APOBEC and Cancer Viroimmunotherapy: Thinking the Unthinkable

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ABSTRACT

The apolipoprotein B mRNA editing enzyme catalytic polypeptide (APOBEC) family protects against infection by degrading incoming viral genomes through cytosine deamination. Here, we review how the potential to unleash these potent DNA mutagens comes at a price as APOBEC DNA mutagenesis can contribute to development of multiple types of cancer. In addition, because viral infection induces its expression, APOBEC is seen as the enemy of oncolytic virotherapy through mutation of the viral genome and by generating virotherapy-resistant tumors. Therefore, overall APOBEC in cancer has received very poor press. However, we also speculate how there may be silver linings to the storm clouds (kataegis) associated with APOBEC activity. Thus, although mutagenic genomic chaos promotes emergence of ever more aggressive subclones, it also provides significant opportunity for cytotoxic and immune therapies. In particular, the superpower of cancer immunotherapy derives in part from mutation, wherein generation of tumor neoantigens—neoantigenesis—exposes tumor cells to functional T-cell repertoires, and susceptibility to immune checkpoint blockade. Moreover, APOBECs may be able to induce suprathreshold levels of cellular mutation leading to mitotic catastrophe and direct tumor cell killing. Finally, we discuss the possibility that linking predictable APOBEC-induced mutation with escape from specific frontline therapies could identify mutated molecules/pathways that can be targeted with small molecules and/or immunotherapies in a Trap and Ambush strategy. Together, these considerations lead to the counterintuitive hypothesis that, instead of attempting to expunge and excoriate APOBEC activity in cancer therapy, it might be exploited—and even, counterintuitively, encouraged.

Introduction: APOBECs—Where Viruses Fear to Tread But Cancers Progress

The apolipoprotein B mRNA editing enzyme catalytic polypeptide (APOBEC) family of enzymes are antiviral factors with cytosine deamination activity that leads to mutation of viral genomes thereby reducing their infectivity (1–3). They are named after APOBEC1, which edits both single-stranded DNA cytosines and cellular mRNA cytosines. Human cells express 11 family members, including seven APOBEC3 proteins, AID, APOBEC1, APOBEC2, and APOBEC4 (4–6). Although the human genome encodes seven distinct APOBEC3 enzymes (APOBEC3A though H), the mouse genome encodes a single gene (7). The seven APOBEC3 proteins catalyze single-stranded DNA cytosine to uracil (C-to-U) deamination (4) serving a major function in their antiviral activities, such as deamination of HIV type 1 cDNA replication intermediates (8). However, as well as mutating incoming viral genomes, APOBEC activity can have significant mutagenic effects upon the cellular genome. This leads predominantly to cytosine to thymine (C-to-T) transition mutations (9–11), although cytosine to guanine (C-to-G) transversions and other mutations can result from APOBEC activity during DNA repair (11).

Tumor cells readily escape from therapy through multiple genetic and epigenetic mechanisms derived from the genetic instability inherent to cancer (9, 12–14). This genetic instability confers phenotypic plasticity and allows for the selection and expansion of therapeutically resistant subclones (15, 16). While there are multiple mechanisms of DNA mutagenesis associated with development of cancer—including spontaneous methylation-associated errors, ultraviolet light, oxidative damage, environmental carcinogens, errors in DNA polymerase/repair activity—it is now clear that dysregulation of the APOBEC family of cytosine deaminase enzymes contributes a major endogenous source of DNA damage, which drives the initiation and evolution of cancers either alone, or in the presence of response to different therapies (9, 17). Evidence of APOBEC mutation exists in approximately half of all human cancers (9) and APOBEC mutational signatures are associated with poor prognosis and therapeutic resistance in multiple cancer types (18–27). For example, high levels of *APOBEC3B* expression have been reported to act as an independent prognostic factor to predict both worse overall survival and disease-free survival of patients with ovarian cancer (21). High levels of APOBEC3A and APOBEC3B expression have been associated with aggressive tumor phenotypes in breast cancer (22). Similarly, mutational patterns associated with APOBEC deregulation have been observed at the sites of translocations of the MYC gene in patients with multiple myeloma which occur in patients with very poor clinical outcomes (18, 21, 22). In addition, cancer subtypes that contain the specific APOBEC3B mutation pattern express high levels of APOBEC3B relative to other APOBEC family members (9, 28, 29) and APOBEC3B levels in primary estrogen receptor–positive (ER^+) breast tumors inversely correlated with the clinical benefit of tamoxifen in patients with metastatic ER^+ disease (19).

Overall, therefore, it seems clear that the unavoidable tax that APOBECs impose for their service of protecting against invading pathogens is an inherent mutability of our own genomes.

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Therapy-Driven, APOBEC-Induced Mutational Escape

A common clinical observation is that, regardless of the treatment, a subset of patients initially develops promising clinical responses, followed by aggressive recurrence resulting in uncontrolled, lethal, tumor growth. These treatment-resistant tumors differ significantly from primary tumors immunogenically, genetically, and phenotypically (9, 12, 13, 30–34) and arise as the result of high mutational plasticity. This genomic instability allows tumor cells to escape frontline treatment through evolution of a pool of mutated tumor cells from which highly aggressive, treatment-resistant clones are selected (9, 12, 13, 15, 16, 32–35). We have shown that APOBEC3 induction following frontline treatment with adoptive T-cell therapy, HSVtk/ganciclovir gene therapy, or oncolytic virotherapy has profound consequences for the generation of escape variants from all three types of therapy (32–34). For example, tumor cells recently infected with vesicular stomatitis virus (VSV) induced human APOBEC3B, or murine APOBEC3, in a type I IFN–dependent manner (33). This led to mutation of both the target tumor cell genome, allowing selection of clones resistant to oncolysis (34), as well as degradation of the infecting viral genome, leading to loss of oncolytic efficacy of the virus (33). Those tumor cells which escaped oncolysis by VSV, and were resistant to subsequent VSV-mediated killing (VSV-ESC; ref. 33), carried stable APOBEC3B mutational signatures in multiple genes (34). Some of those mutations which were selected at the highest frequency in VSV-ESC cells play critical roles in replication of VSV, and their mutation was a key mechanism by which cells would escape VSV replication/oncolysis. Similarly, suboptimal HSVtk/ganciclovir gene therapy or T-cell immunotherapies which did not kill all of the tumor cells directly triggered induction of APOBECs which subsequently mediated tumor cell mutation, adaptation, and escape (32–34).

Therefore, APOBECs represent the smoking gun which links known initiators of carcinogenesis (pathogen infection, inflammation, environmental, epigenetic, and physiologic factors which deregulate their expression) with at least a subset of those mutations which play a pivotal role in the initiation and progression of many cancer types (9–11, 17), as well as in the ability of tumors to evade ongoing therapies (30). Hence, inhibition of multiple APOBECs has been explored as a way to slow the evolution of aggressive tumors and to prevent escape from therapy (36) and APOBEC inhibition may be an important adjuvant to many therapies to reduce the risk of treatment failure and prevent recurrence, such as with oncolytic viral therapies (**Fig. 1**; ref. 33). Conversely, in the case of the treatment of virally induced cancers, such as those associated with Epstein-Barr virus or human papillomavirus (HPV; ref. 37) infection, to which APOBEC induction is a natural response to restrict viral infection, it may be that APOBEC inhibition might be more detrimental than beneficial.

Finding a Silver Lining

So far, so bad then for APOBECs because high mutational plasticity in tumor cells is regarded as deleterious (9–11, 36). However, several strands of experimental and clinical data suggest maybe the APOBECs have some redeeming features, which may change our perspective of them in the field of cancer therapies of the future.

Central to how the image of the APOBECs might receive a significant boost is the meteoric rise of cancer immunotherapy. It is now clear that high mutational loads within tumor cells correlate very well with response to cancer immunotherapy, especially immune checkpoint blockade (ICB; refs. 38–41). Mutation of either passenger or driver (15, 16, 35) genes generate novel neoepitopes within cancer cells, which may be sufficiently different from the unmutated, self-epitopes to prime, and activate, preexisting T cells which have not been tolerized or deleted (40, 42, 43). Activation of these neoepitope-specific T cells can then lead to potentially therapeutic antitumor T-cell responses (44–46). Neoepitopes are encoded by nonsynonymous mutations, splice variants, or genome rearrangements that transform a self-peptide into a non–self-peptide. Identification of neoepitopes from patients, and strategies to activate cognate T cells against them, have provided effective clinical therapy (44–46). So in this context, high levels of genomic instability and mutation begin to look rather more positive and the question arises as to where exactly do these immune-uncloaking mutations come from? Although DNA mutation in cancer cells originates from a wide variety of both natural and therapy-induced sources (47), at least part of the answer to this question is from APOBEC activity. Not only do those cancers which do well with ICB often have high mutational burdens (40, 41), they are often also closely associated with either high detectable levels of APOBEC expression/activity, or with mutational signatures which suggest that "APOBEC was here" (9). So, for example, both cutaneous melanoma and squamous cell lung carcinoma typically have high levels of coding somatic mutations per Mb of DNA and are in the upper echelons of response rates with ICB (20%–40%; **Fig. 2**; ref. 41). These two tumor types also score very well in the levels of detectable APOBEC expression (**Fig. 2**; ref. 9), consistent with APOBEC3B expression correlating with increased frequency of tumor-infiltrating lymphocytes(48, 49). In contrast, glioblastoma and gliomas typically score among the lowest numbers of coding mutations per Mb, are notoriously unresponsive to ICB, and have the lowest levels of APOBEC expression (**Fig. 2**). Of all tumor types, uveal melanoma is at the rock bottom of mutational load and very rarely responds to ICB (**Fig. 2**; refs. 41 and 50). Intriguingly, however, in a rare example of uveal melanoma actually responding to anti–PD-1 ICB, a MBD4-related hypermutator phenotype was identified which was associated with a high CpG>TpG mutation burden, exactly as has been observed with APOBEC in other cancers (9, 50).

At the other end of the spectrum, Merkel cell cancer has one of the highest response rates to ICB but is only intermediate in its levels of mutational burden (**Fig. 2**; ref. 41). Risk factors for Merkel cell cancer include either polyoma virus infection (in which tumors have very low mutational burdens) or UV irradiation (in which tumors have very high mutational burdens and UV mutational signatures; refs. 51 and 52). It is tempting to speculate that the sensitivity of this type of cancer to ICB relies upon either heightened immunogenicity associated with immunogenic residual polyoma proteins and/or virus infection [which interestingly also induces APOBEC3B activity (53)], or with increased mutational burden associated with UV irradiation (51, 52, 54, 55)—which is the sun's equivalent of APOBEC mutagenesis. Therefore, the intermediate mutational load score for Merkel cell cancers represents a combination of both polyoma virus positive and polyoma virus negative tumors. However, the high response rate to ICB reflects the fact that both such tumor types are excellent candidates for T-cell–mediated tumor control (and, hence, response to ICB). Similarly, in other cancer types such as head and neck, the association with viral infection (in this case HPV) is also likely to affect APOBEC induction, levels, mutational load and response to ICB.

Therefore, it may be that levels of APOBEC expression in individual tumors may be as important as, for example, the levels of exposure to sunlight, or environmental carcinogens, in predicting response to ICB, and other types of immunotherapy.

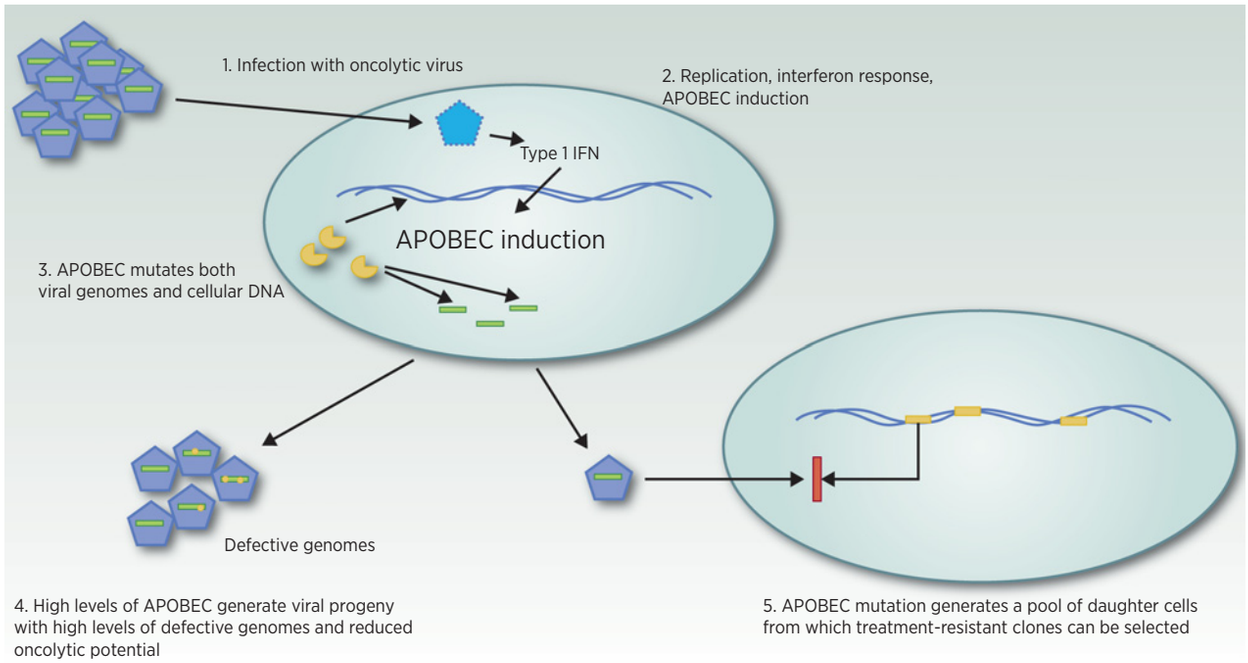


Figure 1.

APOBEC3B mediates resistance to oncolytic virotherapy through both cellular and viral mutation. Infection of tumor cells induces type I IFNs which, in turn, induce APOBEC family members. APOBEC activity degrades the viral genomes, making them less fit as replication competent viruses and decreasing their efficacy as oncolytics. In addition, APOBEC mutagenesis of the cellular genome generates a pool of daughter subclones from which virus resistant cells can be selected, thereby generating more aggressive, treatment escape variants.

Neoantigenesis

These observations imply that induction/overexpression of APOBECs may have two therapeutically opposing consequences. In the first, actively driving mutation of transcriptionally active regions of the genome generates clones with the potential to progress and/or evade most applied therapies. In addition, APOBEC and other mutational inducers could generate critical mutations in the antigen presentation machinery, which would abrogate tumor cell immunogenicity. Conversely, APOBEC-mediated mutation of the genes encoding the cellular immunopeptidome might generate neoepitopes—neoantigenesis—which would prime T-cell responses against the newly immunogenic tumor cells. If these two counteracting effects both exist, the implication is that there must be a sweet spot level of mutation for cancer cells which allows for sufficient diversity generation without exposing the new clones to punitive levels of immune surveillance (**Fig. 3**).

This concept of a "just right" amount of mutation mediated by APOBECs, and other mutagens, has already been proposed from a cell biology, as opposed to an immunologic, perspective. Thus, although cancers thrive on the genomic diversity induced by mutagenesis to adapt and evolve away from selective pressures, too much mutation will ultimately lead to cell lethality resulting from catastrophic disruption of the cell's essential genetic information (**Fig. 3**). This suggests a "just right" model for tumor diversity in which neither too little, nor too much, mutation is optimal for cancer cell survival/adaptation/progression (9, 56, 57). This model is supported by clinical data showing that the most aggressive cancers can be associated with intermediate levels of chromosomal instability rather than either higher, or lower levels (58, 59). Therefore, there is a cytotoxic rationale for the active enhancement of genetic instability to push "just right" levels of mutation in cancers over the top into the zone where so much mutation occurs that cell viability is lost (**Fig. 3**; ref. 9).

To address whether this same "just right" level of mutation also holds from an immunotherapeutic standpoint, we used a model in which B16 tumors expressing the *HSVtk* gene are rendered sensitive to chemotherapy with ganciclovir (GCV) treatment (60). When mice bearing B16tk tumors were treated with suboptimal levels of GCV, tumors could be treated effectively but without long-term cures. These same B16tk tumors, in the absence of chemotherapeutic killing, were completely insensitive to treatment with ICB with anti–PD-1 or anti–CTLA-4 antibodies, presumably because the B16 tumors are poorly immunogenic and do not raise potent T-cell responses in the host mice (ref. 34; **Fig. 4**). In the presence of GCV chemotherapy, inflammatory killing (60, 61) augmented the immunogenicity of the tumor cells and conferred some limited sensitivity to ICB (34). When the B16tk tumors were engineered to overexpress APOBEC3B, an extensive mutational burden was induced as detected by whole-genome sequencing. Murine melanomas that overexpressed hAPOBEC3B grew at equivalent rates to parental, unmodified tumors in the absence of applied therapeutic selective pressure as described in ref. 32. However, upon treatments of different types (oncolytic virotherapy, adoptive T-cell therapy, HSVtk/GCV-mediated gene therapy), the APOBEC overexpressing tumors escaped from treatment faster than the control, non–APOBEC-overexpressing tumors, as detailed in refs. 32–34. Thus, as predicted, B16tk-APOBEC3B tumors rapidly escaped GCV therapy and grew faster than the parental tumors (34)—consistent with APOBEC3B-driving mutation and selection of clones which can acquire resistance (9). However, when B16tk-APOBEC3B tumors were treated with GCV and ICB (such as

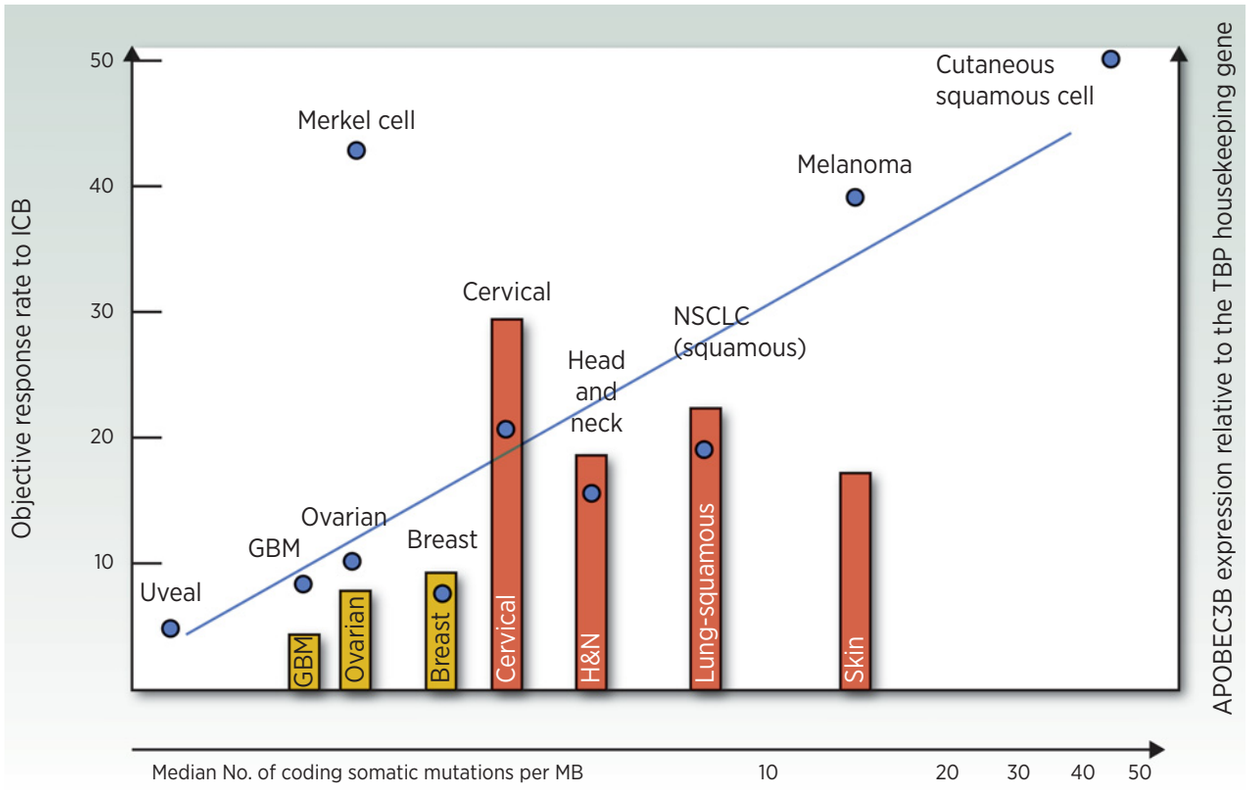


Figure 2.

High levels of APOBEC activity in tumors correlates well with a high mutational load, ICB-sensitive, tumor phenotype. Cancer types which have a high level of mutations (per Mb of DNA) are typically sensitive to ICB therapies; in general, there is also a good correlation between tumors types with high levels of APOBEC activity and those with high mutational loads—suggesting a link between APOBEC activity, genomic mutagenesis and acquired sensitivity to ICB therapies. (Adapted from refs. 15 and 41).

anti-CTLA4), we observed complete cures (**Fig. 4**; ref. 34). So, on the one hand, APOBEC3B mutational activity converted the melanomas into a significantly more aggressive tumor in the face of frontline chemotherapy; however, it also conferred upon the same tumor a phenotype which was exquisitely sensitive to immune ($CD8^+$ T cell) control, although the T-cell response required ICB to be maximally effective. Therefore, in this model at least, chemotherapy's loss was immunotherapy's gain, attributable to the mutations induced by APOBEC3B. The same B16 model has been used extensively by other investigators and has been shown to be sensitive to ICB in the context of tumor cell vaccination—for example, with GM-CSF–modified tumor cells. Therefore, it will be interesting to see just how broad a spectrum of cancer types will respond to increased APOBEC mutagenic activity as a means to confer increased sensitivity to ICB.

These data raise the possibility that the image of APOBECs as agents of genomic chaos might be open to alternative, more positive interpretations (**Fig. 4**).

Tolerating APOBEC Activity in Cancer Therapy APOBEC-driven Trap and Ambush

Comparative genomic data between primary, metastatic, and escaped tumors have defined the difference between "trunk and branch" mutations, which should be targeted by therapy (9, 15, 16, 35). Although it is not possible to target APOBEC expression right at the root of the cancer until it becomes clinically evident, these data suggest that cutting off the supply of mutation as early as possible upon detection (just above the roots at ground level) could target the specific genes/proteins which drive progression, metastasis, escape, and recurrence (9). Conversely, endogenous, even cancer-driving, levels of APOBEC activity in tumors might represent a distinct silver lining surrounding the mutagenic storm cloud by providing predictable, actionable mutations (**Fig. 5**). For example, tumor cell killing by oncolytic viruses can lead to immunogenic priming of antitumor T-cell immunity (62, 63). So, while APOBEC-induced mutation *reduces* direct oncolytic virus efficacy by mutating the incoming virus (**Fig. 1**; ref. 33), this same mutagenic activity turned upon the cellular genome may, in part at least, contribute to the enhanced immunogenicity of tumors undergoing virotherapy (**Fig. 5**).

Therefore, if a frontline therapy, such as oncolytic virotherapy, is known to induce APOBEC-driven mutations which allow for selection of escape variants with certain defined phenotypes, then it should be possible to exploit those mutations for follow-up therapy. This would be the equivalent of shepherding tumor cells into a treatment-driven, highly predictable, phenotype (a trap), which could then be ambushed with an escape-specific, targeted therapy. This ambush could be pharmacologic, by targeting APOBEC-specific mutations in proteins to generate an engineered synthetic lethality for escape tumors.

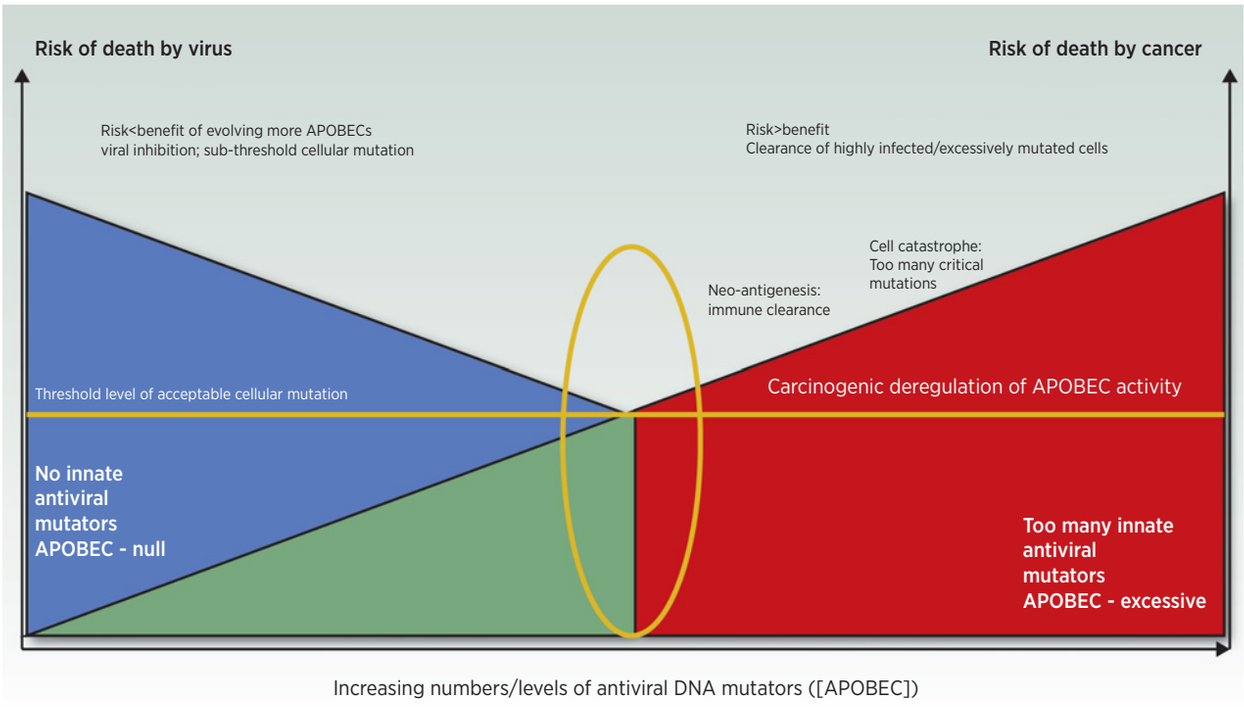


Figure 3.

The "just right" model for the levels of allowable cellular mutation leading to effective cellular protection without catastrophic genomic chaos. There is an inherent contradiction with the presence of so many DNA mutators in the cell's arsenal to counteract infection with the proven contribution that these mutators can make to carcinogenesis. Activation of broad acting anti-pathogen, innate defenses is clearly worth the effort, and associated risks, of evolving multiple APOBECs to complement the complex immune networks that have coevolved to fight virus infections. Upon viral infection, broad spectrum antiviral APOBEC activation would lead to increasing levels of bystander cellular mutation. Below a certain threshold of mutation per kb of DNA, this level of self-harm could be tolerated and may stochastically present an overall favorable risk/benefit balance for the cell. However, if infection continues to escalate, the gloves may come off. Increasing levels of APOBEC activation required to dispose of the intruding viral genomes (on both a cell and population level) may trigger suprathreshold levels of cellular mutation; at these levels of infection/APOBEC induction, so much DNA mutation may accumulate that cells lose their viability. In addition, neoantigenesis generates so many neoepitopes that there is direct recognition of cells which have been mutated to unacceptably high levels, leading to immune clearance. If this is true, it should be possible to correlate levels of APOBEC induction with immune recognition and clearance both *in vitro* and *in vivo*. This, in turn, may help to understand if, and how, forced expression of APOBEC can be exploited to promote neoantigenesis while minimizing tumor promotion.

Alternatively, the ambush could be immunologic in nature—targeting neoepitopes generated as a result of APOBEC-induced mutations in HLA-presented epitopes of escape-promoting proteins as described in ref. 34 (**Fig. 5**). In this respect, tracking cancer evolution in real time in patients using circulating DNA could be a key supporting weapon in the ambush strategy.

Therefore, instead of necessarily seeking to inhibit APOBEC mutation while administering frontline therapy, there may be a case for at the very least tolerating APOBEC to facilitate the active sculpting of predictable phenotypes of escape variants which can then be targeted as they emerge. In this scenario, APOBEC would act as a covert double agent carrying out ostensibly highly collaborative activities within the therapy evading cancer cell, but simultaneously informing on the most likely mechanisms by which it will escape.

Exploiting APOBEC Activity in Cancer Therapy

These data raise the rather counterintuitive hypothesis that it might be possible to use the APOBECs as an immunotherapeutic adjuvant by uncoupling the full catastrophe of their mutagenic activity from their potential to drive sensitivity to ICB.

The least controversial way to exploit the immunogenicity of APOBEC-induced neoantigenesis, while uncoupling mutation from cancer evolution, would be in the context of an *ex vivo* therapeutic vaccine. To achieve this, APOBEC-mutated cells would have to be able to raise T-cell responses against unmutated tumors. The majority of tumor-associated antigens (TAAs) are very poorly, if at all, recognized by T cells because central and peripheral tolerance deletes most T cells which could recognize self-antigens (64, 65). However, a subset of undeleted, nontolerized T cells can survive and recognize native TAA but with very low T-cell receptor (TCR) affinities (64, 65). An additional set of potentially tumor-reactive T cells may exist, which can recognize neoantigens which are significantly different from the native antigens by mutation of the neoepitope (40, 42, 66). Both of these classes of T cells form the targets of ICB following their chronic exposure to tumors in patients (38, 39). We hypothesized that those T cells, which escape central tolerance, but are only weakly reactive to self TAA, could be significantly activated by an epitope that is modified from the weakly HLA-binding wild-type epitope. This neoepitope would bind to the HLA molecule more strongly—thereby sending a more potent T-cell activation signal through the erstwhile weak-

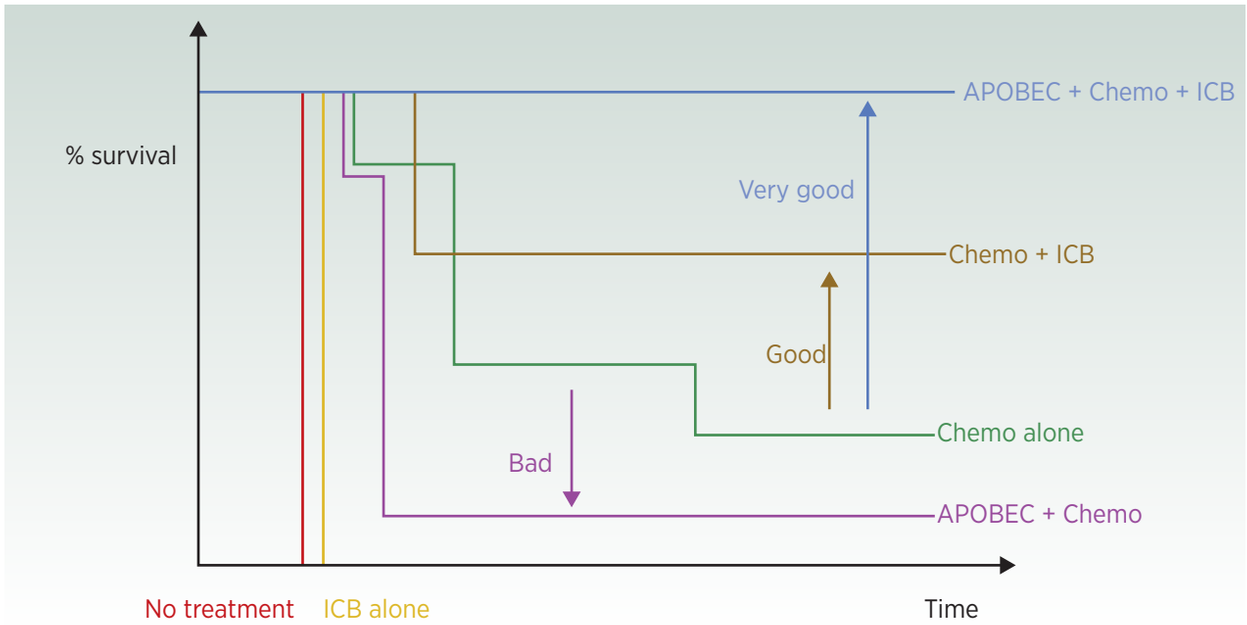


Figure 4.
APOBEC3B activity enhances escape from frontline chemotherapy but confers sensitivity to ICB. Tumors expressing APOBEC3B (purple line) escaped HSVtk/ganciclovir gene therapy/chemotherapy more quickly than parental tumors (green line), consistent with a mutational load generating a pool of clones from which escape variants could be selected. *In vivo* inflammatory killing with chemotherapy increased the sensitivity of the tumor to anti–CTLA-4 ICB (brown line compared with yellow and green lines) consistent with chemotherapy being an effective immune adjuvant. However, tumors expressing APOBEC3B exposed to both chemotherapy and anti–CTLA-4 ICB (blue line) were cured by a $CD8^+$, $CD4^+$ T cell–dependent immune mechanism—showing that APOBEC3B-induced mutations provided a significantly improved immunogenic landscape for the immune system to work with to mediate tumor rejection. (Figure adapted from ref. 34).

affinity TCR or through a completely new high-affinity TCR on a nontolerized T cell. This concept of heteroclitic epitope formation explains how xenogeneic proteins can be more immunogenic than autologous proteins and has been used in experimental cancer vaccination strategies (**Fig. 6**; refs. 67–70), although to date, its significance in clinical treatments remains unclear. Therefore, we tested whether heteroclitic neoepitopes induced by APOBEC3B mutation would activate weakly reactive anti-TAA T cells which would then react back against a host tumor expressing the native protein (71–74). We hypothesized that global APOBEC3B expression would generate a library of (subtly) mutated antigens, raising multiple clones of previously inactive tumor-reactive T cells, thereby reducing the chances of antigen escape. If these T-cell responses are truly heteroclitic, the hope would be that some will target epitopes from proteins which are as close to the trunk of the cancer phenotype as possible. Alternatively, and more probable, heteroclitic T-cell responses will be generated which will target multiple branch mutations—immune control of which may control, but not eradicate, the developing tumor population. In this scenario, APOBEC mutagenesis might allow clearance of "pockets of branches" within the overall tumor. Although the APOBEC3B-mutated tumor cell vaccines themselves were poorly immunogenic, when used with anti–PD-1 ICB, we saw highly effective therapy (34), dependent upon CD4, CD8, and NK cells (**Fig. 6A**). In our experiments, antitumor therapy correlated strongly with detection of CD8 T-cell responses against the APOBEC3B-modified vaccine cells but also, and more importantly, against the unmodified, parental cells, consistent with clinical findings that the major predictor of success across immunotherapeutic protocols is a response in patients to autologous cancer cells (66). Interestingly, even in the presence of ICB, we did not observe any signs of autoimmunity. This heteroclitic epitope activated therapy (HEAT) was broadly applicable to treatment of cancers of different histologic types and locations (34) and does not require identification of specific neoepitopes on a patient-by-patient basis (75, 76). Thus, we would predict that APOBEC activity will generate a spectrum of potential neoepitopes which can be selected by each patient's own immune system based on HLA binding and heteroclitic activity (i.e., different neoepitopes would be relevant for different HLAs).

Thinking the Unthinkable—Hypermutagenic Immunotherapy?

HEAT insulates the mutagenic activity of APOBEC from direct mutation of the tumor itself, which is a big safety plus in its favor. An extension of the HEAT, and Trap and Ambush, philosophies of exploiting, or at least tolerating, APOBEC mutagenesis raises a much more controversial hypothesis. This is that actively driving APOBEC expression in tumors themselves—hypermutagenesis—may induce either (i) a genetic catastrophe leading directly to cell lethality (exceeding the "just right" rule); (ii) a sufficiently high mutational load to render poorly immunogenic tumors susceptible to targeted immunotherapy with ICB; (iii) both or (iv) a rapidly evolving tumor that is capable of killing the patient even quicker.

Although initially alarming as a concept, there is precedent for both (i) and (ii) above. As discussed earlier, clinical evidence supports the existence of a "just right" level of mutation to support cancer aggressiveness (9, 56–59). Too much mutation may induce both

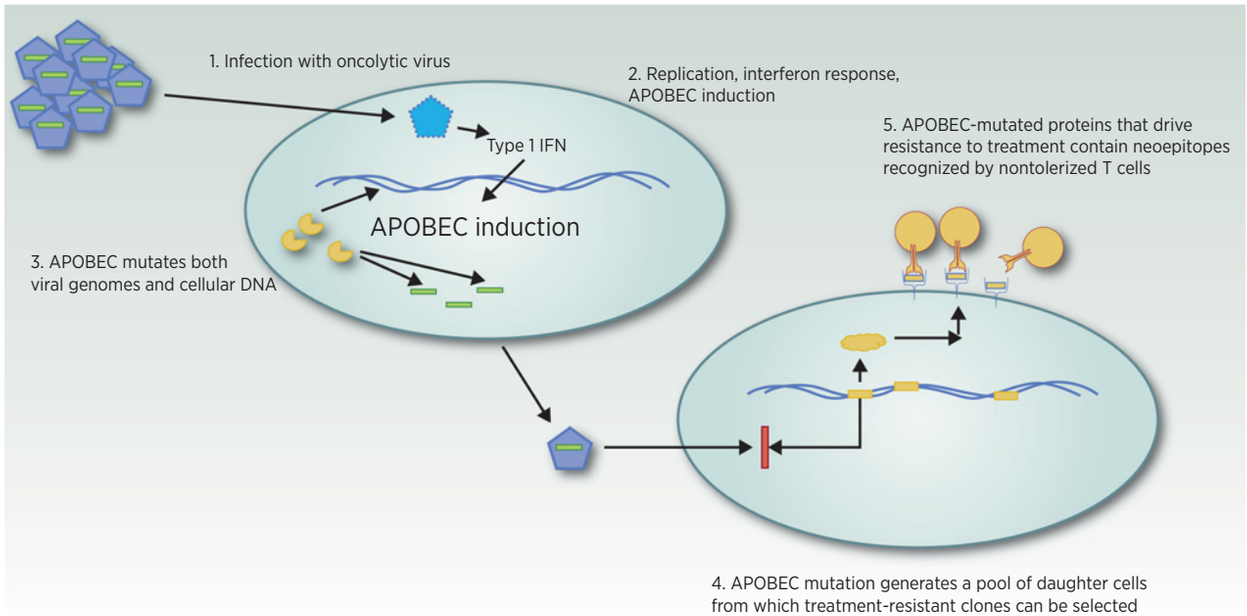


Figure 5.

Trap and ambush viroimmunotherapy through APOBEC-mutation driven neoepitope formation (neoantigenesis). Oncolytic infection of tumor cells induces type I IFNs which, in turn, induce APOBEC family members. APOBEC activity degrades the viral genomes, decreasing their efficacy as oncolytics (**Fig. 1**). In addition, APOBEC mutagenesis of the cellular genome generates a pool of daughter subclones from which virus resistant cells can be selected, thereby generating more aggressive, treatment escape variants. However, a proportion of the APOBEC-mutated cellular proteins that allow for escape from the viral infection may also express new, immunogenic epitopes as a direct result of the mutation. If those mutated proteins are critical to confer the escape phenotype, the frontline oncolytic virotherapy can be used as a trap to drive the predictable and reproducible escape-mediating mutations. These mutations can then be targeted (ambushed) by second line therapies (in this case vaccination against the escape-specific neoepitope) actioned against the APOBEC-driven, escape-driving mutation. (Figure adapted from ref. 34).

mitotic catastrophe through genomic chaos and neoantigenesis, which would trigger an immune surveillance failsafe mechanism. Within the immunotherapy context, hypermutagenesis by inhibition of DNA repair mechanisms led to generation of neoantigens which were effective targets for immune control (46).

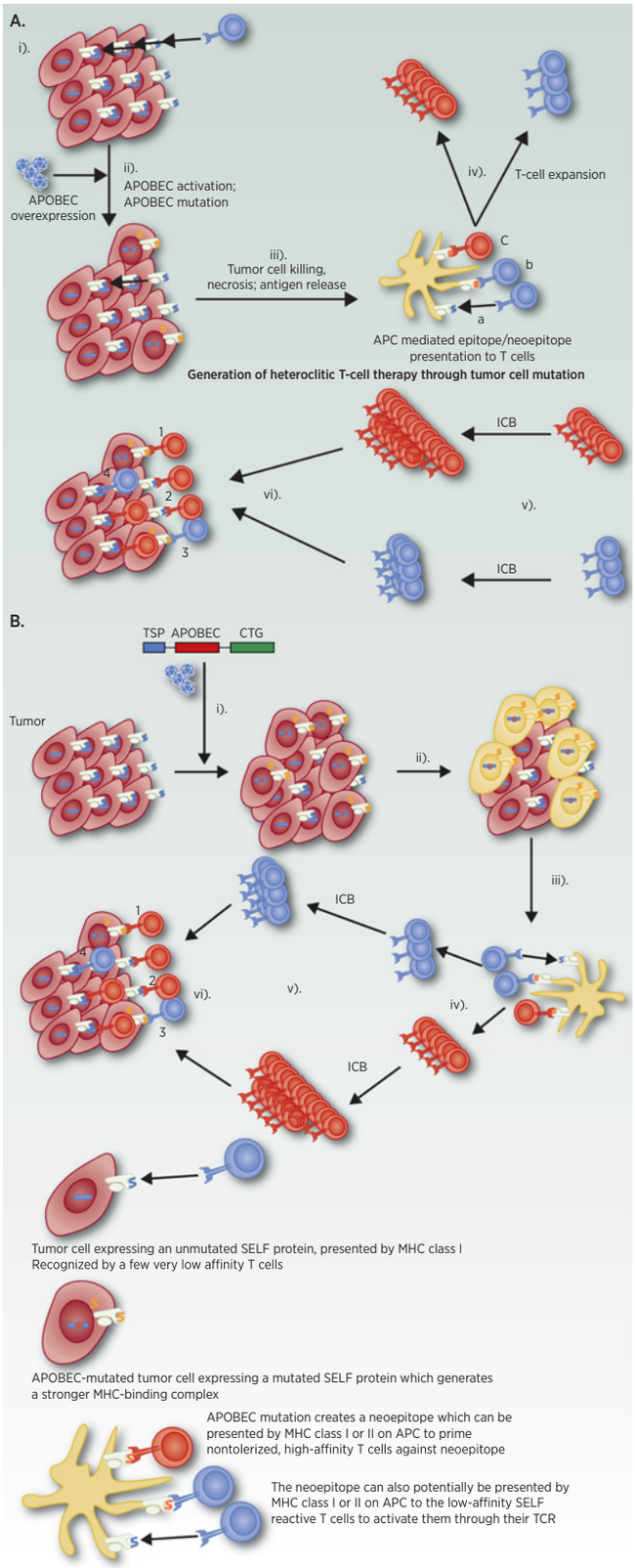
Therefore, we hypothesize that it may be possible to deliver vectors into tumors in which tumor-targeted expression of an APOBEC, such as APOBEC3B, is linked inextricably to that of an inducible potent cytotoxic gene (**Fig. 6B**). Tumor targeting would have to be provided by the use of either surface-, or transcriptionally, targeted vectors; and inducibility of cytotoxicity could be provided by a system such as the inducible Caspase 9 system, in which provision of a chemical inducer of dimerization will induce apoptosis in tumor cells transduced with the vector. APOBEC expression would then wreak mutational havoc within the tumor cells (hypermutagenesis). This could cause direct cytotoxicity by breaking the "just right" rule. Such direct cytotoxicity could itself result in potent immunization as a result of immunogenic cell death and release of tumor antigens, quite separate from the effects of any APOBEC-induced mutations. In addition, hypermutagenesis would induce neoepitopes, a proportion of which would be heteroclitic, exposing the tumor to T-cell attack. Before any surviving, hypermutated cells could evolve into more aggressive, metastatic mutation-driven variants, induction of the cytotoxic gene would ensure that all hypermutated cells were killed (**Fig. 6B**). *In situ* cell killing, along with ICB, would enhance the priming of neoepitope reactive direct and heteroclitic T cells. This hypermutagenic immunotherapy (HIT) would, in principle, allow for a combination of local cytotoxic therapy, combined with T-cell–mediated killing of both mutated (directly T cell recognized) and parental, unmutated (recognized by heteroclitic T cells) tumor cells both locally and systemically (**Fig. 6B**). In addition, such an *in situ* therapy would not require 100% transduction of the tumor cells—only enough to ensure killing of sufficient numbers to prime the appropriate heteroclitic T-cell response.

The concept of actively driving a mutator phenotype in tumor cells flies in the face of the received wisdom of APOBECs as drivers of mutational chaos from which only an evil panoply of aggressive clones can emerge. It also requires an equally great faith in the power of immunotherapy, and would require profoundly impressive preclinical validation. Indeed, the strategy has been likened to telling a patient with melanoma to go and sunbathe, or a patient with lung cancer to smoke more cigarettes.

However, in defense of the unthinkable. . ..We have seen that APOBEC3B expression can convert a completely ICB-insensitive tumor into a profoundly ICB-sensitive and curable phenotype (**Fig. 4**; ref. 34) and the potential of APOBEC to drive sensitivity to ICB is well supported (**Fig. 2**; ref. 9). In addition, two of the major stalwarts of cytotoxic cancer therapy used in the clinic today—radiotherapy and chemotherapy—are both highly mutagenic. Despite being mutagens, or possibly even because of being mutagens, both of these therapies are perceived now as having the capacity to be powerful immune adjuvants (77–79). If the trade-off between tumor cell killing and potential mutagenicity is both acceptable, and increasingly valuable, for radiation and chemotherapy—why not for APOBEC? Unquestionably, novel effective strategies able to convert ICB-insensitive tumors into ICB-sensitive tumors would be highly

Figure 6.

Generation of heteroclitic T-cell therapy through APOBEC-mediated tumor cell mutation. **A,** HEAT. (i). In most cases, only a few T cells with low-affinity TCR (blue T cell) for TAA (blue peptides) survive central and peripheral tolerance mechanisms. These are insufficient to clear tumors. (ii). Tumor cells, taken *ex vivo* as a vaccine can be subjected to APOBEC-mediated mutation (via transfer of an APOBEC-expression vector) which generates neoepitopes (orange peptides). Administration of the mutated vaccine cells *in vivo* leads to (iii). cross-presentation by APC (yellow) of (a). unmodified epitopes (blue peptides) to the low-affinity, preexisting T cells; (b). APOBEC-generated neoepitopes to the low-affinity, preexisting T cells which now become activated through a stronger HLA-epitope-TCR interaction; and (c). neoepitopes to nontolerized T cells with high-affinity HLA-neoepitope-TCR interactions. (iv). Immunostimulatory presentation of these epitopes lead to T-cell activation and expansion *in vivo*, which (v). can be augmented in the presence of appropriate ICB. (vi). A proportion of the neoepitope-specific, high-affinity T cells will also be able to bind the unmutated self-epitopes expressed by unmodified tumor cells. These heteroclitic T cells (red) will recognize both mutated (1) and unmutated (2) epitopes; a proportion of the SELF epitope-specific, low-affinity T cells (blue) will recognize the mutated neoepitopes (3) and will also recognize the self-epitopes more strongly having been activated by presentation of the neoepitopes (4). **B,** HIT. (i). Vector-based delivery of a tissue-specific promoter (TSP) driving an APOBEC3B gene, linked to expression of an inducible cytotoxic gene (CTG), would lead to hypermutation of a proportion of the tumor cells, generating neoepitopes (orange peptides). (ii). After APOBEC mutagenesis, induction of the cytotoxic gene would kill all the mutated cells (yellow), releasing both self-epitopes as well as neoepitopes for cross-presentation by APC. (iii)–(vi) as in **A.**



valuable—to make ICB accessible to those majorities of patients who do not currently respond. Data showing that large established tumors can be made to shrink by APOBEC expression combined with ICB would be required to validate these ideas, and the results of such experiments in the preclinical arena will establish whether this proposal has translational value.

Conclusion—APOBECs in Cancer Therapy: Batman or Joker? Red, Yellow, or Green Light for APOBECs?

APOBEC mutation can occur in the cellular genome in localized clusters of hypermutational activity—often referred to as kataegis, from the Greek for thunderstorm (11, 80). Three radically different philosophies see the role of such kataegis in cancer therapy in very different lights. The red light school of thought views them simply as ominous, threat-filled thunderstorms from which no good can come. The red lighters would inhibit APOBEC induction and activity at all costs to prevent evolution of more aggressive tumors and inhibit escape from treatment. The yellow light strategy would neither block, nor encourage, APOBEC activity. Seeing the silver lining to kataegis permits the exploitation of APOBEC mutagenesis —by understanding the mutations which drive tumor adaptation, by predicting sensitivity to immunotherapies such as ICB, and by targeting any predictable and reproducible mutations which are critical to driving escape and progression (Trap and Ambush). Finally, the much more controversial green light faction would actively drive APOBEC mutagenesis—to exceed the "just right" level of allowable mutation for cell viability, to generate vaccines in which cancer progression by mutation can be uncoupled from increased immunogenicity by neoantigenesis (HEAT), or by linking enforced mutation with tumor cell killing *in situ* to confer susceptibility to ICB therapies (HIT).

The fact that APOBECs could be given the red, yellow, or green light, shows that they currently have a major public relations image problem in the cancer field. Although they protect the cell by neutralizing invading pathogens they are increasingly vilified as agents of genomic chaos. However, cancer immunotherapy can thrive on genomic chaos via triggering of increased immunogenicity resulting from neoantigenesis. As controversial as it may be, the exploitation of the APOBECs as controlled agents of chaos could yield major benefits for cancer immunotherapy.

Authors' Disclosures

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