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

Neeraj Agarwal, MD

Associate Professor

Huntsman Cancer Institute, University of Utah, Salt Lake City, UT

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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

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Genomic Imbalance Associated with VEGF-TKI Response

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**

CNA Genomic Coordinates (hg19)*	CNA	LTR WAF (%)†	STR WAF (%)†	# Genes
chr2:187,336,028-190,827,254	Gain	60	19	24
chr3:9,384,734-45,926,839	Loss	100	71	327
chr3:56,598,114-58,663,954	Loss	100	67	25
chr3:86,942,253-89,560,080	Loss	67	29	12
chr5:86,387,177-89,619,066	Gain	62	24	17
chr10:3,259,317-4,542,161	Gain	13	48	8
chr13:54,651,604-55,500,545	Gain	27	0	5
chr13:75,768,595-77,876,659	Gain	27	0	19
chr14:58,941,997-62,235,410	Loss	67	29	27
chr14:78,277,956-78,303,795	Loss	80	43	1

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

Frequently Mutated Genes
VHL
PBRM1
SETD2
BAP1
ARID1A
TP53
MTOR
PIK3CA
TSC1

Mutated Genes Mapping to Regions of Genomic Imbalance		
PGLYRP3	PCNA	EPHB4
BRINP2	SRC	PIK3CG
UBE2D1	PCK1	MET
PTEN	CDH4	ZNF800
SFXN4	SCARB2	SMO
CCND2	AFF1	CUL1
ING4	BMPR1B	RHEB
RALGAPA1	TET2	FGFR1
HIF1A	NPNT	ZFPM2
DIO2	SOX4	NDRG1
GOLGA5	DAXX	JAK2
HSP90AA1	MAPK14	IFNB1
AKT1	DST	PTCH1
SPRED1	FYN	TNC
TSC2	ROS1	ABL1
AKT2	CARD11	RAPGEF1
AXL	RBAK	CCDC120
ALK	DFNA5	KDM5C
SMOX	GLI3	STAG2
RASSF2	GUSB	MAGEC1

Targets for FDA-Approved Drugs
BRAF
RAF1
EGFR
ERBB2
PDGFRB
KIT
FLT3

Prognostic SNPs
rs3834129 (CASP8)
rs9582036 (VEGFR1)
rs1332018 (GSTM3)
rs7121 (GNAS)
rs11549465 (HIF1A)
rs2057482 (HIF1A)
rs3814055 (NR1I2)
rs6785049 (NR1I2)
rs1054190 (SXR)
rs699947 (VEGFA)
rs833061 (VEGFA)
rs1570360 (VEGFA)
rs3025039 (VEGFA)
rs1126647 (IL8)
rs4073 (CXCL8)
rs11762213 (MET)

PLUS **SNPs placed every 3Mb to serve as backbone for genomic gain/loss**

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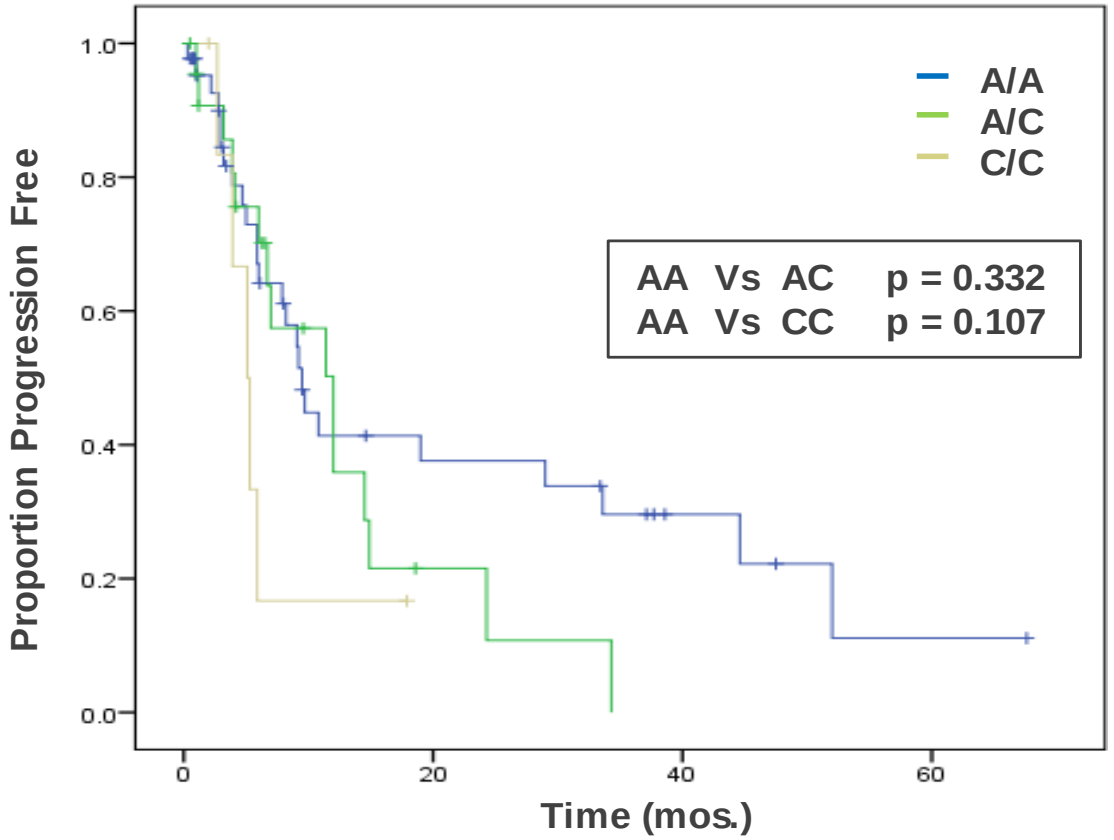
Germline SNPs Associated with VEGF-TKI Response

Comparative analysis performed:

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

FLT1 (VEGFR1) [rs9582036] (*p*=0.025)

Tested for association with TKI PFS (n=74)



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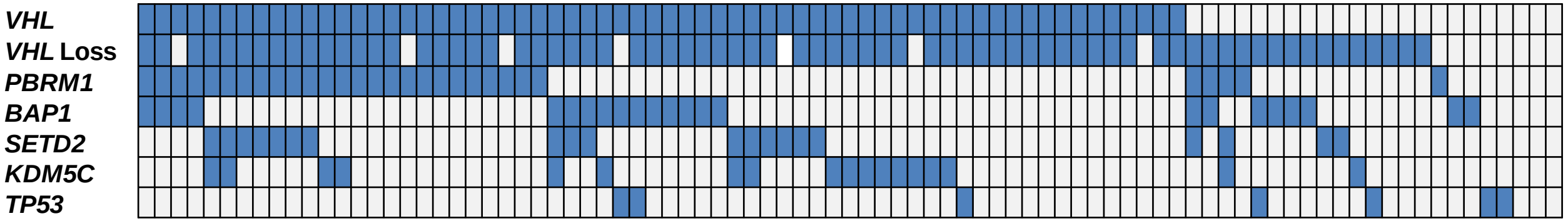
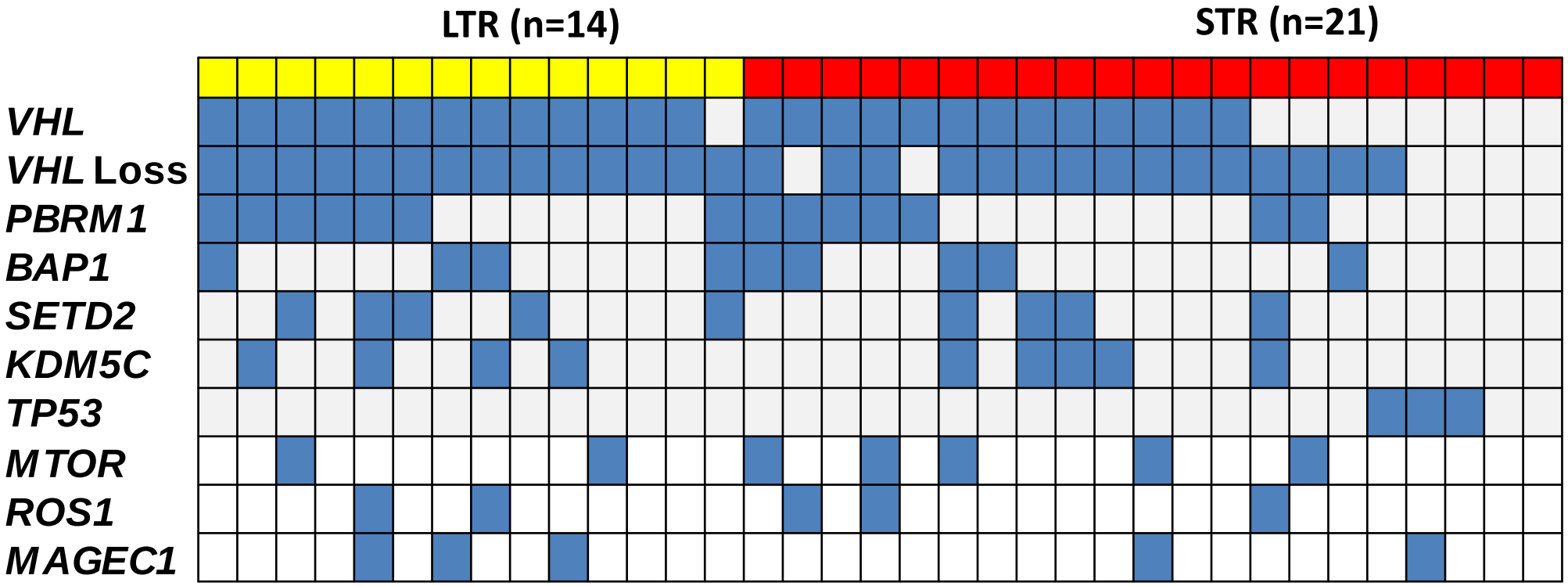
Somatic Variants and VHL Loss in 87 “metastatic” ccRCC



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**

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Conclusions

- Several copy number alterations in metastatic ccRCC were predictive of response and outcomes to treatment with VEGF-TKIs
- 9 genomic loci was 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 moelcualr signature predicting response

Acknowledgements

RUTHERFORD, NJ

CGI Headquarters
Meadows Office Complex
201 Route 17 North, 2nd Fl.
Rutherford, NJ 07070

Phone: +1 201-528-9200
Fax: +1 201-528-9235

RALEIGH, NC

Research Triangle Park
133 Southcenter Court, S.400
Morrisville, NC 27569

Phone: +1 919-465-0100
Fax: +1 919-465-0554

HYDERABAD, INDIA

#3-1-135 / 1A CNR Complex
Mallapur Main Road, R.R. Dst.
Hyderabad – 500 076, Telangana

Toll-free: +91 040-2717-8178
Fax: +91 040-2717-8176

SHANGHAI, CHINA

781 Cai Lun Road, Room 803
Shanghai 201203
P.R. China

Toll-free: +91 040-2717-8178
Fax: +91 040-2717-8176