Biomarkers for Head and Neck Cancer: A Look to the Future

Ranee Mehra, MD
Attending Physician
Fox Chase Cancer Center Partners
Philadelphia, PA
Disclosure

Dr. Mehra has the following relevant financial relationships with commercial interests to disclose:
• Spousal Employment (not relevant to content): GlaxoSmithKline
Overview

- DNA repair biomarkers
  - ERCC1
  - RAD51
  - XPF
  - XRCC1
- Aurora Kinase
- Disruptive p53 mutations
DNA Repair Biomarkers
Nucleotide excision repair

• Responsible for repair of helix distorting lesions through multi-step mechanism of excision of damaged DNA, followed by repair via DNA synthesis

• Repair of UV photodimers
Platinum and NER

- Platinum compounds induce cytotoxic effects by binding to DNA molecules in the form of platinum DNA Adducts.
- The intrastrand and interstrand DNA adduct interferes with DNA transcription and replication.
- Nucleotide excision repair (NER) is responsible for resistance by increasing platinum DNA adduct removal.
- Excision repair cross complementing group 1 (ERCC1) gene has the leading role in the NER pathway because of damage recognition and excision ability.
Analysis of ERCC1 in SCCHN

- 107 patients – 96 samples analyzed
- Locally advanced SCCHN
- All received induction cisplatin/5FU followed by RT
Percentage and intensity of immunostains

## Results

<table>
<thead>
<tr>
<th>H score</th>
<th># patients (%)</th>
<th>Odds ratio (response)</th>
<th>Median survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>68 (71%)</td>
<td>1</td>
<td>27.7 m</td>
</tr>
<tr>
<td>&lt;3</td>
<td>28 (29%)</td>
<td>2.9 4.3</td>
<td>40.5 m</td>
</tr>
</tbody>
</table>

Hazard ratio for cancer death: ERCC1 score 0.42
63% patients overall had an objective response
ERCC1 in SCCHN

- 34 patients with locally advanced SCCHN
- Treated with 3 cycles of docetaxel and cisplatin, followed by definitive radiotherapy with concurrent cisplatin.
- Evaluated the tissue for ERCC1 expression by standard IHC
- ERCC1 high expression in 65% of patients
- Those who were classified as being ERCC1 positive had a decreased progression-free survival compared to ERCC1-negative patients (p = .03)

# Predictive value of ERCC1 IHC

<table>
<thead>
<tr>
<th>Study</th>
<th>Primary</th>
<th># Patients</th>
<th>ERCC1 high %</th>
<th>Treatment</th>
<th>HR (ERCC1 – death)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handra-Luca et al.</td>
<td>SCCHN</td>
<td>107</td>
<td>71%</td>
<td>PF</td>
<td>0.42</td>
</tr>
<tr>
<td>Jun et al.</td>
<td>SCCHN</td>
<td>45</td>
<td>73%</td>
<td>Cisplatin-RT</td>
<td>0.12</td>
</tr>
<tr>
<td>Fountzilas et al.</td>
<td>SCCHN</td>
<td>34</td>
<td>65%</td>
<td>Induction then cisplatin-RT</td>
<td></td>
</tr>
</tbody>
</table>
AQUA analysis of ERCC1

 Representative spot:
 AQUA = 1696.393
Analysis of 5 FCCC TMA’s

• ERCC1 antibody (8F1, Lab Vision) was utilized to study protein expression by IHC with AQUA

• TMA’s were constructed from SCCHN tissue from the Fox Chase Cancer Center Biosample Repository.
  – Tissue was collected from 1990-2002 at Fox Chase Cancer Center; all were surgical specimens.

• Clinical data was annotated for treatment and outcome.

• ERCC1 expression levels were determined in the tumor subcellular compartments
  – tumor mask, cytoplasm and nuclear compartment.

• The 30th percentile was determined to be the cutpoint.
  – High and low ERCC1 expression was correlated with survival and T stage.
## Patient Characteristics

<table>
<thead>
<tr>
<th>Primary Site</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral cavity</td>
<td>36</td>
</tr>
<tr>
<td>Tongue</td>
<td>34</td>
</tr>
<tr>
<td>Tonsil</td>
<td>5</td>
</tr>
<tr>
<td>Glottic</td>
<td>15</td>
</tr>
<tr>
<td>Retromolar Trigone</td>
<td>10</td>
</tr>
<tr>
<td>Pyriform Sinus</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gender</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Female</td>
<td>39</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AQUA score in (nuclear)</th>
<th>30th percentile</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>262.37</td>
<td>0 – 2774.64</td>
</tr>
</tbody>
</table>

- 76/109 patients received adjuvant RT
- 16 received chemotherapy during treatment course (7 patients received concurrent chemo-RT).
### AQUA scores

#### Nuclear AQUA scores

<table>
<thead>
<tr>
<th>ERCC1</th>
<th>Cytokeratin</th>
<th>DAPI</th>
<th>combination</th>
<th>IHC-DAB</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
<tr>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
</tbody>
</table>

- ERCC1: 2502
- Cytokeratin: 331

**Head and Neck Cancer**

7th Annual Multidisciplinary Symposium

November 19th, 2011 • Hyatt at the Bellevue • Philadelphia, PA
Overall survival based on nuclear ERCC1 level in patients treated with surgery (n=33)
Overall survival based on nuclear ERCC1 level in patients who received surgery plus adjuvant RT (n=76)

median OS 75.6 versus 28.4 months (p=0.02)
## Correlation with T stage

<table>
<thead>
<tr>
<th>Path T stage; N(%)</th>
<th>All</th>
<th>Low Score</th>
<th>High Score</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15(17)</td>
<td>4(14)</td>
<td>11(19)</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>25(29)</td>
<td>4(14)</td>
<td>21(36)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14(16)</td>
<td>4(14)</td>
<td>10(17)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>33(38)</td>
<td>16(57)</td>
<td>17(29)</td>
<td></td>
</tr>
</tbody>
</table>

| Tumor size; mean(std) | 2.8 (1.4) | 3.1 (1.7) | 2.6 (1.3) | 0.14 |

**Path T stage; N(%)**: 1. 15(17); 2. 25(29); 3. 14(16); 4. 33(38).

**Tumor size; mean(std)**: 2.8 (1.4); 3.1 (1.7); 2.6 (1.3).
Overall survival based on nuclear ERCC1 level in patients treated with surgery (n=33)

8F1 antibody (Sigma)
Overall survival based on nuclear ERCC1 level in patients who received surgery plus adjuvant RT (n=76)
• DNA repair protein that serves in the homologous recombination pathway.
• 12 patients treated with induction chemotherapy (paclitaxel and carboplatin) followed by RT given concurrently with chemotherapy (paclitaxel, fluorouracil, hydroxyurea)
• Patients with high RAD51 levels in their pre-treatment tumor biopsies had worse cancer-specific survival rates than patients with lower RAD51 levels (33.3% vs. 88.9% at 2 years; p=0.025).
• Normal p53 expression: non-significant trend toward better induction chemotherapy RR in the tumors with lower RAD51 levels

Nuclear staining of RAD51
Panel of DNA repair biomarkers

- Analysis by IHC of tumor from 73 patients who received induction carboplatin and paclitaxel followed by 5-fluorouracil, hydroxyurea and RT.
- Markers included ERCC1 (8F1), XPF, pMK2, PARP, MLH1, FANCD2, pH2AX, and PAR1.
- **Low levels of XPF were associated with a significant response to induction therapy.**
- ERCC1 was not found to be significantly associated with response to induction treatment. In spite of the small sample in the study, there was still a trend towards improved survival based ERCC1 levels (p=.085).
- Biomarker was analyzed individually and not in multiplexed analyses.
- Definitive chemoradiation treatment did not include cisplatin.

Seiwert et al. *J Clin Oncol* 26: 2008 (May 20 suppl; abstr 6003)
XPF

- XPF is one subunit of the ERCC1-XPF complex that repairs DNA damage induced by RT or platinum compounds.
- Investigators analyzed tumors from 99 patients with non-recurrent HNSCC treated with platinum compound and/or radiation therapy.
- XPF protein expression was measured in tumor sections using automated quantitative immunohistochemistry.

- XPF expression level was grouped into quartiles for evaluation of time to treatment failure (TTF) and overall survival.

Vaezi et al. J Clin Oncol 28:15s, 2010 (suppl; abstr 5520)
TTF and OS

- low XPF (median TTF, 90 months)
- Intermediate XPF (median TTF, 29 months \([p = 0.007]\))
- high XPF (median TTF, 9 months \([p=0.001]\)).

Vaezi et al. J Clin Oncol 28:15s, 2010 (suppl; abstr 5520)
XRCC1

- XRCC1 is involved in base excision repair and single strand break repair; may have role in repair of DNA damage caused by chemoradiation.
- Retrospective analysis of samples from 137 patients treated at the University of North Carolina at Chapel Hill from 2002-06
- Variety of treatments, T and N stages
- XRCC1 analyzed by IHC; median was cutpoint

Ang et al. J Clin Oncol 28:15s, 2010 (suppl; abstr 5541)
XRCC1 expression

Ang et al. J Clin Oncol 28:15s, 2010 (suppl; abstr 5541)
XRCC1 and overall survival

Ang et al. J Clin Oncol 28:15s, 2010 (suppl; abstr 5541)
p16 and XRCC1

Ang et al. J Clin Oncol 28:15s, 2010 (suppl; abstr 5541)
Aurora Kinase
Aurora kinases and mitosis

- Family of mitotic serine/threonine kinases
- Aurora A kinase - involved in centrosome maturation and separation; regulates spindle assembly and stability

## Aurora kinase and mitosis

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Gene location</th>
<th>Cell location</th>
<th>Role in mitosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aurora A</td>
<td>20q13</td>
<td>Peri-centriole Spindle pole Mitotic spindle</td>
<td>Centrosome function Mitotic spindle assembly Mitotic entry Chromosome alignment cytokinesis</td>
</tr>
</tbody>
</table>
Aurora kinase A (AURKA) expression in (HNSCC) and normal tissues

Activation of AURKA in human HNSCC

- Increased expression of AURKA in tumor tissue.
- In vitro kinase assay of AURKA from immunoprecipitates of normal (N) and tumor (T) specimens shows increased AURKA activity in tumors versus normal tissue.

Effect of AURKA mRNA levels on OS in patients with HNSCC

- 66 HNSCC tumors were analyzed for expression of Aurora kinase A with real-time quantitative reverse transcription-PCR and immunohistochemistry (34/66) (P < 0.001).

Aurora-A expression with AQUA

- Tumor tissue from 89 patients with SCCHN at the Fox Chase Cancer Center was analyzed for Aurora-A expression.
- T stage was inversely related to Aurora-A expression when adjusted for p16 status (p = 0.04).
- Aurora-A expression was not related to N stage.
- OS was 36 months for over-expressers of Aurora-A and 92 months for patients with low levels of Aurora-A expression (HR 1.9, 95% CI 1.05-3.46).
- 69 p16 negative patients: OS was 93.6 months for patients with low levels of Aurora-A expression and 35.9 months for with elevated levels of Aurora-A (HR 1.84, 95% CI 0.89-3.79).

Golemis et al.
Aurora kinase expression in p16-
Aurora kinase expression in p16+
Disruptive p53 mutations
TP53 Mutations and Survival in Squamous-Cell Carcinoma of the Head and Neck

M. Luana Poeta, M.D., Judith Manola, M.S., Meredith A. Goldwasser, Sc.D., Arlene Forastiere, M.D., Nicole Benoit, B.A., Joseph A. Califano, M.D., John A. Ridge, M.D., Jarrard Goodwin, M.D., Daniel Kenady, M.D., John Saunders, M.D., William Westra, M.D., David Sidransky, M.D., and Wayne M. Koch, M.D.

N Engl J Med
Volume 357(25):2552-2561
December 20, 2007
TP53 Mutations and Survival in Squamous-Cell Carcinoma of the Head and Neck

- TP53, the gene for the tumor-suppressor protein p53, is the most commonly mutated gene in cancer cells.
- In this study of 560 patients who had surgery for SCCHN, about half the tumors had a TP53 mutation (53%).
- The presence of mutations that could disrupt the binding of p53 to a DNA target had the strongest association with decreased survival.
- The results indicate that a disruptive mutation of TP53 is an independent risk factor for death among patients with head and neck cancer (hazard ratio, 1.7; 95% CI, 1.2 to 2.4; P=0.003).
Overall Survival

Cumulative incidence of death according to mutation category

Evaluation of disruptive TP53 Mutation in an Orthotopic Murine Model of Oral Tongue Cancer

• 48 SCCHN cell lines were characterized for p53 status

• Each cell line was used to generate an orthotopic mouse model of SCCHN

• Tumor behavior such as tumor volume, and survival were assessed based on p53 status

Survival time and tumor volume in the orthotopic xenograft model according to TP53 mutation status and level of p53 protein expression.

Conclusions

• In retrospective analyses, low levels of ERCC1, XRCC1, RAD51 and XPF in tumor samples was associated with improved outcomes
• Low Aurora Kinase A levels was also associated with improved OS and DFS
• Disruptive p53 is associated with decreased survival (biomarker to be evaluated in planned ECOG study) and a more aggressive tumor phenotype
• Need for prospective validation with biomarker driven clinical trials