Investigation of aberrant EGFR splice variants in clear cell renal cell carcinoma

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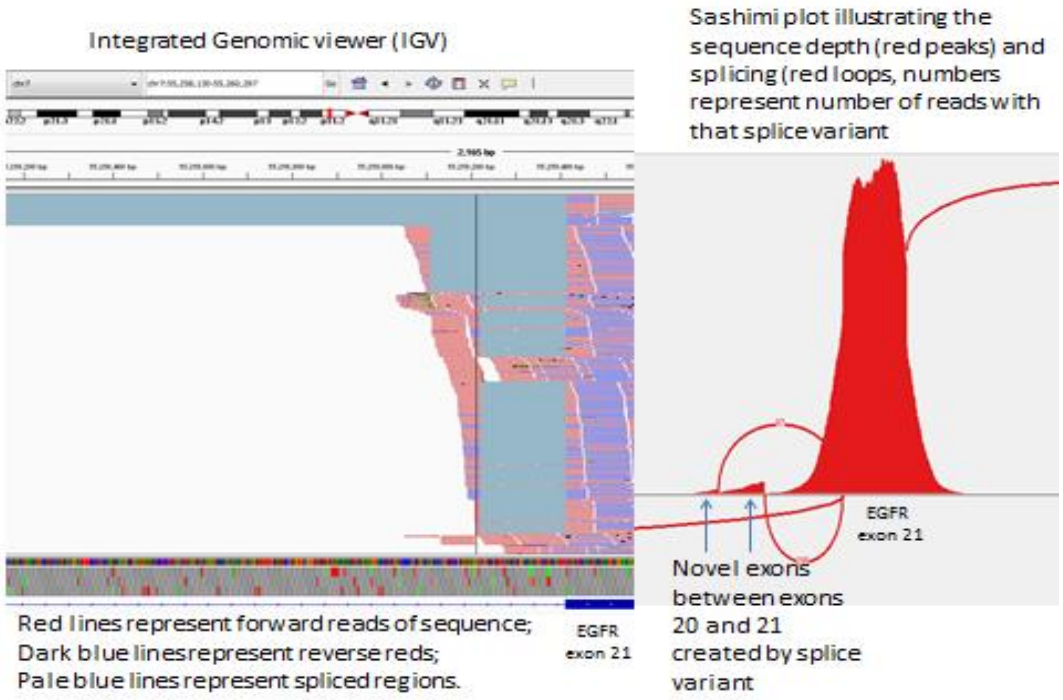
Observation: recurrent EGFR splice variants/alternative start site in clear cell renal cell carcinoma (ccRCC) tumor



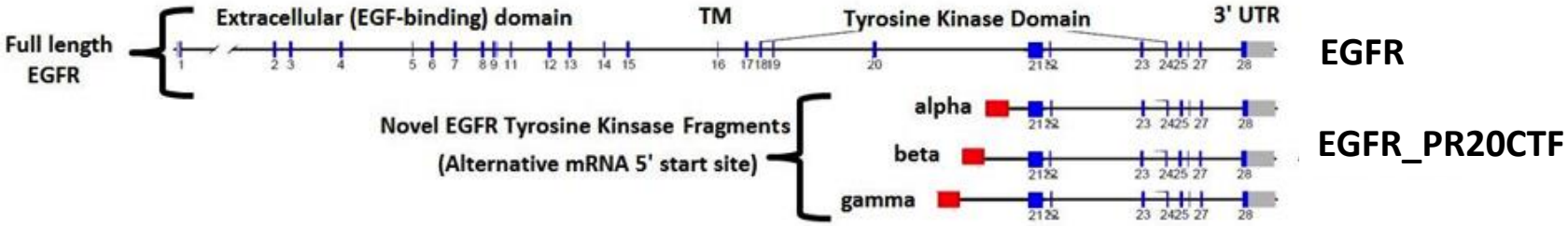


Figure 1. Exon map for *EGFR* splice variant C-terminus fragment isoforms

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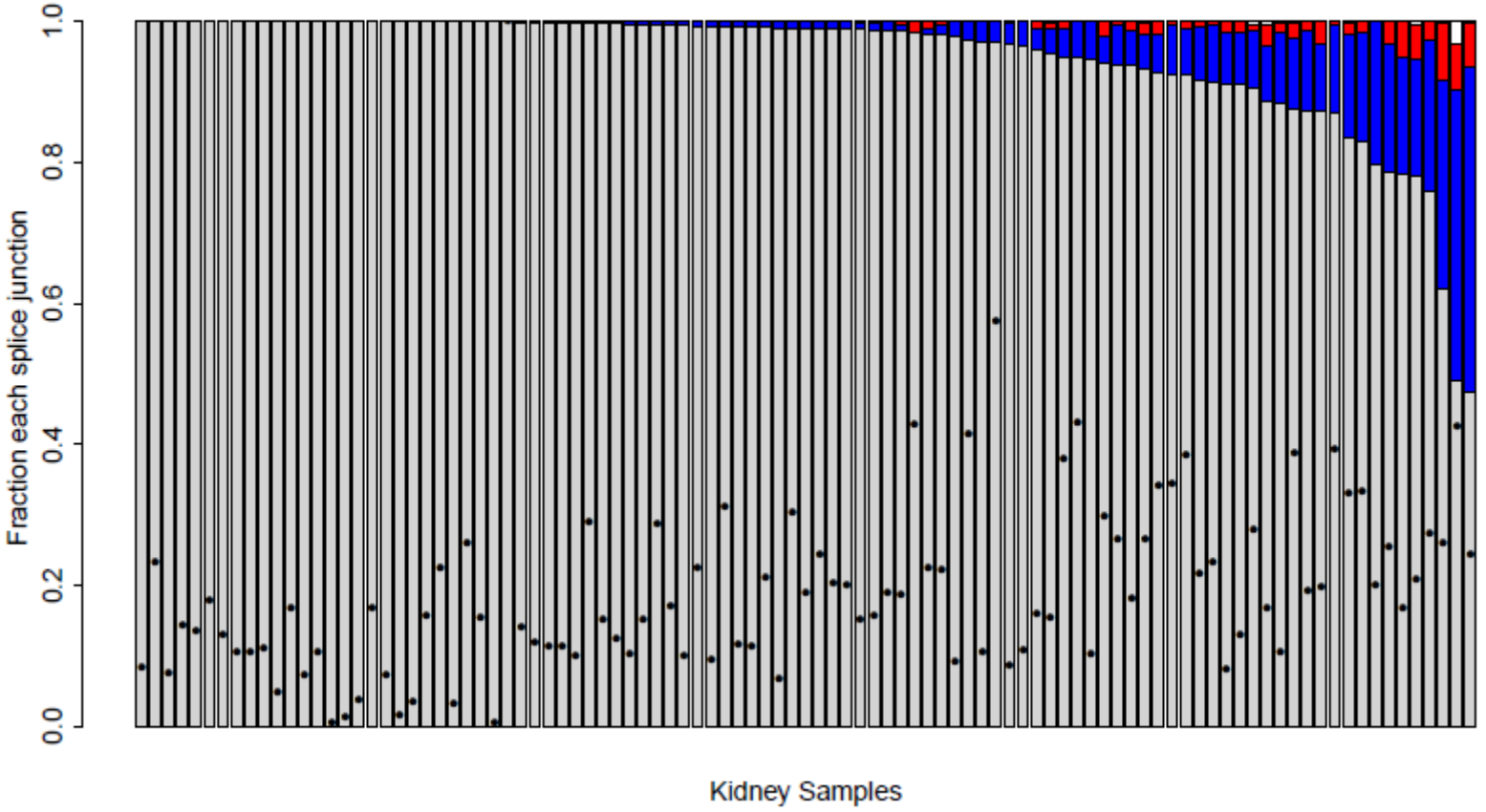
Frequent alteration in ccRCC



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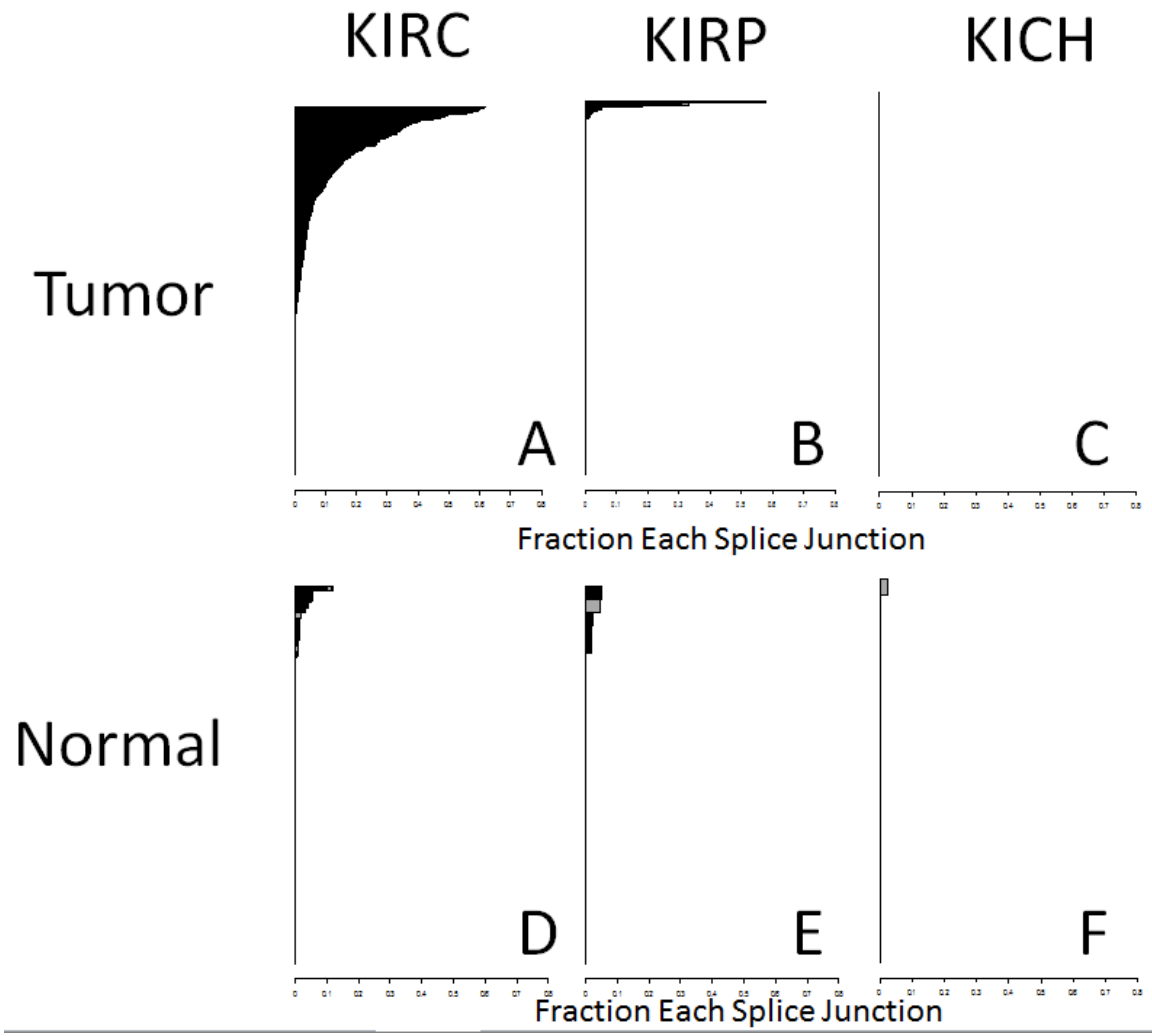
Frequent alteration in ccRCC-TCGA



Relatively Specific to ccRCC

Table. Non-Renal Cell Carcinoma TCGA tumors types analyzed for EGFR-20-CTF. Up to 100 samples from each tumor type were targeted for tumor type, including normal samples when present. Only samples with greater than 10 total reads crossing the EGFR junctions of interest were included in the analyses

Tumor Type	Cohort	No.	Samples demonstrating EGFR-20-CTF, no. (%)
H&N Tumor	TCC	189	0
Breast Tumor	TCC	164	0
Sarcoma Tumor	TCC	117	1 (0.85%)
Ovarian Tumor	TCC	62	0
Bladder Tumor	TCC	60	0
Prostate Tumor	TCC	4	0
Multiple Myeloma/Heme	TCC	4	0
Glioma (GBM) Tumor	TCGA	92	0
Normal	TCGA	-	-
Colon (COAD) Tumor	TCGA	53	0
Normal	TCGA	9	0
Rectal (READ) Tumor	TCGA	53	0
Normal	TCGA	2	0
Lung squamous (LUSC) Tumor	TCGA	93	0
Normal	TCGA	7	0
Lung adenocarcinoma (LUAD) Tumor	TCGA	86	1 (1.2%)
Normal	TCGA	9	0
Stomach (STAD) Tumor	TCGA	79	0
Normal	TCGA	8	0
Total		**1091**	**2 (<0.2%)**

Abbreviations: EGFR, epidermal growth factor receptor; TCC, Moffitt Total Care Cohort; TCGA, The Cancer Genome Atlas

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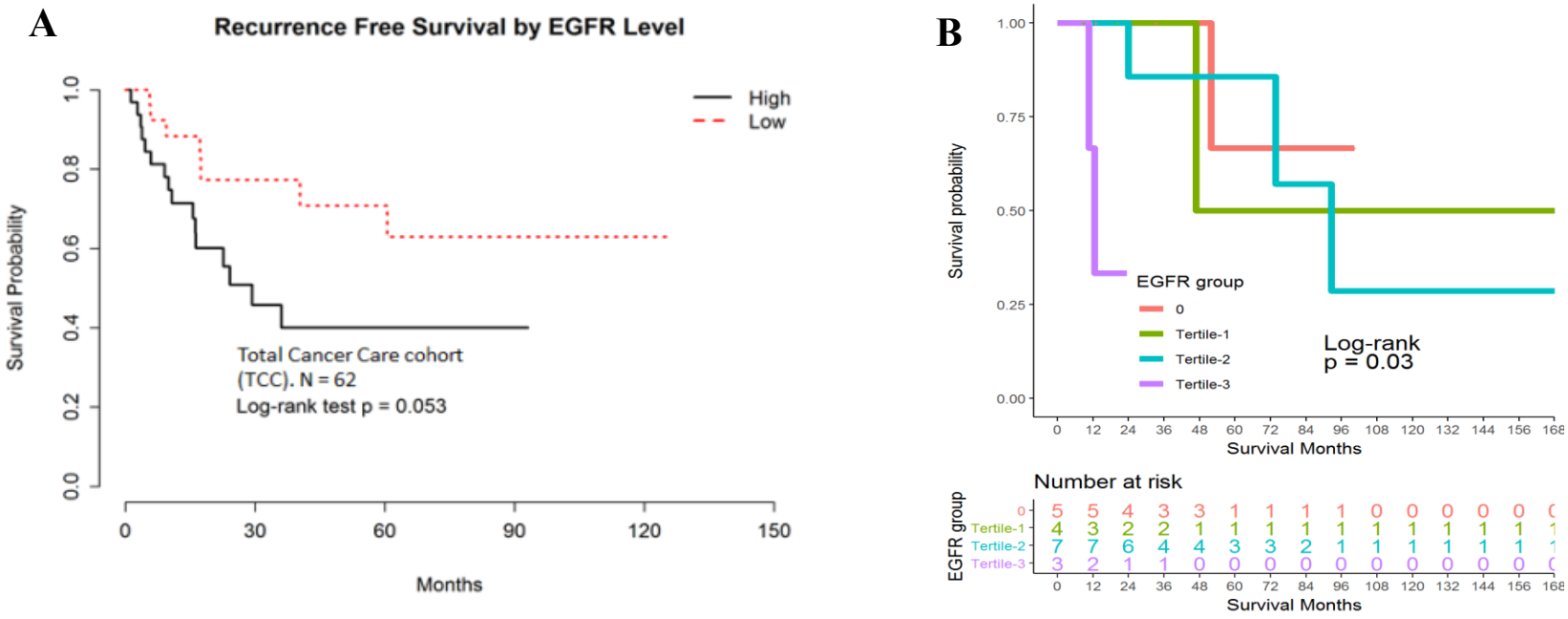
Clinical Associations



A. *Kaplan-Meier curves for recurrence free survival analysis in ccRCC patients (n=62) using 1% expression cutoff values (High vs. Low) for presence of EGFR-20-CTF* **B.** *Analysis of survival probability (months) from date of first immunotherapy treatment, stratified by EGFR_PR20CTF expression*. Group 0 = no EGFR_PR20CTF expression; Tertile-1 = Patients with the lowest tertile of expression levels of EGFR_PR20CTF(1- <33%); Tertile -2 = Patients with the middle tertile of expression levels of EGFR_PR20CTF(33-66%); Tertile-3 = Patients with the highest tertile of expression levels of EGFR_PR20CTF (>66%).

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Specific Aims

Aim 1. **Validate association of EGFR_PR20CTF expression in ccRCC tumors with increased risk of primary resistance to ICB for first line treatment of metastatic disease.** To investigate primary resistance to ICB, defined by progression free survival (≤15 weeks) requiring change in treatment, we will use tumor samples from patients with metastatic ccRCC who receive first line treatment with ICB. Droplet Digital PCR (ddPCR) will be used to characterize EGFR_PR20CTF expression in tumor samples.

Secondary Objective(s):

- Analyze objective radiographic treatment response according to RECIST 1.1 and associations with EGFR_PR20CTF expression
- Assess correlation of EGFR_PR20CTF expression in primary vs. metastatic tumor samples and ICB response.
- Identify associations with EGFR_PR20CTF expression levels with secondary resistance as defined by progression free survival ≤30 weeks necessitating a change in treatment regimen
- Associations with dose limiting drug toxicities and the presence of EGFR_PR20CTF alterations.

Aim 2. **Define the presence of novel protein products from EGFR_PR20CTF and examine signaling of EGFR_PR20CTF to Develop Drug Repurposing Strategies for ccRCC.**

This aim focuses on the verification of protein expression for EGFR_PR20CTF and its consequences. The **primary objective** of this aim to detect the proteoform associated with EGFR_PR20CTF in cell lines and tumors and determine whether the presence of this protein variant impacts kinase signaling cascades in ccRCC, which would indicate that it can be developed as a therapeutic target.

Secondary Objective(s):

- Determine whether EGFR_PR20CTF interacts with full length WT EGFR and what are the consequences for kinase signaling.
- Perform phenotypic assays (e.g. Matrigel invasion, colony formation, etc.) to see what processes are enhanced by EGFR_PR20CTF expression.
- Determine whether EGFR_PR20CTF expression can rescue ccRCC cells after siRNA Knockdown or CRISPR Knockout of full length WT EGFR.