MOLECULAR BIOLOGY, BIOMARKERS, AND TCGA

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Advisory board member or speaker with honoraria (managed by my Institution): Pfizer, Roche, Astra/Zeneca, Bayer, Innate, Merck Serono, Boehringer, BMS, Novartis, Janssen, Incyte, …

Travel expenses: Amgen, BMS, Pfizer, MSD, …

Data safety monitoring board with honoraria: Debio, Nanobiotix

Institutional conflict of interest (Funding to institution for research support): all companies

Uncompensated advisory role: MSD

EORTC: investigator and study coordinator
Learning objectives

• Key **molecular biology features** of HPV-negative and positive head and neck cancer

• **Prognosis** value of p16 and EGFR

• **Predictive** biomarkers: EGFR, p16 and PD-L1
Squamous cell carcinoma of the head and neck

The seventh most common form of cancer
600,000 cases per year worldwide

➔ Alcohol & tabacco
    (oral cavity, larynx and pharynx)

➔ Human Papillomavirus (HPV+)
    (oropharynx)
Presentation outline

- Key molecular features
- EGFR and p16 as **prognosis** biomarkers
- EGFR and p16 as **predictive** biomarkers
- Microenvironment: immunology
Candidate Therapeutic Targets

Analysis - Tanguy Seiwert, Niki Schultz

<table>
<thead>
<tr>
<th>Receptor/Tyrosine Kinase</th>
<th>HPV(−) N=244</th>
<th>HPV(+) N=35</th>
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<tbody>
<tr>
<td>EGFR</td>
<td>16%</td>
<td>6%</td>
</tr>
<tr>
<td>FGFR1</td>
<td>10%</td>
<td>0%</td>
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<tr>
<td>MET</td>
<td>5%</td>
<td>3%</td>
</tr>
<tr>
<td>ERBB2</td>
<td>5%</td>
<td>3%</td>
</tr>
<tr>
<td>EGFR1</td>
<td>4%</td>
<td>0%</td>
</tr>
<tr>
<td>EPHA2</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>DDR2</td>
<td>3%</td>
<td>6%</td>
</tr>
<tr>
<td>FGFR2</td>
<td>2%</td>
<td>0%</td>
</tr>
<tr>
<td>FGFR3</td>
<td>2%</td>
<td>6%</td>
</tr>
<tr>
<td>CCND1</td>
<td>30%</td>
<td>3%</td>
</tr>
<tr>
<td>MYC</td>
<td>14%</td>
<td>3%</td>
</tr>
<tr>
<td>HRAS</td>
<td>4%</td>
<td>0%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>18%</td>
<td>3%</td>
</tr>
<tr>
<td>PTEN</td>
<td>51%</td>
<td>37%</td>
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<tr>
<td>PIK3R1</td>
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<td>3%</td>
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<tr>
<td>NF1</td>
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<td>9%</td>
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<tr>
<td>TP53</td>
<td>82%</td>
<td>3%</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>80%</td>
<td>9%</td>
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</table>

Amplification | Homozygous Deletion | Heterozygous Deletion | mRNA Downregulation | Mutation | RPPA Downregulation | RPPA Upregulation

The Cancer Genome Atlas

TSeiwert, draft 5/26/2012
**HPV-negative genomic alterations**

<table>
<thead>
<tr>
<th>Cellular process</th>
<th>Gene</th>
<th>Protein</th>
<th>Type of gene</th>
<th>Mutation frequency (%)</th>
<th>CNA frequency (%)</th>
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</thead>
<tbody>
<tr>
<td>Cell cycle</td>
<td>CDKN2A</td>
<td>p16INK4A</td>
<td>Tumour suppressor</td>
<td>22</td>
<td>32</td>
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<tr>
<td></td>
<td>TP53</td>
<td>p53</td>
<td>Tumour suppressor</td>
<td>72</td>
<td>1.4</td>
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<tr>
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<td>G1–S-specific cyclin D1</td>
<td>Oncogene</td>
<td>0.6</td>
<td>25</td>
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<td>Growth signals</td>
<td>EGFR</td>
<td>EGFR</td>
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<td>11</td>
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<td>Survival</td>
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<td>Catalytic p110α subunit of class 1 PI3Ks</td>
<td>Oncogene</td>
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<td>21</td>
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<tr>
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<td>PTEN</td>
<td>PTEN</td>
<td>Tumour suppressor</td>
<td>3</td>
<td>4</td>
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<tr>
<td>WNT signalling</td>
<td>FAT1</td>
<td>Protocadherin FAT1</td>
<td>Tumour suppressor</td>
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<td>8</td>
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<td>AJUBA</td>
<td>LIM domain-containing protein AJUBA</td>
<td>Tumour suppressor</td>
<td></td>
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<tr>
<td></td>
<td>NOTCH1</td>
<td>NOTCH1</td>
<td>Tumour suppressor</td>
<td>18</td>
<td>4</td>
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<tr>
<td>Epigenetic regulation</td>
<td>KMT2D</td>
<td>Histone-lysine N-methyltransferase KMT2D</td>
<td>Tumour suppressor</td>
<td>16</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>NSD1</td>
<td>Histone-lysine N-methyltransferase NSD1</td>
<td>Tumour suppressor</td>
<td>12*</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Data from TCGA. Mutation data were taken from The Cancer Genome Atlas (TCGA) [Ref. 3] using the cBioPortal. CNA, copy number alteration; EGFR, epidermal growth factor receptor; *Putative passenger mutation that requires further functional studies.

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Leemans R et al, Nature Reviews 2018
HPV negative

Leemans R et al, Nature Reviews 2011
HPV positive

Leemans R et al, Nature Reviews 2011
Gene expression profile

MS = Mesenchymal
CL = Classical

Keck et al, Clin Cancer Res 2014
Leemans R et al, Nature Reviews 2018
**HER family**
- *EGFR* amplification/mutation in 15%
- *ERBB2* amplification/mutation in 5%

**FGFR pathway**
- *FGFR1* amplification in 10% HPV neg disease
- *FGFR3* mutations in up to 10% HPV pos disease

**PI3K pathway**
- *PIK3CA* mutation/amplification in up to 56% HPV positive SCCHN
- PTEN loss in up to 12%

**HRAS** mutated in 4-5%

**Homologous recombination deficiency in HPV+ or in HPV-platinum sensitive disease?**
HPV negative

Leemans R et al, Nature Reviews 2011
Presentation outline

- Key molecular features
- EGFR and p16 as **prognosis** biomarkers
- EGFR and p16 as **predictive** biomarkers
- Microenvironment: immunology
• The gold standard to detect HPV infection is detection of E6/E7mRNA

• In Situ Hybridization for HPV DNA

• p16 is a good surrogate marker of HPV infection in oropharyngeal cancer: sensitivity and specificity around 90%

• Diagnosis and prognosis value of p16 outside the oropharynx is controversial

• p16 positivity + HPV PCR DNA
Survival by HPV or p16 status for oropharyngeal cancer

Ang KK NEJM 2015
Rischin JCO 2010
Prognosis: p16 and recurrent disease
Prognosis: p16 and recurrent disease

A. Locoregional relapse
B. Distant metastases
C. Salvage surgery
D. No salvage surgery
EGFR expression and prognosis


Ang et al. Cancer Res 2002
Presentation outline

- Key molecular features
- EGFR and p16 as **prognosis** biomarkers
- EGFR and p16 as **predictive** biomarkers
- Microenvironment: immunology
HER1 or EGFR targeting

- Monoclonal Antibodies
  - Cetuximab
  - Panitumumab
  - Zalutumumab

- Tyrosine kinase Inhibitors
  - Gefitinib (EGFR)
  - Erlotinib (EGFR)
  - Lapatinib (EGFR + HER2)
  - Afatinib, dacomitinib (pan-HER)

Tumor cell cytoplasmic membrane

EGF receptor

Tumor proliferation
Who is going to respond?

EGFR overexpression: NO

$EGFR$ polysomy or amplification: NO

RAS mutations Rare in H&N

p16 or HPV ?

Licitra et al, Annals of Oncology 2010
Cetuximab versus MEHD after platinum?

Measures of HER3 activation correspond with tumor shrinkage in both treatment arms.

A. An inverse expression pattern of NRG1 and ERBB3, suggesting HER3 activity in patients with significant tumor shrinkage in both arms.
B. Corresponding EGFR and NRG1 ligand co-expression associated with tumor shrinkage.
C. HPV(+) patients tended to have lower HER family ligand expression and no response. Penuel et al, AACR 2015

Courtesy of A. Pirzkall, Oncology Biomarker development, Genentech
Radiotherapy +/- cetuximab: stage 3 and 4

![Overall survival graph]

- Hazard ratio = 0.74 (95% CI: 0.57–0.97)
- Log-rank p=0.03

Bonner, Lancet Oncology 2010
Figure 3: Overall survival by pre-treatment characteristics: 5-year update
AJCC=American Joint Committee on Cancer; KPS=Karnofsky performance score. EGFR=epidermal growth factor receptor.

Bonner et al, Lancet Oncology 2011
**p16 as a predictive marker**

- **p16 +**
  - 3-year LRC rate: 87% vs 65%
  - HR: 0.31 (95%, CI, 0.11-0.88)

- **p16 -**
  - 3-year LRC rate: 31 vs 19%
  - HR: 0.78 (95% CI, 0.49-1.25)

Rosenthal, J Clin Oncol 2016
Who is going to response? HPV or p16 no clear answer

<table>
<thead>
<tr>
<th>Study</th>
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<th>Population</th>
<th>Benefit</th>
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<tr>
<td>Rosenthal ASCO 2014</td>
<td>Radiotherapy/cetuximab</td>
<td>p16+ = 44; p16- = 109</td>
<td>Stage III/IV</td>
<td>p16- : HR: 0.9 (0S)</td>
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<td>Radiotherapy</td>
<td>p16+ = 39; p16- = 120</td>
<td></td>
<td>p16+: HR: 0.45</td>
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<td>Vermorken Ann Oncol 2014</td>
<td>Platin/5-FU/Cetuximab</td>
<td>p16+ = 18; p16- = 178</td>
<td>Recurrent/metastatic: first-line</td>
<td>p16- : HR: 0.82 (0S)</td>
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<td>p16+ = 23; p16- = 162</td>
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<td>p16+: HR: 0.63</td>
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<td>Platin/5-FU/Panitumumab</td>
<td>p16+ = 57; p16- = 179</td>
<td>Recurrent/metastatic first-line</td>
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<td>Platin/5-FU</td>
<td>p16+ = 42; p16- = 165</td>
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<tr>
<td>Machiels Lancet Oncol 2015</td>
<td>Afatinib</td>
<td>p16+ = 31; p16- =141</td>
<td>Recurrent/metastatic second-line</td>
</tr>
<tr>
<td></td>
<td>Methotrexate</td>
<td>p16+ = 11; p16- = 42</td>
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MEHD7945A

EGFR  HER-2  HER-3  HER-4

Lapatinib

Afatinib
Dacomitimb
Who is going to response? HPV or p16 no clear answer

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<td>p16+ = 18; p16- = 178; p16+ = 23; p16- = 162</td>
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<td>p16+ = 57; p16- = 179; p16+ = 42; p16- = 165</td>
<td>Recurrent/metastatic first-line p16-: HR: 0.73 (0S) p16+: HR: 1</td>
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<tr>
<td>Machiels Lancet Oncol 2015</td>
<td>Afatinib Methotrexate</td>
<td>p16+ = 31; p16- =141; p16+ = 11; p16- = 42</td>
<td>Recurrent/metastatic second-line p16-: HR: 0.69 (PFS) p16+: HR: 0.95</td>
</tr>
<tr>
<td>Study</td>
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<td>N</td>
<td>Population</td>
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<td>p16-: HR: 0.69</td>
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<tr>
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<td></td>
<td>p16+: HR: 0.95</td>
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</table>

- Very low number of patients in the p16 positive group
- p16 positive group included non-oropharyngeal sites
- p16 cut-off different in the EXTREME (10%)
Presentation outline

- Key molecular features
- EGFR and p16 as **prognosis** biomarkers
- EGFR and p16 as **predictive** biomarkers
- Microenvironment: immunology
T cell

anti-tumor
T lymphocytes

tumor antigens

Genes encoding tumor antigens:
1. mutated
2. cancer-germline (IMAGE)
3. viral
4. differentiation
5. over-expressed

Tumor cell

Courtesy of P. Coulie and S. Lucas
Institut de Duve, UCLOUVAIN
Antigens resulting from mutations

Non-synonymous mutations result in amino acid change in a protein that can be recognized by T-cells

Vogelstein et al. Science 2013
CTL = cytotoxic T cell.

Spontaneous anti-tumor T cell responses exist in cancer

- Antitumor T cells are present in patients with cancer prior to any treatment: blood and tumor

- The T cell responses are insufficient
Mechanisms to evade or suppress the immune system

1. Inhibition of tumor antigen presentation
e.g. down regulation of MHC I

2. Secretion of immunosuppressive factors
e.g. TGF-β

3. Inhibition of attack by immune cells
e.g. through T-cell checkpoint pathways

4. Recruitment of immunosuppressive cell types
e.g. T-reg

MHC = major histocompatibility complex; TGF-β = tumor growth factor-β.

TCR = T-cell receptor; PD-L1 = programmed death-ligand 1.
This is only the beginning

We have to learn how to fill this gap

Seiwert et al. Lancet Oncology 2016
PD-L1 assessment

PD-L1+ immune cells

PD-L1+ tumour cells
PD-L1 testing

**Tumor Proportion Score (TPS)**

TPS is calculated by dividing the number of tumor cells showing partial or complete PD-L1 membrane staining by the total number of all viable tumor cells present in the sample x 100.

The score is reported as a percentage.  

\[
\text{TPS} = \frac{\# \text{PD-L1 positive tumor cells}}{\text{Total \# viable tumor cells}} \times 100
\]

**Combined Positive Score (CPS)**

CPS is calculated by dividing the number of PD-L1-positive tumor cells and tumor-associated immune cells (including lymphocytes and macrophages) by the total number of all viable tumor cells in the sample x 100.

The score is reported as a numeric value and not a percentage because the result may exceed 100.

\[
\text{CPS} = \frac{\# \text{PD-L1 staining cells} \text{ (tumor cells, lymphocytes, macrophages)}}{\text{Total \# viable tumor cells}} \times 100
\]
The benefit is clinically relevant in CPS $> 20$

<table>
<thead>
<tr>
<th></th>
<th>HR (95% CI)</th>
<th>$P$</th>
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<tr>
<td>Pembro alone</td>
<td>0.61 (0.45-0.83)</td>
<td>0.0007</td>
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**EXTREME**

Median (95% CI)
- 14.9 mo (11.6-21.5)
- 10.7 mo (8.8-12.8)

<table>
<thead>
<tr>
<th>Months</th>
<th>No. at Risk</th>
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<tbody>
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<td>0</td>
<td>133</td>
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<tr>
<td>5</td>
<td>106</td>
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<td>10</td>
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<td>35</td>
<td>2</td>
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<td>40</td>
<td>0</td>
</tr>
</tbody>
</table>

No. at Risk:
- 133
- 122
- 106
- 100
- 85
- 64
- 65
- 42
- 47
- 24
- 22
- 12
- 11
- 5
- 2
- 0
Change in target lesion from baseline by PD-L1

Patients with PD-L1 high tumours

Patients with PD-L1 low/negative tumours

Change in target lesion from baseline by PD-L1

Data cutoff: 29 April, 2016.
Pembrolizumab in NSCLC

Also linked with:
- Molecular smoking signature
- Mutations in DNA repair mechanisms
1. **IR-group: Inflamed – Responders**
   - Gamma-IFN Inflamed
   - Benefitting from anti-PD1 therapy

2. **INR-group: Inflamed – NonResponders**
   - Gamma-IFN Inflamed
   - Not Benefitting from anti-PD1 therapy
   - Given biologic signal - Can these patients be converted into responders e.g. via combinations, vaccine etc.

3. **NI-group: Non-Inflamed**
   - Very high negative predictive value
   - Not benefiting from anti-PD1 therapy
   - Clinically potentially useful: Identify patients who shoult NOT receive PD-1 therapy
   - Unclear whether non-inflamed phenotype can be converted into inflamed phenotype
THANK YOU!

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