Molecular Profiling in Head and Neck Squamous Carcinoma

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Disclosures

- Paid consultant for Merck, Amgen, Castle Biosciences, Prometheus Laboratories
- Travel grant from Novartis, Bristol-Myers Squibb
- Research support from Merck, Amgen
- I am also a melanoma medical oncologist, not head and neck oncologist!!
Aims
The Cancer Genome Atlas Project and what it can do for your own research/curiosity
Discuss molecular landscape of HNSC by reviewing high throughput analysis (nucleic acid sequencing) and protein expression of HNSC alone and in relation to other cancer types
- Understand inter-patient and intra-patient heterogeneity
- Primary vs. Relapsed/Metastatic HNSC
- This molecular “stuff” is research to ultimately benefit the patient, not some geeky bioinformaticians
- How the HNSC field is incorporating progress on molecular profiling for prognostic purposes and predictors of response to novel systemic treatments (targeted therapies, immunotherapies)
Cancer Genome Atlas Project (TCGA)
>30 Cancer Types, >10,000 samples total

Requirements:
- Sufficient quantity to complete all 6 platforms
- Sufficient quality (<20% necrosis)
- Sufficient tumor (>60% tumor nuclei)
- No prior treatment
- Only snap-frozen
- Sample to be processed for downstream analysis is macrodissected

Limitations
- Follow-up for overall survival is usually available from time of original diagnosis of cancer (not from the time of specimen procurement)
- Treatments (local, systemic) following specimen acquisition are not known
- There is no immunohistochemical or contextual information apart from a hematoxylin and eosin-stained picture of the processed specimen
- Same patient primary-metastatic specimens are rare
TCGA data are PUBLICLY AVAILABLE (1)

Any Reliable Hypothesis in 2016 Cancer Research Cannot be Substantiated Unless There is Some Mining of TCGA Datasets

The Difficult Route (you need a bioinformatician!)
TCGA data are PUBLICLY AVAILABLE (2)

The Easy Route (you can do it yourself)

But if you do not like the output you cannot change it!

Cbioportal.org
An Example of How cBioportal (the Easy Way) Can Help Answer Your Questions in a Powerful Fashion

Research Question: Does any of the genes that were found to be significantly altered in the HNSC TCGA correlate, or anti-correlate, with density of tumor-infiltrating CD8 cells?

Cbioportal Visuals/Output

Cbioportal Biostatistics
- Significant positive correlation between CD8A expression and NOTCH1, KMT2D
- Significant positive correlation between CD8B expression and KMT2D
- Significant anticorrelation between CD8A/CD8B expression and CDKN2A

N=496 tumor samples
Genetic Alterations in HNSC
There are **Log-scale** Differences in Total Somatic Mutation Burden Across Different Cancers
Intratumoral Heterogeneity in HNSC
Not just a Bioinformatical Finding

Burrell Nature 2013

Morris Oncotarget 2016
Mutational Landscape Across 12 Major Cancer Types

- Oncogenes >> Tumor suppressor Genes
  - TP53 42%
  - PIK3CA >18%

- Chromatin Remodeling
  - MLL2/KMT2D
  - MLL3/KMT2C

- Methylation
  - DNMT3A

- Cancer Type-Specific
  - VHL (renal)
  - APC (colorectal)
  - PTEN/PIK3R1 (uterine corpus)
  - DNMT3A/NPM1 (leukemia)

- HNSC
  - MLL2
  - NSD1
  - TP53
  - CDKN2A
  - PIK3CA
  - NOTCH1

Kandoth Nature 2013
Cancer Genome Atlas in HNNC

**Untreated**, n=279, male (73%), smokers (81%), few HPV+ (14%), oral cavity/larynx (62%/26%)

Preferentially mutated genes in HPV- vs. HPV+ cancer (*)
High genomic instability (recurrent focal gene copy number alterations, *)

**Known prognostic factors**

- **HPV(-) n = 243**
- **HPV(+) n = 36**

**Unknown prognostic factors**

TCGA Investigators Nature 2015
Genetic Aberrations In Recurrent/Metastatic HNSCC
Implications for Clinical Decisions

N=19 Same-Patient

N=53, more HPV+ and recurrent (MSK-IMPACT)

HPV+ metastatic/recurrent could evolve like HPV-
Targeted therapies in Head and Neck Cancer
Will they be developed with more rational biomarkers other than TP53, HPV?

**Everolimus**

- No PIK3CA mutations!

Geiger Head & Neck 2016

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**Buparlisib + Paclitaxel (n=71)**

- No PIK3CA mutation analysis reported!

Soulieres (abstr 6008) ASCO 2016

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**Buparlisib (n=79)**

- Median OS, months: 10.4
- Hazard Ratio (90% CI): 0.72 (0.58–0.92)
- One-sided p-value*: 0.041

**Placebo (n=79)**

- Median OS, months: 6.5
- Hazard Ratio (90% CI): (0.49–1.94)

Protocol-specified criteria for success were:
HR $\geq 0.77$ and posterior probability of (HR $< 1$) $> 80.0\%$

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**Michel (abstr 6043) ASCO 2015**

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**Study Schema:**

- Imaging p16 Status:
  - Cycle 1 D1, D8, D15
  - Cycle 2 D1, D8, D15
  - Imaging (RECIST 1.1)

- Palbociclib:
  - Dose level 1 = 100 mg/d; dose level 2 = 125 mg/d

- Cetuximab Infusion:

**Treatment Response Summary**:

1. Best Treatment Response: 2 PR and 6 SD
2. Mean TTP = 112 days (range: 28-224)
Expression Profiling in HNSC
Classification of HNSC According to Gene Expression Profiling
A UNC-CH First!

1: Basal-like (EGFR signaling)
2: Mesenchymal (EMT transition)
3: Atypical (low EGFR signaling)
4: Classical (oxid stress, xenob metab)

RNA subtypes are prognostic!

And correlate with disease site.

Especially for non-HPV positive patients.

Chung Cancer Cell 2004
TCGA Investigators Nature 2015
Walter PLoS One 2013
Clinical Utility of Gene Expression Profiling in HNSC

Clinical Questions
A. 20% of T1/T2 cancers of tongue have pN+ at the time of neck dissection
   • How to avoid unnecessary surgery in 70% of patients who are destined to have pN- disease?
B. Predict innate radioresistance (add chemotherapy?)
   • Classical subtype?
   • TP53, CCND1, RAS, BCL2 mutations?

How to develop a molecular diagnostic that will define high-risk signature?
• Validate gene-expression tumor subtyping diagnostic assay (reduce the 800-gene classifier to < 100 genes)
• Targeted gene panel sequencing

UNC-CH Trial Proposal (Hayes, Zevallos)

But the Biology of Oral Cavity Cancers is Diverse and Prognostically Heterogenous

TCGA Investigators Nature 2015
HPV subgroups are prognostically heterogeneous and may differentially respond to therapies.

- Basal category includes hypoxia genes (true hypoxia? EGFR signaling? MYC ampl)
- Immune response can occur in HPV negative tumors (how do we make it better?)
- Do certain RNA subgroups predict better response to certain therapies (chemotherapies, immunotherapies)?
- Are we looking for an OncotypeDx type of assay for HNNC?
Protein Expression in HNSC Tissues
(or rather multiplatform?)
Biology Has to be Considered in Systemic Treatment Selection in 2016

Because we will Bankrupt the Health Care System!

LUX-Head & Neck 1 Example; A Drug with Borderline Clinical Benefit, but Better for Some

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>No. of patients</th>
<th>Percentage of total, n/N (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Afatinib</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>EGFR amplified</td>
<td>50</td>
<td>16</td>
</tr>
<tr>
<td>EGFR non-amplified</td>
<td>53</td>
<td>27</td>
</tr>
<tr>
<td>PTEN &gt;150</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>PTEN ≤150</td>
<td>82</td>
<td>33</td>
</tr>
<tr>
<td>HER3 &gt;50</td>
<td>64</td>
<td>26</td>
</tr>
<tr>
<td>HER3 ≤50</td>
<td>49</td>
<td>17</td>
</tr>
<tr>
<td>p16-positive</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>p16-negative</td>
<td>135</td>
<td>64</td>
</tr>
<tr>
<td>VeriStrat®: good</td>
<td>127</td>
<td>69</td>
</tr>
<tr>
<td>VeriStrat®: poor</td>
<td>70</td>
<td>35</td>
</tr>
</tbody>
</table>

*Percentage based on total patients with specific biomarker available

Cohen abstr 6023 ASCO 2015
Machiel Lancet Oncol 2015
Rigorous Immunohistochemical Test Development For Various Targets May Refine Use of Different Chemotherapies in HNSC

Academia-Industry Partnership (Caris Life Sciences), n=735
- NGS of 47 genes with MiSeq (Illumina TruSeq)
- ISH for 5 genes (cMET, EGFR, HER2, PIK3CA, TOP2A)
- IHC for 24 proteins

<table>
<thead>
<tr>
<th>Test</th>
<th>Modality (IHC/ISH/NGS)</th>
<th>Alteration</th>
<th>Interpretation</th>
<th>Drug associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGP</td>
<td>IHC</td>
<td>0+ 100% (intensity and cell staining)</td>
<td>Negative</td>
<td>Benefit from taxanes (docetaxel, paclitaxel, and nab-paclitaxel)</td>
</tr>
<tr>
<td>SPARC</td>
<td>IHC</td>
<td>2+ 35% (intensity and cell staining)</td>
<td>Positive</td>
<td>Use nab-paclitaxel</td>
</tr>
<tr>
<td>TLE3</td>
<td>IHC</td>
<td>2+ 35% (intensity and cell staining)</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>TS</td>
<td>IHC</td>
<td>1+ 1% (intensity and cell staining)</td>
<td>Negative</td>
<td>Benefit from fluoropyrimidines (fluorouracil, pemetrexed)</td>
</tr>
<tr>
<td>TOP01</td>
<td>IHC</td>
<td>2+ 40% (intensity and cell staining)</td>
<td>Positive</td>
<td>Benefit from camptothecin Derivatives (topotecan, irinotecan)</td>
</tr>
<tr>
<td>TOP2A</td>
<td>IHC</td>
<td>1+ 2% (intensity and cell staining)</td>
<td>Negative</td>
<td>Lack of benefit from TOP2A-targeted agents (doxorubicin)</td>
</tr>
<tr>
<td>APC</td>
<td>NGS</td>
<td>L1129S</td>
<td>Variant of unknown significance</td>
<td>Clinical trials</td>
</tr>
<tr>
<td>TP53</td>
<td>NGS</td>
<td>R213X</td>
<td>Pathogenic mutation</td>
<td>Clinical trials</td>
</tr>
</tbody>
</table>

Abbreviations: IHC, immunohistochemical; ISH, in situ hybridization; NGS, next-generation sequencing; PGP, permeability glycoprotein; SPARC, secreted protein acidic and rich in cysteine.

Feldman Head & Neck 2016
The One Biomarker-One Platform Approach May NOT be Enough Even for Predicting Response to Immunotherapies

Multi-Platform Approach to Develop Predictive Biomarkers of Response; EAY161 Study (PI: Moschos)

Immunoscore® HalioDx

Primary objective: Assess Antitumor Response Rate at Week 12 by RECIST 1.1 Criteria for each Cancer Type and as an Entire Cohort. Correlate Response Rate with Immunoscore® and PD-L1 IHC 28-8 PharmDx Status

Archived FFPE option
1. H&E stain (GG and Lymphocyte Density-H&E)
2. Immunoscore® (plus control slide)
3. PD-L1 IHC 28-8 PharmDx Test (plus control slides)
4. Multiplex IF Vectra® Panels (plus control slides)
5. WES (plus H&E for guide to macrodissect and backup slides)
6. RNAseq (plus H&E for guide to macrodissect and backup slides)
7. Backup slides for studies 2 thru 6

Fresh Biopsy option
1. DC, guide slide
2. 4-micron-test slide
3. 10-micron-test slide
4. Backup slide
5. Core 3, snap-frozen
6. Core 4 OR 5, snap-frozen or paraffin BACKUP

Immunoscore® HalioDx

Multiparameter Immunofluorescence

Courtesy Ignacio Wistuba MD
MD Anderson Cancer Center
Conclusions

• Cancer follows the Anna Karenina Principle of Success, or Lack of, within families

• “Happy families are all alike; each unhappy family is unhappy in its own way”

  Lev Nikolayevich Tolstoy

• Much like family therapy counseling aims to understand “where is the dysfunction”, cancer patients and clinicians have the right to know before they are treated

• Investment in a good biomarker saves lives and prevents health care bankruptcy

• There are dysfunctions in multiple levels in HNSC (genetic aberrations, expression profiling, protein expression) and the more we know the better we treat and predict

• We can no longer afford the “if you have a hammer every problem looks like a nail” approach
Acknowledgements

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