Trends in Cancer

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Opinion

Prospects for understanding and exploiting the consequences of hyperactivation lethality

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Cancer cells optimize oncogenic signaling to maintain a defined range for survival. The success of targeted therapeutic inhibitors is based on suppressing signaling below this optimal fitness zone. Conversely, cancers are also susceptible to a clinically underutilized vulnerability – oncogenic hyperactivation. Cytotoxic hyperactivation is observed across diverse cancers, with direct small-molecule activators and inhibitors of negative regulators inducing lethal pathway activation. Deep characterization of the cancer genome and unbiased screening approaches have yielded multiple targets vulnerable to hyperactivation; however, translation into the clinical setting will require defining signaling thresholds, discovering biomarkers, and developing appropriate trial designs. By exploiting cancer's intrinsic vulnerabilities, activation lethality offers a promising therapeutic strategy to expand the treatment landscape and overcome resistance to targeted inhibition.

Highlights

Cancer cells optimize and maintain oncogenic signaling within a 'Goldilocks' zone. Like pathway inhibition, excessive activation impairs cancer cell viability, creating new therapeutic opportunities.

Mechanisms to enact activation lethality can work through signaling pathways, driver addiction, cellular stress, or transcriptional rewiring.

The genomic landscape of cancer reveals evolutionary pressures that limit pathway hyperactivation; thus, analyses of genomic patterns, such as mutual exclusivity, coupled with unbiased screening can enable target identification.

Therapeutic strategies exploiting activation lethality include direct functional activators and inhibitors of negative regulators. Resistance to inhibition and hyperactivation is likely to be non-cross-resistant, suggesting that sequential or cyclical targeting of both vulnerabilities could offer a curative strategy.

Do not fly too high: exploiting activation lethality in cancer

The possibility that excessive activation of certain oncogenic pathways might be damaging to cancer cells is intriguing to pursue at a time when the benefits of targeted cancer therapies are generally limited by drug resistance. Such therapies predominantly block key oncogenic signals, but select mutations and transcriptional plasticity in cancer cells enable them to evade inhibitors, escape through bypass routes, and evolve to survive. New strategies are needed to break a relentless cycle of treatment and resistance. One such strategy might be to exploit rather than merely suppress cancer dependencies, using hyperactivation of oncogenic signaling pathways to disrupt the altered homeostasis in cancer cells and augment current therapeutic approaches.

Building upon the early observations of oncogene-induced senescence or apoptosis [1,2], several laboratories have published studies in recent years that have brought to the fore the idea that hyperactivation of selected oncogenic signaling pathways might be lethal in certain cancers. To compare these findings, to look for common mechanisms of hyperactivation, and to discuss strategies that might produce therapeutically beneficial toxicity in cancer cells, William Sellers, Harold Varmus, Kimberly Stegmaier, and René Bernards organized an Activation Lethality Conference that was held on 9 and 10 May 2024, at the Dana-Farber Cancer Institute in Boston, MA, USA. The meeting brought together about 40 investigators, an equal mix of laboratory leaders and early career scientists from diverse disciplinary backgrounds, to listen to presentations from each laboratory and engage in extended discussions. The talks and discussions led to the emergence of several key themes – mechanisms underlying activation-induced lethality, strategies for the discovery of targets to induce hyperactive signaling, and approaches for the development of novel therapies.

Mechanisms underpinning activation lethality

Underlying diverse mechanisms of activation lethality is the pervasive observation of the 'Goldilocks' phenomenon: like Goldilocks searching for a 'just right' porridge that is neither too

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cold nor too hot, cancer cells require a precise balance of oncogenic signaling to maintain their survival (Figure 1). Either insufficient signaling or, as initially described for aberrant oncogene induction, excessive activation of signaling pathways, can lead to growth arrest, senescence, or apoptotic cell death. Activation-induced lethality exploits cancers' own adaptive mechanisms in a variety of ways – by exaggerating oncogenic signaling pathways, by subscribing to oncogene addictions, by altering cellular metabolism, or by rewiring transcriptional networks in ways that violate sustainable thresholds.

Signaling pathways regulate cancer's altered homeostasis

The precise regulation of signaling pathways is crucial for cellular homeostasis. This is especially true for cancer cells, which depend on finely tuned signaling to support their unchecked proliferation while avoiding cellular stress or death. Multiple meeting presentations demonstrated that disruption of cancer's altered homeostasis through hyperactivation of oncogenic pathways induces lethality across diverse cancer types. Consistent mechanistic patterns across studies of the MAPK, PI3K, and WNT pathways reveal actionable vulnerabilities and inform therapeutic strategies.

The mutual exclusivity of *EGFR* and *KRAS* oncogenic mutations in the context of lung adenocarcinoma is the result of excessive activation of the downstream protein serine-threonine kinase ERK, leading to impaired cell fitness, as reported by the Varmus laboratory [3,4]. Inhibition of the protein phosphatase, DUSP6, a negative feedback regulator of ERK, can also result in loss of fitness in a human lung adenocarcinoma cell line driven by a mutant *KRAS* allele, suggesting that the hyperactivation state may be inducible. Using phosphoproteomic methods, comparison of distinct MAPK signaling activity levels in cancer cells, including growth-compatible pERK levels, excess KRAS signaling, and rescue of excess KRAS signaling by MEK inhibition, uncovered targets of ERK that potentially mediate lethality (A. Unni and H. Varmus, unpublished) [4].

Importantly, thresholds for inducing lethality through ERK hyperactivation are dynamic and environmentally sensitive. In melanoma cells, successive cycles of BRAF or MEK1/2 inhibitor removal and rechallenge change the cellular transcriptomic state and the sensitivity to ERK activation. ERK hyperactivation upon drug removal reduces the viability of drug-resistant cells and creates a distinct transcriptomic state that resensitizes cells to drug, despite sustained elevation of

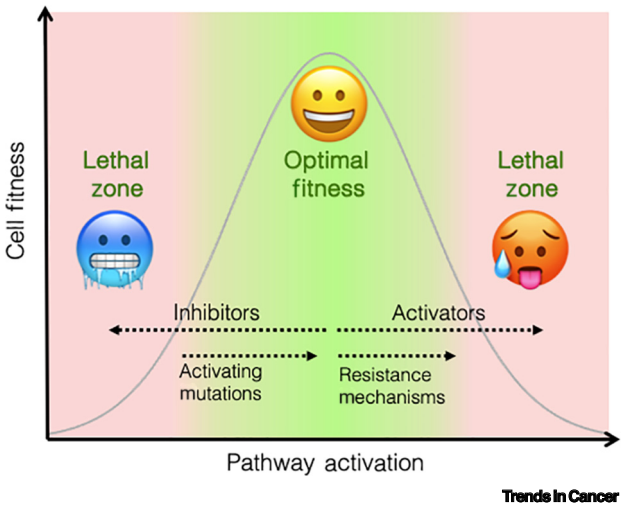

Figure 1. The Goldilocks concept of cancer growth and survival. Cancer evolution leads to signaling outputs that are optimized for growth. Gain-of-function or loss-of-function mutations often lead to increased signaling outputs that drive cancer growth and survival. Therapeutic inhibitors act to reduce signaling, leading to loss of cell fitness. Under pressure from inhibitors, mechanisms of resistance lead to persistent or increased signaling outputs restoring cell fitness. Therapeutic activators, or in some cases withdrawal of inhibitors, also lead to a loss of cell fitness through pathway hyperactivation.

resistance gene transcripts [5]. Ongoing work in the Ahn laboratory queries the specific molecular events modulated by signaling thresholds, investigating phosphosites that respond to high versus low thresholds of ERK activation (K. Hayashi and N. Ahn, unpublished).

Beyond the ERK pathway, other key networks confer cancer cell fitness only within a specific range of signaling. Negative regulation of the PI3K pathway in B cell malignancies points to tight control of PI3K pathway signaling, with inhibition of PI3K regulators such as PTEN and SHIP1 inducing PI3K hyperactivation and driving negative B cell selection [6,7]. The Wnt pathway similarly exhibits a Goldilocks zone of signaling, with activation of Wnt signaling through inhibition of the β-catenin destruction complex reducing tumor formation. The Vermeulen group found that the GSK3β inhibitor lithium destabilizes the fitness of APC-mutant colorectal cancer cells relative to normal cells in the tumor vicinity [8]. In keeping with these findings, APC-dependence in *APC*-mutant colorectal cancer has been previously linked to the control of β-catenin levels [9]. Together, these studies demonstrate that multiple oncogenic pathways, when pushed beyond their optimal thresholds, become liabilities for cancer cells. It will be of significant interest to understand how hyperactivation of more than one pathway in genetically relevant cancers (e.g., *APC* and *PIK3CA* dual-mutant colorectal cancers) might or might not act cooperatively.

Driver addiction points to key molecular dependencies

Lethality can also be enacted in a context-dependent manner by activating, rather than suppressing, driver oncogenes to which cancer cells are known to be 'addicted'. In the context of Ewing sarcoma, addiction to EWS–FLI1 fusion proteins drives the disease but also causes a vulnerability. The Stegmaier laboratory discovered and characterized the E3 ligase TRIM8 and ETS transcription factor ETV6 as selective dependencies in Ewing sarcoma. Repression of either TRIM8 or ETV6 increases EWS–FLI1 activity to the detriment of Ewing sarcoma models. In the case of TRIM8, this happens through increased total EWS–FLI1 protein levels, and in the case of ETV6 through increased EWS–FLI1 on chromatin, suggesting cancer cells maintain 'just right' levels of the oncogenic fusion protein [10,11]. Maintenance of this Goldilocks zone through transcriptional control can be visualized at the molecular level using single-molecule tracking and super-resolution photoactivated localization microscopy techniques. The Chong laboratory reported that multivalent interactions of its intrinsically disordered, low-complexity domains concentrate EWS–FLI1 at target genes in Ewing sarcoma cells [12]. These low-complexity domain interactions promote efficient transcriptional activation within a narrow range, with endogenous EWS–FLI1 expression producing multivalent interactions within this optimum. Increasing interactions beyond this range, toward putative phase separation, leads to transcriptional repression [13].

Addiction to mutant IDH2 as an oncogenic driver underpins activation lethality in leukemia, with excessive activity of mutant IDH2 leading to mitochondrial dysfunction and impaired growth in cell models. Somatic mutations in *IDH1/2* result in neomorphic production of the oncometabolite 2-hydroxyglutarate (2HG) [14]; notably, Intlekofer *et al.* showed that resistance to enasidenib, an allosteric inhibitor of mutant IDH2, arises through *trans* mutations in the wild-type allele [15]. Experimentally inducing the same mutations in *cis* results in hyperproduction of 2HG and consequent metabolic toxicity. Chemical screening uncovered small molecules that bind mutant IDH2 and recapitulate this activation-induced toxicity selectively in IDH2-mutant leukemia cells, corroborating the therapeutic potential of direct IDH2 activators (A. Intlekofer and S. Hou, unpublished).

Cellular stress causes vulnerability to pathway activation

Metabolic and perhaps many other stresses induced by hyperactivation provide another means to overwhelm the ability of cancer cells to adapt and survive. Using stress-focused drug screens and genome-wide clustered regularly interspaced short palindromic repeats (CRISPR) screens in

colorectal cancer cells, Dias *et al*. observed synthetic lethality between the protein phosphatase 2A (PP2A) inhibitor LB-100, which overactivates multiple oncogenic pathways, and adavosertib, an inhibitor of the WEE1 kinase. The two inhibitors were synergistic in various colorectal, pancreatic, and bile-duct cancer cell models and suppressed the growth of orthotopic patient-derived colon tumors *in vivo*. Interestingly, in colon cancer cells, resistance to the combination of PP2A and WEE1 inhibitors mitigated the cancer phenotype to the extent that drug-resistant cells lost oncogenic capacity *in vivo* by reducing aneuploidy, suppressing transcription of oncogenic signaling target genes, and promoting expression of tumor suppressor genes [16].

Transcriptional rewiring helps cancer cells maintain homeostasis

Given that cancer cells rely on lineage-specific transcriptional states and tight epigenetic control to maintain growth and survival, overactivation of transcriptional regulators and chromatin remodeling complexes can be leveraged to induce cell death. For example, the androgen receptor (AR) drives a gene-expression program that supports survival of prostate cancer cells, but cancer growth shows a biphasic response to AR ligands, with cells proliferating at normal androgen levels but not at castrate or supraphysiological levels. In contrast to androgen deprivation therapy, so-called 'bipolar androgen therapy' or BAT administers supraphysiological levels of testosterone in a cyclical fashion to capitalize on this biphasic response, achieving response rates of 20–40% in clinical testing [17]. Studies in the Nelson laboratory of supraphysiological androgen exposure in androgen-addicted prostate cancers reveal consequences that include DNA damage, repression of genes involved in DNA repair, and cell cycle arrest [18,19]. Small-molecule AR agonists recapitulate the effects of supraphysiological androgen, while also downregulating MYC expression and suppressing prostate cancer growth *in vitro* and *in vivo* [20]. Bernasocchi and Theurillat discussed how thresholds of sensitivity to AR signaling vary depending on specific genomic features. Prostate cancers with dominant negative mutations in the ubiquitin ligase adaptor SPOP promote and require high levels of AR signaling, precluding sensitivity to supraphysiologic levels of testosterone. By contrast, prostate cancers with *TMPRSS2–ERG* fusions require wild-type SPOP to prevent AR-mediated hyperactivation of ERG and thus are highly sensitive to elevated androgen levels [21].

Allelic bias, or preferential expression of one allele, can regulate gene expression, thus avoiding toxic overexpression of onco-fusions and promoting cancer cell survival. In subsets of pediatric B cell acute lymphoblastic leukemia, structural rearrangements cause abnormal activation of *DUX4*, a gene normally silenced in differentiated cells. Zhang and Ma showed that the *IGH–DUX4* translocation occurs in the silenced IGH allele and that this allelic bias optimizes dosage of DUX4 to avoid toxic overexpression [22]. Building on the requirement of precise epigenetic control to maintain cancer cell survival, work from the Liau lab demonstrates that lymphoma cells exhibit a biphasic dependency on EZH2 activity and H3K27me3 levels. Gain-of-function mutations in the histone methyltransferase gene *EZH2* increase H3K27me3 levels and contribute to lymphomagenesis. However, introduction of additional hyperactivating mutations in *EZH2*, as well as inhibition of methyltransferase SETD2, induces overspreading of H3K27me3 on chromatin, aberrant gene silencing, and ultimately cell death, indicating an upper threshold of H3K27me3, beyond which lymphoma cells cannot survive [23].

Transcriptional regulation is critical to maintaining 'just right' signaling not only in oncogene-driven cancers but also in those driven by tumor suppressor loss. Malone and Roberts reinforced the Goldilocks theme in cancers with loss-of-function mutations in the SWI/SNF chromatin remodeling complex. Such cancers require precise levels of chromatin remodeling activity and exhibit sensitivity both to rescue of SWI/SNF activity and to further impairment of remodeling. For example, in cancers with mutations in the *SMARCB1* subunit of SWI/SNF, inactivation of DCAF5 and the subsequent

loss of ubiquitin-mediated degradation of SWI/SNF reverses the cancer phenotype [24]. Collectively, these data illustrated how precise control of epigenetic and transcriptional regulators maintains the Goldilocks zone of cell fitness crucial for cancer cell survival across diverse contexts.

Strategies for the discovery of targets to induce hyperactive signaling

By exploiting the fragile balance that is maintained by cancer cells, activation lethality can be strategically induced, potentially opening new avenues for cancer therapy. The cancer genome itself carries intrinsic information about vulnerabilities to hyperactivation encoded in its patterns of mutual exclusivity, structural rearrangements, and copy-number alterations. These genomic insights, combined with high-throughput experimental efforts such as genetic screens, transcriptomics, and phosphoproteomics enable the systematic identification of targets where hyperactivation might drive cell death. Together, these strategies provide a roadmap for uncovering the key dependencies that underlie hyperactivation lethality.

Patterns in the genomic landscape reveal targets for inducing activation lethality

While the genetic evolution of cancer is largely driven by the positive selection of gain-of-function alterations in oncogenes and loss-of-function alterations in tumor suppressor genes, cancer genotypes are also shaped by selection against cells with mutations in genes essential for survival or growth. Of potentially greater interest, this negative selection may occur against cancer cells that acquire mutations that are detrimental specifically in cells that have previously acquired certain oncogenic mutations.

This scenario produces a pattern of mutations often referred to as 'mutual exclusivity,' in which two or more mutations appear together less frequently than expected based on their individual mutational frequencies. Strikingly, patterns of mutual exclusivity have been observed with mutation combinations that each activate the same oncogenic signaling pathway, raising the possibility that excessive activation of such pathways produces toxic effects in cancer cells in a fashion that could restrict cancer growth and benefit the host. The mutual exclusivity of oncogenic mutations in *EGFR* and *KRAS* points to toxicity of ERK hyperactivation in lung cancer [3,4]. Thus, as noted earlier, hyperactivation of the RAS–ERK pathway can also be leveraged to induce cell death in B cell malignancies, mainly leveraging mutual exclusivity of the ERK pathway with other 'diverging' oncogenic drivers, including JAK–STAT signaling [25,26]. In prostate cancer, mutual exclusivity in *SPOP* mutations and *TMPRSS2–ERG* fusions demonstrates tight regulation of AR signaling [21].

Compensation mechanisms to limit overexpression can also be informative about potential hyperactivation targets. Using an algorithm that detects genomic loci under positive and negative selection when amplified or deleted, the Beroukhim laboratory identified 41 loci that were under negative selection for gains, indicating the presence of toxic genes [27]. Efforts to overexpress genes whose amplification does not substantially increase expression has generated a compendium of hundreds of toxic amplification-related gain of sensitivity, or 'ARGOS', genes [28]. Together, these findings support the idea that gene overexpression lethality to cancer cells is a widespread phenomenon and can significantly shape genome evolution by exerting strong selective pressures.

Tools for actively hunting activation lethality targets

The identification of activation lethality targets requires tools capable of systematically probing the signaling pathways, genetic dependencies, cellular stress responses, and epigenetic states in cancer. A number of orthogonal experimental approaches such as genetic screens, transcriptomics, and phosphoproteomics have been proposed to identify actionable, context-specific dependencies to hyperactivation.

CRISPR-based genetic screens have proven invaluable in uncovering targets for activation lethality across various cancer contexts. In collaboration with the Broad Institute's Dependency Map team, Stegmaier's group conducted genome-scale CRISPR screening in Ewing sarcoma models. Comparison of these data to gene dependency data from over 1000 other cancer cell lines revealed TRIM8 as a key regulator of the EWS–FLI1 fusion protein in Ewing sarcoma, where its knockdown destabilizes cancer cells through hyperactivation of the fusion protein [10]. In colorectal cancer, the Bernards laboratory employed CRISPR screens to identify synthetic lethal interactions with PP2A inhibitors, leading to the discovery that WEE1 and PP2A inhibition synergize to induce hyperactivation lethality [16].

Building on these insights, gain-of-function screens extend the search for activation lethality targets by identifying genes and pathways that, when directly hyperactivated, disrupt cancer cell survival. The Sellers laboratory activated ten key nodes in MAPK, PI3K, and WNT pathways in ~500 cell lines using lentiviral open reading frames (ORFs) to drive exogenous protein expression. This gain-of-function screening approach revealed that hyperactivation of WNT selectively induced lethality in *APC*-mutant colorectal cancers [9].

In sessions of our meeting devoted to open discussion, several participants noted gain-of-function screens as a key tool in future efforts to identify targets for activation lethality-based therapeutics, with the understanding that current methodologies still require further development. ORF overexpression screens suffer from the lack of a comprehensive, synthetic, fully validated human ORF collection. However, overexpression of wild-type genes, driven by CRISPR activation, might not be sufficient for discovery of activation lethalities that depend on gain-of-function mutations. Additionally, future work should consider how hyperactivation affects the tumor microenvironment as well as normal tissue. Defining optimal activation thresholds, the required duration of hyperactivation, and the contexts in which these dependencies uniquely affect cancer cells will be critical to advancing the discovery of appropriate targets for therapy.

While genetic screens have been pivotal in identifying dependencies, they provide an incomplete picture of the dynamic processes underlying activation lethality. Complementary approaches, such as phosphoproteomics, kinase activity profiling, and transcriptomics, can map key phosphorylation events and kinase activation states that drive cancer survival and yield further resolution to identify dependencies that are lethal only when activation exceeds specific thresholds. Work from the Varmus and Ahn laboratories, described above, employ phosphoproteomics to pinpoint critical kinases, identify phosphorylation sites, and define ERK activity thresholds that mediate hyperactivation lethality. By integrating analyses of CRISPR-derived sensitivity data with gene expression profiles of *KRAS*-mutant cancer cells, the Sebti laboratory has developed a transcriptomic KRAS dependency signature that predicts KRAS addiction in lung and pancreatic cancer. A subset of genes that were upregulated in KRAS-addicted tumors was identified [29], highlighting potential targets for drugs that might produce toxic hyperactivity. By integrating these complementary methods, researchers can uncover nuanced dependencies and actionable vulnerabilities across diverse oncogenic contexts.

Exploiting activation lethality as a therapeutic paradigm

Activation lethality addresses a critical gap in cancer therapy by targeting vulnerabilities that emerge when oncogenic signaling surpasses tolerable thresholds. This paradigm offers a unique advantage in tackling resistance to targeted therapies, exploiting the adaptations that allow cancer cells to evade conventional treatments. The development of small-molecule activators represents a promising frontier in activation lethality, offering the potential to directly hyperactivate key oncogenic signaling pathways or enzymes. The Vanhaesebroeck laboratory demonstrated that a small-

molecule activator that works by binding directly to PIK3CA, the catalytic subunit of PI3Kα [30], induced lethality in cancer cell lines carrying activating mutations in PIK3CA but less so in cancer cells without these mutations or in untransformed cells; moreover, cancer cell death was AKT/mTORC1-dependent and occurred only in stress conditions (B. Bilanges and B. Vanhaesebroeck, unpublished). The Intlekofer group discovered small-molecule binders of mutant IDH2 that selectively induce hyperactivation toxicity in *IDH2*-mutant leukemia cells (A. Intlekofer and S. Hou, unpublished).

The development of drugs that can serve as direct activators of signaling molecules is an exciting but ambitious undertaking, owing to multiple factors – such as the complexity of designing allosteric binders compared to orthosteric blockers, the precise protein conformations required for protein activation, and the current focus of chemical libraries built around inhibitors. Alternatively, the development of 'inhibitors of inhibitors' provides a practical route to hyperactivation in some contexts. The modulation of negative regulators, such as TRIM8 or ETV6 in Ewing sarcoma [10,11] and DUSP6 in lung adenocarcinoma [4] or DUSP4/6 in melanoma [31], can be leveraged to drive hyperactivation of EWS–FLI1 and ERK pathways, respectively, to induce lethality.

Combining chemical inhibitors of PP2A and WEE1 induces excessive oncogenic signaling, effectively suppressing colon tumor growth *in vivo* [16]. Building on this therapeutic concept, drug development efforts and clinical trials of inhibitors of negative regulators are advancing. Cooke and Malek described the development of small molecules at Novartis that disrupt the interactions between ERK and its negative regulators PEA15 and DUSP4, inducing ERK hyperactivation. ERK2-dependent hyperactivation leads to increased cell stress, cell cycle arrest, and apoptosis in *BRAF* V600-mutant melanoma cells *in vitro*, as well as durable tumor regression *in vivo* [32]. The inhibition of GSKB by lithium increases Wnt signaling and reduces tumor formation in *APC*-mutant colorectal cancer [8]; a Phase 2 clinical trial evaluating the effect of lithium in preventing progression of polyps into colon cancer in patients with familial adenomatous polyposis is underway [33]. Given that many precancerous lesions bear activated oncogenic alleles (e.g., *BRAF* mutation in benign moles) and often undergo oncogene-induced senescence, it is also possible that such lesions might be eradicated upon pathway hyperactivation. Thus, activation lethality could serve as a broader therapeutic strategy for cancer prevention.

Non-cross-resistant combinations of activators and inhibitors, delivered sequentially or cyclically, show curative potential. The strong 'addiction' cancer cells manifest toward the maintenance of appropriate signaling levels leads to mechanisms of pathway re-activation as a major driver of resistance and escape from pathway inhibitors. To this end, Cho and Sellers shared ongoing work demonstrating that *BRAF*-mutant melanoma cells can be evolutionarily steered toward resistance to MAPK inhibitors, resulting in maximal sensitivity to and complete loss of cell viability in response to MAPK hyperactivation through inducible ERK2 overexpression. Additionally, clonal dynamics was observed through high-complexity cellular barcode tracing, showing that inhibition and subsequent hyperactivation of the MAPK pathway results in more dramatic loss of clonal diversity than either inhibition or hyperactivation alone. Notably, there were no shared clones between pathway-inhibiting and hyperactivating conditions, confirming a lack of cross-resistance and underscoring the curative potential of a cyclical treatment strategy (E. Cho and W. Sellers, unpublished).

Work from the Beijersbergen laboratory supported the idea that hyperactivation of the MAPK pathway could enhance the effects of drug withdrawal. Hyperactivation of the MAPK pathway, using phorbol-12-myristate-13-acetate (PMA) or Prostratin, leads to detrimental growth effects in BRAF/MEK-inhibitor-resistant *BRAF*-mutant melanoma cells. MAPK hyperactivation upon withdrawal of a BRAF/MEK inhibitor causes mitotic catastrophe and cell death. The introduction

of genetic alterations commonly associated with resistance, such as oncogenic *RAS*, loss of *NF1*, or EGFR activation, induce sensitivity to PMA or Prostratin, even without pre-treatment with a BRAF inhibitor. Notably, *BRAF*-mutant melanoma cells surviving PMA treatment regained sensitivity to combined BRAF and MEK inhibition (J. Poell and R. Beijersbergen, unpublished). Together, these studies motivate a therapeutic rationale for cyclical inhibition and hyperactivation of the MAPK pathway. Moreover, cyclical therapy has already demonstrated clinical efficacy in certain cancers. Alternating supraphysiological doses of testosterone and periods of androgen deprivation in castration-resistant prostate cancer has yielded response rates of 20–40% in clinical testing [17].

Concluding remarks

Activation lethality is an emerging orthogonal strategy for exploiting the oncogenic rewiring that drives cancer forward. While the scope of this phenomenon was initially observed in ERK and AR pathways, it now appears that many diverse cancer pathways exhibit tuned optima that can be exploited by both inhibitors and activators. Leveraging activation lethality for therapeutic benefit will require answering key questions about its mechanisms and clinical implementation (see Outstanding questions).

Maximizing the therapeutic potential of activation lethality will require elucidating activation thresholds and clinically relevant biomarkers. Thresholds for activation lethality will likely vary among oncogenic contexts and tissue lineages. Distinct cancers or subtypes, even those driven by common oncogenic pathways, may require different levels of pathway activation to cross the threshold from survival to lethality. Moreover, as discussed earlier in melanoma and prostate cancer, substantial variability in sensitivity to activation-induced lethality can occur within cancers of the same lineage. Variable responses within and across cancer types reflect differences in genomic landscape, epigenetic state, signaling biology, and tolerance to cellular stress. An interesting advantage for clinical development might arise in inhibitor-resistant tumors, where elevated oncogenic signaling places cells nearer to a critical threshold for inducing lethality, thus enhancing sensitivity to additional activation; by contrast, treatment-naive tumors may lie farther from this threshold and require more potent or sustained activation to achieve the same effect. Defining these thresholds will require tools that can capture signaling states in human cancers, such as phosphoproteomic and transcriptomic methods.

Translation of activation lethality to clinical settings will require thoughtful trial designs.

As is the case with inhibitor-based strategies, inter- and intra-tumoral heterogeneity may pose difficulties, as a single patient might present with subclones within primary tumors or with metastatic lesions that display varied sensitivity to activation lethality. However, such heterogeneity may be substantially reduced following therapy with pathway inhibitors, leading to more uniform activator susceptibility.

Dose finding in Phase 1 will likely require different strategies. Importantly, subtherapeutic doses, typically experienced by patients in the early dose-escalation cohorts, might accelerate rather than repress cancer growth, leading to premature clinical trial termination. Consequently, trial designs may need to depart from traditional dose-escalation models in favor of designs that rapidly attain biologically active doses or even start at an anticipated therapeutic dose with dose escalation or de-escalation in subsequent cohorts. Similarly, it is likely that activator therapeutics that emerge in this paradigm will require distinct pharmacologic properties, perhaps trending toward short-acting, pulsatile administration profiles. In some cases, such profiles would be best achieved by intravenous routes of administration. Clearly, engagement with regulatory agencies will be essential to design trials that balance safety, efficacy, and translational feasibility of this emergent modality.

Outstanding questions

How do we find new targets for activation-induced lethality? Can mutual exclusivity resulting from activation conflict be systematically interrogated and/or validated? What advances in screening are necessary to find targets?

How do we find direct activators of key oncogenes? Are there therapeutic modalities that lend themselves well to activators beyond agonistic antibodies or molecular glues?

What impact will hyperactivation have on normal tissues? How should intertumoral heterogeneity be addressed? What if one lesion in a patient is primed for hyperactivation, but the other lesions are not?

What is the ideal pharmacologic profile of an activator? How is the dosing schedule determined for cyclical therapy of pathway inhibition and hyperactivation? Would different schedules be required to increase the therapeutic index of such an approach? What is needed to capitalize on the inhibition/activation resistance cycle? What dosing schedule of a hyperactivating therapeutic agent would be needed for complete coverage?

Can we anticipate what kinds of mechanisms will arise to circumvent activation lethality? Are cells as susceptible to developing resistance to activation lethality agents as conventional targeted therapies? Will resistance to activation lethality agents reduce oncogenic signaling?

How should a clinical trial be designed? How can the maximum tolerated dose be determined while ensuring that hyperactivation is enacted to the point of lethality?

A key concern regarding the activation paradigm is the potential risk of inducing cancer. Of course, this risk is not unique to the concept of pathway activation and accompanies highly effective yet genotoxic chemotherapies (e.g., alkylating agents and etoposide). In these cases, the clinical priority is to induce lasting remissions in the existing cancer, with the risk of secondary malignancies managed by careful, long-term surveillance. In the absence of direct, drug-induced genotoxicity, *de novo* induction of cancer is unlikely, as cancers are typically induced by multiple mutational events. Thus, the greatest risk of activation therapeutics would be inducing the progression of, rather than eradicating, pre-existing precancerous lesions. Presumably, this risk would be greatest during active treatment and would subside after treatment cessation. As noted above, it remains possible that some precancerous lesions would undergo oncogene-induced or 'activation-induced' senescence instead.

Therapeutics that induce activation lethality may also promote different evolutionary trajectories in response to treatment. Unlike resistance to traditional inhibitors, cells escaping activation-induced lethality may downregulate their oncogenic signaling pathways, potentially reverting to a less aggressive state. This raises the possibility that hyperactivation therapies might select for cell populations with reduced oncogenic activity. Still, it remains unclear whether such cells would persist over time or if hyperactivation merely resets cancer to an earlier evolutionary state or, at a minimum, restores sensitivity to inhibitors. In this latter regard, we are attracted to the notion that cancer subclones that are cross-resistant to both inhibitors and activators might be rare – or even non-existent – suggesting the curative potential of this emerging therapeutic strategy.

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Declaration of interests

R.B. receives or has received research funding from Heparegenix, Verastem, Lixte, and Boehringer Ingelheim. R.B. holds equity in Oncosense, Agendia, Lixte, and Erasca and is a member of the advisory board for Amphista, Boehringer Ingelheim, Erasca, Isu/Abxis, Roche China Innovation Center, and Tessellate. R.B. is a co-patent holder on PP2A inhibition for cancer therapy. W.R.S. is a Board or SAB member and/or holds equity in CJ Bioscience, Delphia Therapeutics, Ideaya Biosciences, Pierre Fabre, Red Ridge Bio, Scorpion Therapeutics, and has consulted for Array, Astex, Epidarex Capital, Ipsen, Merck Pharmaceuticals, Sanofi, Servier and Syndax Pharmaceuticals and receives or received research funding from Bayer Pharmaceutical, Bristol-Myers Squibb, Boehringer Ingelheim, Ideaya Biosciences, Calico Biosciences, and Servier Pharmaceuticals. W.R.S. is a co-patent holder on EGFR mutation diagnostic patents. K.St. is a member of the SAB and has stock options with Auron Therapeutics. H.V. is a member of the SAB for DragonFly and Surrozen. K.S. has no conflicts of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, GPT-4o was used to track content organization and provide feedback on language clarity. After using this tool, the authors reviewed and edited the content and take full responsibility for the content of the publication.

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