Molecular predictors of response and survival outcomes in patients with metastatic clear cell renal cell carcinoma (mccRCC) treated with VEGF-TKIs

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Disclosures

• None
Background

• VEGF receptor-tyrosine kinase inhibitors (VEGFR-TKIs) are the standard therapeutic option for front line treatment of patients with metastatic renal cell carcinoma

• mccRCC patients have variable clinical outcomes on treatment with targeted therapies

• Molecular markers predictive of response to therapy will allow better patient selection and guide towards more individualized therapy
Patient Characteristics

Number of Patients: 89 (all with mccRCC)

Treated at the Huntsman Cancer Institute at the University of Utah

TKI front-line therapy: n=77 (objective response: n=65)

Long-term TKI responders (>18m on treatment): n=15

Short-term TKI responders (<6m treated due to progression or death): n=21
Methods

DNA extraction: from “macro-dissected FFPE sections” using, Epicentre FFPE DNA Extraction Kit

Whole genome array Comparative genomic hybridization (aGCH):

• aCGH (4x180K, Agilent Technologies, Inc.) with differential gain/loss assessed using Nexus Copy Number Analysis (Biodiscovery, Inc.) [n=88]
  (Differential gain/loss considered significant at p<0.05 for >25% difference for weighted average frequency (WAF)

Next Generation Sequencing (NGS):

• Custom-designed capture-based panel (Nimblegen), sequenced on a Miseq (Illumina, Inc.) with alignment and variant detection using CLC Biomedical Genomics Workbench with annotation using Wannovar [n=87]

  Median coverage = 368x (range 187x-1337x), Target Drop-out (>100x for >90% target) = 1-2%
  Variants considered: >5% allele variant frequency (AVF) in coding regions
  Nonsynonymous SNVs, indels
  SNPs at <0.0.5% in ExAC or 1000KGenomes

Significance between LTR and STR tested using Chi square test

Association with PFS or OS tested using the log rank statistic and plotted using the Kaplan Meier method
Comparative analyses on array CGH indicate,

- LTR (n=15) vs. STR (n=21)
  - 9 genomic loci more frequent in LTR, 1 in STR
- CR, PR, SD (n=45) vs. PD (n=20)
  - 17 genomic loci more frequent in CR, PR, SD
- CR, PR (n=26) vs. SD, PD (n=39)
  - 8 genomic loci more frequent in CR, PR

One gain and one loss common to all 3 analyses (more frequent in LTR)

Two losses common to LTR vs STR and one of the other analyses (more frequent in LTR)
Custom RCC Next Generation Sequencing Panel

Targets included for diagnostic, prognostic, and theranostic clinical utility in clear cell renal cell carcinoma

<table>
<thead>
<tr>
<th>Frequently Mutated Genes</th>
<th>Mutated Genes Mapping to Regions of Genomic Imbalance</th>
<th>Targets for FDA-Approved Drugs</th>
<th>Prognostic SNPs</th>
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</thead>
<tbody>
<tr>
<td>VHL</td>
<td>PGLYRP3</td>
<td>BRAF</td>
<td>rs3834129 (CASP8)</td>
</tr>
<tr>
<td>PBRM1</td>
<td>BRINP2</td>
<td>RAF1</td>
<td>rs9582036 (VEGFR1)</td>
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<tr>
<td>SETD2</td>
<td>UBE2D1</td>
<td>EGFR</td>
<td>rs1332018 (GSTM3)</td>
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<td>BAP1</td>
<td>PTEN</td>
<td>ERBB2</td>
<td>rs7121 (GNAS)</td>
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<td>ARID1A</td>
<td>SFXN4</td>
<td>PDGFRB</td>
<td>rs11549465 (HIF1A)</td>
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<tr>
<td>TP53</td>
<td>CCND2</td>
<td>KIT</td>
<td>rs2057482 (HIF1A)</td>
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<tr>
<td>MTROR</td>
<td>ING4</td>
<td>FLT3</td>
<td>rs3814055 (NR112)</td>
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<tr>
<td>PIK3CA</td>
<td>RALGAPA1</td>
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<td>HIF1A</td>
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<td>rs699947 (VEGFA)</td>
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<td>rs1126647 (IL8)</td>
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<td>SPRED1</td>
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<td>rs4073 (CXCL8)</td>
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<td>TSC2</td>
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<td>rs11762213 (MET)</td>
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<td>RASSF2</td>
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<tr>
<td></td>
<td>PLUS SNPs placed every 3Mb to serve as backbone for</td>
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<tr>
<td></td>
<td>genomic gain/loss</td>
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Germline SNPs Associated with VEGF-TKI Response

Comparative analysis performed:

- LTR (n=14) vs. STR (n=21)

- FLT1 (VEGFR1) [rs9582036] ($p=0.025$)

Tested for association with VEGF-TKI PFS (n=74)
Somatic Variants and VHL Loss in 87 “metastatic” ccRCC

<table>
<thead>
<tr>
<th>VHL</th>
<th>VHL Loss</th>
<th>PBRM1</th>
<th>BAP1</th>
<th>SETD2</th>
<th>KDM5C</th>
<th>TP53</th>
</tr>
</thead>
</table>

Genomic landscape of frequently mutated genes in ccRCC according to COSMIC (>5% frequency)

- **VHL** loss and/or mutation detected in 79/87 (91%) (43% mutation rate in COSMIC)
- **PBRM1** mutations detected in 30/87 (34%) (31%)
- **BAP1** mutation detected in 23/87 (26%) (only 6 overlap with **PBRM1**) (11%)
- **SETD2** mutations detected in 20/87 (23%) (10%)
- **KDM5C** mutations detected in 18/87 (21%) (6%)
- **TP53** mutations detected in 7/87 (8%) (5%)
Somatic Variants and VHL Loss in 14 LTR and 21 STR mccRCC

Genes with variants in at least 3 specimens in either STR or LTR

- No significant association was found, but VHL mutation was enriched in LTR and TP53 in STR
Conclusions

• Several copy number alterations in metastatic ccRCC were predictive of response and outcomes to treatment with VEGF-TKIs

• 9 genomic loci were more frequent in LTR, while 1 in STR

• 17 genomic loci more frequent in CR, PR, SD, as compared to PD

• Study limitations: sample size

• Future directions: 1) further validation in an independent cohort, 2) development of a molecular signature predicting response
Acknowledgements