Molecular Tumor Boards

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Chemotherapy vs. targeted therapy vs. immunotherapy:

DNA / Cisplatin
- Low specificity
- High toxicity

Bcr-Abl / Imatinib
- High specificity
- Lower toxicity

TCR / pMHC
- Very high specificity
- Lower (off target) toxicity
Personalized Oncology: a new paradigm

Mutations
- RAS, RAF, PI3K, POLE, P53, ...

CNV
- HER-2, TGFβ, FGFR, MET, ...

RNA-seq classification
- CMS I-IV

RNA-seq
- RNA sig?

Immuno
- PD-L1, LAG, ...
- CD8 TCR, ...

...
The rapidly evolving personalized oncology landscape
The big data transformation of personalized oncology

Lung cancer

Histological subtypes: adenocarcinoma, squamous cell, ...

Molecular subtypes: EGFR, ALK, ...

Standard chemo → Response 15-20%
- Oncologist

Adapted chemo → Response 25-30%
- Oncologist
- Pathologist

Targeted therapies → Response 40-80%
- Oncologist
- Pathologist
- Geneticist
- Biologist

Personalized therapy → Response +++
- Oncologist
- Pathologist
- Geneticist
- Biologist
- Bioinformatician

-Omics
- EGFR Mutations (15%) • Response 70%
- ALK Rearrangement (4%) • Every patient unique!
- BRAF Mutation (2%) • Response 40%

Every patient unique!
Personalization in immuno-oncology

Unselected tumor

- Oncologist

Tumor selected on immune infiltrate
- Immune desert
- Oncologist
- Pathologist
- Geneticist
- Biologist
- Bioinformatician

Tumor selected on immune escape mechanism

Each patient unique!

Unique immuno

Adapted immuno

Specific immuno

Personalized immuno

Response

Response

Response

Response

- Oncologist
- Pathologist
- Biologist

- Oncologist
Applications of NGS in molecular oncology

1. Large coverage of genomic regions (introns, exons, …): somatic/germline alterations

2. Deep coverage (driver mutations and rare variants)

3. Large patient populations (GWAS, PheWAS, signatures, …):

4. Tumoral heterogeneity (single cell analysis)

5. Therapeutic follow up (adaptive resistance)
NGS technologies: tissue requirements

Typical requirements for NGS:

- **Exome sequencing:** 3 μg of DNA
- **RNAseq:** 1 μg of RNA

This corresponds to:

- **500’000 cells for exome** (6 pg of DNA per cell)
- **50’000 cells for RNAseq** (10-30 pg of RNA per cell)

### Fresh frozen:

- **Quantities:**
  - 5x5x4 mm$^3$ (100 mm$^3$ ≈ 100 mg) equals about 10 million cells
- **Quality:**
  - time to freeze is crucial, conservation issues

### FFPE:

- **Quantities:**
  - 1 cm$^2$ x 10 μm (1 mm$^3$ ≈ 1 mg) equals about 100’000 cells
- **Quality:**
  - processing time is crucial, conservation usually fine
  - Exome can be of good quality, but RNA is very fragmented

### Other:

Circulating tumor cells (CTC)? Cytologies?
Genomic and transcriptomic analyses:

1. Whole genome
   - Predominant applications:
     - Structural variants
     - Point mutations
     - Copy number variation

2. Whole-exome (1%)
   - Predominant applications:
     - Structural variants
     - Point mutations
     - Copy number variation

3. PCR amplicon
   - Predominant applications:
     - Point mutations
     - Deletions

4. Transcriptome RNA
   - Predominant applications:
     - Gene expression
     - Gene fusions
     - Splice variants

5. Exon capture transcriptome
   - Predominant applications:
     - Gene expression
     - Gene fusions
     - Splice variants

An illustrative example: MAPK alterations in melanoma
Targeted therapy of BRAF\textsuperscript{V600E} mutation

- Selective inhibitors have produced spectacular clinical results

- But the disease inexorably evolves towards progression within 6-12 months
BRAF is a clear oncogenic driver in melanoma and provides a growth advantage to cell harboring the V600E/K mutations.

BRAF V600E/K mutation is an early event in melanoma genesis and is conserved throughout disease progression.

But

- It is not necessary:
  - 50% of melanoma are BRAF WT
- It is not sufficient:
  - Mouse models suggest that additional mutations are required for melanoma genesis (Dankort & al. *Nature Genetics* 2009)
  - Most naevi are BRAF V600E (Ichii-Nakato & al. *J. Invest. Dermatol* 2006)
Example: genomic landscape of melanoma

BRAFi + MEKi

MEKi

Targeted therapies

BRAFi
NRAS
NF1
WT

Age
Subtypes
BRAFi
NRAS
HRAS
KRAS
NF1
CDKN2A
TP53
PPP6C
ARID2
PTEN
IDH1
MAP2K1
DDX3X
RAC1
RB1

Tissue
Primary
Metastatic

**Non-MAPK alterations associated with outcome**

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Baseline frequency</th>
<th>Impact on RR</th>
<th>Impact on PFS</th>
<th>Resistance mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3K/AKT/mTOR Pathway</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>8.5%(^1)</td>
<td>Yes(^2)/No(^3)</td>
<td>Yes(^3)</td>
<td>Yes(^8)</td>
</tr>
<tr>
<td>Pi3K / AKT / mTORC1</td>
<td>3/3/10%(^1)</td>
<td>Yes(^12)</td>
<td>Yes(^8,11)</td>
<td></td>
</tr>
</tbody>
</table>

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**PI3K / AKT / mTORC1**

- Vem PTEN+ (N = 82)
- Vem PTEN loss (N = 32)
- Vem + cobi PTEN+ (N = 92)
- Vem + cobi PTEN loss (N = 32)

HR 0.36 (95% CI, 0.19-0.65) for PTEN loss Vem + Cobi vs. Vem

**G. McArthur & al. ESMO 2015:**
Addition of cobi to vem overcomes the negative impact of PTEN loss on PFS

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Are all mutations equal?

• Different mutations might have similar impact on protein function, whereas others might induce divergent biological effects
• Example: MEK1 - E203K, P124Q, P124L and P124S

This could imply that MEK1 E203K and P124Q act through the same mechanism and would be interesting to pool in the analysis in order to increase statistical power

Carlino CCR 2015; Nikolaev Nat. Gen. 2012; Zoete unpublished data
Clinical and molecular biomarkers for personalized oncology

Clinical
• LDH
• # sites
• ECOG
• Sex
• ...

Molecular

Baseline biomarkers

On treatment biomarkers

Selective pressure

Tumor cells / μ-environment

First line I-O

Other lines

RR

PFS

OS
Strategies to identify driver mutations in melanoma:

- **p-value < 1e-5**
- Positive Selection YES

Candidate driver mutations

Hodis et al. *Cell* 2012
Paradigm shift: genomic landscape integration

• We are witnessing a major shift of paradigm
  • from a simplified model « one gene, one therapy »
  • to a model of system biology where all alterations are taken into account for the therapeutic decision

Figure:
BRAF and 20 most frequently altered neighbor genes. Network computed from the TCGA melanoma dataset.

• Such a change will require the integrated action of a large number of competences and specialists

Patient ➔ Oncologist ➔ Pathologist ➔ Radiologist ➔ Surgeon ➔ Geneticist ➔ Bioinformatician
First results of large prospective trials
Results of first large scale studies:

• Wheler, *Cancer Research* 2016:
  - MD Anderson single institution experience
  - 500 patients enrolled
  - 138 received either matched (65%) or unmatched (35%) treatments
  - Improvement in RR, PFS, OS

• Le Tourneau, *Lancet Oncol* 2015:
  - Multicentric French Randomized trial (SHIVA)
  - 741 patients enrolled
  - 195 received either matched (50%) or unmatched (50%) treatments
  - No improvement in PFS
First clinical results: the MOSCATO study

• 1035 patients included, 948 biopsied
• 411 actionable mutations discovered in the 843 patients with molecular profile (49%)
• A total of 199 patients were treated with a targeted therapy matched to a genomic alteration
• Objective responses were observed in 22 of 194 patients (11%; 95% CI, 7–17%), and median overall survival was 11.9 months (95% CI, 9.5–14.3 months)
• The PFS2/PFS1 ratio was >1.3 in 33% of the patients (63/193), where PFS2 is the PFS on the molecularly matched treatment and PFS1 that of the previous line

1 Massard, Cancer Discovery 2017
Better patient selection: predictive biomarkers!
Principle of personalized oncology

100% benefit in this subpopulation if we can identify it!
Complex nature of candidate biomarkers in melanoma

Most current biomarkers:
- LDH
- BRAF
- PD-L1
- Ulceration
- ...

Adapted from Dafni Urania, ESMO Edu.
Expected benefit from personalized I-O strategies

Adapted from A. Ribas, WCM 2013
Complex biomarkers factoring many aspects will be needed.

The cancer immunogram assess many aspects of tumor immunology to predict I-O response.
Checkmate-038 prospective biomarker study\textsuperscript{1}

- 68 advanced melanoma patients
- Multidimensional biomarker analysis at baseline and on treatment reveals molecular actions of anti-PD-1 therapy
  a) Anti-PD-1 therapy induces changes in the mutational burden of tumors
  b) Distinct changes in gene expression programs associate with clinical response
  c) Shifts in the TCR repertoire occur following immune checkpoint blockade
- This study is one example of the level of information that need to be integrated for complex biomarker research
- Many more to come!

\textsuperscript{1}Riaz, Cell 2017
Deploying a molecular TB in a regional health care environment: Romand Network of Oncology
Expertise required at our molecular TB

- Oncologist: therapeutic decision
- Pathologist: tissue used for analysis
- Surgeon: sample's surgical procedure
- Geneticist: germline mutations
- Bioinformatician: -omics analysis
- Biologist: cell lines analysis

17th ESO-ESMO Masterclass in Clinical Oncology
Organization of the Romand Network of Oncology

Regional Romand Hospital

Clinical trial (I-III)

Off Label

-omics platforms

CHUV/HUG

Autres

Data Mining

Electronic patient record

Flying Data manager
Reaching out to a population of 1.9 Mio habitants
Molecular tumor-board and videoconferencing
Details of current and upcoming genes panel

- 52 gene panel covers known hot-spots on known drivers
  - No need for constitutive DNA
- A 400+ full length gene panel is also in use since early 2017
  - Constitutive DNA is required
- Full exomes and genomes will follow...

<table>
<thead>
<tr>
<th>Targets</th>
<th>Hotspot regions, including ~2,800 COSMIC mutations of 50 oncogenes and tumor suppressor genes, with wide coverage of the KRAS, BRAF, and EGFR genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplicon length</td>
<td>111–187 bp, average 154 bp</td>
</tr>
<tr>
<td>Primer pool size</td>
<td>207 primer pairs in 1 tube</td>
</tr>
<tr>
<td>Input DNA required</td>
<td>Only 10 ng per DNA sample</td>
</tr>
<tr>
<td>Time-to-results</td>
<td>10 hours (DNA to annotated variants)</td>
</tr>
</tbody>
</table>
Molecular tumor board: activity details

- 69% of external patients
- Regular participation of 26 external referring medical oncologist and 20 internal
- Therapies: off label 53%, clinical trials 62%
- 350+ patients referred to the network in 2017!
Somatic mutations observed in the Romandie Network

350+ patients enrolled in 2017!
Romand Network of Oncology: early signs of efficacy

- Clinical responses to the molecularly selected treatment options are seen regularly and statistical analyses are ongoing.
- Such responses involve targeted therapies and immunotherapies.

1. Urothelial carcinoma having failed all standard therapies. HER-2 amplification detected at molecular tumor board and off label treatment with Trastuzumab Emtansine.

2. Histiocytic sarcoma having failed all standard therapies, showing 2 deep responses to 2 successive targeted therapies (MEK and KIT).
Thank you for your attention!