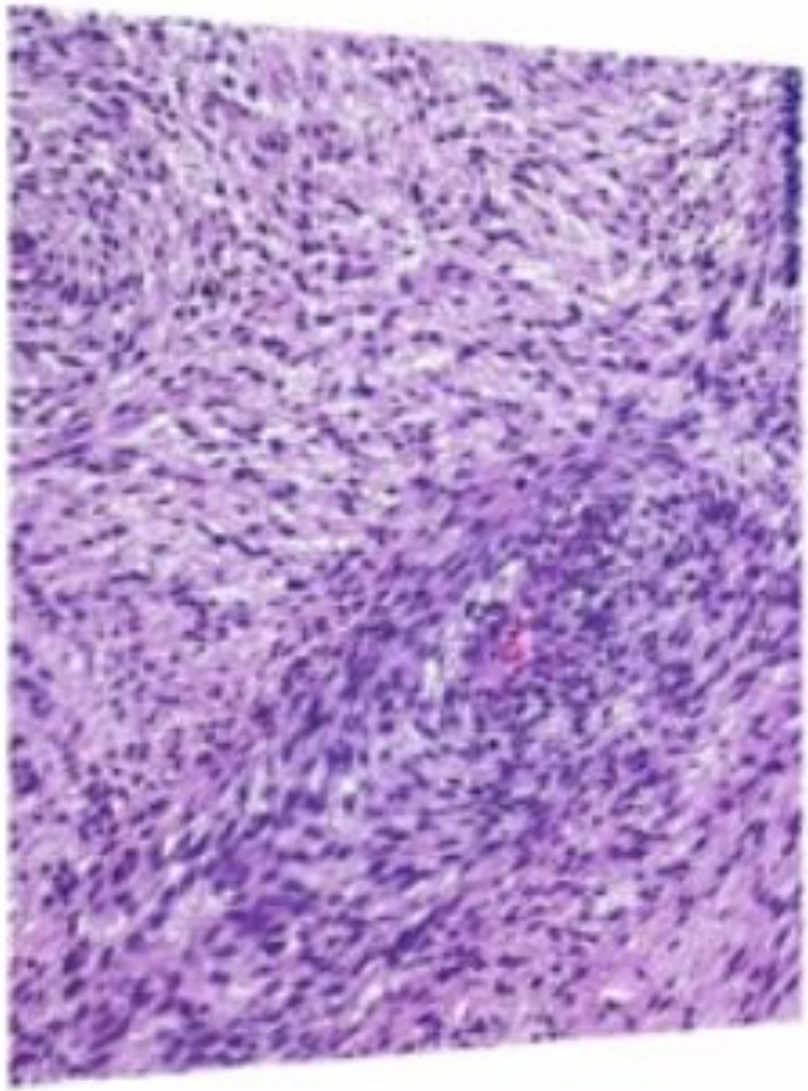
Introduction

- Sarcomatoid differentiation is observed in 5%-8% of renal cell carcinomas.
- Originates from any of the RCC histological subtypes but most common in ccRCC due to its frequent occurrence.
- The tumor has an aggressive biological behavior.
- Microscopically the tumor is characterized by a spindle cell histological appearance.

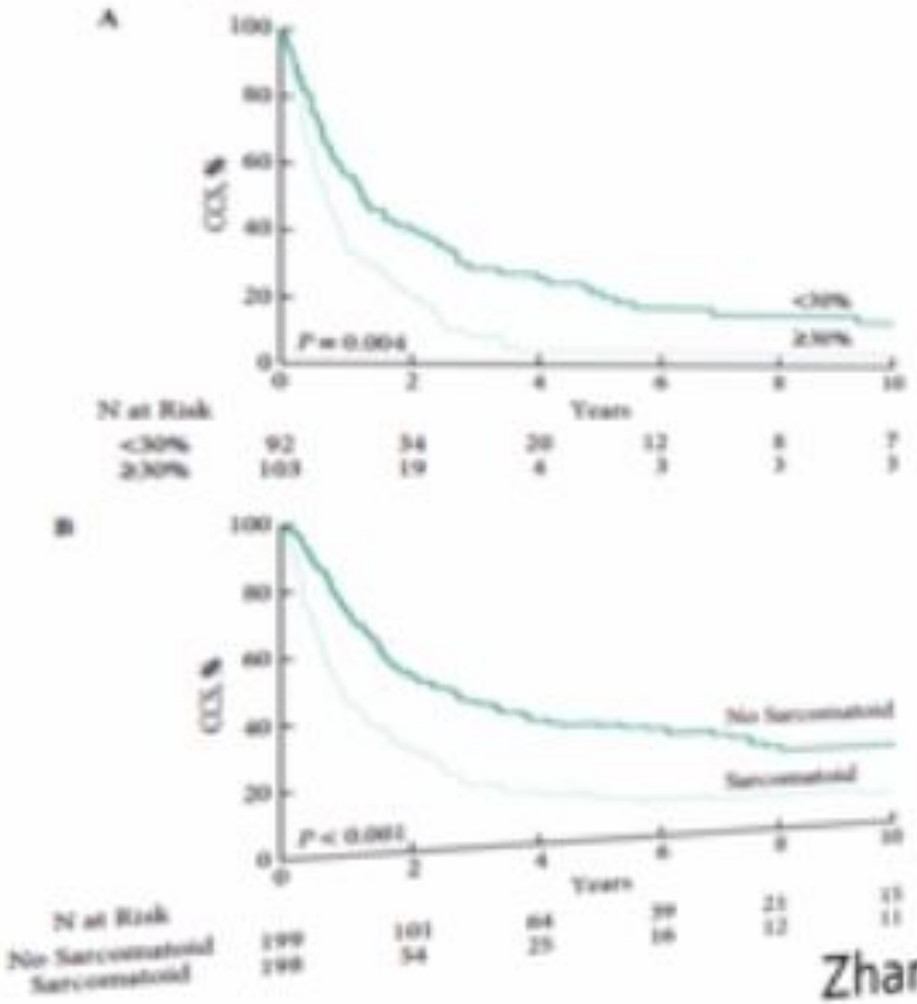


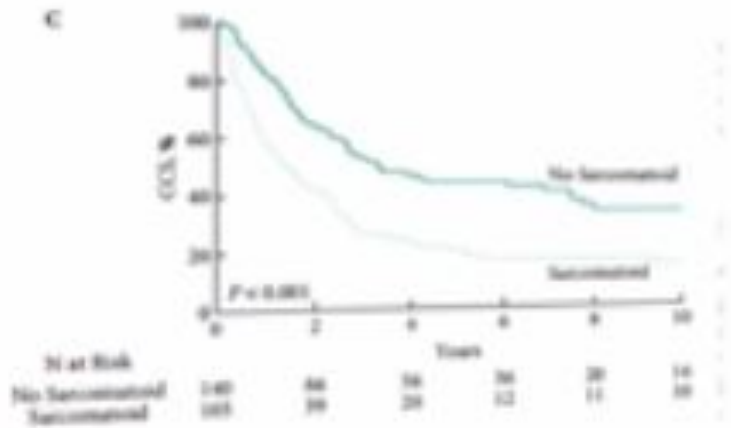
Introduction

- High risk tumor characteristics such as necrosis (90%) and microvascular invasion (30%) are common.
- Immunohistochemical staining shows evidence of epithelial and mesenchymal differentiation:
  - Cytokeratin AE1/AE3 (97%).
  - Vimentin (56%).
  - Additional mesencyhmal markers such as desmin and actin are infrequently expressed.

DeLong et al. Arch Pathol Lab Med. 1993 Jun;117(6):636-40.
Krishnan et al. Hum Pathol. 2002 Jan;33(1):68-79.

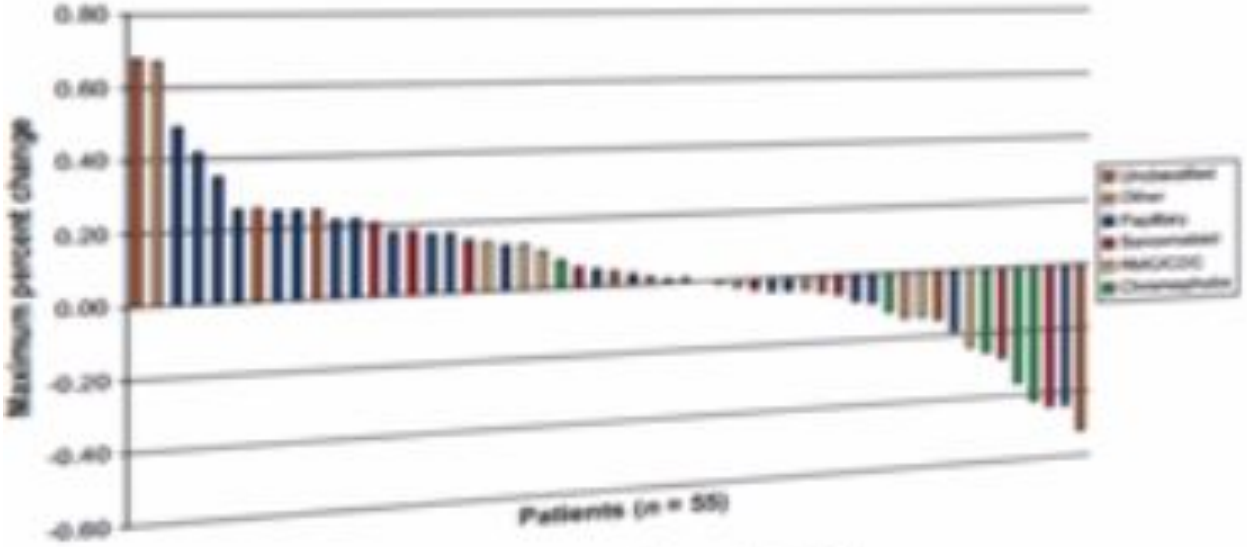
Prognostic Role



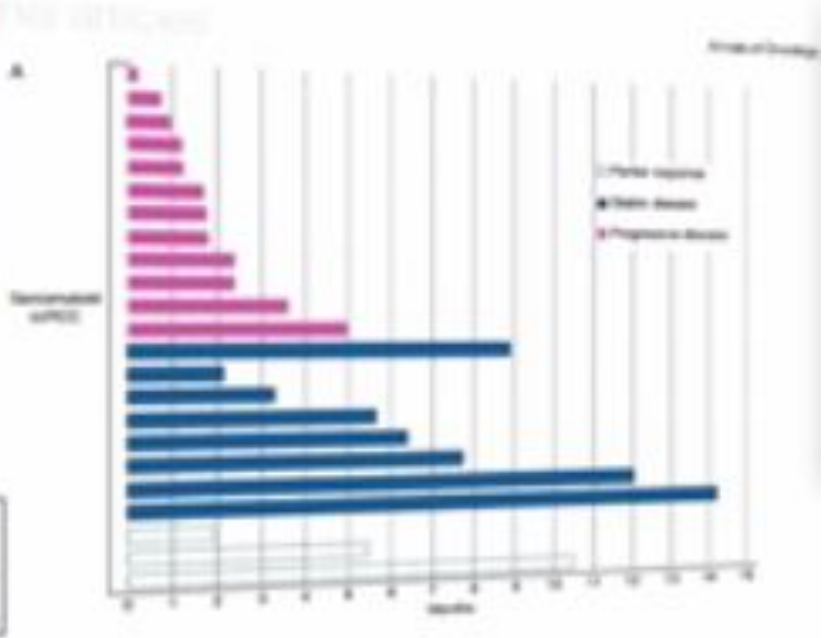


Zhang et al BJUi 2014

Some objective response to targeted agents



Tannir et al Eur Urol 2012



Voss et al Ann Onc 2014

Introduction – Molecular Characteristics

- **Prior studies** evaluating the molecular characteristics of sRCC are limited
  - Studies evaluated whole tumors rather than the sarcomatoid component alone.
  - Multiple histologies rather than a specific subtype were analyzed, complicating outcome interpretation.
  - No study to date has performed a broad search assessing the whole genome for genetic alterations in sRCC.

huch et al. Oncologist. 2012;17(1):46-54.

Introduction – Molecular Characteristics

- **Cytogenetic Alteration**
  - Initial studies showed most sarcomatoid ccRCC had a loss of chromosome 3p.
  - Comparative genomic hybridization (CGH)
    - Large number of chromosomal changes (losses > gains).
    - Common losses in 13q (75%) and 4q (50%).

Molecular Characteristics - A common origin?

- A study assessing selected allelic loss and X-chromosome inactivation in matched pairs of sarcomatoid and ccRCC showed
  - Different patterns of allelic loss in 70% of clear cell and sarcomatoid pairs.
  - Similar X-chromosome inactivation in more than 90%.

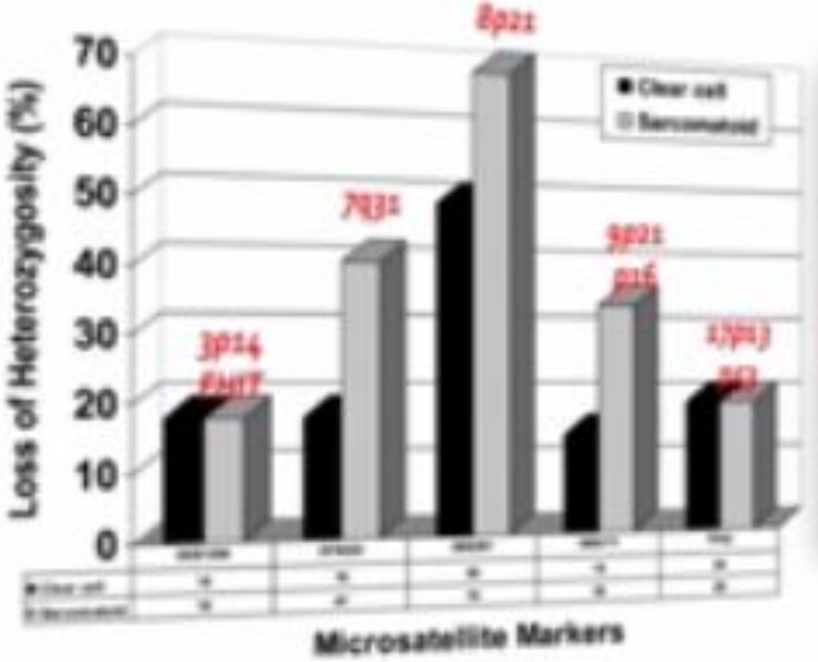
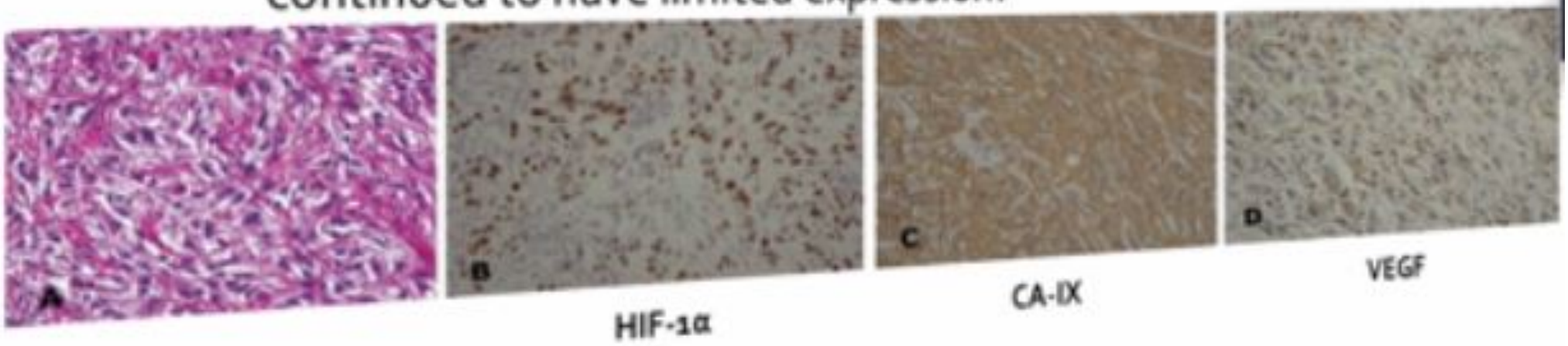


FIGURE 3. Comparison of frequency of loss of heterozygosity across five polymorphic microsatellite markers between clear cell and sarcomatoid components of renal cell carcinomas.

...es et al. Cancer. 2005 Sep 15;104(6):1195-203.

Introduction – Molecular Characteristics

- Protein Signature
  - Sarcomatoid tumors arising from ccRCC maintain high HIF pathway expression including HIF-1α, GLUT-1 and CA-IX.
  - Tumors originating from non-ccRCC epithelioid components continued to have limited expression.



Tickoo et al. J Urol. 2007 Apr;177(4):1258-63.

Introduction – Molecular Characteristics

- **Mutational Analysis**
  - Sarcomatoid transformation in RCC has been associated with *TP53* gene alteration.
    - Genetic sequencing found 79% of the sarcomatoid component showed *TP53* gene mutation compared to 17% of carcinomatous tissue.
    - All mutations were missense mutations.
    - Two mutational hot spots were found in codons 278 (57.1%) and 244 (42.9%).
    - Finding has not been replicated

da et al. Cancer Res. 1995 Feb 1;55(3):658-62.

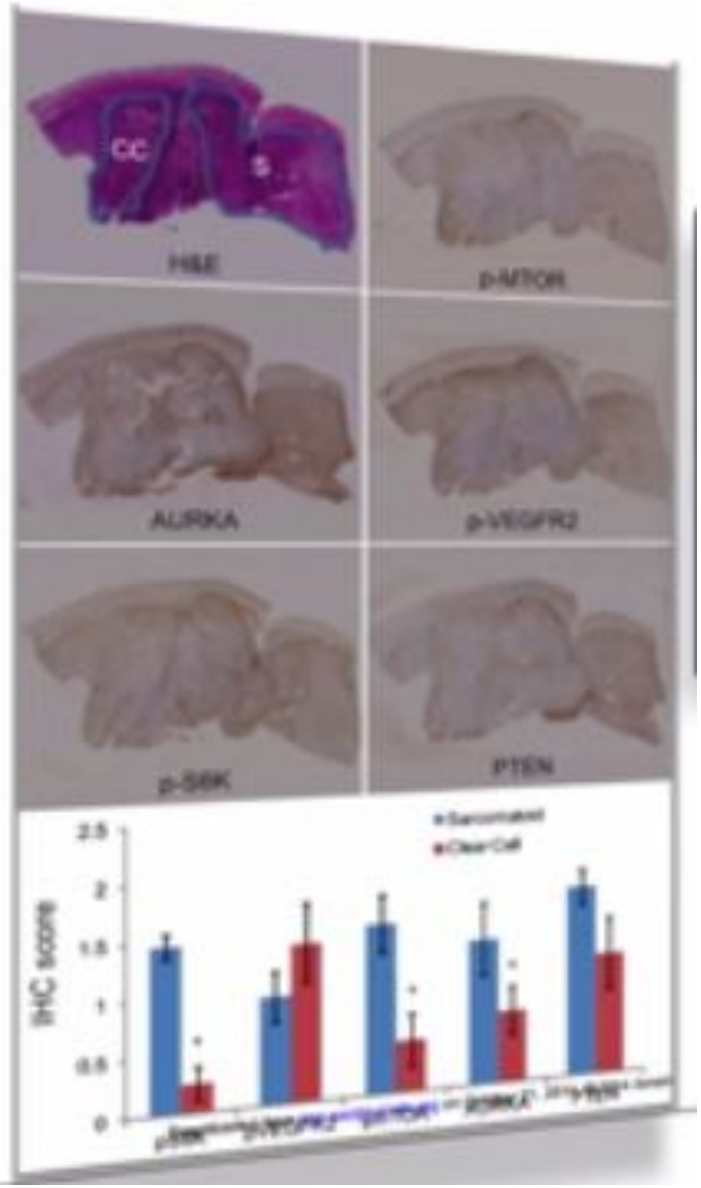
Molecular Cancer Research

RNA-seq Reveals Aurora Kinase Driven-mTOR Pathway Activation in Patients with Sarcomatoid Metastatic Renal Cell Carcinoma

Sumanta K. Pal, Miaoling He, Tommy Tong, et al.

Mol Cancer Res Published OnlineFirst September 2, 2014.

- Comparative RNA-seq of adjacent sarcomatoid and clear cell histology renal cell carcinoma
- Detected a proliferative phenotype and increased AURKA-dependent activation of mTOR signaling in sarcomatoid RCC



Hypothesis

- 1. Sarcomatoid RCC is distinct from other high grade RCC
- 2. Sarcomatoid represent a further evolution of tumorogenesis
  - % seems to be clinically relevant

Rationale – Distinct Entities

[1075] The Epithelioid and Sarcomatoid Components of Sarcomatoid RCC Are Molecularly Similar to Each Other but Distinct from Fuhrman Grade 4 Non-Sarcomatoid RCC

Kanishka Sircar, Suk-Young Yoo, Ted Majewski, Lalit Patel, Horatiu Voicu, Khalida Wani, Pheroze Tamboli, Keith Baggerly, Ken Aldape, Bogdan Czerniak. UT MD Anderson Cancer Center, Houston, TX; Baylor College of Medicine, Houston, TX

Design: DNA copy number was assessed using the Illumina HumanOmniExFFPE platform for which FFPE samples from 9 patients with sRCC (stage 4, n=7; stage 3, n=2) were macrodissected for sarcomatoid (S) and epithelioid (E) areas. As controls, we used TCGA copy number data from 158 patients with nsRCC of advanced stage (stage 4, n=57; stage 3, n=101) and Fuhrman grade 4 nsRCC (stage 4, n=15; stage 3, n=4). The transcriptome was examined by expression microarray (Illumina HumanRef-8) using FFPE samples from 17 patients with sRCC (stage 4, n=14; stage 3, n=2; stage 2, n=1) and from 8 patients with grade 4 nsRCC (stage 4, n=6; stage 3, n=2). Subsequently, RNA-Seq (Illumina HiSeq) was performed on independent patient samples: 7 sRCC (stage 4, n=6, stage 3, n=1) and 6 grade 4 nsRCC (stage 4, n=5; stage 3, n=1).

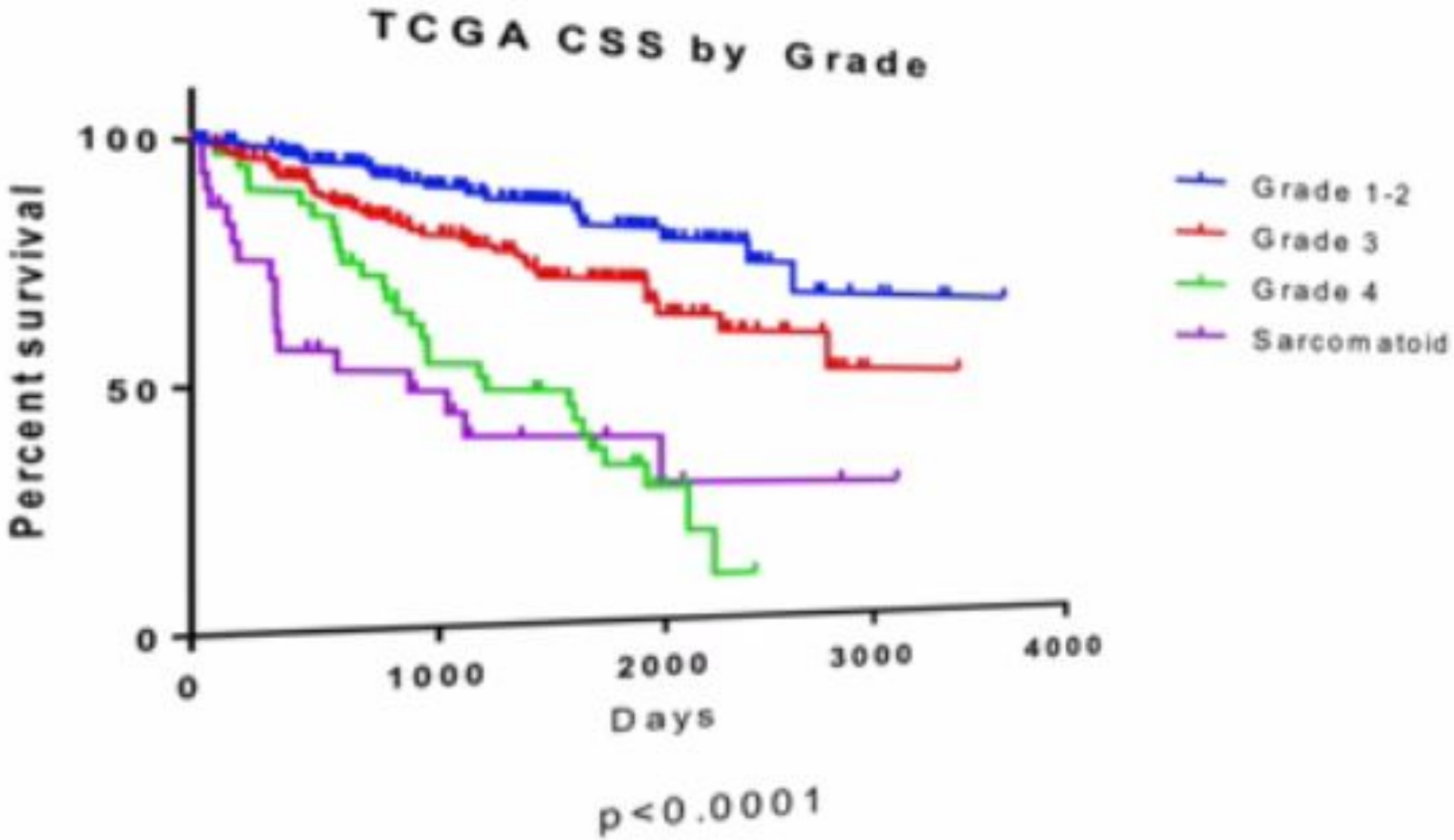
Results: Multiple copy number and transcriptomic aberrations were seen in sRCC, including known poor prognostic changes (9p/14q loss, ccB expression profile). Copy number and transcriptomic differences between the E and S components of sRCC were negligible. When comparing sRCC (E/S) to nsRCC, however, many genes were significantly associated with copy number variations (1079 genes, FDR <0.05). Subset analysis of Fuhrman grade 4 nsRCC also showed significant differences when compared to sRCC, both in terms of copy number (347 genes, FDR < 0.05) and transcript abundance (1396 genes, FDR < 0.05). RNA-seq confirmed the transcriptomic similarity of E and S components and their distinctiveness from Fuhrman grade 4 nsRCC using independent samples (n=659 genes, FDR <0.05).

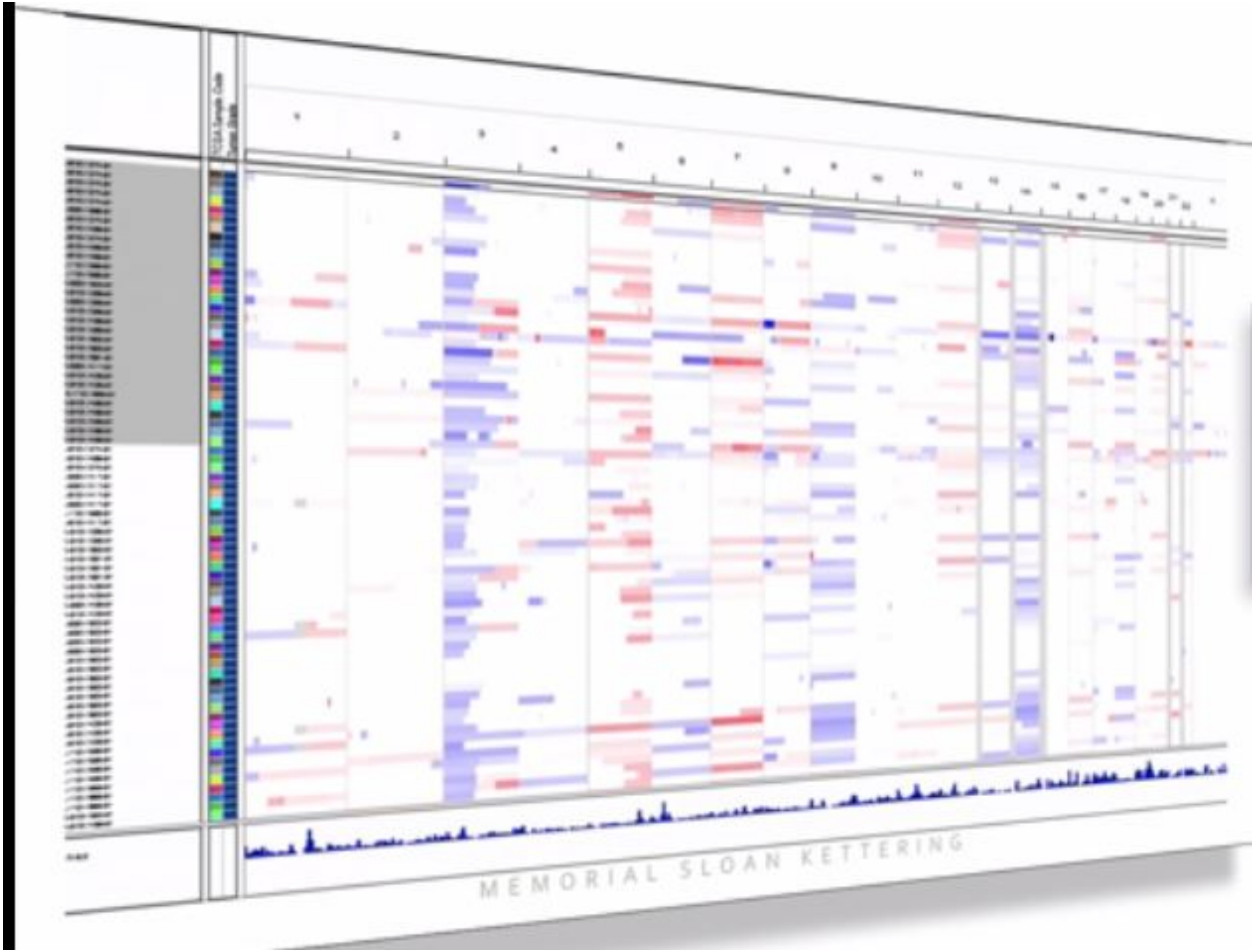
Conclusions: The discrete DNA copy number and gene expression signatures of sarcomatoid RCC suggests that it should be categorized separately from non-sarcomatoid Fuhrman grade 4 RCC. Despite stark morphologic differences, the E and S components of sarcomatoid RCC are molecularly similar suggesting that its genetic programming is largely embedded in its epithelioid component.

USCAP 2014

Hypothesis 1: sRCC is molecularly distinct from high grade ccRCC

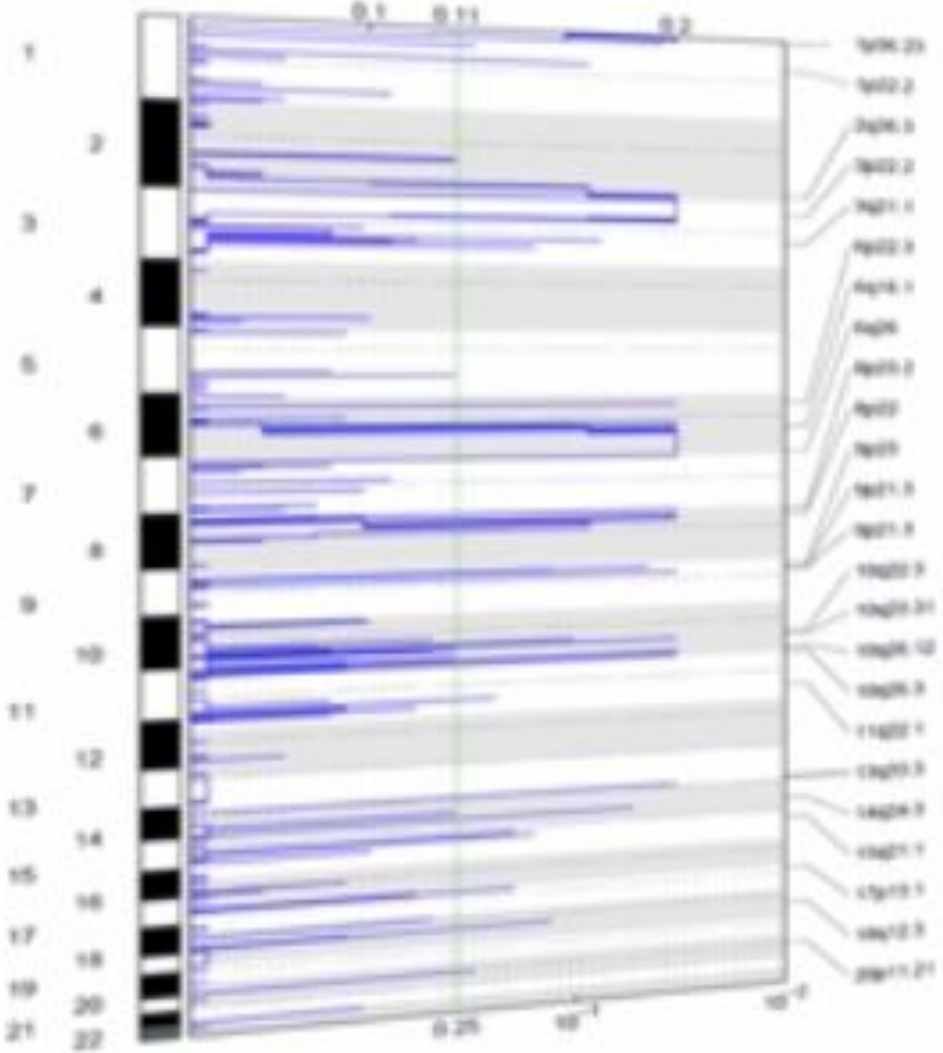
- Reviewed pathology reports for KIRC TCGA
- 31 sRCC with available genomic data
- Compared to 43 Grade IV's
  - Mutations
  - Copy Number
  - Expression
    - GSEA



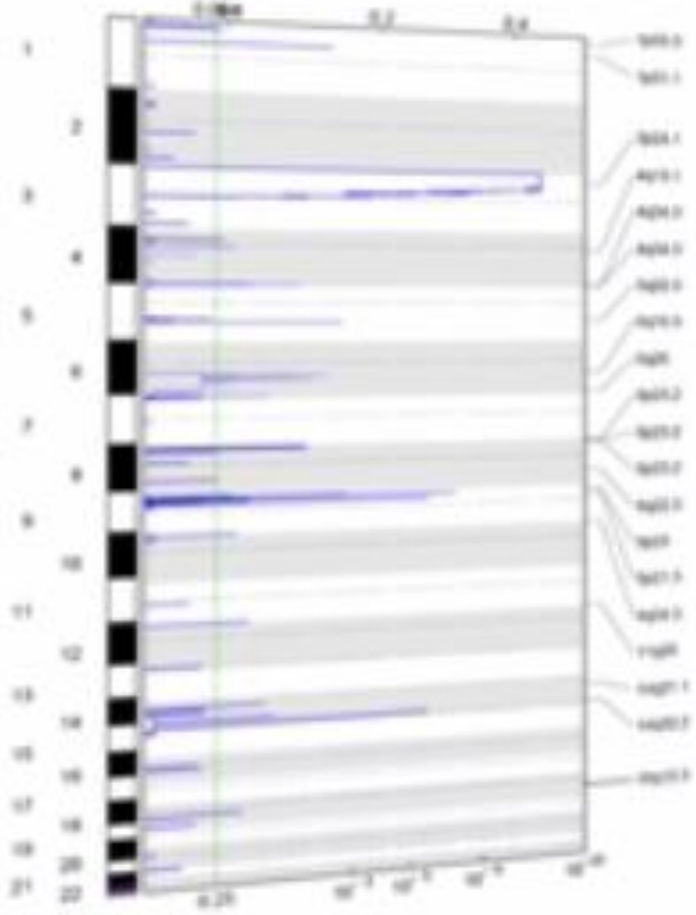

MEMORIAL SLOAN KETTERING

Gistic

sRCC

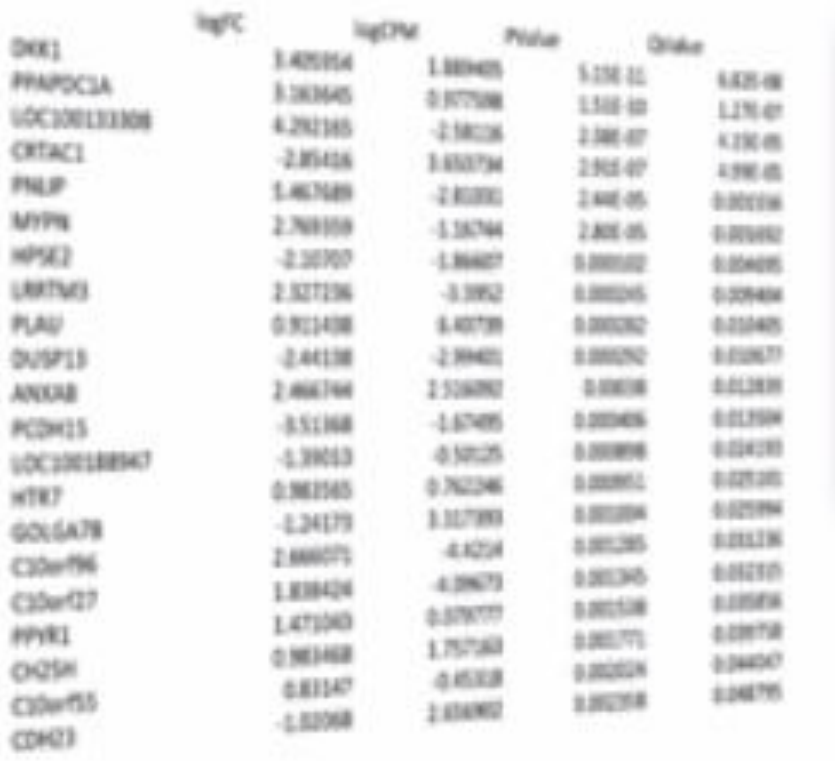


Grade IV



10q deletions

- 10q deletions seem to be enriched in sRCC compared to Grade IV
  - Expression overlap shows:
    - Downregulation of DKK1 (WNT signaling antagonist)

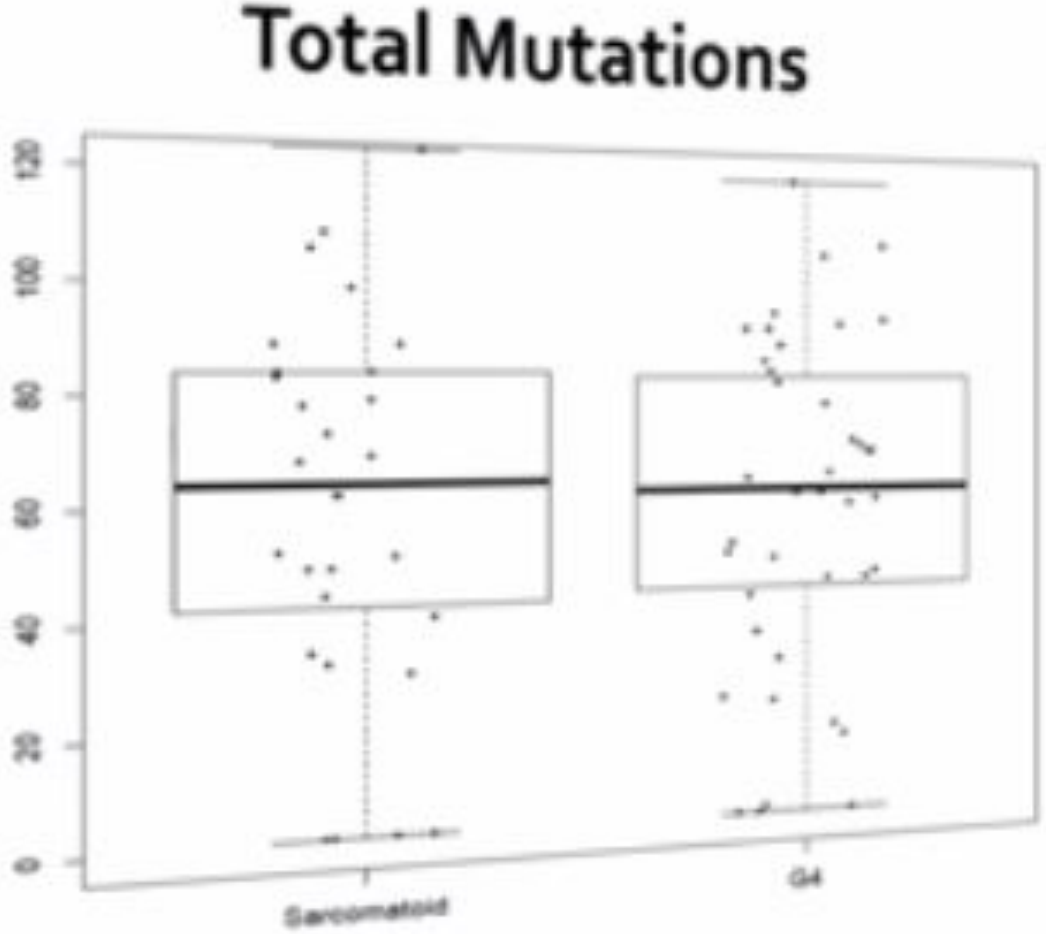


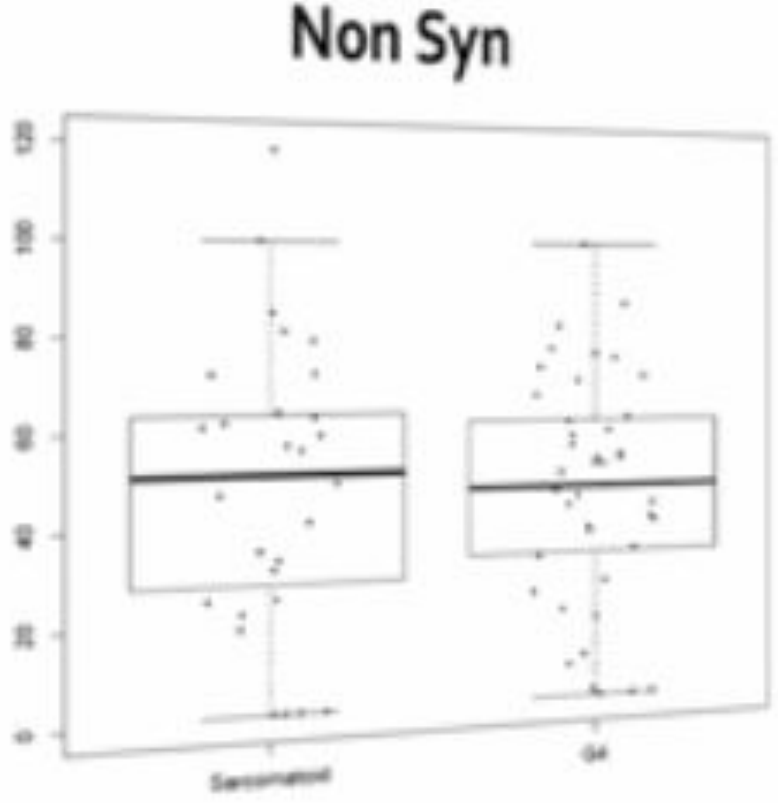
	logFC	logCPM	PValue	DValue
DKK1	3.405054	1.889405	5.15E-11	6.82E-08
PPARGC1A	3.283645	0.977508	1.51E-10	1.27E-07
LOC100133308	4.292165	-2.58228	2.38E-07	4.23E-05
ORTAC1	-2.85416	3.653734	2.91E-07	4.39E-05
PNLIP	5.467689	-2.81031	2.44E-05	0.003156
MYPN	2.769359	-1.16744	2.80E-05	0.003492
HPSE2	-2.10707	-1.86607	0.000102	0.004695
LRRTM3	2.327236	-3.3952	0.000245	0.009404
PLAU	0.921408	6.40739	0.000282	0.010405
DUSP13	-2.44138	-2.99401	0.000292	0.010677
ANXA8	2.466744	2.516092	0.00038	0.013939
PCDH15	-3.51368	-1.67495	0.000406	0.013504
LOC100188947	-1.39013	-0.50125	0.000898	0.024193
HTR7	0.983565	0.762246	0.000951	0.025101
GOLGA7B	-1.24173	3.317393	0.001094	0.025994
C10orf96	2.866071	-4.4224	0.001285	0.031236
C10orf27	1.838424	-4.39673	0.001345	0.032157
PPYR1	1.471043	0.070777	0.001538	0.035858
CH25H	0.983468	1.757163	0.001771	0.039718
C10orf55	0.83147	-0.45318	0.002024	0.044047
CDH23	-1.02068	2.658492	0.002358	0.048795

Specific mut

Gene	# mut, G4	# mut, Sarc.	% altered, G4	% altered, Sarc	logOdds
VHL	19	11	0.454545455	0.315789474	-0.5254615
PBRM1	17	3	0.409090909	0.105263158	-1.9584209
BAP1	8	3	0.204545455	0.105263158	-0.9584209
MUC4	8	8	0.204545455	0.236842105	0.2115041
MUC16	5	2	0.136363636	0.078947368	-0.7884959
SETD2	5	3	0.136363636	0.105263158	-0.3734584
ALMS1	4	0	0.113636364	0.026315789	-2.110424
MST1P2	4	1	0.113636364	0.052631579	-1.110424
MTOR	4	1	0.113636364	0.052631579	-1.110424
CDKN2A	3	2	0.090909091	0.078947368	-0.2035334
KDM5C	3	2	0.090909091	0.078947368	-0.2035334

Total Mutations Burden





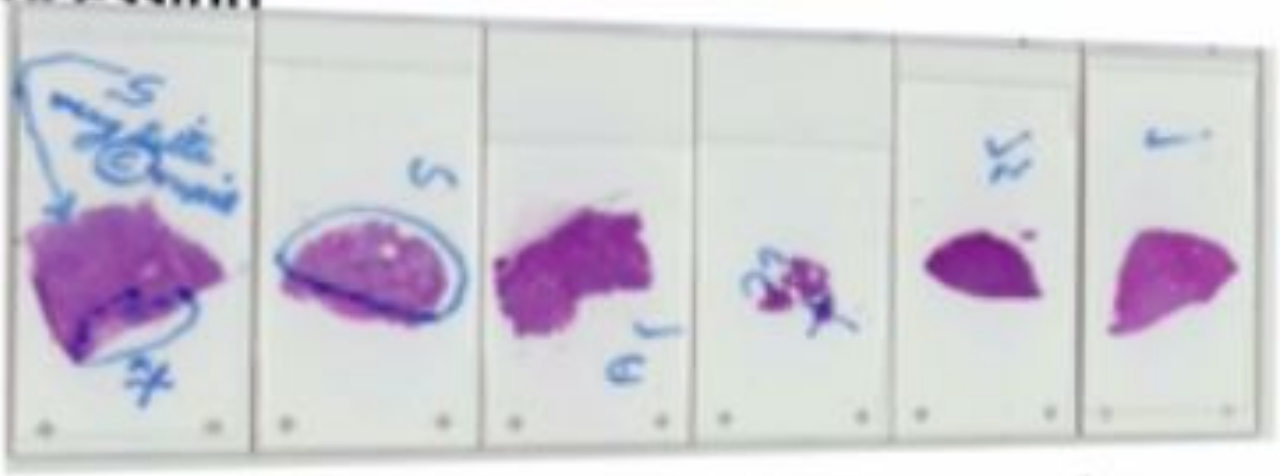
Hypothesis 2 – sRCC evolves from underlying carcinoma

- Whole geno sequencing on 7 fresh frozen triplets - Discovery
  - Normal
  - Clear cell component
  - Sarcomatoid.
- Targeted mutation and copy number assessment on larger FFPE cohort – Validation
  - MSK-IMPACT assay

Methods and Materials - Discovery Phase

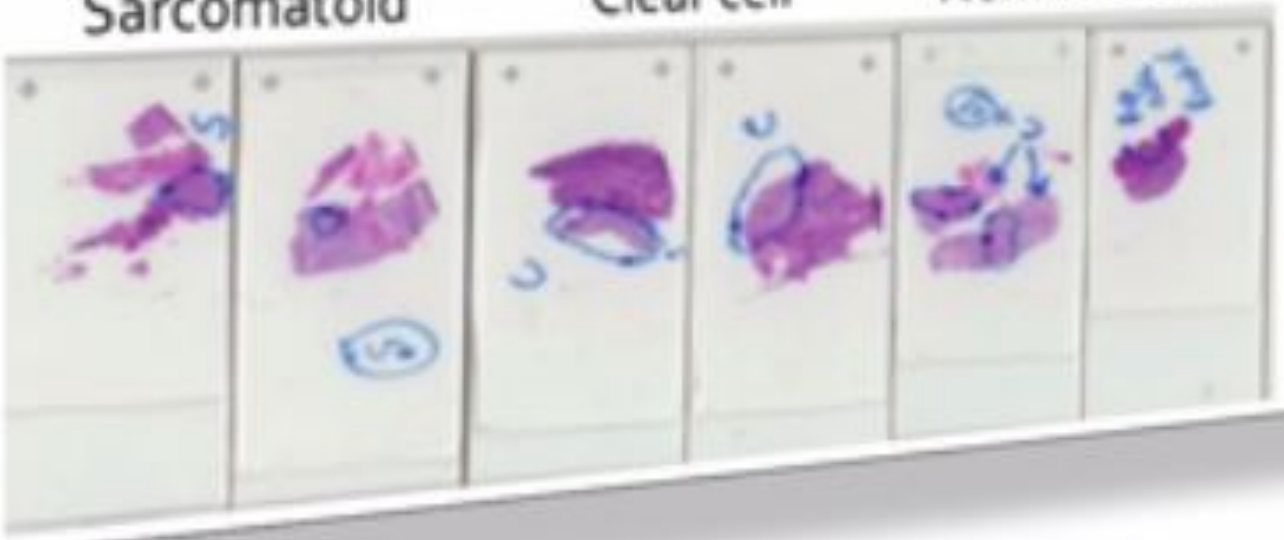
> Sample processing

Macrodissection



Sarcomatoid Clear cell Normal Tissue

Laser Capture Microdissection (LCM)



Methods and Materials - Discovery Phase

- Whole genome sequencing performed by NYGC.
- Point mutation were called on:
  - Sarcomatoid vs. Normal.
  - Clear Cell vs. Normal.
  - Sarcomatoid vs. Clear Cell.
- Copy number and loss of heterozygosity (LOH) analysis using FREEC (read-depth based).
- Structural variance (SV) (CREST)