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EDITORIAL



# Drug-based cancer therapy to overcome immune resistance by steering tumor evolution

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## 1. Introduction

Genomic instability is a hallmark of cancer. It enables the tumor to evolve, adapt, and develop resistance to treatment [1,2]. Thus, cancer represents a moving target. Current therapies select for resistance and therefore do not effectively deal with the hurdle. Even the most promising immunotherapies that block tumor-induced immunosuppression via the exploitation of immune checkpoint inhibitors (ICIs) [3] are eventually neutralized by the selection of immune-evasion phenotypes [4,5]. To overcome resistance, we advocate for a choreographed (timed) hybrid immune-drug-based therapy, where the clonal dynamics of cancer cells are subject to a variable selection pressure arising by periodically switching the drug-based targeting of the immune-evasion phenotypes. We propose to exploit ICIs, combine them with immune-priming epigenetic drugs to promote responsiveness [6], and finally incorporate an alternating targeted therapy to steer the evading phenotype into an immunogenic trap. As tumor-induced immunosuppression is blocked utilizing an ICI, resistance needs to be overcome. This is so because the turned-on immune surveillance imposes selection pressure, while the epigenetic drugs induce genetic instability as shown below [7], creating an evolutionary scenario for the selection of resistance that ultimately leads to relapse.

The proposed therapeutic strategy interferes with clonal evolution, perturbs the fitness landscape, and pushes the cell population down an immune-evasion pathway toward extinction, inviting clinical development. In a preliminary stage, therapeutic efficacy needs to be established on a humanized murine model with patient-derived tumor engraftment [8], validated vis-à-vis a cogent model of clonal dynamics of cancer cells exposed to a tuned selection pressure. The drug-based evolution-steering strategy outlined may well be combined *mutatis mutandis* with alternative ways of imposing immune surveillance, namely cancer vaccines or adoptive T-cell therapy. An assessment of such combinations is outside the scope of this work.

Despite its therapeutic potential, patient responsiveness to ICI is considered low, since it is mostly restricted to patients with highly immunogenic conditions resulting from hypermutability, which in turn arises from specific conditions such as DNA-mismatched repair deficiency or the rare Lynch syndrome [3]. Other culprits for low responsiveness arise from a dearth of cytotoxic tumor-infiltrating lymphocytes (TILs) in tumor microenvironments [9]. Even when impressive results are achieved, restoration of T cell function is transient, usually followed by relapse as a consequence of the development of resistance to treatment [5]. Overcoming resistance in this therapeutic context can be daunting because the genomic instability that typically promotes immunogenicity also provides the evolutionary substrate for immune evasion. In this context, we address the challenge of achieving lasting cure, overcoming resistance through a timed immunotherapy that evolves concomitantly with the disease, steering the immune-evasion phenotype toward extinction. By contrast with standard immune treatments, the proposed therapy is choreographed to exert a variable (steering) selection pressure promoting transitions between immune-evasion phenotypes.

## 2. Epigenetically induced responsiveness to ICI therapy

To enhance ICI-responsiveness, we propose combinations with epigenetic drugs (epidrugs) [6] in a first stage of treatment. Such epidrugs typically consist of chromatin-unraveling promoters like inhibitors of DNA methyltransferases (DNMTi) or inhibitors of histone deacetylases (HDACi) that impact significantly the regulation of chromatin restructuring, with multiple immune-priming effects. Thus, epidrugs upregulate expression of checkpoint ligands PD-L1/PD-L2 (programmed death ligands 1 and 2) in cancer cells, embryonic antigens like the cancer testis antigen not expressed in adults, and genes involved in antigen processing and presentation along the major histocompatibility complex (MHC) pathway. In particular, the DNMTi reactivates endogenous retroviral (ERV) repetitive elements that restore interferon (IFN) pathways that in turn lead to expression of checkpoint ligands, antigen-presentation complexes, and pro-apoptotic and anti-proliferative genes [10]. In spite of such remarkable immune-priming effects conducive to the enhancement of ICI efficacy, tumors subject to ICI + epidrug combination therapy are likely to develop resistance, especially since epidrugs

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typically promote genomic instability [7], creating an evolutionary substrate for immune evasion through the selection of resistance. This last assertion becomes evident since epigenetic marks and the enzymes that create them are involved in multiple aspects of maintaining genomic integrity [7]. Thus, alterations in DNA methylation and chromatin structure strongly impinge on microsatellite stability, DNA damage repair, the onset of mutations, and chromosomal rearrangements.

## 3. Overcoming immune evasion by alternating drug-based treatments

To overcome immune evasion in the immune-priming context described, we outline an evolving therapy that avoids maintaining a fixed selection pressure by cycling the tumor through the two most common possibilities for immune evasion (Figure 1): (a) recruitment of immunosuppressive regulatory T cells ($T_{regs}$) and/or tumor-associated myeloid suppressive cells (TAMSCs) within the tumor microenvironment (TME) [11]; and (b) impairment of antigen presentation through beta2-microglobulin (B2M) mutation [12] or mutational deactivation of the JAK1/2 kinase, a signal transducer in the IFN pathway that induces expression of MHC-I [10]. There is evidence that under a suitable evolutionary substrate enabling selection and adaptation, i.e. genetic instability provided by the Lynch syndrome [3], the (T reg, TAMSC)-recruiting 'adaptive' phenotype *a* and the antigen-presentation-deficient phenotype *b* are not only complementary but also in competition with each other [12]. Thus, expression levels of mutated B2M in cancer cells are anticorrelated with expression levels of the transcription factor FOXP3 in tumor-adjacent $T_{regs}$ that controls gene expression programs for T reg function. In principle, nonadaptive immune evasion may take alternative pathways, such as the JAK1-impairing mutation. However, this impairment of the IFN pathway has not been observed in representative models of ICI responsiveness [12].

Alternative less common ways of achieving resistance have been documented (Figure 1). Thus, tumor-promoted apoptosis of cytotoxic TILs has been reported in one specific context [13], and we cannot discard a priori the possibility of developing a hindrance to the infiltration of effector T cells [9]. Yet, these resistance strategies are less common and at any rate, not encountered in ICI-responsive patients [12].

The immune-evading phenotype may be steered into pattern *b* by complementing the ICI + epidrug combination with a PI3K inhibitor specific for γ,δ-isoforms, such as *duvelisib*. This targeted agent enables the immune system to eliminate cancer cells that adopted evasion pattern *a* [14], while the residual population that survived by adopting pattern *b* may be partly eliminated by switching to a β-isoform-specific PI3K inhibitor, like GSK2636771 or AZD8186, that promotes elimination of phenotype *b* [15]. Thus, the proposed evolving therapy cycles the tumor back and forth between the two immune-evading phenotypes by alternating between the PI3K(γ,δ)-inhibitor and the PI3Kβ-inhibitor, so that the selected phenotype becomes susceptible in the next iteration (Figure 2). In this way, we outline a timed immunotherapy tuned to steer cancer evolution into a lethal immunogenic trap furnished by alternating the selection of immune-evasion phenotypes with dwindling populations.

The choice of evasion-steering agents is justified. Pharmaco logical inactivation of PI3Kγ reduces integrin-mediated adhesion of myeloid-derived cells and $T_{regs}$, hampering their infiltration into

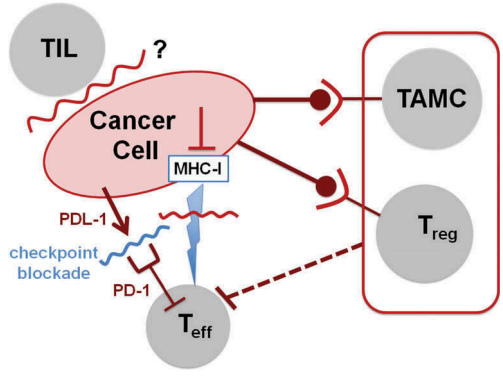


Figure 1. Patterns of cancer resistance to an adaptive immune attack elicited by a blockade of the immune checkpoint PD-1 receptor in the effector T cell ($T_{eff}$). The resistant phenotypes may be characterized by recruitment of immune-modulatory T cells such as regulatory T cells ($T_{regs}$) and tumor-associated myeloid cells (TAMCs) which suppress $T_{effs}$, or alternatively by impairment of MHC-I-mediated antigen presentation that stimulates the activation of $T_{eff}$, or possibly, by hindrance of the recruitment of tumor-infiltrated lymphocytes (TILs). The latter should be regarded only as an a-priori possibility.

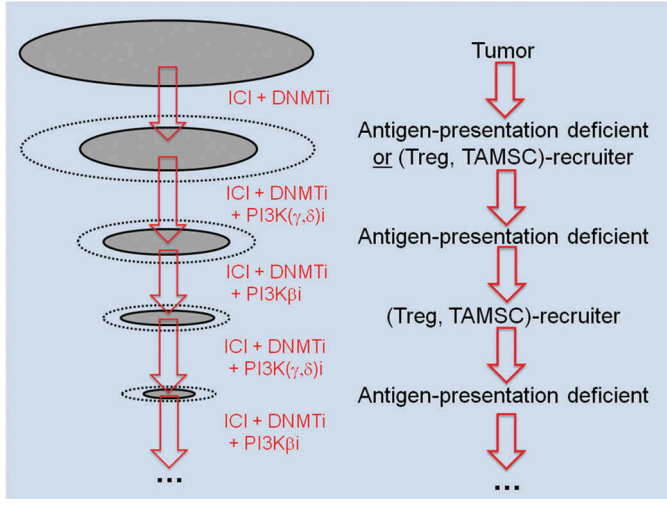


Figure 2. *Lethal Immune Complementarity, choreographed therapy to steer immune evasion*. Evolving therapy promoting immune attack and steering the tumor back and forth between two immune-evading phenotypes with dwindling surviving population. The therapy involves three components: (1) ICI-induced blockade of tumor-induced immunosuppression; (2) immune priming by an epidrug, here represented by DNA methyltransferase inhibitor (DNMTi); (3) alternation between two isoform-selective PI3K inhibitors (PI3K(γ,δ)i, PI3Kβi) needed to, respectively suppress two competing resistant phenotypes arising from immunoediting. Immune surveillance creates a selection pressure that promotes adaptive change in the tumor. Adaptive change is realized by two immune-evading competing phenotypes: (a) The recruiter of immunosuppressive T cells (Tregs) and tumor-associated myeloid suppressive cells (TAMSCs); (b) the antigen-presentation-deficient cell. After the initial immune attack fostered by the ICI + DNMTi combination, the tumor is cycled between the two immune-evasion phenotypes, so that phenotype *b* prevails in the (ICI + DNMTi)-resistant population when the PI3K(γ,δ)i is administered placing selective pressure on phenotype *a*, while prevailing phenotype *b* in the smaller (ICI + DNMTi + PI3K(γ,δ)i)-resistant population is under selection pressure when PI3K(γ,δ)i is switched to PI3Kβi. The alternation between PI3K(γ,δ)i and PI3Kβi funnels the cancer cell population toward extinction. The prevailing phenotype at each stage is indicated.

the TME, while PI3Kδ-inhibition in $T_{regs}$ is known to impair their maintenance and function within the TME [14]. On the other hand, PI3Kβ-inhibition in tumor cell impairs MHC-I-mediated antigen presentation, while stimulating the innate immune response of natural killers (NK), which are themselves responsive to cells lacking MHC-I antigen presentation [15].

The centrality of the PI3K-AKT-mTOR pathway in maintaining homeostasis in normal cells makes pan-PI3K inhibitors toxic and therapeutically inefficient, since the tolerable dose is insufficient to fulfill their inhibitory role [14]. Furthermore, non-isoform-specific PI3K inhibition is certain to cause counterproductive effects, since PI3Kβ-inhibition suppresses the adaptive immune response elicited by PI3K(γ,δ)-inhibition [15]. These opposing effects justify the need to use isoform-specific PI3K inhibitors in the proposed alternating manner.

## 4. Lethal immune complementarity

Rather than merely promoting cancer evolution, the outlined immune cycling trap is expected to overcome resistance by funneling the tumor into a resistance pathway that leads to extinction. To deal with the moving-target nature of the disease, the therapy does not maintain a single pattern of selection pressure imposed by immune surveillance, rather it steers the tumor back and forth through two complementary immune-evasion phenotypes with progressive shrinking of the selected population. We propose cycling between two drug-based therapies within an immune-priming background, so that the cell subpopulation resistant to one therapy becomes susceptible to the other. Immune resistance provides a selective advantage with one drug therapy but turns into a fitness handicap as drug therapies are switched, so the phenotype selected by one therapy is subject to selection pressure in the following iteration (Figure 3). In this way, the alternating therapy sculpts an evolutionary path within an immunogenic trap that leads to extinction. We name this paradigm Lethal Immune Complementarity (LIC). LIC is somewhat akin to collateral sensitivity [2], but adapted to immunotherapy *mutatis mutandis*.

## 5. Conclusion

We have argued that therapy must lead rather than follow vis-à-vis cancer evolution. Clinical experience suggests that the evading phenotype resulting from treatment can never be fully anticipated or eliminated when a single source of selection pressure is applied during treatment. Thus, therapy must steer cancer evolution and LIC may well become the underlying paradigm to overcome resistance in a therapeutic context where immune surveillance is maintained.

## 6. Expert opinion

The key point upheld is that it is possible to overcome resistance to cancer ICI-based immunotherapy by a second-stage drug-based steering of tumor evolution into an immunogenic trap. This may be achieved through a hybrid immune-drug-based therapy that alternates the targeting of immune-evasion phenotypes. The proposed hybrid therapy unleashes the adaptive immune response and subsequently chases the evading phenotype into an immunogenic trap wherein the tumor is driven to extinction. This trap involves steering the tumor into an alternation between the adaptive and nonadaptive routes of immune evasion through the periodic switching of two isoform-specific PI3K inhibitors. The competing/complementary routes of evasion have been identified by examining the evolutionary substrate of a representative cancer for ICI responsiveness [12]. The evolution-steering treatment may be applied *mutatis mutandis* in other therapeutic contexts where immune surveillance is maintained, making use for example of cancer vaccines or adoptive T-cell therapy.

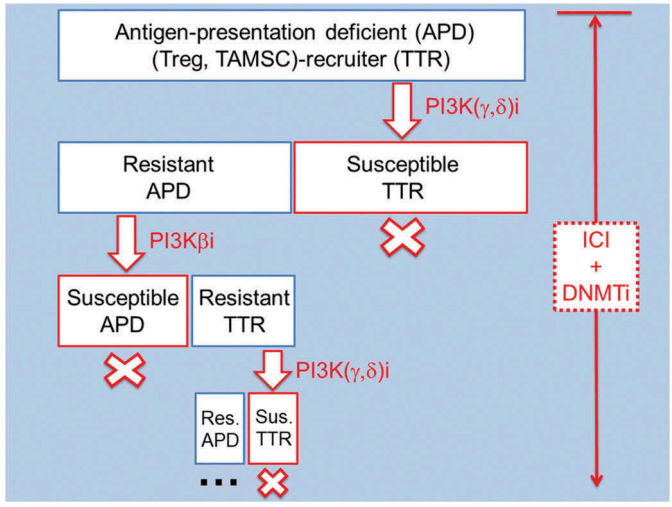


Figure 3. Alternating targeted therapies (PI3K(γ,δ)i, PI3Kβi) within an immune-priming background (ICI + DNMTi) setting an immunogenic trap, so that the cell subpopulation selected by one targeted therapy becomes susceptible to the other. The initial batch contains the evasion phenotypes selected by immune surveillance. During subsequent alternating treatment, immune resistance provides a selective advantage with one therapy but the selective advantage turns into a handicap as the therapy is switched. In this way, the selected cancer phenotype becomes susceptible to drug treatment in the next iteration, and therefore subject to selection pressure. For each iteration that follows after immune priming, the susceptible and resistant subpopulations and their respective fate are indicated.

The main weakness of the proposed therapeutic paradigm arises from the possibility that alternative unforeseen and unfamiliar routes of immune evasion may arise in other therapeutic contexts different from the representative case for ICI-responsiveness described in [12]. Such routes may involve recruitment of unfamiliar T-cell modulators different from $T_{regs}$ or TAMCs, uncommon routes to suppress NK activity on antigen presentation-deficient cells, or even hindrance of the infiltration of effector T cells into the tumor microenvironment [9,13] (Figure 1). These possibilities may decrease the efficacy of drug-based steered evolution and may be assessed through examination of bioinformatics annotation complemented by preclinical models of immune evasion fostered by ICI-based therapy.

The emergence of resistance represents a major hurdle in the development of cancer therapies. Most cancers have an evolutionary substrate and the adapted phenotypes arising imply that evolution selects for resistance. Overcoming resistance is precisely the overarching goal of this proposal. The proposed hybrid immunotherapy exploits ICIs in a priming context, the most

promising treatment to-date, and deals effectively with resistant phenotypes arising from immunoediting. We note however that the applicability of the proposed evolution-steering therapy is restricted to the common patterns of immune evasion for ICI-responsive cancers described previously and excludes contexts where, for instance, the tumor becomes resistant by hampering the infiltration of cytotoxic T cells in its periphery [9,13].

Intensive scrutiny of systems biology and bioinformatics annotation as well as preliminary assays on humanized murine models with patient-derived tumor engraftment [8] will be required to assess the feasibility of alternative routes of immune evasion that may diminish the efficacy of the proposed evolution-steering strategy. A major challenge in adopting PI3K inhibitors to steer the resistance pathway arises because of the relative universality of the PI3K signaling axis for maintenance of cell homeostasis [14]. Therefore, toxicity issues resulting from off-target effects may arise as pan-PI3K inhibitors are adopted to steer immune evasion. For this reason, we advocate the use of isoform-specific or isoform-sparing kinase inhibitors, as indicated in Figure 2.

Future directions inspired by the proposed research involve assays on humanized murine models required to preliminarily assess the efficacy of the immunogenic trap and to identify possible unfamiliar routes of immune evasion. These animal models may provide guidance in determining clinically relevant endpoints and may guide the development of models of clonal evolution. The latter are useful to assess the efficacy of the evolution-steering therapy, to examine the resistance selection process, and to optimize pharmacokinetics/pharmacodynamics (PK/PD) parameters. Clinical development reliant on properly identified endpoints and proper scheduling of drug inoculation upon the immune-priming substrate will then become an essential future direction. The dosing schedule optimized to steer cancer evolution will need to be established vis-à-vis endpoints defined by adequate biomarkers, such as FOXP3 expression and mutated B2M levels, of the anticipated evasion phenotypes.

New areas of interest will likely emerge, focusing on the immunologic counterpart of therapeutic strategies based on synthetic lethality and collateral sensitivity [2]. We argued that it is possible to steer cancer evolution when the latter enables immune evasion. This idea will invite innovative ways of implementing lethal complementarities in a manner akin to collateral sensitivity for targeted therapy.

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## Declaration of interest

A Fernandez has his own consultancy company - Ariel Fernandez Innovation. He has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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