡️ RNAseq: fusion? specific BCOR-Fused Cluster ➡️ Fusion YWHAE-NUTM2A

💡 **Case 3**
M 65 yo, supraclavicular lymph node, unknown primary site

Ki67 30%, CD99+, poorly differentiated tumor  
Ewing Sarcoma?

➡️ RNAseq: fusion? specific SLC45A3-ERG fusion  
➡️ Prostate AdenoCarcinoma

TP53 p.R175H 96%
Daily practice exemples

- **4 months baby girl**
  Abdominal lesion, skin biopsy
dermal & hypodermal infiltration (a)
monotonous spindle cells, fascicular and storiform pattern, honeycomb pattern (fat) (b)
scattered collagen bundles (c)
CD34+ (d)
AE1/AE3; desmin; PS100; SOX10; STAT6 neg
KI67 15

COL1A-PDGFB -> neg
ETV6-NTRK3 ? -> neg

- **RNAseq: KHDRBS1-NTRK3 fusion**

Novel KHDRBS1-NTRK3 rearrangement in a congenital pediatric CD34-positive skin tumor: a case report.

Talayes F1, Frankel S2, Brista A3, Orbach D4, Jourdain A5, Raymond S6, Freeman S6, Nachet MC1,2, Marnari A3,5,12.

- **Targeting NTRK fusion**

- **Mutations characterization**
  **Resistance**
  (G623R in NTRK3 & G595R and G667C in NTRK1)

  **Driver**
  (NTRK1 I699V, NTRK2 P716S, and NTRK3 R745L)

  In primary, relapse & for follow up
Daily practice examples

- **Targeting ALK**

- More than 30 fusions partners
  - Classic or new ones
  - In Sarcomas ... or not
  - almost exhaustive for fusion detection

- **Hot spot Mutation detection**
  - RMSa : PAX3–FOXO1 + ; Cluster RMSa
  - ALK p.(F1174L)

- **High levels of transcript** :
  - FUS-TFCP2 + ;
  - Cluster TCFP2
  - OverExpression of ALK Kinase domain
Integrated Signatures for Metastatic Children & AYA

Exome Tumor and Germline RNAseq

Copy number Profile
Mutationnal Overview
Translocation identification
Signature assessment

A single Molecular report for Clinical use
Targeted Therapy orientation

MAPPYACTS
Molecular biology incidental finding at Germline level

- **MAPPYACTS**
  - Young Girl
  - Nephroblastoma in 2015
  - Relapse may 2016

- **SMARCA4 Mutation**
  - Homozygous somatic (isodisomy)
  - Germline

- **Kidney Rhabdoid tumor**
- **Genetic counseling**
TP53 mutation, MAF 77% : isodisomy of chr 17

somatic event
MAPPYACTS

MAP178
♂ 18 ans
Ostéosarcoma in 2007
Relapse in January 2017

- Very complexe profile
  TP53 Mutation

- Variation at 53% in tumor

- Tumoral cells content estimation 30%

- Validation at germline level
  Genetic counseling
  Li Fraumeni

- Expression 90%
Molecular Diagnosis of Sarcoma for Integrative patient management care

- Help for tumors classification
  - Diagnostic practice

- Discovering new tumoral entity
  - Specific Pathways
  - Therapeutic targets

- Atypical cases identification
  - Pathology follow up, and response to treatment
  - Interaction between Clinicians-Radiologists-Pathologists-Biologists
\[ \int_{RNA}^{DNA} f(\sqrt{\text{sample}}) \frac{\text{dtime}}{\varepsilon} = e^{\text{Informations}} \]
Thanks