Perspective

The immune activation model for targeted inhibitors of mutated oncogenic kinases

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Abstract

Targeted cancer therapies by small-molecule inhibitors of receptor tyrosine and other kinases have achieved great success in recent years. Most targeted medications specifically inhibit a protein kinase mutated in the patient's tumor. Although many possible mechanisms have been investigated, the drugs' astounding efficacies are not well understood. Here we propose a unifying mechanism of action. Strong binding by the inhibitor could lead to increased ubiquitination and degradation by the proteasome, boosting the presentation of kinase-associated neoantigen peptides. This could facilitate tumor cell recognition by T cells, leading to a sustained immune attack. We discuss implications for experimental and clinical cancer research.

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Abbreviations: CHIP, C terminus of Hsp70 interacting protein; CPI, immune checkpoint inhibitor; Cdc37, cell division cycle 37 cochaperone; EGFR, epidermal growth factor receptor; FDA, federal drug administration; HLA-I: human leukocyte antigen class I, genes coding for MHC-I; HER2, human epidermal growth factor receptor 2; Hsp70/90, heat shock protein 70/90; KI, kinase inhibitor; MHC-I, major histocompatibility complex class I; NSCLC, non-small-cell lung cancer; RTK, receptor tyrosine kinase; TCGA, The Cancer Genome Atlas.

What's new: Targeted cancer therapy by small-molecule inhibitors of oncogenic, mutated kinases leads to rapid tumor shrinkage in a significant fraction of patients. The prevailing model explains the cell death with oncogene addiction of tumor cells. Here we propose a complementary model: Inhibitor-bound kinases are ubiquitinated and degraded by the proteasome, leading to tumor antigen presentation, T cell recognition, and immune activation.

Background

All cancers require self-sufficiency in mitogenic growth signals, which they commonly achieve by constitutive activation of a growth signaling pathway.[1] Most cancers acquire oncogenic mutations in a specific receptor tyrosine kinase (RTK) transducing the growth signals into the tumor cell[2] or in an intracellular kinase transmitting and amplifying those signals within the cell[3] (Figure 1a). Oncogenic mutations typically lead to constitutive activation or overexpression of the kinase. Targeted cancer therapy with small-molecule inhibitors against the mutated kinases have been a main driver behind improvements in cancer therapy.[4] RTK inhibitors have proven particularly potent in the treatment of metastatic non small cell lung cancer (NSCLC), chronic myelogenous leukemias, and renal cell carcinoma.[5] Fifty-five targeted kinase inhibitors against neoplasm have been approved by the FDA, nine in the last year, most of them directed against mutated or fused RTKs.[6]

The mechanism of action of kinase inhibitors is often explained by blocking mitogenic signalling and inhibition of cell proliferation.[4] However, this mechanism alone cannot explain the massive cell death manifested in the rapid shrinkage of tumors often observed within a few weeks of treatment initiation. The prevailing model of *oncogene addiction* holds that tumor cells become addicted to the prosurvival signal from the oncogen. When the prosurvival signal is removed, apoptotic signals dominate, leading to tumor cell apoptosis.[7] While this model has been clearly confirmed for specific cases,[8,9,10] it does not account for the fact that in cell culture direct inhibition of the oncogene or inhibition of its expression in most cases resulted merely in growth arrest but rarely in cell death,[11,1213] (see their Table I for an overview). Also, even after intense efforts it has been challenging to delineate clear mechanisms of addiction for most cancer types that are successfully treated with kinase inhibitors.[10]

In the last decade the central role of the immune system and the tumor microenvironment have come into the limelight, underscored by the great progress in long-term survival afforded by immune checkpoint inhibitors.[14] Here we propose a complementary mechanism of action of targeted kinase inhibitors that could provide the missing link to the immune system and thereby explain their surprising efficacy (Figure 1b).

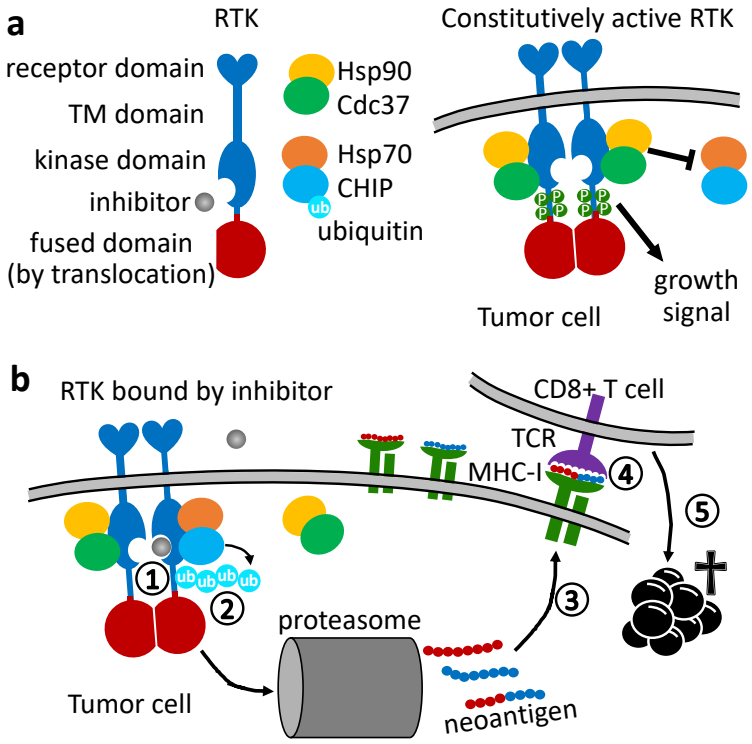

Figure 1: Model of tumor-directed immune activation by small-molecule inhibitors of oncogenic kinases. **a** In many cancers, a kinase in a growth signaling pathway is constitutively activated by an oncogenic mutation. In the illustrated example, a receptor tyrosine kinase (blue) was fused to an activating domain (red) by a genetic translocation. **b** The model: ① The kinase inhibitor binds to the kinase domain, thereby reducing the affinity to the molecular chaperone Hsp90.[15,16] ② The kinase thus loses protection of Hsp90 against binding of Hsp70 and its cochaperone CHIP, an E3 ubiquitin ligase, which marks the mutated kinase for proteasomal degradation.[17,18,16] ③ Peptide fragments are transported to the plasma membrane for antigen presentation on the MHC-I complex. ④ Neoantigens from the mutated kinase are recognized by T-cell receptors (TCR) as non-self, ⑤ directing T cells to mount an immune attack on the tumor cell. Upon its death, further neoantigens are released and taken up by antigen-presenting cells, leading to a sustained immune attack on the tumor.

Model of immune activation by targeted kinase inhibitors

Hsp70 chaperone recognizes many shortlived and abnormal proteins and, through its cochaperone CHIP, an E3 ubiquitin ligase, marks them for degradation by the proteasome.[18] Most kinases in the human proteome exhibit conformational flexibility similar to Hsp70 clients. They would be triaged for degradation by Hsp70 if they were not protected by the Hsp90/Cdc37 chaperone complex, which binds and protects over 60% of the human kinases and 85% of RTKs.[15] According to the proposed model in Figure 1b, ① the binding of small-molecule inhibitors has been shown to stabilize the structure of the targeted kinases in a way that the kinase binding affinity to Hsp90/Cdc37 is reduced.[15] ② This deprives them of protection against binding by Hsp70/CHIP and ubiquitination mediated by CHIP, leading to their degradation by the proteasome.[17,19] (For RTKs this is preceded by endocytosis of the activated kinases.[20]) Indeed, binding of various small-molecule inhibitors to ErbB-2 receptor tyrosine kinases leads to its ubiquitylation and degradation by the proteasome.[16] ③ Peptide fragments are translocated to the lumen of the endoplasmic reticulum by TAP protein, where they are loaded onto MHC-I complexes and transported to the cell surface by exocytic vesicles for antigen presentation.[21,22] ④ Some peptides of the mutated oncogenic kinase can be recognized as non-self by T cells with matching T-cell receptors. ⑤ Cytotoxic CD8+ T cells mount an immune attack on the recognized tumor cells. Upon their death, further neoantigens are released and taken up by antigen-presenting cells. These migrate to lymph nodes, where they instruct CD4+ T helper cells to seek out cells carrying these antigens, thus mounting a sustained immune attack on the tumor.

Further supporting evidence

It is striking that the majority of the 55 approved small-molecule protein kinase inhibitors are indicated for patients with mutations in exactly those kinases, mostly RTKs.[6] Only a minority of kinase inhibitors target overexpressed but unmutated proteins, and some that do are used in combination with other inhibitors targeting a mutated kinase (such as MEK1/2 inhibitors used in combination with $BRAF^{V600E/K}$ inhibitors). The proposed model would add a complementary, unified mode of action to oncogen addiction to explain the efficacy of inhibitors targeted against mutated kinases.[10]

Evidence for the involvement of the immune system is supplied by the observation that, in a cohort of 293 patients with EGFR-mutant advanced NSCLC, even in patients with ≤ 5 of the cells harboring the mutation in their EGF receptors, the response rate to the EGFR inhibitor was still as high as 34%.[23] This is not compatible with the main mode of action being only mediated by the inhibition of EGFR, since at least 95% of cells would not be expected to be directly affected.

Evidence for the importance of neoantigen presentation on MHC-I complexes in tumor control is contributed by a study on 36 patients from the lung adenocarcinoma cohort in TCGA (treated with the standard of care) with two specific EGFR mutations. Twelve patients had HLA class I alleles predicted to be protective for these mutations. The 12 patients with protective alleles had considerably longer disease free and overall survival times, with hazard ratios of 0.2 (p=0.012) and 0.13 (p-value = 0.005), respectively.[24]

Implications for cancer research

According to the model of immune activation by targeted kinase inhibitors, these drugs are not merely growth inhibitors but *immune-active agents*. The model emphasizes the importance of the immune system for the therapeutic success of these drugs and might give guidance how to modulate it for maximum efficacy. In fact, several phase I trials in NSCLC and melanoma have been conducted combining RTK inhibitors with checkpoint inhibitors. Unfortunately, all showed severe immune-related adverse effects,[26] which in one study, however, disappeared for the reverse order of drug administration.[27] This is most likely due to autoimmune reactions against tissues with similar antigens to the tumor,[28] possibly combined with epitope drift, and again underscores the need to elucidate the interplay between kinase inhibitors and the immune system (Figure 2a). The new model might also help us in investigating the immunity-related mechanisms underlying the so far inevitable resistance to kinase inhibitors.[29]

If indeed MHC-I-presented neoantigens of the mutated kinase lead to immune activation, we might be able to predict the relative efficacy of targeted kinase inhibitors for each patient by predicting how well the kinase

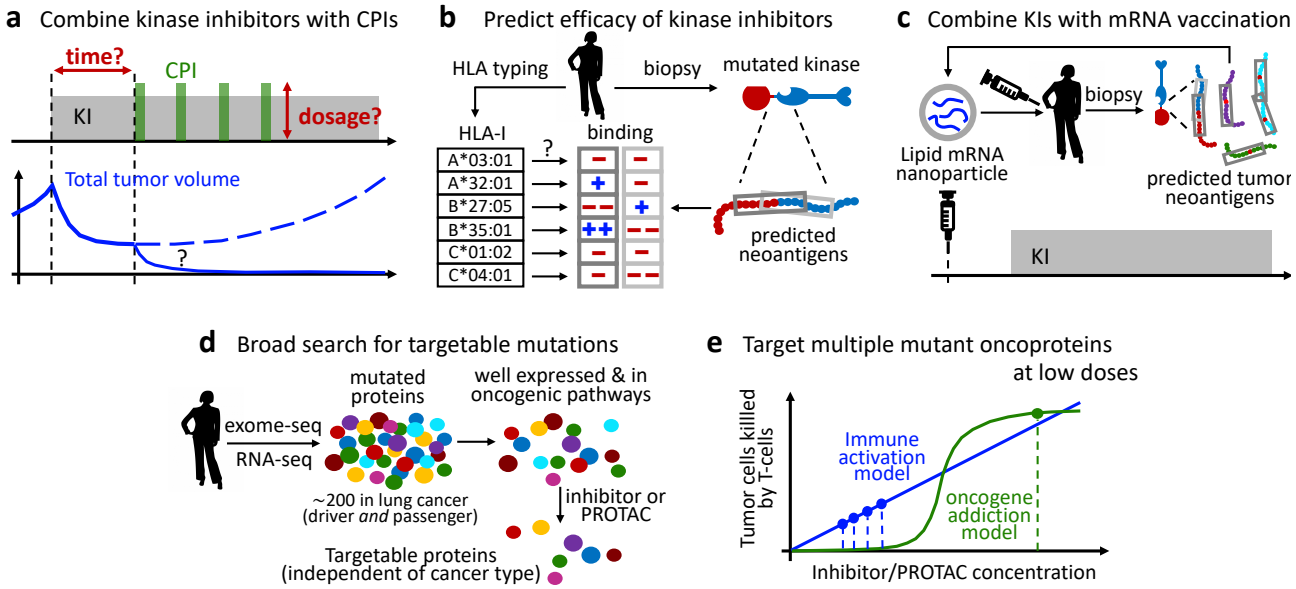

Figure 2: Implications for cancer research. **a** The strong adverse effects observed when combining immune checkpoint inhibitors (CPI) with inhibitors against mutated oncogenic kinases point to an exploitable synergy between CPIs and the immune-activating effect of the kinase inhibitors (KIs). Finding the optimal dosages and relative timing might be crucial. Adverse effects might be reduced while maintaining synergy by administering CPIs a few months after the start of the kinase inhibitor therapy, when the absolute rate of tumor cell shrinkage has subsided. **b** The efficacy of targeted therapy for individual patients might be predicted from their MHC/HLA class I alleles by predicting the binding affinity of the neoantigens to their HLA-I protein variants.[24] **c** Targeted kinase inhibitors might synergize with mRNA-based vaccination against tumor neoantigens[25] if the mRNA vaccine includes neoantigens of the targeted kinase. **d** Targeting passenger mutations could elicit just as effective an immune response as targeting driver mutations. This suggests sequencing each patient's exome and transcriptome to identify mutated proteins in oncogenic pathways that can be degraded with targeted medication. **e** The immune activation model implies that there might not be a threshold effect for the inhibitor concentration. If true, all drugable mutatant proteins in oncogenic pathways could be targeted jointly, spreading out the dose among them and dispersing adverse effects while reducing the risk of resistance.

neoantigens are bound by the patient's HLA class-I proteins[24] (Figure 2b). The model also suggest that combining targeted kinase inhibitors with mRNA-based vaccination against tumor neoantigens[25] might be particularly powerful when the mRNA contains neoantigens from the targeted kinase (Figure 2c).

The model predicts that small-molecule inhibitors might be effective against any mutated kinase in an oncogenic pathway, *independently of cancer type*, as long as the kinase is sufficiently expressed and the tumor microenvironment is permissive to immune stimulation. To date, tumors are screened only for a small panel of specific oncogenic mutations that are frequent in the patient's specific type of cancer, for instance EGFR, BRAF, KRAS, ALK, ROS1, RET, MET in NSCLC, even though other mutated kinases such as HER2 could be targeted.[30,31] Cancers often have mutations in tens to hundreds of proteins.[32] It seems high time to institute procedures to discover all mutated kinases in oncogenic signalling pathways that could be targeted with an approved inhibitor, independent of cancer type. In contrast to current practice, the model predicts that addressing these mutations might be effective *even if they are not driver but merely passenger mutations* in an oncogenic pathway (Figure 2d). Widening the focus to include non-driver mutations might thus open up a much greater spectrum of actionable mutations. In this sense, the Achilles heels of cancer might be easier to find than once thought[7] – via exome and transcriptome sequencing.

The idea of cancer type-independent and oncogene-specific indications is already gaining a foothold. The small molecule inhibitors entrectinib and larotrectinib were approved by the FDA in 2019 for the treatment

of any solid cancer harboring NTRK1/2/3 fusion proteins, regardless of organ, tissue, or histology type.[33] The defragmentation of treatment indications along the latter dimensions also has the potential to translate to higher efficiency and increased robustness in clinical research.[34]

The model further suggests that there might not be a strong threshold effect below which inhibitors become ineffective; ineffective as inhibitors of their target kinase, but not necessarily as immune agents inducing the tumor cells to present neoantigens to T cells (Figure 2e). If true, this would offer the possibility to *combine multiple inhibitors at low doses* targeting kinases mutated in the patient's tumor, with their effects adding up. The lower doses would decrease and disperse off-target, adverse effects while the combination of multiple inhibitors should suppress the development of resistance .

Finally, if the efficacy of small-molecule kinase inhibitors for cancer treatment is indeed at least in part explained by their immune-related effect, the same could be expected of *proteolysis-targeting chimeras* (PROTACs).[35,36] Like kinase inhibitors, PROTACs are small molecules that lead to the proteasomal degradation of the protein they target. They consist of a high-affinity ligand of the target protein linked to a high-affinity small-molecule ligand of an E3 ubiquitin ligase such as cereblon. The model presented here predicts a double-whammy effect of PROTACs directed against oncogenic protein targets with somatic mutations: one mediated by repression of the oncogenic protein and the other by neoantigen presentation and immune stimulation.[21,22]

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Conflicts of interests
The author declares that he has no competing interests.

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