Harnessing Molecular Pathways in Cancer: A New Paradigm in Urothelial Carcinoma

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Overall survival in patients with locally advanced/metastatic UC with chemotherapy

Von der Maase et al, JCO 2005

Targeted Therapy in Solid Tumor Oncology

Tumor Type: TUR, cystectomy, metastatic biopsy

Peripheral Blood DNA

NSCLC

CRC

RCC

Medullary Thyroid Cancer

Breast Cancer

Melanoma

Personalized Medicine in Bladder Cancer

The Promise of Targeted Therapy

Pre-treatment 15 weeks of PLX4032 (vemurafenib)

Wagle et al, J Clin Oncol 2011; 29(22): 3085-96

Mutations in Bladder Cancer: Correlation with Grade and Stage

Genetic Alterations in Bladder Cancer

Knowles MA. Carcinogenesis 2006
Bladder Cancer Oncogenome Project

- An integrated genomics approach to define the molecular aberrations that characterize bladder cancer
- Identify prognostic and/or predictive molecular biomarkers
  - Low grade versus high grade tumors
  - Primary versus metastatic lesions
  - Likelihood of recurrence
- Identify novel therapeutic targets

The Landscape of Genomic Alterations in Bladder Cancer

The Spectrum of Copy Number Alterations Across Cancer Types

MAPK Pathway Alterations in Bladder Cancer

Cell Cycle Pathway Alterations in Bladder Cancer
**Pl3K/Akt Pathway Alterations in Bladder Cancer**

**Predictive Biomarkers of Response to Everolimus in Bladder Cancer: An N of 1 Approach**

**Phase II Trial of Everolimus (RAD001) in Advanced Bladder Cancer**

45 patients enrolled with 37 eligible for primary endpoint
Median PFS: 2.2 mo (95% CI 1.3, 3.2)
Median OS: 9.8 mo (95% CI 7.8, 12.2)
2 partial responses: 10% and 94% reduction in target lesions

**N of 1 Paradigm in Bladder Cancer**

- 73 year old woman with metastatic bladder cancer
- Achieved a complete response to everolimus on MSKCC protocol 08-123
- The patient remains on drug with no evidence of disease 36 months after beginning treatment
- This patient was one of only 2 patients who responded to drug

**Why did this patient respond so well to this targeted inhibitor?**

**N of 1 Paradigm in Bladder Cancer**

- Cystectomy
- PBMCs
- Paired-end sequencing 2x100 bp mate pairs

**Candidate mutations mediating sensitivity to mTOR pathway inhibition?**

- TSC1 c.615T>C
- PI3KCA c.868A>T
- PIK3CA c.2410G>A
- AKT1 c.1076G>A
- NFKBIA c.286C>T
TSC1 and NF2: A Possible Basis for Everolimus Sensitivity

Exon Capture in Solution by Hybrid Selection (Agilent SureSelect)

Advantages of Targeted Sequencing

More comprehensive than Sequenom
- Entire exons rather than pre-specified hot spots
- Suitable for tumor suppressor genes as well as oncogenes
- Copy number alternations and select rearrangements
- Greater sensitivity for low allele frequency events

More practical than whole genome, whole exome
- Works for frozen and FFPE
- Lower DNA input (30 ng)
- Higher throughput and lower cost through multiplexing
- Smaller computational demands (processing and storage)
- Higher coverage and sensitivity in most important exons

TSC1 and NF2 alterations in bladder cancer

What is the frequency of NF2’s alterations?

What is the pattern of co-mutated genes associated with TSC1 alterations?

Exon Capture Assay

Focal Amplification of ERBB2
The Pattern of Co-Altered Genes in TSC1 Mutant Bladder Cancer

Responders vs. Non-responders by TSC1 mutation status

Conclusions
- Outlier analyses is a novel paradigm that can accelerate targeted drug development
- Next Generation technologies provide unique tools to identify actionable alterations
- mTOR inhibitor therapy possesses anti-tumor activity in bladder cancer patients with specific mutation profiles

Functional impact of TSC1 and NF2 loss in urothelial cell lines

Expansion of oncogenome project
- Prospectively collect tumor and germline tissue from all patients treated for bladder cancer
  - IRB-approved protocol (89-076)
  - Radical cystectomy +/- neoadjuvant chemotherapy
  - Transurethral resection of bladder tumor
- Establish bladder cancer “tumor bank”
  - Patients with confirmed disease at cystectomy, TUR
  - Consented for blood draw at follow up visits
- Planned analyses
  - Exon capture assay
  - Select samples for whole genome/exome analysis
  - MiSeq assay

Differences from TCGA

<table>
<thead>
<tr>
<th>BC Oncogenome</th>
<th>TCGA</th>
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<tr>
<td>All patients with bladder cancer</td>
<td>Only patients with urothelial carcinoma</td>
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<tr>
<td>Assess response/non-response to local or systemic therapy</td>
<td>No prior treatment</td>
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<tr>
<td>Tumors across all stages</td>
<td>Only muscle-invasive tumors</td>
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<td>Targeted/multiplatform analysis</td>
<td>Multiplatform analysis</td>
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The Challenge of Targeted Therapy

23 weeks of PLX4032

Pre-treatment 23 weeks of PLX4032 ( vemurafenib)

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