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Forum

# Making Targeted Therapy Compatible with Checkpoint Immunotherapy

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**Immune checkpoint blockades induced by antibodies are revolutionizing cancer therapy. Combinations of checkpoint immunotherapies with kinase inhibitors (KIs) are being clinically evaluated as oncogenic mutations arise. Off-target KI cross-reactivity will often compromise synergistic efficacy, with KIs suppressing T-cell functionalities that checkpoint blockers are purportedly boosting. This incompatibility may be removed through molecular optimization.**

## Checkpoint Blockades for Treating Cancer

With the striking success of immune checkpoint blockades in cancer treatment, the recruitment of immune-based anticancer activity has become a key asset in the therapeutic arsenal[i,ii] [1,2]. Checkpoint blockade is achieved through antibody recognition of the programmed death-1 (PD-1) receptor, which promotes downregulation of the adaptive immune response. Thus, humanized antibodies like (i) nivolumab (Opdivo®) [1] or (ii) pembrolizumab (Keytruda®) [2] prevent the PD-1-receptor from binding its natural ligand PDL-1 and thereby prevent the receptor from recruiting the repressive phosphatase SHP1. When recruited, SHP1 dephosphorylates and thereby deactivates ZAP70, a downstream signal transducer. In this way, the biologics hinder the suppression of antigen-triggered T-cell activation (Figure 1). PD-1 blockade is meant to restore the adaptive immune response that is suppressed by tumors capable of expressing PD-1 ligands. Other T-cell-repressive receptors like CTLA4 may be also targeted (Figure 1) with similar therapeutic efficacy. This general strategy has proven successful for treating metastatic melanoma and metastatic non-small cell lung cancer (NSCLC), and has succeeded as a second-line treatment for head and neck squamous cell carcinoma and renal cell carcinoma[i,ii].

Combinations of checkpoint immunotherapies with targeted therapies based on KIs are being vigorously pursued, particularly when oncogenic mutations or other genetic accidents occur at the onset of the disease [3,4]. Although KI-based treatment often improves prognosis, most patients relapse due to the emergence of secondary mutations as drug resistance develops. This effect demonstrates the limitations of targeted therapy as monotherapy, suggesting the need for combination therapies. For example, there have been reports indicating that the therapeutic efficacy of the KI dasatinib (Sprycel®) in the c-KIT mutant P815 mastocytoma relies significantly on components of the immune response [5]. Thus, combinations of dasatinib with immuno-stimulators have been pursued. Such combinations lead to a potent therapeutic synergy with demonstrated tumor clearance in animal models [5]. The rationale for such combinations is that they mitigate the broad relatively short-lived effect of targeted therapy with the narrower but longer-term impact of immunotherapy. Additionally, immune stimulation harnesses the immunogenic molecular debris arising from cancer cell death promoted by targeted therapy. Examples of combinations that are currently being clinically evaluated include: (i) imatinib/pembrolizumab in metastatic melanoma with mast/stem cell growth factor receptor (c-KIT) mutation or amplification (ClinicalTrials.gov Identifier NCT02812693); (ii) crizotinib/pembrolizumab in anaplastic lymphoma kinase (ALK)-positive advanced NSCLC (ClinicalTrials.gov Identifier NCT02511184); and (iii) dabrafenib/nivolumab in BRaf-mutated metastatic melanoma (ClinicalTrials.gov Identifier NCT02910700).

However, the off-target cross-reactivity of many KIs against the lymphocyte-specific kinase (LCK) is likely to compromise the

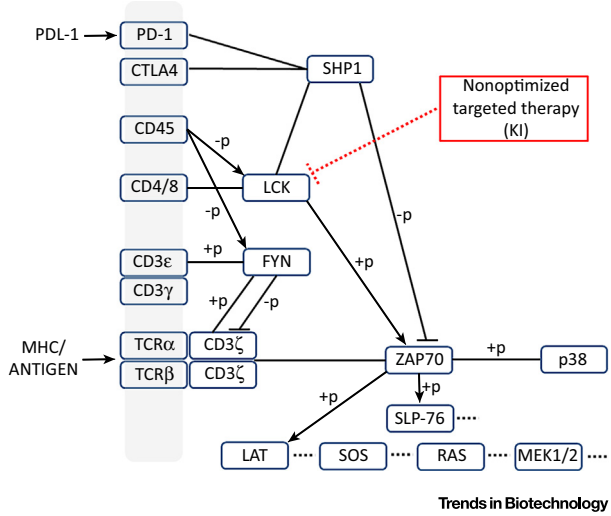


Figure 1. Signaling Network for the Activation and Downregulation of Antigen-Triggered T-Cell Activation. Adapted from www.genome.jp/kegg-bin/show_pathway?hsadd04660.

efficacy of such combinations because, via LCK inhibition, KIs suppress precisely the antigen-triggered T-cell response that the PD-1 inhibitors are supposed to boost. As shown in Figure 1, while PD-1 blockade prevents the PD-1-recruitable phosphatase SHP1 from suppressing the antigen-triggered T-cell response, drug-induced upstream LCK inhibition exerts an equivalently suppressive activity. This finding suggests that KIs with nanomolar LCK affinity are *a priori* incompatible with checkpoint immunotherapy.

Recent kinome-wide screening revealed that imatinib, crizotinib, and dabrafenib are nanomolar LCK inhibitors, with dissociation constants ($K_D$) of 40, 20, and 180 nM, respectively[iii]. This pharmacological-level affinity, particularly for imatinib and crizotinib, makes them intrinsically unsuitable to synergize with checkpoint blockers. Dasatinib, possibly the most powerful LCK inhibitor known ($K_D$ = 0.2 nM)[iii], would be *a priori* the worst candidate to synergize with a PD-1 blocker, notwithstanding past efforts to combine them [5]. This article argues that molecular optimization of the KIs is required to synergize them with immune-based PD-1 blockers.

## Optimizing KIs for Combination with Checkpoint Immunotherapy

While homologous on-target and off-target kinases are structurally similar and therefore difficult to tell apart through molecular recognition, their patterns of packing defects make them distinguishable. Packing defects refer here to water-exposed backbone hydrogen bonds, the so-called 'dehydrons' [6]. Interfacial water molecules surrounding dehydrons are constrained and hence easily removable and a payoff for such dehydration is the strengthening and stabilization of the underlying electrostatic interaction [6]. This observation has prompted the design of highly selective drugs that act as 'dehydron wrappers', removing water upon drug-target association from around unique non conserved dehydrons. This design principle has been used to rework imatinib into a more selective drug, named WBZ_4. This compound has enhanced affinity against the primary imatinib target c-KIT, while presenting a significantly reduced affinity against LCK ($K_D$ = 800 nM, compared with $K_D$ = 40 nM for imatinib) [6]. Compound WBZ_4 is a methylated version of imatinib capable of wrapping the dehydron that pairs Cys673 with Gly676 in the KIT kinase (PDB file 1T46). Since this dehydron aligns with the well-wrapped backbone hydrogen bond Met319-Gly322 in LCK, the affinity of WBZ_4 for LCK is compromised, while its affinity for on-target c-KIT is enhanced [6]. Based on this

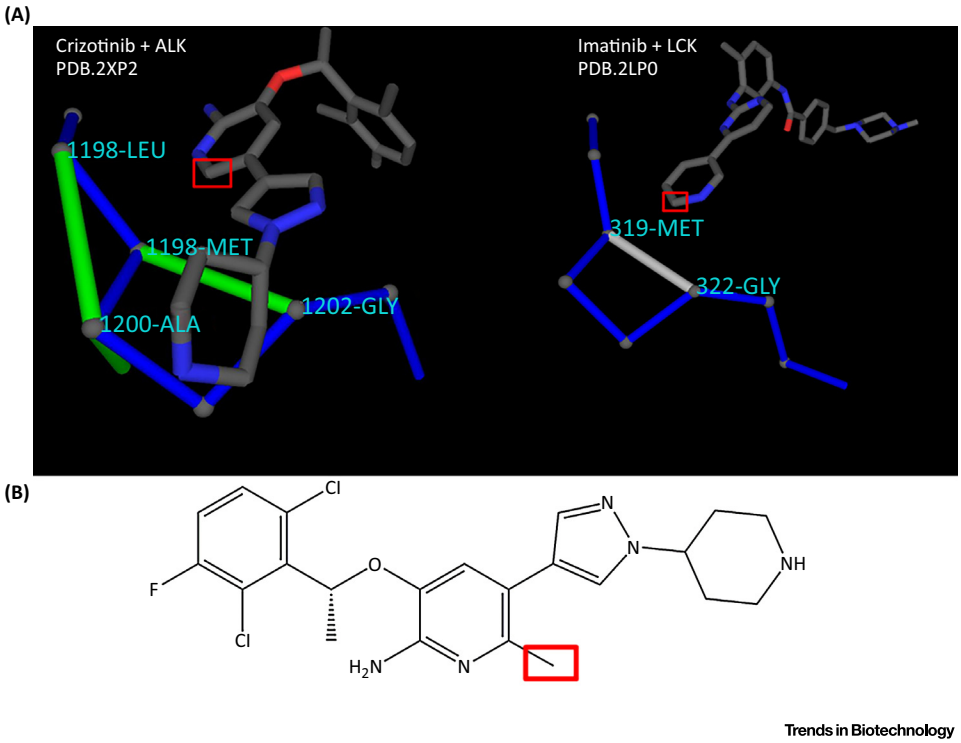


Figure 2. Differences in Dehydron Patterns Apparent upon the Structural Alignment of Anaplastic Lymphoma Kinase (ALK) and Lymphocyte-Specific Kinase (LCK) Structures Enable the Redesign of the Kinase Inhibitor (KI) Crizotinib to Retain Activity against On-Target ALK While Significantly Reducing Activity against LCK. (A) The protein backbone region is represented by virtual bonds joining α-carbons of aligned residues, dehydrons are marked as green lines joining the α-carbons of the paired residues, and well-wrapped (dehydrated) backbone hydrogen bonds are marked by white lines. The sites of the LCK-discriminating methylation in crizotinib and imatinib are highlighted by red boxes. (B) The chemical structure of the crizotinib redesign able to tell ALK apart from LCK.

analysis, WBZ_4 is *a priori* expected to be a better KI than imatinib to synergize with pembrolizumab in the treatment of KIT-dependent melanoma, an observation that invites clinical evaluation of the novel combination.

Generally, we argue that the anti-LCK–anti-PD-1 antagonism may be removed through redesign of the KI by adopting the unique dehydron pattern of the target as a selectivity filter. In this way, an optimized KI that avoids LCK while retaining on-target activity at parental-drug levels may be engineered to synergize with checkpoint blockers. The optimization strategy may be implemented by structurally aligning the dehydron pattern of the on-target protein with that of LCK. For example, due to its potency as an LCK inhibitor, crizotinib is likely to be incompatible for combination with immune-based checkpoint therapy. It would be desirable to redesign crizotinib into a compound that retains the parental nanomolar affinity for ALK, while significantly reducing LCK affinity. Structural alignment of ALK and LCK reveals that the dehydron Met1199-Gly1202 in ALK aligns with the well-wrapped Met319-Gly322 backbone hydrogen bond in LCK (Figure 2). While the residues (Met and Gly) paired by the backbone hydrogen bonds are conserved, the dielectric environments around the bonds are different, with the ALK hydrogen bond significantly more exposed to water. The pre-existing wrapping difference prompts a redesign of crizotinib in order to contribute upon binding to the wrapping of dehydron Met1199-Gly1202 in ALK. This wrapping modification may be realized, for example, through methylation at the position highlighted in Figure 2. The wrapping modification is expected to enhance affinity for ALK beyond parental levels since exclusion of interfacial water surrounding an electrostatic amide–carbonyl interaction enhances and stabilizes the preformed hydrogen bond, and drug affinity is controlled by the free energy content of the drug-target complex. On the other hand, just like in the case of WBZ_4 versus imatinib, the proposed wrapping modification of crizotinib will decrease affinity for LCK, a desirable outcome to create a synergic combination with the anti-PD-1 antibody.

## Concluding Remarks

The adaptive arm of the immune response is a key ally of KI-based targeted therapy in the cure for cancer. Immunogenic cellular debris arising from drug-induced apoptosis needs to be harnessed by the immune system, therefore immune stimulation through checkpoint blockade appears *prima facie* to provide a suitable complement to targeted therapy. Yet, the undesired cross-reactivity of KIs may create incompatibilities between the two therapeutic modalities, particularly if the KI shows nanomolar affinity towards LCK, a key signal transducer in T-lymphocyte activation. Molecular optimization of KIs to control their specificity has proven possible [6,7], and may be essential to avert anti-LCK activity while retaining affinity towards clinically relevant targets. This article advocates for the implementation of structure-based selectivity filters in drug redesign capable of telling LCK apart from on-target kinases. Only compatibility-optimized KIs should be utilized in combination therapies with immune-based PD-1 blockers. Antagonistic effects in the antigen-triggered activation of the T-cell must first be removed to guarantee an effective synergy.

Resistance patterns are likely to emerge for the optimized combinations, impairing KI or antibody affinity, or competitively enhancing kinase or receptor affinity for their natural ligands. On targeted kinases, resistance is likely to be promoted by mutations that are not necessarily the same as those identified when the KI is used as a monotherapy. In fact, differences in resistance patterns may be informative of the synergistic mechanism of the combination therapy. Molecular wrapping redesigns of the KI may be successfully introduced to address the 'moving target' problem posed by the emergence of kinase resistance mutations [7], as long as the latter alter the targetable pattern of packing defects (dehydrons) of the protein.

## Resources

(i) FDA Report: nivolumab/Opdivo® Highlights of prescribing information (revised 11/2015) url: http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/125554s012lbl.pdf

(ii) FDA Report: pembrolizumab/Keytruda® Highlights of prescribing information (revised 10/2016) url: http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/125514s012lbl.pdf

(iii) Harvard Medical School – Library of Integrated Network-based Cellular Signatures (HMS-LINCS) Kinome Scan Data/Last Update: September 15, 2016. url: http://lincs.hms.harvard.edu/kinomescan/

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