MOLECULAR BIOLOGY, BIOMARKERS, AND TCGA DATA

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DISCLOSURE OF INTEREST

Advisory role: MSD (uncompensated), Innate, Debio, Astra-Zeneca, Nanobiotix

Research grants: Novartis, Janssen.
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

The Cancer Genome Atlas
# 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>
</tr>
</thead>
<tbody>
<tr>
<td>Cell cycle</td>
<td>CDKN2A</td>
<td>p16(^{INK4A})</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>
<td></td>
<td>CCND1</td>
<td>G1–S-specific cyclin D1</td>
<td>Oncogene</td>
<td>0.6</td>
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<td>Growth signals</td>
<td>EGFR</td>
<td>EGFR</td>
<td>Oncogene</td>
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<td>Survival</td>
<td>PIK3CA</td>
<td>Catalytic p110(\alpha) subunit of class 1 PI3Ks</td>
<td>Oncogene</td>
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<td>21</td>
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<td>PTEN</td>
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<td>Tumour suppressor</td>
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<td>4</td>
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<td>WNT signalling</td>
<td>FAT1</td>
<td>Protocadherin FAT1</td>
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<td>AJUBA</td>
<td>LIM domain-containing protein AJUBA</td>
<td>Tumour suppressor</td>
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<td>NOTCH1</td>
<td>NOTCH1</td>
<td>Tumour suppressor</td>
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<td>Epigenetic</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>regulation</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 Ref. 5. Mutation data were taken from The Cancer Genome Atlas (TCGA) (\(n=304\)) using the CBioportal. CNA, copy number alteration; EGFR, epidermal growth factor receptor. *Putative passenger mutation that requires further functional studies.

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?
Amplified in 30%
Inactivated in 90%
Mutated in 70%

HPV negative

CDK inhibitors: Palbociclib (LEE011, LY2835219)

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 status for oro pharyngeal cancer

RTOG 0129

TROG 02.02

HR = 0.36, P = 0.004
2-y OS: 74% & 91%

Hazard ratio 95% CI

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

Carole Fakhry et al. JCO 2014;32:3365-3373
Prognosis: p16 and recurrent oropharyngeal cancer

- Locoregional relapse
- Distant metastases
- Salvage surgery
- No salvage surgery

Fakhry et al. J Clin Oncol 2014
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
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
Cetuximab vs 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 responses were seen in these patients.

Courtesy of A. Pirzkall, Oncology Biomarker development, Genentech

Penuel et al, AACR 2015
Radiotherapy +/- cetuximab: stage 3 and 4

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 positive as a predictive biomarker?

Rosenthal, J Clin Oncol 2016

- **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)
Panitumumab + RT versus chemoradiation

Concert 2: Chemoradiation vs radiotherapy plus panitumumab

Mesia et al, Lancet Oncology 2015
Giralt et al, Lancet Oncology 2015
Panitumumab + RT versus chemoradiation

Concert 2: Chemoradiation vs radiotherapy plus panitumumab

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<th>2-Y survival</th>
<th>Chemoradiation</th>
<th>RT+ panitumumab</th>
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<td>71%</td>
<td>63%</td>
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Mesia et al, Lancet Oncology 2015
Giralt et al, Lancet Oncology 2015
Panitumumab + RT vs chemoradiation

NCIC CTG HN6
Stage III/IV

Conventional Radiation (70 Gy in 7 weeks)
Cisplatin 100 mg/m2 Day 1, 22, 43
N=160

Primary endpoint = PFS

Accelerated Radiation (70 Gy in 6 weeks)
Panitumumab 9 mg/kg one week before RT and on days 15 and 36
N=160

L Siu et al. ASCO 2015
Panitumumab + RT vs chemoradiation

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L Siu et al. ASCO 2015
This supports the investigation of treatment de-escalation in favorable HPV positive by replacing chemotherapy with anti-EGFR mAbs
Maybe, cetuximab or anti-EGFR mAb could be useful in p16+ oropharyngeal cancer in combination with RT: de-intensification strategy.

favored HPV positive by replacing chemotherapy with anti-EGFR mAbs.
### Who is going to response? HPV or p16 no clear answer

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<th>Population</th>
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<td>Rosenthal ASCO 2014</td>
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<td>Recurrent/metastatic: first-line</td>
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<td>p16+ : HR: 1</td>
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<td>Machiels Lancet Oncol 2015</td>
<td>Afatinib</td>
<td>Recurrent/metastatic second-line</td>
<td>p16- : HR: 0.69 (PFS)</td>
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<td>Methotrexate</td>
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<td>p16+ : HR: 0.95</td>
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Who is going to response? HPV or p16 no clear answer
MEHD7945A

EGFR  HER-2  HER-3  HER-4

Lapatinib

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

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<td>p16+ = 57; p16-</td>
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<td>FU/Panitumumab</td>
<td>p16- = 179</td>
<td>first-line</td>
<td>p16+: HR: 1</td>
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<td>Platin/5-FU</td>
<td>p16+ = 42; p16-</td>
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<tr>
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<td>p16- = 165</td>
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<td>Afatinib</td>
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<td>Recurrent/metastatic:</td>
<td>p16- : HR: 0.69 (PFS)</td>
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<td>Methotrexate</td>
<td>p16- = 141</td>
<td>second-line</td>
<td>p16+: HR: 0.95</td>
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<td>p16+ = 11; p16-</td>
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- 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
Genes encoding tumor antigens:
1. mutated
2. cancer-germline (MAGE)
3. viral
4. differentiation
5. over-expressed

T cell

T lymphocytes

anti-tumor

Tumor cell

tumor antigens

TCR

peptide

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

Antigens resulting from mutations

Non-synonymous mutations
Vogelstein et al. Science 2013
The cancer-immunity cycle

- Priming and activation (APCs and T cells)
- Cancer antigen presentation (dendritic cells/APCs)
- Release of cancer cell antigens (cancer cell death)
- Infiltration of T cells into tumours (CTLs, endothelial cells)
- Recognition of cancer cells by T cells (CTLs, cancer cells)
- Killing of cancer cells (immune and cancer cells)
- Trafficking of T cells to tumours

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
Tumors use complex, overlapping mechanisms to evade and 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

IC, immune cells; PD, progressive disease; TCGA, The Cancer Genome Atlas.
## Objective response rate

- **PD-L1 high** = ≥25% of tumour cells with membrane staining
- **PD-L1 low/negative** = <25% of tumour cells with membrane staining

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>PD-L1 high</th>
<th>PD-L1 low/negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RECIST response (ORR), n/N (%)</strong></td>
<td>7/62 (11)</td>
<td>4/22 (18)</td>
<td>3/37 (8)</td>
</tr>
<tr>
<td>95% CI</td>
<td>4.7–21.9</td>
<td>5.2–40.3</td>
<td>1.7–21.9</td>
</tr>
<tr>
<td><strong>DCR 12 weeks, n/N (%)</strong></td>
<td>18/62 (29)</td>
<td>7/22 (32)</td>
<td>10/37 (27)</td>
</tr>
<tr>
<td>95% CI</td>
<td>18.2–41.9</td>
<td>13.9–54.9</td>
<td>13.8–44.1</td>
</tr>
</tbody>
</table>
Change in target lesion size from baseline by PD-L1

Patients with PD-L1 high tumours

Patients with PD-L1 low/negative tumours

Data cutoff: 29 April, 2016.
Pembrolizumab in NSCLC

Also linked with:
- Molecular smoking signature
- Mutations in DNA repair mechanisms

Rizvi et al. Science 2015
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 shout NOT receive PD-1 therapy
   - Unclear whether non-inflamed phenotype can be converted into inflamed phenotype
Take home messages

• p16+ oropharyngeal has a better outcome: de-escalation ongoing but NOT STANDARD today? Cetuximab?

• EGFR is prognosis

• Unfortunately, we are missing validated biomarkers and the treatment is still based on disease stage, tumor location, and centre expertise and not on tumor biology