Special Report

Do inhibitors targeted against mutant oncogenic kinases act via kinase degradation-induced immune activation?

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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. 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 would facilitate tumor cell recognition by T cells, leading to a sustained immune attack. The model suggests that the as yet inevitable failure to shrink tumors further after a few months might be caused by a transition to chronic inflammation. If true, the model has a multitude of implications for cancer and clinical research.

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Abbreviations: CHIP, C terminus of Hsp70 interacting protein; Cdc37, cell division cycle 37 cochaperone; EGFR, epidermal growth factor receptor; 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 results in 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] 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.[5] While first-generation inhibitors blocked multiple related receptor kinases, third generation drugs are much more specific and have weaker adverse effects.

The mechanism of action of kinase inhibitors is explained by blocking mitogenic signalling and inhibition of cell proliferation.[4] However, this mechanism alone struggles to explain the massive cell death manifested
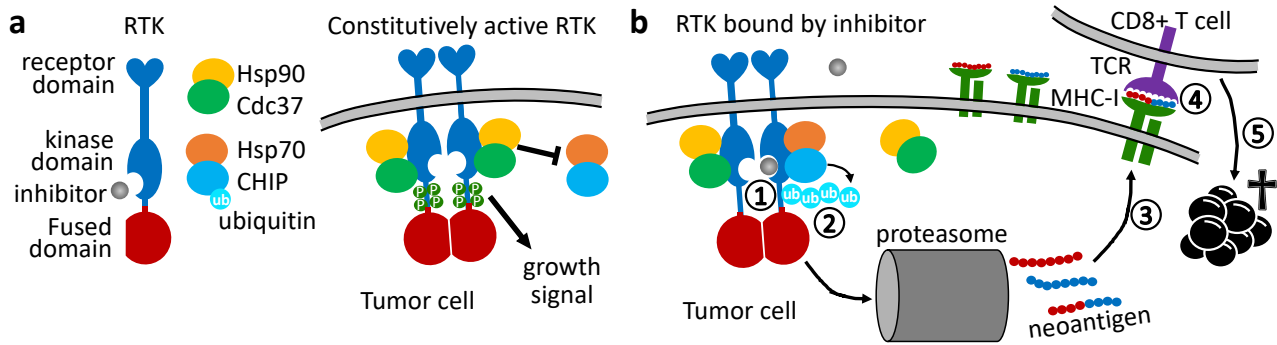
Figure 1: Model of tumor-directed immune activation by small-molecule inhibitors of oncogenic kinases. **a** In many cancers, a kinase in a growth signaling pathway is constitutively activated by an oncogenic mutation. In the illustrated example, a receptor tyrosine kinase (blue) was fused to an activating 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.[16,17,18] 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,[19] 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, systemic immune attack on the tumor.

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.[6] While this model has been clearly confirmed for specific cases,[7,8,9,10] it does not account for the fact that in cell culture direct inhibition of the oncogene or inhibition of its expression in many cases resulted merely in growth arrest rather than cell death[11,12] (see Table I in ref.[13] for an overview). Also, even after intense efforts it has been challenging to delineate 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 a link to the immune system and thereby explain their surprising efficacy (Figure 1b).

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 human kinases 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] The model relies on the following observations (Fig. 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] (For RTKs this is preceded by endocytosis of the activated kinases.) For the Erb2 receptor this has been shown explicitly: binding by various small-molecule inhibitors 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.[20,21] ④ Some peptides of the mutated oncogenic kinase are recognized as non-self neoantigens by T cells with matching T-cell receptors.[19] ⑤ Cytotoxic CD8$^+$ T cells recognize and kill the tumor cells. Upon their death, further neoantigens are released and taken up by antigen-presenting cells. These migrate to lymph nodes where they activate CD4$^+$ and CD8$^+$ T cells. The cancer antigen-specific T cells proliferate and attack the tumor.[22]
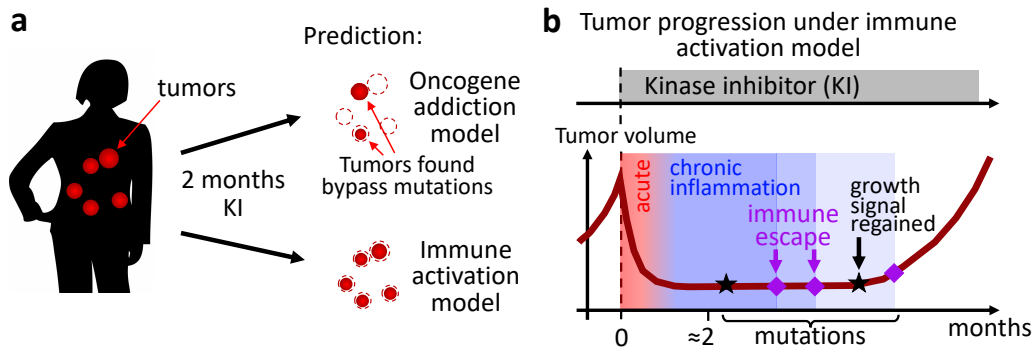
Figure 2: Tumor size development upon kinase inhibitor (KI) treatment points to immune system as main cause of tumor cell death. **a** KI treatment tends to shrink tumors relatively homogeneously, against expectations from the oncogene addiction model but in accordance with the immune activation model. **b** According to the immune activation model, KI treatment could trigger an acute inflammation (red) that shrinks the tumor volume considerably within a few weeks. The immune reaction might become chronic (blue) by self-limiting negative immune feedback, including expression of PD-1 on T cells. The cancer would then have ample time to accumulate muations to evade the immune system (pink stars) and regain growth signals (black diamonds).
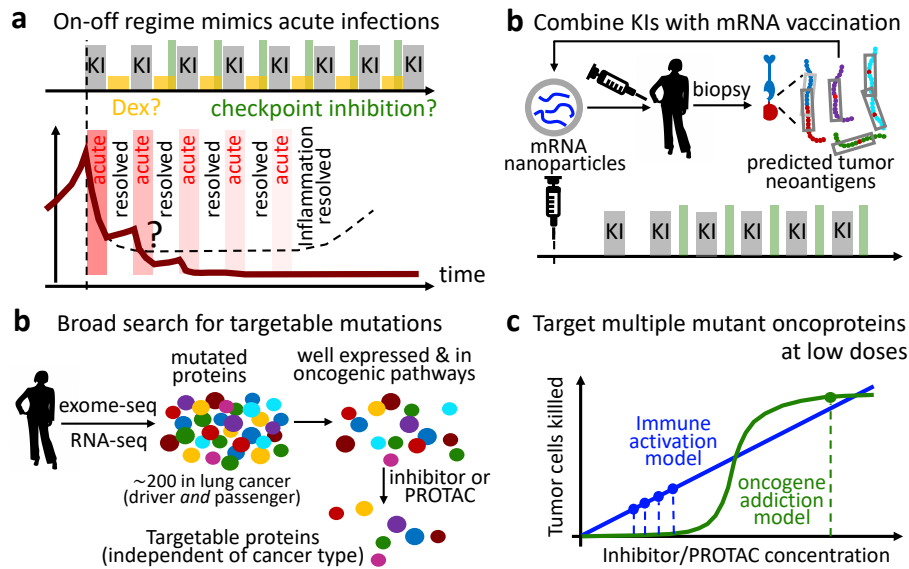
Further supporting evidence

It is striking that the majority of the 55 approved small-molecule protein kinase inhibitors are indicated for patients with mutated kinases, mostly RTKs,[5] while only a minority target overexpressed wildtype kinases, and some that do are combined with 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 mode of action to oncogen addiction to explain the efficacy of inhibitors targeting mutated kinases.[10]

Evidence for the importance of RTK neoantigen presentation is provided 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 MHC-I/HLA class I alleles predicted to be protective for these mutations. The 12 patients with protective alleles indeed had considerably longer overall survival times (hazard ratio 0.13, p-value = 0.005),[23] indicating that the effective presentation of the EGFR neoantigens conferred a substantial advantage.

Evidence for the key role of T cells directed against the mutated oncogenic kinase in patients treated with targeted kinase inhibitors is provided by three studies. In the first, ten lymphoblastic leukemia patients with BCR-ABL fusion were treated with imatinib, an inhibitor of the oncogenic bcr-abl kinase. In all patients, bcr-abl-specific CD8$^+$ and CD4$^+$ effector T lymphocytes were detected in bone marrow or blood.[24] In a follow up, three lymphoblastic leukemia patients undergoing maintenance tyrosine-kinase inhibitor therapy were treated with T cells against bcr-abl kinase. All patients achieved complete remission.[25] In the third study, a patient with exon 19 deletion of EGFR was treated with EGFR inhibitor.[26] After relapse and successful immune checkpoint therapy, T-cell responses were measured in vitro. Two of the three strongest responses were against neoantigens derived from the exon 19 deletion of EGFR.

Indirect yet hard-to-dismiss evidence against oncogene addiction as the dominant cause of tumor cell death is summarized in Figure 2: With this model, we would expect only a small fraction of a patient's tumors to survive a few months of therapy, those which in time can find mutations bypassing the growth signal inhibition, while all others should disappear. This is not what is observed in the clinic. In most patients responding well to kinase inhibitors, the various metastatic tumors shrink in a relatively homogeneous way, and larger metastases rarely disappear (Fig. 2a bottom). In oncogene addiction, the patient's tumors would have to find escape mutations independently of each other at similar times, a very unlikely scenario.

These observations are however in accordance with the immune activation model (Fig. 2b). The inhibitor would make tumor cells visible to T cells recognizing the kinase neoantigens. Further cancer neoantigens from dead tumor cells would be presented in lymph nodes, enabling systemic activation of T-cells against other neoantigens. In this acute phase of inflammation, many tumor cells are killed, resulting in relatively homogeneous shrinkage across metastases. However, when an infection cannot be resolved within several weeks, the immune response transitions from acute to chronic to limit collateral tissue damage. At this

a On-off regime mimics acute infections

b Combine KIs with mRNA vaccination

b Broad search for targetable mutations

c Target multiple mutant oncoproteins at low doses

Figure 3: Implications for cancer research. **a** An *on-off* kinase inhibitor (KI) regime might mimic a series of acute infections. If during *off* phases cancer cell death and pro-inflammatory cytokines subsided, this would signal the successful resolution of the inflammation. Resolution is required for the formation of memory T cells, resets negative feedback mechanisms, and enables a fresh acute response in the next *on* phase. Possibly, immunosuppressive drugs (e.g. dexamethasone, Dex) during off-phases might help to resolve inflammations.[27] **b** Targeted KIs might synergize with mRNA-based vaccination against tumor neoantigens[28] if the vaccine includes neoantigens of the targeted kinase. **c** 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. **d** The immune activation model suggests that there might not be a threshold effect for the inhibitor concentration. If true, all drugable mutant proteins in oncogenic pathways could be targeted jointly, spreading out the dose and dispersing adverse effects while reducing the risk of resistance.

time, tumors stabilize. Over time, mutations to evade control by the immune system (magenta diamonds) and to bypass inhibition of growth signal (black stars) accumulate, until relapse occurs.

Implications for cancer research

To prevent the transition to chronic inflammation, an intermittent *on-off* drug regime might help (Fig. 3a). Each *on* phase might simulate an acute viral outbreak. Each *off* phase might mimic the resolution of the infection and prepare the immune system for a renewed, acute inflammatory response. A phase-2 study in 206 advanced melanoma patients investigating a 5+3 weeks *on-off* cycle with targeted inhibitor against mutated BRAF was conducted with a different motivation, to delay resistance development.[29] Overall survival was the same, and progression-free survival was unfortunately even lower than with continuous schedule. Insufficient resolution of the inflammation during *off* phases is a possible culprit. An immunosuppressive drug might help to resolve inflammations, as observed in chronic HIV infection.[27] Also, immune checkpoint inhibitors might reenergize anergic T cells before *on* phases.

If indeed targeted kinase inhibitors are not merely growth inhibitors but immune-active agents, synergies might be expected from combining them with immune checkpoint inhibitors. Several phase I trials testing such combinations have been conducted.[30] Unfortunately, most showed severe immune-related adverse effects. However, these largely disappeared in retrospective analyses if checkpoint inhibitors were given after RTK inhibitor therapy. The adverse effects might be due to flooding of lymph nodes with cancer neo- and self-antigens from dying tumor cells. Dampened negative feedback by checkpoint inhibition together with a high density of dendritic cells carrying danger signals and presenting self-antigens would trigger antigen spreading from neo- to self-antigens. This is in accord with the observation that the autoimmune reactions are directed against tissues sharing antigens with the tumor cells.[31] If true, adverse effects might be minimized by administering checkpoint inhibitors well after the initial, rapid tumor shrinkage (Fig. 3a).

The model suggests that combining targeted kinase inhibitors with mRNA-based vaccination against tumor neoantigens[28] might be particularly powerful when including neoantigens from the targeted kinase (Fig.

3b). It further 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, even though other, less frequent mutations (such as in HER2 for NSCLC) could be targeted.[32,33] Cancers often have mutations in tens to hundreds of proteins.[34] It seems high time to institute procedures to discover all mutated kinases in oncogenic signalling pathways that could be targeted with approved inhibitors, 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 (Fig. 3c). 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 anticipated[6] – 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.[35] The defragmentation of treatment indications along the latter dimensions also has the potential to translate to higher efficiency and increased robustness in clinical research.

The model 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 (Fig. 3d). If true, we might *combine multiple inhibitors at low doses* targeting kinases mutated in the patient's tumor, with their effects adding up.

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).[36,37] PROTACs are small molecules that trigger proteasomal degradation of the protein they target. They consist of a high-affinity ligand for 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.[20,21]

Given these broad implications for cancer and clinical research, we hope that this report will stimulate experiments to test the proposed model of immune activation.

Conflicts of interests
The author declares to have no competing interests.

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