

Representative images of UPFL tumors.

(A) Gross images of UPFL tumor bearing bladders.
(B-E) Photomicrographs of four (4) representative H&E stained UPFL bladder tumors. Each tumor is shown at low magnification with an inset box indicating the area of the related high magnification image.



A



Genomic and transcriptomic features of UPFL tumors - (A)

Co-occurence rates of *FGFR3* DNA calling based mutations and RNA based *TP53* Pathway alterations for the UROMOL cohort. **(B)** Gene set enrichment analysis (GSEA) for the Hallmark expression signatures were performed on the log2 - fold change between UPFL and UPPL tumors. The top ranking signatures by normalized enrichments score for UPFL and UPPL were plotted. Signature with an FDR <0.05 are indicated by teal bars.



T cell signature immune gene signatures scores.

Signatures representing T cells were calculated for the UROMOL cohort and plotted by NMIBC class. All boxplots are represented by the IQR and midline at the median. Error bars represent Q1/Q3 \pm 1.5*IQR. Two-sided t-tests followed by Bonferroni correction, to account for multiple comparisons, were preformed with the p-values shown above the given comparison.



UPFL derived cell line (UPFL3) is sensitive to FGFR inhibition - Immunoblots with the indicated antibodies in whole cell extracts of UPFL3 cells treated with erdafitnib for the indicated dose and time.



С







D

Histologic assesment of UPFL allograft

tumors (A) UPFL tumors sections from mice treated with vehicle (n=7), anti-PD-1 antibody (n=5), erdafitinib (n=7), or anti-PD-1+erdafitinib (n=6) were stained for either Ki67 or Cleaved Caspase 3. (B) The percent of stong staining (3+) nuclei were plotted by group (C) along with the percent of cells positive for Cleaved Caspase 3. Boxplots are represented by the IQR and midline at the median. Error bars represent Q1/Q3 ± 1.5*IQR. Two-sided t-tests followed by Bonferroni correction, to account for multiple comparisons, were performed with the p-values shown above the given comparison. (D) Immune phenotype calls were made based on the location and intestity of CD8+ cells, with representive image shown above.



Supplementary Figure 6 (A) Box plots of percentage of CD45+ cells that are PD1+ in CD8+ (left) and CD4+ (right) for the indicated treatment group as well as **(B)** CD45+, CD3+, and CD8+ cytotoxic T cells after 1 week of treatment. **(C)** The T cell subset of cells identified by SingleR from control or erdafitinib treated tumor were plotted by the scRNA expression values for *Ptprc* (CD45), *Cd3e* (CD3), and *Cd8a* (CD8) **(D)** *II1r1*. All boxplots are represented by the IQR and midline at the median. Error bars represent Q1/Q3 \pm 1.5*IQR. Two-sided t-tests followed by Bonferroni correction, to account for multiple comparisons, were preformed with the p-values shown above the given comparison.



Gating strategy of flow cytometry on UPFL1 allografts Example of gating strategy used for flow cytometry analysis. Cells were first gated for aqua live/dead staining to select live cells and then gated as single cells. CD45 staining was used to identify the total immune cells. T cells were selected as positive to CD3 staining and then, sub-sequentially, identified as cytotoxic T cells (CD8+), Helper T cells (CD4+) and Tregs cells (CD4+, Foxp3+). In addition, the expression of PD-1, CTLA4 in T cells was analyzed.







Gating stratagy and quantification for the isolation for Treg proliferation assay. (A) FACS analysis was perferformed on the CD4+/Foxp3-GFP+ population. Cells positive for crystal violet tracer (CVT) were identified as the non-proliferative population, where as CVT+ cells were considered proliferative. (B) Boxplot of the percent living cell Tregs following the 3 day proliferation assay, regardless of CVT status. (C) RNA expression of FGFR1, FGFR2, FGFR3 in T cell subsets. All boxplots are represented by the IQR and midline at the median. Error bars represent Q1/Q3 ± 1.5*IQR. Two-sided t-tests followed by Bonferroni correction, to account for multiple comparisons,were preformed with the p-values shown above the given comparison.

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FGFR family expression by immune cell type (Schmiedel et. al.)

