APOBEC-mediated mutagenesis in urothelial carcinoma is associated with improved survival, mutations in DNA damage response genes, and immune response

Alexander P. Glaser MD, Damiano Fantini PhD, Kalen J. Rimar MD, Joshua J. Meeks MD PhD

APG, DF, KJR, JJM: Northwestern University, Department of Urology, Chicago, Il, 60607

Running title: APOBEC mutagenesis in bladder cancer

*Corresponding author:
Joshua J. Meeks, MD PhD
303 E. Chicago Ave.
Tarry 16-703
Chicago, IL 60611
Email: joshua.meeks@northwestern.edu

Keywords (4-6):
- Urinary bladder neoplasms
- APOBEC Deaminases
- Mutagenesis
- DNA damage
- Interferon

Abbreviations and Acronyms:
TCGA – The Cancer Genome Atlas
ssDNA – single stranded DNA
APOBEC – apolipoprotein B mRNA editing catalytic polypeptide-like
GCAC – Genome Data Analysis Center
MAF – mutation annotation format
“APOBEC-high” – tumors enriched for APOBEC mutagenesis
“APOBEC-low” – tumors not enriched for APOBEC mutagenesis
Abstract:

Background:

The APOBEC family of enzymes is responsible for a mutation signature characterized by a TCW>T/G mutation. APOBEC-mediated mutagenesis is implicated in a wide variety of tumors, including bladder cancer. In this study, we explore the APOBEC mutational signature in bladder cancer and the relationship with specific mutations, molecular subtype, gene expression, and survival. We hypothesized that tumors with high levels of APOBEC-mediated mutagenesis would be enriched for mutations in DNA damage response genes and associated with higher expression of genes related to activation of the immune system.

Methods:

Gene expression (n=408) and mutational (n=395) data from the Cancer Genome Atlas (TCGA) bladder urothelial carcinoma provisional dataset was utilized for analysis. Tumors were split into “APOBEC-high” and “APOBEC-low” tumors based on APOBEC enrichment score. Analysis was performed with R.

Findings:

Patients with APOBEC-high tumors have better overall survival compared to those with APOBEC-low tumors (38.2 vs 18.5 months, p=0.005). Tumors enriched for APOBEC mutagenesis are more likely to have mutations in DNA damage response genes (TP53, ATR, BRCA2), and chromatin regulatory genes (MLL, MLL3), while APOBEC-low tumors are more likely to have mutations in FGFR3 and KRAS. APOBEC3A and APOBEC3B expression correlates with total mutational burden, regardless of bladder tumor molecular subtype. APOBEC mutagenesis and enrichment is associated with increased expression of immune-related genes, including interferon signaling.
**Interpretation:**

Tumors enriched for APOBEC mutagenesis are more likely to have mutations in DNA damage response genes and chromatin regulatory genes, potentially providing more single-strand DNA substrate for *APOBEC3A* and *APOBEC3B*, leading to a hypermutational phenotype and the subsequent immune response.
Highlights:

- ABPOEC enzymes, particularly APOBEC3A and APOBEC3B, are responsible for the predominant pattern of mutagenesis in bladder cancer.
- Tumors enriched for APOBEC-mediated mutagenesis are more likely to have mutations in DNA damage response genes and chromatin regulatory genes, while tumors not enriched for APOBEC-mediated mutagenesis are more likely to have mutations in KRAS and FGFR3.
- APOBEC enrichment is associated with upregulation of genes involved in the immune response.
1. Introduction

Urothelial carcinoma has one of the highest mutation rates of any sequenced cancer to date. High-throughput next generation sequencing analyses such as The Cancer Genome Atlas (TCGA) and others have identified a mutational signature characterized by a TCW>T/C mutation thought to be attributable to the apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC) family of enzymes. This mutational pattern is the predominant pattern in bladder cancer and is also frequently found in breast, cervical, head and neck, and lung cancers.

The APOBEC family consists of 11 members, including AID, APOBEC1, APOBEC2, APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3D, APOBEC3F, APOBEC3G, APOBEC3H, and APOBEC4. These enzymes function as cytosine deaminases and are involved in C>U deamination in single-stranded DNA (ssDNA), and likely function physiologically in antiretroviral defense. However, in tumor cells, these enzymes are likely responsible for hypermutation at cytosine bases in exposed ssDNA, known as kataegis. The APOBEC3 family, and particularly APOBEC3A and APOBEC3B, are the predominant APOBEC enzymes theorized to contribute to cancer mutagenesis.

Several studies have linked APOBEC3B expression with mutagenesis, but expression alone does not fully explain this mutational signature, and APOBEC3A may also play a significant role. In breast cancer, DNA replication stress and mutations in DNA repair genes have been linked to APOBEC-mediated mutagenesis, potentially due to increased availability of ssDNA substrate for enzymatic deamination. However, less is known about the downstream effects of APOBEC mutagenesis in bladder cancer.

In this study, we explore the APOBEC mutational signature in bladder cancer and its relationship with specific mutations, molecular subtype, gene expression, and survival. We hypothesized that tumors
with high levels of APOBEC-mediated mutagenesis would be enriched for mutations in DNA damage response genes and express genes related to activation of the immune system at higher levels.

2. Methods:

2.1 The Cancer Genome Atlas data

The Cancer Genome Atlas (TCGA) bladder urothelial carcinoma data was downloaded from the Broad Institute Genome Data Analysis Center (GDAC) (http://gdac.broadinstitute.org) and from cBioPortal (http://cbioportal.org). Data from GDAC was downloaded on November 8, 2016, from the analysis timestamp “analyses_2016_01_28” (doi:10.7908/C19G5M58). Downloaded data includes clinical and demographic data (age, sex, tumor stage, overall survival), mutation annotation files (MutSig 2CV v3.1; MAF file; Mutsig_maf_modified.maf.txt) and mRNA expression (Immumina HiSeq RNAseqV2). RNA-seq mRNA expression levels are presented as RNA-seq by expectation-maximization (RSEM) values.

Clinical information was available on 412 samples, RNA-seq data was available on 408 samples, and mutation information was available on 395 samples for TCGA BLCA data version 2016_01_28. Overlap between the 412 patients with clinical information, 408 patients with RNA-seq data, and 395 patients with mutation annotation information yields 391 patients. Three outliers were removed from mutational analysis (TCGA-DK-A6AW, >150 mutations/Mb; TCGA-XF-AA N8 and TCGA-FD-A43, both with ≤5 total mutations).

2.2 Mutation analysis and APOBEC enrichment

Analysis and visualization of mutations from mutation annotation format (MAF) files was performed using R version 3.3.3, Bioconductor version 3.4 (http://www.Bioconductor.org), and
Mutation rates per sample were calculated using MutSig2CV v3.1 from the Broad Institute GDAC. APOBEC enrichment score based on the frequency of TCW>T/G mutations was calculated as previously described. Samples were classified into two groups: “APOBEC-high” based on APOBEC enrichment > 2 and Benjamini-Hochberg false-discovery-rate corrected p-value < 0.05; and “APOBEC-low” based on an APOBEC enrichment < 2 and/or Benjamini-Hochberg false-discovery-rate corrected p-value ≥ 0.05. Survival outcomes between patients with APOBEC-high-enrichment and APOBEC-low-enrichment was performed using log-rank test and Kaplan-Meyer curves (R packages survival v 2.41-2, survminer v0.3.1, ggplot2 v2.2.1).

Significantly differentially mutated genes between APOBEC-high-enrichment and APOBEC-low-enrichment groups was performed using MAFtools as previously described and visualized with oncoplots and forest plots.

2.3 Molecular Subtyping

Molecular subtyping of 408 bladder urothelial carcinoma samples with RNA-seq data was performed using R, Bioconductor, and multiclust version 1.4.0. Samples were classified as luminal, p53-like, basal, or claudin-low as previously described with hierarchical clustering using Euclidean distance and Ward’s linkage method (ward.D2; hierarchical clustering analysis shown in Supplementary Figure 1). Differences in mutational load and expression of APOBEC3 enzymes (RSEM) between tumor subtypes was compared using ANOVA.

2.4 Differential gene expression and gene expression associated with APOBEC enrichment

Differential expression analysis between APOBEC-high and APOBEC-low tumors was performed using R, Bioconductor, limma version 3.30.13 and edgeR version 3.16.5. Association of gene expression with numeric APOBEC enrichment score was performed using Spearman’s rho. Functional
annotation of genes was performed with DAVID version 6.8 (http://david.ncifcrf.gov)\textsuperscript{32,33} and visualized with Enrichr (http://amp.pharm.mssm.edu/Enrichr/)\textsuperscript{34,35}.

3. Results:

3.1. APOBEC mutagenesis in bladder cancer

In bladder cancer, as the mutation load increases, the frequency of specific nucleotide conversions changes, and C\textgreater{}G mutations become more common (Figure 1A). Many of these mutations are a specific contextual TCW\textgreater{}T/G mutation attributed to the APOBEC family of enzymes. Of 389 tumors in the provisional TCGA bladder urothelial carcinoma dataset, 324 are enriched for APOBEC mutagenesis (“APOBEC-high”) vs. 64 with low or no enrichment (“ABPOEC-low”). APOBEC-high tumors have improved overall survival compared to APOBEC-low tumors (median overall survival 38.2 vs 18.5 months, \(p=0.0050\), Figure 1B).

APOBEC-low tumors are more likely to be low-grade (17\% vs 3\%, \(p<0.0001\)), but there is no significant difference in age at diagnosis, tumor stage, tumor subtype, or patient smoking history category between groups (Supplementary Table 1). Interestingly, a higher frequency of Asian patients was noted in the APOBEC-low group (26\% vs 7\%), and \(APOBEC3B\) was expressed at a lower level in Asian patients vs. patients not of Asian ethnicity (\(p<0.0001\), Supplementary Figure 2).

APOBEC-high tumors have a higher number of variants per sample and a higher proportion of C\textgreater{}T and C\textgreater{}G mutations (Figure 1C-D). Despite an association of APOBEC enrichment score with total mutations, 42\% of APOBEC-high tumors have a mutational burden below the median (Supplementary Figure 3), and APOBEC-high and APOBEC-low tumors share many of the commonly mutated genes in bladder cancer (Figure 1E).
3.2. Frequency of mutations in APOBEC-high and APOBEC-low tumors

To determine what mutations were associated with APOBEC mutagenesis, we next compared significantly mutated genes between APOBEC-high and APOBEC-low tumors. After correction for multiple comparisons, APOBEC-high tumors were more likely to have mutations in TP53, PIK3CA, ATR, BRCA2, MLL, MLL3, and ARID1A, among others; while APOBEC-low tumors were more likely to have mutations in KRAS and FGFR3 (Figure 2A-B; complete list of differentially mutated genes in Supplementary Table 2).

Functional annotation of differentially mutated genes demonstrates that APOBEC-high tumors are enriched for mutations in DNA damage repair genes (e.g. TP53, ATR, BRCA2, POLQ) and chromatin modification genes including MLL, MLL3, ARID1A, NCOR1, BPTF, CHD7, and others. A full list of gene ontology terms associated with genes mutated in APOBEC-high tumors can be found in Supplementary Table 3. APOBEC-low tumors were significantly more likely to harbor mutations in KRAS and FGFR3. As in the original TCGA dataset (n=131) \(^{36}\), non-synonymous mutations in KRAS and FGFR3 are mutually exclusive (Figure 2C).

3.3. Expression of APOBEC3A and APOBEC3B correlate with mutational burden

We next investigated the role of expression of APOBEC3 enzymes with total mutations in both the entire TCGA bladder cancer dataset and in the four molecular subtypes (luminal, p53-like, basal, and claudin-low). Expression of APOBEC3A and APOBEC3B correlate with the total mutation burden in bladder cancer (Figure 3A-B), while expression of APOBEC3F and APOBEC3G correlate weakly with total mutations and APOBEC3C, APOBEC3D, and APOBEC3H do not correlate with total mutations (Supplementary Figure 4).

Total mutations, APOBEC enrichment score, and the percentage of tumors classified as APOBEC-high vs APOBEC-low are not different between subtypes of bladder cancer (Supplementary Figure 5).
However, APOBEC3A is expressed at a significantly higher level in the basal subtype than in luminal, p53-like, or claudin-low subtypes (Figure 3C), while APOBEC3B is evenly expressed across subtypes (Figure 3D). Despite this, APOBEC3A expression levels correlate with total mutations in every subtype, as do APOBEC3B expression levels (Supplementary Figure 6).

3.4. Gene expression associated with APOBEC enrichment

We approached gene expression association with APOBEC enrichment by two methods. We first analyzed differentially expressed genes between APOBEC-high and APOBEC-low tumors. APOBEC-high tumors were enriched for expression of genes related to regulation of the immune response and lymphocyte-mediated immunity (Figure 4A and Supplementary Table 4), whereas APOBEC-low tumors with higher expression of genes related to transcription and translation (Figure 4B and Supplementary Table 5). Similarly, correlation between numeric APOBEC enrichment score and gene expression revealed a positive relationship between APOBEC enrichment score and gene families involved in IFN-gamma signaling, antigen presentation, and regulation of the immune response, including immune checkpoint HAVCR2 (also known as TIM-3; r=0.229, p<0.0001) (Figure 4C and Supplementary Table 6), while genes inversely correlated with APOBEC enrichment score were enriched in gene families related to transcription and translation (Figure 4D and Supplementary Table 7).

4. Discussion:

APOBEC mutagenesis is the predominant mutational pattern in bladder cancer. In this paper, we demonstrate that tumors enriched for APOBEC mutagenesis (APOBEC-high tumors) have better survival and are more likely to have mutations in DNA damage repair genes and chromatin regulation genes. Expression of APOBEC3A and APOBEC3B correlates with overall mutation load in bladder cancer, regardless of molecular subtype. In addition, APOBEC enrichment is associated with upregulation of
immune-related genes including interferon signaling. Unexpectedly, we also demonstrate that tumors not featuring the APOBEC mutational pattern (APOBEC-low tumors) are significantly more likely to harbor mutations in FGFR3 and KRAS, which are mutually exclusive.

Our work is consistent with several prior studies linking APOBEC expression to mutational burden. APOBEC3B expression is upregulated in breast cancer and is associated with overall mutations\(^1\). Our group also demonstrated the relationship between APOBEC3B expression and mutations in bladder cancer\(^3\). Overexpression of APOBEC3B in breast cancer cell lines results in DNA fragmentation, increased C>T mutations, delayed cell cycle arrest, and eventual cell death\(^1\). Furthermore, knockdown of APOBEC3B with short hairpin RNA in breast cancer cell lines decreases total number of uracil lesions, TP53 mutations, and C>T mutations\(^1\).

APOBEC3A expression was not initially detectable in breast cancer cell lines\(^1\), and APOBEC3B expression correlates strongly with overall mutations in multiple malignancies\(^1,15\), leading many to believe that APOBEC3B is responsible for the majority of these mutations. However, APOBEC3A expression is also correlated with mutational burden\(^3\), as we demonstrate again here, APOBEC3A is highly proficient at cytidine hypermutation and creation of DNA double-strand breaks\(^38,39\), and may have a larger role in mutagenesis than previously recognized\(^10\).

Expression alone of APOBEC enzymes is insufficient to explain APOBEC-mediated mutagenesis. In this study, we demonstrate that although the basal subtype of bladder cancer has significantly higher expression of APOBEC3A than other subtypes, basal tumors do not have greater number of mutations or APOBEC enrichment compared to the other subtypes, and APOBEC3A expression correlates with total mutation burden even in subtypes with lower levels of APOBEC3A expression. This suggests that a baseline level of APOBEC expression is required for APOBEC mutagenesis, but above a certain threshold other factors are also influential\(^4\). Post-translational modification regulating APOBEC enzymatic activity
may play a role. Alternatively, as with any enzymatic reaction, increasing substrate availability may move equilibrium toward the accumulation of mutated DNA products.

The proposed substrate for APOBEC mutagenesis is ssDNA, a common DNA repair intermediate that may accumulate in cells with defects in DNA repair pathways. Interestingly, APOBEC-high tumors are more likely to have mutations in genes related to DNA repair and chromatin regulation. In breast cancer, tumor cell lines with high levels of APOBEC3B expression are more likely to have mutations in TP53. Furthermore, APOBEC-high breast cancers are more likely to have mutations in TP53, NCOR1, MLL3 and other genes involved in DNA replication stress. In this study, we demonstrate that APOBEC-high bladder tumors are more likely to have mutations in TP53, NCOR1, MLL3 (KMT2C), MLL (KMT2A), ATR, BRCA2, and other genes related to DNA repair and chromatin modification.

We also demonstrated a higher frequency of PIK3CA mutations in APOBEC-high bladder tumors. PIK3CA has been previously reported to be mutated at a high frequency in specific TCW-containing helical motifs across a number of tumor types. Our analysis supports these results, with the majority of PIK3CA mutations in APOBEC-high tumors occurring in the helical domain at E542 and E545; no mutations in the helical domain were seen in APOBEC-low tumors (Supplementary Figure 7). These specific mutations in E542 and E545 have also been reported as hotspot mutations in breast cancer.

Interestingly, APOBEC-low tumors in this study were more likely to have mutations in the oncogenes FGFR3 and KRAS, which are mutually exclusive. This suggests that tumors not enriched for the APOBEC mutational pattern may be driven by oncogenes which may dysregulate cellular homeostasis via mechanisms that do not result in accumulation of ssDNA intermediates used as substrate for APOBEC mutagenesis.

APOBEC enrichment was associated with overall survival and expression of immune related genes. APOBEC3A expression measured by Nanostring has previously been associated PD-L1 expression on
tumor-infiltrating mononuclear cells in bladder cancer.\textsuperscript{44} APOBEC mutagenesis is associated with overall mutational burden in bladder cancer, which likely reflects downstream neoantigen burden and subsequent immune response.\textsuperscript{45-47}

Based on our results and the above discussion, we propose a working model of mutagenesis and the immune response in bladder cancer (Figure 5), in which a urothelial cell acquires one or more driver mutation(s). Accumulation of mutations in $TP53$, $ATR$, $BRCA2$, and/or other DNA damage response genes or chromatin regulation genes may result in the accumulation of ssDNA substrate for to $APOBEC3A$ and $APOBEC3B$, leading to a high level of APOBEC-mediated mutagenesis and a hypermutation phenotype. This hypermutation in turn leads to a large neoantigen burden and the subsequent immune response generated from this increase in neoantigens. In addition, APOBEC enzymes, especially $APOBEC3A$, may be induced and overexpressed in response to interferon\textsuperscript{4,8,39,48}, potentially causing a positive feedback loop. In contrast, other tumors with mutations in the $FGFR3$/$RAS$ pathway or other oncogenes may not expose sufficient substrate ssDNA to APOBEC enzymes to undergo significant APOBEC mutagenesis. These tumors have poor survival, despite an enrichment for $FGFR3$ mutations and low-grade tumors, which were classically considered more benign phenotypes.

Several limitations of this study warrant mention. We utilized the provisional TCGA dataset, for which mutational and expression data is readily available. Results warrant replication in other datasets. The TCGA does not currently include any systemic treatment-related information. However, mutations in $ERCC2$\textsuperscript{49,50} and other DNA repair genes\textsuperscript{51,52} are associated with response to platinum-based therapy, and further investigation into the role of APOBEC mutagenesis and response to both cytotoxic chemotherapy and immunotherapy is warranted. Another limitation is the lack of a specific gene expression signature observed in APOBEC-low tumors other than transcription- and translation-related genes, potentially due to the heterogeneity of this group. In addition, gene expression correlations with
APOBEC enrichment score in APOBEC-low tumors would not be expected to generate a strong signal, as these tumors by definition have a low and heterogeneous numerical APOBEC enrichment score.

In summary, APOBEC enzymes are a major source of mutation in bladder cancer. Tumors enriched for APOBEC mutagenesis have better survival and are more likely to have mutations in DNA damage repair genes and chromatin modifying genes. The APOBEC mutagenesis signature is associated with increased expression of immune-related genes. Bladder tumors not enriched for APOBEC mutagenesis are more likely to have mutations in KRAS and FGFR3, which are mutually exclusive, and these tumors have poor overall survival. Further study of the regulation of APOBEC enzymes, mutagenesis, and response to subsequent therapy may provide further insight into the mutational landscape and potential therapeutics for bladder cancer.

5. Acknowledgements:

The results published here are in whole or part based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/. These results were presented in part at the American Urological Association 2017 meeting (Boston, MA; Abstract #17-3454).

6. Funding Sources

JMJ is funded by a Veterans Health Administration Merit grant BX0033692-01 and the John P. Hanson Foundation for Cancer and Cellular Research at the Robert H. Lurie Comprehensive Cancer Center of Northwestern University. Funding sources had no role in writing of the manuscript or the decision to submit it for publication.

7. Conflicts of Interest

The authors declare no competing interest.

8. Author Contributions
APG and JJM conceived the project ideas. JJM was responsible for project final design, supervision, interpretation of the data. APG, DF, and KR performed data collection and analysis. APG drafted the manuscript. All authors were involved in critical revision of the article and final approval of the version to be published.

9. References:

10. Figure Legends

**Figure 1.** APOBEC-mediated mutagenesis in the TCGA bladder cancer cohort (n=389). (A) Percentage of single nucleotide variations (SNVs) as a function of mutation load. Genomes were binned in groups of 20 samples according to mutation load. (B) Kaplan-Meier survival curve of APOBEC-high and APOBEC-low bladder tumors. (C) Summary of mutagenesis in APOBEC-high tumors, including number of variants per sample, variant classification, and class of SNV. Stacked barplot demonstrates percentage of SNVs in a representative sample of 64 tumors for comparison with APOBEC-low tumors. (D) Summary of mutagenesis in APOBEC-low tumors, including number of variants per sample, variant classification, class of SNV, and stacked barplot of the percentage of SNV per tumor. (E) Oncoplot of the top genes commonly mutated in bladder cancer in APOBEC-high and APOBEC-low tumors.

**Figure 2.** Significantly differentially mutated genes in APOBEC-high and APOBEC-low tumors. (A) Oncoplot of genes differentially mutated in APOBEC-high and APOBEC-low tumors. (B) Forestplot of differentially mutated genes in APOBEC-high and APOBEC-low tumors with log10 odds ratio and 95% confidence intervals and adjusted p-value (MutSig 2CV 3.1; MAFtools v1.0.55). (C) Oncoprint of nonsynonymous mutations in FGFR3 and KRAS (n=395).

**Figure 3.** Correlations of APOBEC3A and APOBEC3B expression with mutational burden and molecular subtype. (A) Spearman correlation between total mutations and APOBEC3A expression. (B) Spearman correlation between total mutations and APOBEC3B expression. (C) Expression of APOBEC3A in the molecular subtypes of bladder cancer. (D) Expression of APOBEC3B in the molecular subtypes of bladder cancer.

**Figure 4.** Barplot of gene ontology biological processes for: (A) genes highly expressed in APOBEC-high tumors (B) genes highly expressed in APOBEC-low tumors (C) genes positively correlated with APOBEC enrichment score (D) genes negatively correlated with APOBEC enrichment score. Figure generated with Enrichr. Bar size based on combined score based on p-value and deviation from expected rank.

**Figure 5.** Working model of APOBEC-mediated mutagenesis in bladder cancer. Accumulation of mutations in TP53, ATR, BRCA2, and/or other DNA damage response genes or chromatin regulation genes may expose more substrate ssDNA to APOBEC3A and APOBEC3B, leading to a high level of APOBEC-mediated mutagenesis and a hypermutation phenotype, with subsequent neoantigen burden, immune response, and survival benefit. Tumors with mutations in FGFR3 and KRAS may not expose enough substrate to APOBEC enzymes to promote APOBEC-mediated mutagenesis.
A
- regulation of cell killing (GO:0031341)
- regulation of innate immune response (GO:0045088)
- regulation of lymphocyte mediated immunity (GO:0002706)
- positive regulation of lymphocyte mediated immunity (GO:0002708)
- regulation of immune effector process (GO:0002697)
- cellular defense response (GO:0006968)
- positive regulation of leukocyte mediated immunity (GO:0002701)
- regulation of leukocyte mediated immunity (GO:0002703)
- positive regulation of cell killing (GO:0031343)
- positive regulation of immunoglobulin mediated immune response (GO:0002891)

B
- nuclear-transcribed mRNA catabolic process, nonsense-mediated decay (GO:0000184)
- translational termination (GO:0006415)
- viral transcription (GO:0019083)
- translational initiation (GO:0006413)
- translation (GO:0006412)
- cellular protein complex disassembly (GO:0043624)
- translation elongation (GO:0006414)
- SRP-dependent cotranslational protein targeting to membrane (GO:0006614)
- cotranslational protein targeting to membrane (GO:0006613)
- protein targeting to ER (GO:0045047)

C
- interferon-gamma-mediated signaling pathway (GO:0060333)
- cellular response to interferon-gamma (GO:0071346)
- response to interferon-gamma (GO:0034341)
- antigen processing and presentation of exogenous antigen (GO:0019884)
- antigen processing and presentation of exogenous peptide antigen (GO:0002478)
- antigen processing and presentation of peptide antigen (GO:0048002)
- antigen processing and presentation of peptide antigen via MHC class I (GO:0002474)
- cytokine-mediated signaling pathway (GO:0019221)
- antigen processing and presentation of exogenous peptide antigen via MHC class I (GO:0042590)
- antigen processing and presentation (GO:0019882)

D
- translational elongation (GO:0006414)
- translational initiation (GO:0006413)
- translation (GO:0006412)
- viral transcription (GO:0019083)
- translational termination (GO:0006415)
- nuclear-transcribed mRNA catabolic process, nonsense-mediated decay (GO:0000184)
- cellular protein complex disassembly (GO:0043624)
- SRP-dependent cotranslational protein targeting to membrane (GO:0006614)
- cotranslational protein targeting to membrane (GO:0006613)
- protein targeting to ER (GO:0045047)
TP53 mutations
DNA damage response mutations
Chromatin regulation mutations

Initial insult and driver mutations

FGFR3 mutation
KRAS mutation
Mutations in other oncogenes

APOBEC-low tumors

TP53 mutations

PD-1 / PD-L1
Other immune checkpoints

IFN

Exposure of ssDNA to APOBEC

Mutations in other oncogenes

Possible upregulation of APOBEC3A and APOBEC3B by IFN

TCW>T/G mutational signature

Neoantigens

Immune Response

APOBEC3A
APOBEC3B

IFN

IFN-1/ IFN-L1
Other immune checkpoints