Oncogene Overdose: Too Much of a Bad Thing for Oncogene-Addicted Cancer Cells

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Supplementary Issue: Signaling Pathways as Biomarkers

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ABSTRACT: Acquired resistance to targeted inhibitors remains a major, and inevitable, obstacle in the treatment of oncogene-addicted cancers. Newer-generation inhibitors may help overcome resistance mutations, and inhibitor combinations can target parallel pathways, but durable benefit to patients remains elusive in most clinical scenarios. Now, recent studies suggest a third approach may be available in some cases—exploitation of oncogene over-expression that may arise to promote resistance. Here, we discuss the importance of maintaining oncogenic signaling at "just-right" levels in cells, with too much signaling, or oncogene overdose, being potentially as detrimental as too little. This is highlighted in particular by recent studies of mutant-BRAF in melanoma and the fusion kinase nucleophosmin–anaplastic lymphoma kinase (NPM–ALK) in anaplastic large cell lymphoma. Oncogene overdose may be exploitable to prolong tumor control through intermittent dosing in some cases, and studies of acute lymphoid leukemias suggest that it may be specifically pharmacologically inducible.

KEYWORDS: oncogene addiction, oncogene overdose, intermittent dosing, the Goldilocks principle, ALK, BRAF

SUPPLEMENT: Signaling Pathways as Biomarkers

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Introduction

For most cancers, the hope that single-molecule targeted therapy would revolutionize treatment has been thwarted by acquired resistance. Mechanisms may be target-dependent, typically mutations or overexpression, or target-independent, such as the activation of parallel pathways.[1] Although rational strategies exist to overcome parallel signaling and resistance-conferring mutations (reviewed elsewhere[2–6]), the intriguing possibility of exploiting target over-expression is now also entering discussion. Here, we review the feasibility of this approach, where target upregulation can cause an overdose of oncogenic signaling that is detrimental to cancer cell survival. By exploiting the "Goldilocks principle"—the idea that even for oncogenes "just-right" levels are required—strategies such as intermittent dosing may permit prolonged tumor control in some patients.

Oncogene Addiction

Though cancer cells accumulate numerous genetic changes, deactivation of individual oncogenes often cause pronounced tumor regressions in mouse-model systems. Bernard Weinstein first used the term "oncogene addiction" in 2002 to describe these findings.[7] Strong clinical support came with the remarkable and durable efficacy of breakpoint cluster region-Ableson kinase (BCR-ABL) tyrosine kinase inhibitors (TKIs) in treating chronic myeloid leukemia (CML) without significant toxicity.[8,9] Such findings revolutionized cancer drug development toward targeted therapies to exploit every tumor's perceived Achilles' heel and ultimately driving a new paradigm of personalization of cancer care through precision medicine.[6,10–12] Unfortunately, however, CML still stands more or less alone in the success of this strategy. Inhibitors targeting other driver kinases produce progression-free survival (PFS) times of typically a few months and have failed to replace frontline chemotherapy in most diseases.[13–16] Even when other inhibitors have moved to the frontline, as the epidermal growth factor receptor (EGFR) inhibitor erlotinib and the anaplastic lymphoma kinase (ALK) inhibitor crizotinib have for appropriate advanced lung-cancer patients, median PFS is ~9–10 months.[17,18] In TKI-treated CML by contrast, median PFS is still unknown because it has not been reached after a decade or more of follow-up.[9,19–22]

Newer Understandings of Oncogene Addiction

Difficulties in recapitulating the success of BCR-ABL kinase inhibitors vs. CML in other cancers have highlighted the deep gaps in our comprehension of oncogene addiction's complexities. Through recent work, however, it is now well understood that the inhibition of a single target can lead to the rewiring of signaling pathways that may rescue the activation of key downstream processes. For example, in triple-negative breast cancer, Duncan et al[23] showed how MEK/ERK inhibition promotes the degradation of MYC, leading to the expression and activation of various receptor tyrosine kinases (RTKs). These RTKs overcome inhibition of MEK2 (but not MEK1), reactivating ERK and culminating in drug resistance. Knockdown of ERK, MYC, AKT, or mTOR recapitulates this reprograming of the kinome, while preventing MYC's proteasomal degradation blocks it.[23–25]

Oncogenic shock. The "oncogenic shock" model provides a framework for conceptualizing such results. Briefly, while oncogenes promote proliferation and survival, they paradoxically activate signals to promote apoptosis or cell cycle arrest.[26,27] This may be because of feedback repression of normal survival pathways (as above) and/or through stress imposed by increased cellular growth rates, similar to oncogene-induced senescence in nontransformed cells.[28] Either way, when an inhibitor of a driver oncogene is introduced to the system, both the growth/survival and the proapoptotic/growth-arrest signals are inhibited. In truly oncogene-addicted cells, abrupt loss of the survival signal destroys the cells before the loss of the apoptotic signal can allow them to survive and vice versa for the cells that survive.[6,26,27]

The oncogenic shock model, which was originally proposed nearly a decade ago, has received strong support from recent results with key clinical targeted inhibitors. In CML, the poster-child disease for oncogene addiction, for example, Asmussen et al[29] found that BCR-ABL represses normal myeloid survival pathways through feedback inhibition mediated by activated MEK, and these pathways come rushing back several hours after BCR-ABL inhibition. However, drug sensitivity is preserved because cells commit to apoptosis before rescue can occur. The story is different for $BRAF^{V600E}$-driven melanoma (Fig. 1A), where inhibitors typically delay progression for only a few months. Here, the time taken for MAPK pathway rebound is a lot quicker than that for CML (2–4 hours vs. 8–24 hours).[29,30] Therefore, resistance arises in mutant BRAF melanomas, as growth factor pathway restoration is established before a significant proportion of cells enter apoptosis.

While apoptosis precedes the restoration of oncogenic signaling through network rewiring in wild-type BCR-ABL cells,[29] complications arise in the context of resistance. For example, the BCR-ABL inhibitors imatinib, dasatinib, and nilotinib have weak off-target activity against RAF that drives RAS-dependent paradoxical BRAF and CRAF activation.[31] Because BCR-ABL is upstream of RAS activation in sensitive cells, inhibition with these agents also suppresses RAS. In drug-resistant cells with the T315I gatekeeper BCR-ABL mutation, however, RAS activity permits the drugs' paradoxical BRAF/CRAF and downstream MEK/ERK activation (Fig. 1A). This results in dependency on this pathway, such that combining BCR-ABL inhibitors with a MEK inhibitor synergistically inhibited resistant cell growth in vitro and in vivo.[31] However, prolonged MEK inhibition can cause autocrine activation of STAT3 via fibroblast growth factor receptors and Janus kinases (JAKs),[32] supporting even further combination therapy, such as adding JAK/STAT inhibitors to the drug cocktail. Combined drug toxicities to patients, however, become a rising concern as more and more drugs are put together. Instead, what may be more practical clinically would be scheduling drug dosing intermittently, carefully pulsed to ensure target inhibition followed by withdrawal to prevent pro-survival pathway reactivation. Important work is being carried out to understand the specific timings of these antagonistic processes and their responses in different contexts based on factors like protein turnover.[6] In addition, a new approach to target the MAPK pathway—the direct inhibition of ERK with the dimerization inhibitor DEL-22379—was recently reported.[33] This drug showed ability to overcome many resistance mechanisms that thwart MEK inhibitors but has not yet been tested in BCR-ABL-driven models.

Oncogene Overdose

While extensive efforts are underway targeting signals to which cancer cells are addicted, the prospect of using these very signals to overwhelm the system is just now entering exploration. Similar to drug addicts overdosing on the very thing they require to avoid withdrawal, cancer cells may be susceptible to the induction of overdoses in oncogenic signaling. Recent studies indicate that this may be feasible in $BRAF^{V600E}$-addicted melanoma, ALK-addicted T-cell lymphoma, and BCR-ABL+ B-cell acute lymphoblastic leukemia (B-ALL).

Oncogene overdose in $BRAF^{V600E}$ melanoma. While $BRAF^{V600E}$-addicted melanomas rely on continual $BRAF^{V600E} \rightarrow$ MEK $\rightarrow$ ERK signaling[34] (Fig. 1A), Das Thakur et al[35] found that the continual administration of vemurafenib caused resistant tumors to actually become dependent on the continued inhibition of this very pathway. Resistance arose through elevated $BRAF^{V600E}$ expression, which permitted survival during drug exposure but proved toxic when the drug was withdrawn. MEK1/2 inhibition with AZD6244 (selumetinib) also rescued from the $BRAF^{V600E}$ overdose signaling. These observations were made in patient-derived xenograft models and confirmed similar findings reported previously in melanoma cell lines.[36] The fitness benefit provided by increased $BRAF^{V600E}$ expression during drug exposure therefore becomes a fitness liability when the inhibitor is withdrawn[35] (Fig. 2). Resistant tumors showed impaired

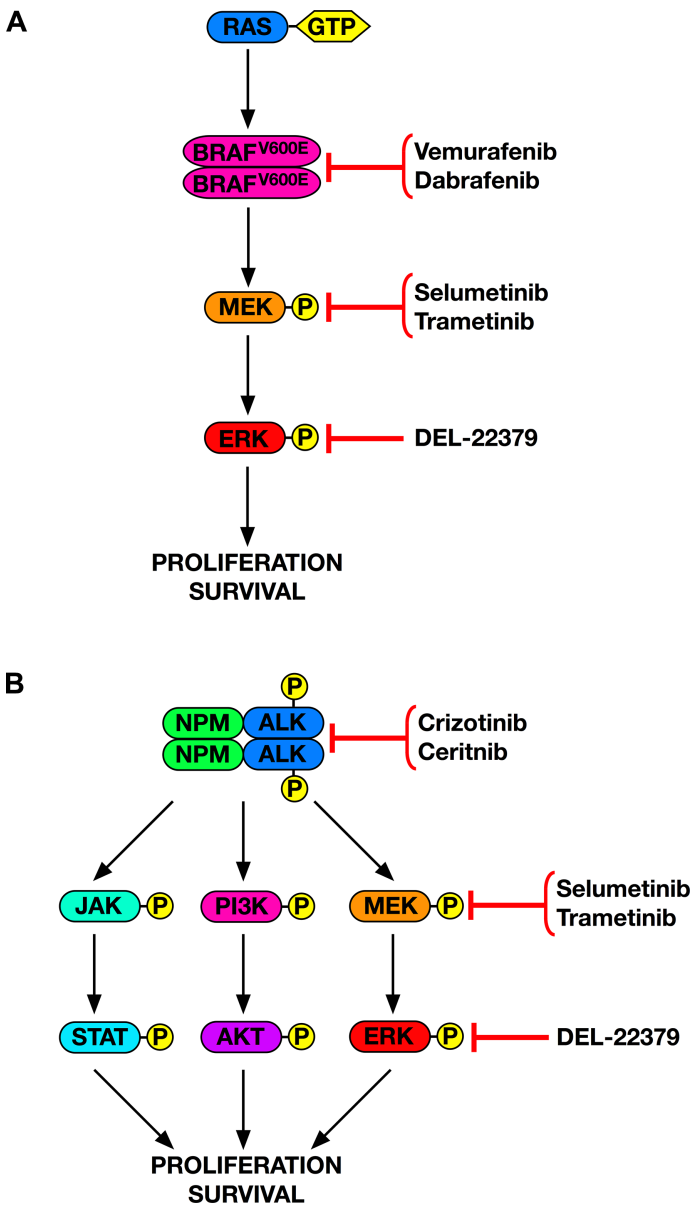

Figure 1. Downstream signaling and targeted pathway inhibition for mutant BRAF in melanoma (**A**) NPM-ALK in ALCL (**B**).
Notes: (**A**) The dimerization of mutant BRAF in melanoma, activated by RAS, turns on several pro-survival and pro-proliferation pathways driving tumor growth in melanoma, which can be targeted using small molecule inhibitors. (**B**) The same pathways are activated through dimerization and subsequent trans-autophosphorylation of several ALK-fusion kinases (NPM-ALK shown above).

engraftment to additional host animals treated with vehicle vs. those treated with vemurafenib. In addition, drug withdrawal in vemurafenib-treated animals produced tumor regressions concomitant with spikes in MEK/ERK activity.

To combat resistance to *BRAF*^{V600E} inhibitors that reestablishes continual BRAF^{V600E} → MEK → ERK signaling despite target inhibition, clinical trials have assessed the efficacy of dual BRAF^{V600E} and MEK inhibitor therapy.[37,38] However, resistance to this dual approach is already reported, arising due to augmentation and/or combination of single-agent resistance mechanisms. Moriceau et al[39] established several cell lines from melanomas that had acquired resistance to dual BRAF^{V600E} and MEK inhibition. One showed ultra-amplification of *BRAF*^{V600E} (>160 copies), which leads to *CRAF* activation. Another showed low copy number gain (20 copies) together with an *MEK1* mutation that increases BRAF^{V600E}-mutant–MEK interactions. Both events led to ERK activation, resulting in growth and survival signals (Fig. 1A). However, both cell lines were also strongly addicted to both inhibitors, as dual inhibitor withdrawal led to a loss of viability via pERK hyperactivation.[39] In fact, a stronger drug addiction phenotype was observed in resistant lines grown ex vivo when patients had been administered dual inhibitors as opposed to single BRAF^{V600E} inhibition. Unfortunately, while looking over the cases of old patients, although the authors were able to identify tumor regression after dual drug

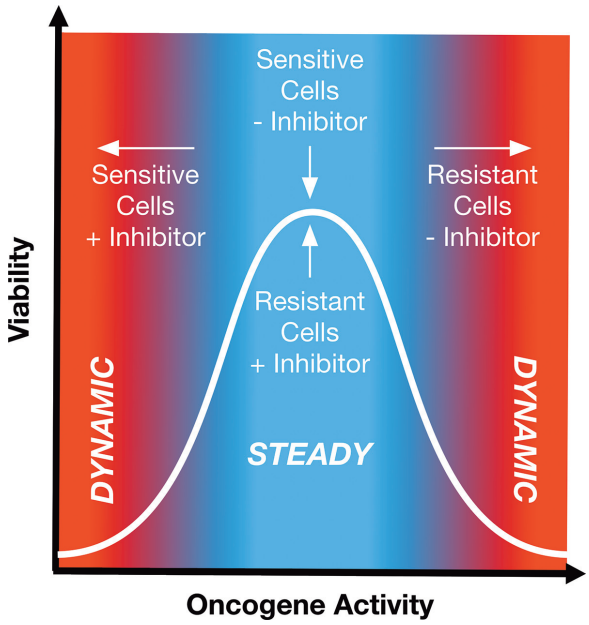

Figure 2. Oncogene-addicted cells can require "just the right" amount of signaling for survival. Oncogene-addicted cells constitutively express the amount of activated oncogene required for growth and proliferation. Targeted inhibition of the oncogene shuts off this signaling and shifts the cells into a dynamic state leading to death. Resistance can be achieved by increased expression and/or activation of the oncogene in question, which allows resistant cells to grow in the presence of the inhibitor originally designed to kill them. Resistance by this means is often accompanied by dependence, such that inhibitor withdrawal causes an overdose of oncogenic signaling that overwhelms the cells and also results in death.

cessation, they did not observe disease stabilization or uniform tumor regression culminating in clinical remission. They only observed decelerated tumor growth in melanomas where single $BRAF^{V600E}$ inhibitor therapy was stopped.

Oncogene overdose in ALK+ ALCL. The anaplastic lymphoma kinase (ALK) is an important new therapeutic target activated through chromosomal translocations that fuse its C-terminal kinase domain to the N-terminus of various constitutively expressed proteins (Fig. 1B). Examples include t(2;5)(p23;q35) creating *NPM* (nucleophosmin)-*ALK*, found in ~70% of anaplastic large cell lymphomas (ALCL), and inv(2)(p21;p23) creating *EML4* (echinoderm microtubule associated protein like 4)-*ALK*, found in 3–5% of non-small cell lung cancers (NSCLC).[40–42] Two U.S. Food and Drug Administration approved ALK TKIs, crizotinib and ceritinib, show activity in both ALK+ NSCLC and ALCL.[16,18,43,44] While resistance in ALK+ NSCLC mainly occurs via the activation of alternative signaling pathways, such as EGFR and c-KIT, as well as the acquisition of second-site mutations, the major mechanism of resistance in ALK+ ALCL reported thus far has been ALK-domain mutations.[45–55]

We recently established, however, that over-expression of *NPM-ALK* reliably arises during resistance selections and was in fact the predominant resistance mechanism initially observed.[56] Through serially plating patient-derived ALK+ ALCL cell lines in increasing concentrations of crizotinib or ceritinib, we generated resistant subclones. Each line acquired resistance by overexpressing NPM-ALK at the levels of both transcription and translation. Surprisingly, however, inhibitor withdrawal induced apoptosis, associated with massive amounts of activated ALK signaling, suggesting that these cells were overdosing on this heightened ALK activity (Fig. 2). This was confirmed by the fact that multiple different ALK TKIs could rescue the toxicity caused by signaling overdose, showing that this is a kinase-dependent consequence of NPM-ALK overexpression. Therefore, similar to the above-mentioned study of mutant BRAF melanoma, these ALK+ ALCL cells had grown to not only become resistant to, but also dependent on, the very inhibitors intended to kill them: a phenomenon we termed "resistance/dependence." Importantly, while three of the five resistant lines established from this study harbored kinase domain mutations, the "resistance/dependence" phenotype, leading to ALK overdose upon drug washout, was seen regardless. This stresses that the upregulation of ALK activity was the major mechanism of resistance acquired by each of these cell lines. The mechanism by which the overdose of ALK activity promoted toxicity is unknown, as individual inhibition of downstream ALK targets (Fig. 1B), including MEK/ERK, failed to rescue from the overdose effects (unpublished observations). Therefore, unbiased approaches are currently underway to ascertain the basis of oncogene overdose in this disease.

In vivo, even stronger than the described observations in melanoma, we found that tumor engraftment of resistant cells was only seen in mice dosed with an ALK TKI (ceritinib).[56] Complete absence of engraftment in vehicle-treated mice underlines the drug dependent phenotype that these resistant cells acquired. Careful passaging of resistant cells in vitro at high confluence, however, allowed us to re-establish lines able to grow in the absence of inhibitor. ALK activity in these lines returned to baseline, and the cells were resensitized to the ALK TKIs. This is similar to observations from the above-mentioned study of melanoma, which showed resensitization through forced knockdown of $BRAF^{V600E}$.[35]

These studies of mutant BRAF melanoma and ALK+ ALCL highlight the importance of having "just the right" amount of signaling with respect to tumor survival (Fig. 2).

Goldilocks Principle

These findings fit well into the "Goldilocks principle," the idea that certain biologic factors require precise levels to promote fitness, with either too much or too little being toxic. For example, the restoration of calcium release in heart cells,[57] the levels of oxygen administered by postcardiac arrest,[58] the amount of vitamin D in the body,[59] the redox environment of the cell with respect to oxidative stress,[60] and the levels of MeCP2 in causing Rett syndrome immune defects[61] must fall within the "Goldilocks zone." Even a person's body mass

index, which should be within the "normal" range, can be thought of in terms of the Goldilocks principle.

MAPK cascade and the Goldilocks principle. Although not usually described in these terms, RAF signaling also follows a Goldilocks paradigm, having dual roles in cell cycle progression or arrest depending on level.[62,63] Studies mostly in mouse fibroblasts, for instance, showed decreases in Raf activity can promote passage through the cell cycle through activation of Cyclin D1/Cdk4 and Cyclin E-Cdk2. Too much Raf, however, can cause cell cycle arrest through the induction of the Cdk inhibitors $p21^{CIP1}$ and $p16^{INK4A}$.[62,63] Interestingly, Raf appears to suppress its own activity to maintain levels in the Goldilocks zone.[64] In addition, further highlighting the paradoxical nature of RAS → RAF → MEK → ERK signaling, the overactivation of any step in the pathway can cause oncogene-induced senescence via replicative stress leading to the activation of $p16^{INK4A}$ and $p19^{ARF}$ tumor suppressor pathways.[28,65–67] In an inducible mouse model with titratable levels of Ras, increased signaling led to senescence, while lower levels induced tumor formation.[68] Therefore, optimum tumor development also requires "just-right" levels of RAS, and indeed, this can be achieved by way of balancing autophagy.[69,70] Furthermore, autophagy itself appears to follow the Goldilocks principle in tumor development. On the one hand, it allows cells to scavenge their own reserves during nutritional and ischemic stress. On the other hand, too much autophagy opposes tumor establishment and development, through nascent tumor death or the prevention of further mutations that favor progression caused by reactive oxygen species that are generated by the buildup of damaged organelles.[71]

Intermittent dosing

The requirement that at least some oncogenes maintain signaling at "just-right" levels may be exploitable using intermittent dosing to delay the onset of fatal resistance (Table 1). As discussed above, pulsed TKI dosing may allow BCR-ABL and its downstream signaling cascade to be effectively turned off on-drug, without permitting the reactivation of growth factor signaling when off-drug.[6,29] This can provide a window where there are only proapoptotic signals and no pro-survival signals present, enhancing cell death. Furthermore, pulsed BCR-ABL inhibition elicits the activation of the proapoptotic BIM protein with the same kinetics seen with continual exposure.[72] This approach, however, is highly specific depending on the oncogene in question. For example, while the inhibition of FLT3-ITD fusion in acute myeloid leukemia turns off oncogenic signaling, growth factor receptor signaling is restored at a faster rate upon inhibitor discontinuation, which means that there is not enough time for the induction of apoptosis before the reactivation of pro-survival signaling.[6,29] It will require meticulous tweaking of dosing schedules to exploit each oncogene to bring about death before the restoration of pro-survival signaling.

Systems in which target overexpression drives both resistance and dependence may be the most amenable to intermittent-dosing strategies. Using patient-derived $BRAF^{V600E}$ melanoma xenografts, Das Thakur et al[35] showed that intermittent vemurafenib dosing prolonged tumor control compared to continuous dosing. Both individualized drug interruptions based on tumor burden and up-front scheduled intermittent dosing were superior to continuous dosing in this report. Considering strategies to combat targeted-drug

Table 1. Studies that favor intermittent dosing to prolong tumor control.

ONCOGENE	INHIBITOR	DISEASE	STUDY	REFERENCE
BCR-ABL	Dasatinib Imatinib	Chronic Myeloid Leukemia	Pulsed dosing favors clinical remission in patients	72
BCR-ABL	Dasatinib Imatinib	Chronic Myeloid Leukemia	Pulsed inhibition prevents downstream growth factor rewiring	29
EGFR	Erlotinib	Chronic Myeloid Leukemia	Pulsed dosing prevents cytotoxicity	72
Mutant BRAF	Vemurafenib	Melanoma	Intermittent dosing prolongs tumor control	35
Mutant BRAF	Dabrafenib Trametinib	Melanoma	Ongoing joint NCI-SWOG randomized trial assessing the feasibility of intermittent dosing in melanoma using dual BRAF and MEK inhibitors	NCT02196181
Mutant BRAF	Vemurafenib Selumetinib	Melanoma	Cells with acquired resistance to dual BRAF and MEK inhibition are sensitive to drug withdrawal	39
Mutant BRAF	Vemurafenib Dabrafenib	Melanoma	Successfully rechallenged 2 mutant BRAF melanoma patients following a drug holiday	73
NPM-ALK	Crizotinib Ceritinib	ALK+ Anaplastic Large Cell Lymphoma	Intermittent dosing forestalls the fatal onset of resistance	56
EML4-ALK	Crizotinib	ALK+ Non-Small Cell Lung Cancer	Patients were successfully rechallenged with crizotinib after a drug holiday	74,75
EGFR	Erlotinib Gefitinib	Non-Small Cell Lung Cancer	Rechallenging with EGFR inhibitors decreased tumor volume	85

resistance, intermittent dosing emerges as one carrying the least amount of both expense and toxicity to patients. Therefore, the melanoma report both established the preclinical proof of principle and highlighted its potential flexibility. A small case series, meanwhile, reported successful BRAF-inhibitor rechallenge in two patients who had previously developed resistance and were then off-drug for several months, but the cause of resistance was not investigated.[73] A joint NCI-SWOG randomized trial specifically comparing intermittent vs. continuous dosing of dabrafenib and trametinib for treating *BRAF*^{V600E}-mutant melanomas is currently enrolling patients (NCT02196181).

Although we found that ALK+ ALCL lines selected for TKI resistance reliably developed resistance–dependence, we have only begun exploring intermittent dosing as a therapeutic strategy. The improvement of ALK+ ALCL mouse models using genome editing with CRISPR/Cas9 should allow this possibility to be more rigorously investigated. However, two case reports have shown that patients may respond positively to rechallenge with crizotinib, lending support to the clinical investigation of discontinuous dosing with ALK TKIs.[74,75]

Pharmacologically Inducing Oncogene Overdose

Another potential way to exploit therapeutically oncogene overdose may be through its forced pharmacological induction. In order to investigate this possibility, the mechanism(s) underlying oncogene overdose must be well understood. In ALK+ ALCL, our knowledge is incomplete, and, as mentioned, the inhibition of downstream targets of ALK failed to rescue cell viability (unpublished observations). We are undertaking several unbiased approaches to elucidate the specific mechanisms underlying overdose in the hope that forced induction may be translatable into a therapeutic strategy.

In another lymphocyte-derived malignancy, however, both the basis for and means to induce an overdose of oncogenic signaling were recently established. Briefly, the Goldilocks principle can be thought of as applying to the positive and negative selection of developing lymphocytes. The positive selection of immature B and T cells requires their newly created B-cell receptor (BCR) or T-cell receptor (TCR) to bind test antigens with a minimum affinity to weed out weak clones not likely to be useful in immune defense. However, negative selection eliminates clones with too strong of an affinity to self-antigens, eliminating clones likely to precipitate autoimmune phenomena. In both the cases, the degree of affinity is translated into the strength of downstream signaling, with too little or too much both being potentially fatal. Therefore, lymphocytes carry a propensity in their life history to die due to the overactivation of their core survival pathways.[76–79]

Approximately 25% of B-ALL cases contain the chromosomal fusion BCR-ABL discussed above, which in this context mimics the constitutive activation of pre-BCR signaling.[80,81] A comprehensive study by Chen et al[82] showed that BCR-ABL ALL cells express increased amounts of several immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing proteins, such as PECAM1, CD300A, and LAIR1. These ITIMs recruit several phosphatases, such as PTPN6 (also known as SHP1) and INPP5D, which downregulate the activation of SYK, an early step in BCR signaling whose activation is normally markedly reduced in BCR-ABL ALL cells. Therefore, the authors used a small molecule inhibitor that targets the inhibitory phosphatase INPP5D to induce SYK activation. This led to the selective death of BCR-ABL+ B-ALL cells, both in vitro and in vivo. Furthermore, this was achieved regardless of the mutational status of BCR-ABL, as SYK induction also caused BCR-ABL^{T315I} ALL cell death. This study highlights the potential of pharmacologically inducing overdose as a form of therapy.

This paradigm may also be applicable to T-ALL. A study of *TEL-JAK2* T-leukemogenesis in transgenic mice crossed these animals with the transgenic Marilyn strain, which expresses an MHC class II-restricted TCRαβ recognizing specifically the H-Y male antigen.[83] Male primary mice, and even male mice engrafted with tumor cells from females, rarely developed tumors, in contrast with females, whose disease onset was the same as baseline *TEL-JAK2* animals. The cause was hypothesized to be self-antigen-induced negative selection but was not specifically established. Nor has a pharmacologic means of inducing such an overdose of signaling in T cells yet been reported.

Conclusions

More work is warranted to assess the impact of oncogene overdose as a general mechanism leading to cell death in the context of acquired resistance, and intermittent dosing to exploit overdose may be fruitful in prolonging tumor control in selected patients. The in vitro and in vivo work discussed above in mutant BRAF melanoma and ALK+ ALCL shows great promise for potentially implementing this treatment paradigm in a patient setting, where intermittent dosing forestalled the onset of resistance.[35,56] The findings of these studies are further corroborated by clinical cases where discontinuous dosing has been highly efficacious (discussed above and summarized in Table 1). However, care must be taken when administering this regimen, as drug withdrawal prior to the onset of resistance, as well as the dose of drug initially administered, could accelerate acquired resistance.[6,84] Therefore, up-front intermittent schedules must be carefully studied and established. Otherwise, individualized approaches may be better, particularly for the short term. Precise characterization of the mechanisms of oncogene overdose will shed clearer light on both the therapeutic potential and appropriate strategies of intermittent dosing and is required before any efforts to pharmacologically induce overdose can be undertaken. It is also important to note that while intermittent dosing is less toxic and expensive than most other treatment strategies, it is not a cure. The in vivo experiments in mutant BRAF melanoma show that although the forestalling of resistance is achieved via intermittent dosing, eventually the cell finds a way and

resistance refractory to intermittent dosing sets in.[35] When feasible, regular tumor screening and genotyping to ascertain the mechanisms at play when tumor burden increases at each drug cycle will help clinicians stay one step ahead of tumor evolution and prevent the fatal onset of resistance.

Author Contributions

Wrote the first draft of the manuscript: ADA and JHS. Contributed to the writing of the manuscript: ADA, SSR, MJG, PP and JHS. Jointly developed the structure and arguments for the paper: ADA and JHS. Made critical revisions and approved final version: ADA, SSR, MJG, PP and JHS. All authors reviewed and approved of the final manuscript.

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