Analysis of EGFR mutant urothelial carcinoma (UC) reveals distinct mutational landscape.

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ABSTRACT

Background: Genomic alterations (GA) of *EGFR*, are well recognized as druggable oncogenic drivers in NSCLC, but the druggable GA *EGFR* L858R and exon 19 deletion (ex19del), are rarely observed in genitourinary cancer. We reviewed the genomic landscape of advanced upper tract and bladder UC (UTUC and BUC) to assess the frequencies of druggable *EGFR* GA.

Results: *EGFR* alterations (*EGFR$_{alt}$*) were present in 17 UTUC and 93 BUC (4.2% and 3.9%). Age distribution between the two subgroups was similar, but UTUC was more prevalent in female patients (47% v 29%). BUC had a higher median TMB (5.2mut/mb v 7.8 mut/mb; p = 0.046) and the prevalence of MSI-H cases was not significantly different. *TERT* (55% v 71%) and *TP53* (59% v 74%) were the most frequently mutated genes in *EGFR$_{alt}$* UTUC and BUC. Within *EGFR$_{alt}$*, amplifications were the most common alterations in both UTUC and BUC (13/17, 76%; 57/93, 61%). Amplifications were mutually exclusive from cases with *EGFR* short variants (SV) in BUC (0/34, 0%), and co-occurred with *EGFR* SV in four UTUC cases (4/8 50%). The majority of *EGFR* SV were *EGFR* exon 20 insertions (*EGFR$_{exon20}$*), which made up a larger proportion of *EGFRalt* in UTUC than BUC (7/17, 41% v 13/93, 14%; p = 0.01). Compared to other *EGFR$_{alt}$*, *EGFR$_{exon20}$* trended towards mutual exclusivity of GA in commonly altered UC genes: *TP53* (UTUC *EGFR$_{exon20}$* v *EGFR$_{alt}$* other: 0% v 100%, p = 5.1E-5; BUC *EGFR$_{exon20}$* v *EGFR$_{alt}$* other: 0% v 86%, p = 2.2E-7), *PIK3CA* (14% v 10%; 0% v 19%), *RAF1* (0% v 10%; 0% v 16%), or *FGFR3* (0% v 10%; 0% v 6.3%) alterations. Only 2.2% (2/93) of BUC *EGFR$_{alt}$* were L858R mutations and none were ex19del (0/93), while neither mutation was detected in UTUC.

Conclusions: *EGFR$_{exon20}$* defines a subset of UC cases, and is the most common non-amplification GA seen in *EGFR* in UC, with some enrichment in UTUC. Consideration should be given to developing a trial for *EGFR*exon20 UC patients given the recent investigational work on inhibitors with activity against *EGFR*exon20, such as poziotinib and TAK-788.

MATERIALS AND METHODS

- CGP was performed using a validated hybrid capture-based CGP assay on 2,402 BUC and 407 UTUC tissue specimens to identify GAs (substitutions, short insertions/deletions, copy number alterations [CNAs], rearrangements) and gene signatures (TMB, MSI)
- CGP was performed on hybridization-captured, adaptor ligation-based libraries to a median coverage depth of 762 for of 287, 324, or 395 cancer-related genes plus select introns from 19, 36 or 31 genes frequently rearranged in cancer. Results were analyzed for substitutions, short insertions/deletions and rearrangements, and copy number changes. Custom filtering was applied to report GAs, and variants of unknown significance were excluded from this analysis
- To determine microsatellite status, 95 or 114 intronic homopolymer repeat were analyzed for length variability and compiled into an overall MSI score via principal components analysis
- TMB was calculated as the number of somatic base substitutions or indels per megabase of the coding region target territory of the test (0.79, 0.83, or 1.1 Mb) after filtering to remove known somatic and deleterious mutations and extrapolating that value to the exome or genome as a whole. TMB was categorized as low (<6 m/mb), intermediate (6–20 m/mb), or high (≥20 m/mb)

RESULTS

	EGFR WT BUC (n = 2,309)	*EGFR$_{alt}$* BUC (n = 93)	*EGFR$_{exon20}$* BUC (n = 13)	*EGFR* WT UTUC (n = 390)	*EGFR$_{alt}$* UTUC (n = 17)	*EGFR$_{exon20}$* UTUC (n = 7)
M:F	2.95:1	2.44:1	2.25:1	1.55:1	1.125:1	2.5:1
Age Median	68	67	61	68	69	62
TMB Median	6.96	7.83	5.22	5.22	5.22	3.48
TMB Mean	9.90	10.65	5.28	10.5	6.12	3.67
% MSI-H	0.7%	2.7%	0%	4.1%	0%	0%
PIK3CA	21.4%	16.1%	0%	17.4%	11.8%	14.3%
FGFR3	19.7%	5.37%	0%	25.9%	5.9%	0%
ERBB2	15.7%	12.9%	7.7%	11.0%	11.8%	0%
TERT	68.4%	70.7%	75%	51.9%	54.5%	80%
Multiple *EGFR*	0%	1.1%	0%	0%	23.5%	57.1%

Table 1. Sex, age, tumor mutational burden (TMB), microsatellite instability (MSI), and relevant cooccurring alterations among *EGFR* WT, *EGFR*-altered, and *EGFR* exon 20 insertion bladder and upper tract urothelial carcinomas.

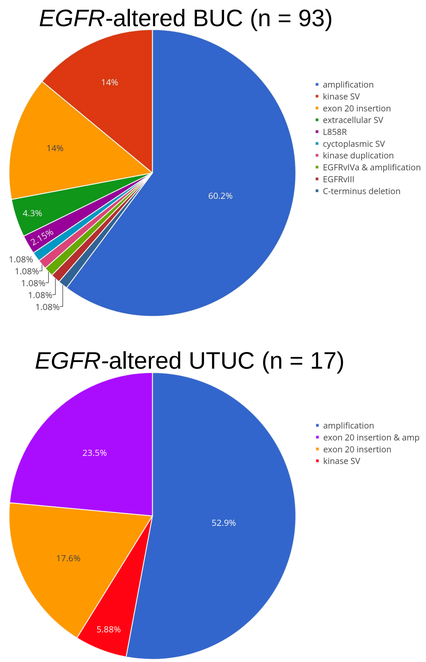

Figure 1: Distribution of distinct *EGFR* alterations in BUC and UTUC. Alterations were more diverse in BUC and exon 20 insertions were mutually exclusive from other alterations

EGFR mutation	Count in Urothelial (N = 2,809)	% Urothelial	Count in Lung (N = 14,483)	% NSCLC	P-value
H773_V774insH	5	22.73%	8	0.36%	9.6E-08
D770_N771insGF	3	13.64%	0	0.00%	1.1E-04
H773_V774insNPH	3	13.64%	20	0.89%	0.014
D770_N771insSVD	2	9.09%	52	2.31%	0.26
D770>ASVH	1	4.55%	0	0.00%	0.1
N771>GF	1	4.55%	0	0.00%	0.1
N771_P772insH	1	4.55%	3	0.13%	0.18
P772_H773insDNP	1	4.55%	2	0.09%	0.18
P772_H773insANP	1	4.55%	0	0.00%	0.1
H773_V774insTH	1	4.55%	2	0.09%	0.18
H773_V774insHV	1	4.55%	6	0.27%	0.23
Other ex20 insertion	0	0.00%	170	7.55%	1
Ex19 deletion	0	0.00%	1058	47.00%	1.3E-05
L858R	2	9.09%	727	32.30%	0.15
Other	0	0.00%	203	9.02%	0.44

Table 2: Frequency of unique *EGFR* activating mutations compared to frequency in NSCLC. *EGFR$_{exon20}$* makes up larger percentage of UC *EGFR* mutations than NSCLC *EGFR* mutations. Mutations included in "other" include S768I, L861Q, and G719X (PMID:29981927)

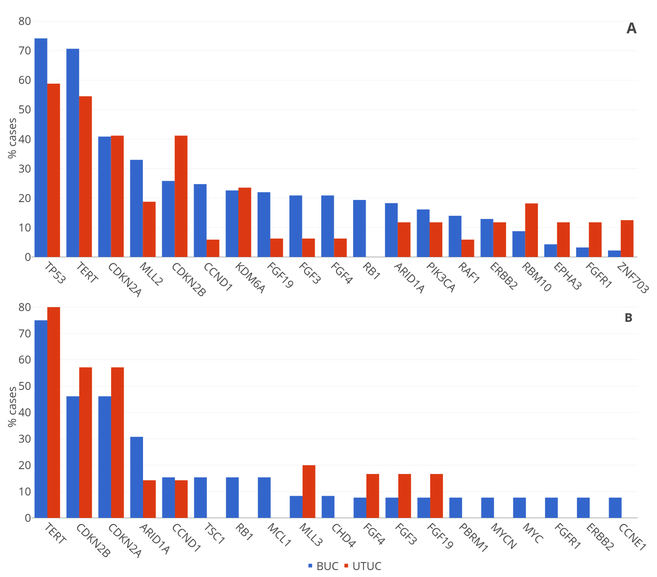

Figure 2: Frequency of cooccurring alterations in A) *EGFR*-altered UC and B) *EGFR$_{exon20}$* UC by subgroup

CASE REPORT

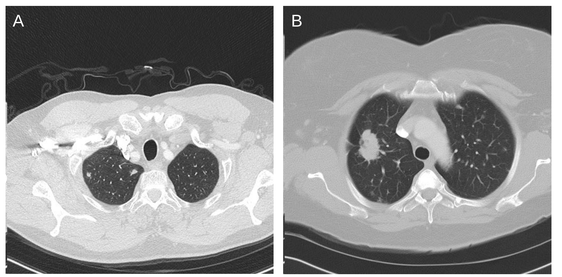

Figure 4: CT scan of lung metastases from two BUC harboring *EGFR$_{exon20}$*

A: A 60-year-old never-smoker male presented with flank pain and hematuria and was found to have a high-grade papillary tumor of the left renal pelvis. A nephrouretectomy was performed and pathology revealed high-grade papillary urothelial carcinoma with in invasion of the renal parenchyma (pT3N0). The patient was treated with adjuvant platinum based chemotherapy, developed acute venous thrombosis. Chemotherapy was discontinued early due to poor tolerance. Seven months after his definitive surgery, he was noted to have lung nodules, which on subsequent scans progressed to increase in size and number signifying metastatic disease. *EGFR$_{exon20}$* detected in specimen taken from primary site (kidney).

B: A 56 year old ex-smoker female presented with hematuria and was found to have a bladder mass. A TURBT revealed a plasmacytoid variant urothelial carcinoma and imaging revealed lung and bone metastases. The patient has received multiple lines of treatment including platinum based chemotherapy (on which she developed arterial thrombosis) and immune checkpoint inhibitors in the PD-1/PD-L1 category over a period of 27 months. *EGFR$_{exon20}$* detected in specimen taken from primary site (bladder).

CONCLUSIONS

- *EGFR* alterations in UC have a distinct distribution when compared to other frequently *EGFR* altered tumors such as NSCLC
- Both *EGFR* altered and *EGFR$_{exon20}$* UC less frequently harbored alterations in putative oncogenic driver *FGFR3* while *EGFR$_{exon20}$* harbored fewer alterations in *ERBB2*.
- *EGFR$_{exon20}$* are found in patients with lower TMB (p = 0.01)
- *EGFR$_{exon20}$* in UC are distinct alterations when compared to NSCLC
- *EGFR$_{exon20}$* UC could be considered for *EGFR* inhibitors with activity against *EGFR$_{exon20}$*, such as poziotinib and TAK-788 or Hsp90 inhibitor luminespib

References:
Journal Thoracic Oncology 2018;13:1560-1568 (PMID:29981927)