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Therapeutic opportunities created by adaptive responses to targeted therapies in cancer

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Abstract

Normal cells explore multiple states to survive stresses encountered during development and self-renewal as well as environmental stresses such as starvation, DNA damage, toxins or infection. Tumor cells co-opt normal stress mitigation pathways to survive stresses that accompany tumor initiation, progression, metastasis and immune evasion. Cancer therapies accentuate tumor cell stresses and invoke rapid non-genomic stress mitigation processes that maintain cell viability and thus represent key targetable resistance mechanisms. In this review, we describe mechanisms by which tumor ecosystems, including cancer cells, immune cells, and stroma, adapt to therapeutic stresses and describe three different approaches to exploit stress mitigation processes: a) interdict stress mitigation to induce cell death, b) increase stress to induce cellular catastrophe and c) exploit emergent vulnerabilities in tumor and microenvironment cells. We review challenges associated with tumor heterogeneity, prioritizing actionable adaptive responses for optimal therapeutic outcomes, and development of an integrative framework to identify and target vulnerabilities that arise from adaptive responses and engagement of stress mitigation pathways. Finally, we discuss the need to monitor adaptive responses across multiple scales and translation of combination therapies designed to take advantage of adaptive responses and stress mitigation pathways to the clinic.

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General introduction.

Tumor cells as well as cells in the tumor microenvironment are subjected to stresses inflicted by altered cell states associated with tumor initiation, progression, metastases and immune evasion[13]. To survive and adapt to these stressors, tumors co-opt endogenous stress mitigation pathways that have evolved to allow normal cells to deal with stresses that occur during development and renewal or as a consequence of environmental perturbations such as those induced by starvation, DNA damage, dietary toxins, infection or cancer therapy[4,5]. Examples of stresses that tumor cells are subjected to during tumor initiation, progression and metastasis are shown in Figure 1A and discussed in more detail by Luo et al[6]. Tumor cells, as well as cells in the tumor microenvironment, must also rapidly implement additional mitigation strategies (Figure 1A) to survive stress created by cancer therapies. Radiation, chemo- and targeted therapies, as well as immunotherapies, augment ongoing oncogenic stress, resulting in further dependence on stress mitigation pathways. The ability of tumor cells and tumor ecosystems to co-opt endogenous stress mitigation pathways and heterotypic cell-cell signaling that maintain tissue homeostasis, constitutes key targetable mechanisms of therapeutic resistance (three different therapeutic approaches to exploit these mechanisms are shown in Figure 1B)[6,7]. Table 1 lists examples of stresses induced by cancer therapies as well as the processes that cellular stress sensors engage to mitigate these stresses.

Several reviews have covered intrinsic drug resistance mechanisms, including pre-existing tumor clones[8,9], as well as adaptive resistance mechanisms, including non-genetic tumor cell state plasticity, microenvironment mechanisms[10–16] and drug target-focused mechanisms (drug efflux, drug inactivation, or alterations in drug targets[17–22]). In this review, we focus on adaptive responses to cancer therapy. These responses represent a unique form of non-genomic resistance that occurs rapidly and is highly reversible and targetable. Importantly, the ability of cells to adapt to the immediate effects of therapeutic stress can facilitate development of irreversible acquired genetic resistance including acquisition of new mutations[23–29] or allow selection of pre-existing drug resistant clones[30,31]. Thus, therapeutic targeting of adaptive resistance provides an opportunity to improve patient outcomes by both immediate inhibition of tumor cell survival, and also by delaying or preventing emergence of genetically resistant clones. However, in order for patients to realize benefit from targeting stress mitigation responses, it is necessary to develop facile approaches to identify the suite of mitigation strategies utilized by individual cells as well as to quantify stress phenotypes and mitigation strategies as they change longitudinally under therapeutic stress. This review highlights examples of stress responses associated with cancer therapies to illustrate concepts that can be generalized across the spectrum of adaptive response and stress mitigation pathways. In addition, we describe three different approaches (Figure 1B) to exploit stress mitigation processes to improve the effectiveness of cancer therapy. Lastly, we discuss opportunities and challenges in implementing these approaches in the clinic. Due to space limitations, the review is designed to provide a framework for broad implementation of therapeutic approaches to capitalize on adaptive responses and engagement of stress mitigation pathways rather than a comprehensive coverage of research and clinical activities.

Section 1: Tumor intrinsic adaptive response programs

Understanding the nature of stresses and adaptive mechanisms (Table 1) that maintain survival of drug-treated tumor cells promises to uncover therapy-induced vulnerabilities and provide rational approaches to target adaptive responses to therapeutic stress to enhance cancer cell death.

Section 1.1: Adaptive responses involving rewiring of signaling pathways

Adaptive responses to signal transduction inhibitors as a special case of stress mitigation: Given the key role of signaling pathways in the development and progression of cancer, major efforts have been deployed to develop targeted therapies that disrupt pro-oncogenic signaling. Unfortunately, the benefit from such drugs is often temporary due to the ability of cells to rapidly adapt through reactivation or bypass of the targeted molecule, allowing cell survival. In normal cells, signaling pathways have a high level of plasticity leading to stable regulatory networks that result in bistable states with cell populations maintaining distinct phenotypes independently of genotype. This plasticity is built on the balance of negative and positive regulators, availability of alternative isoforms, feedback and feedforward loops and crosstalk with other signaling pathways that are required to maintain normal cellular homeostasis. This plasticity, pathway rewiring, and rapid acquisition of adaptive responses allow tumor cells to mitigate stresses caused by inhibition of signaling mediators by targeted therapies. Pathway bypass through signaling rewiring does not engage a “professional” stress mitigation pathway but rather co-opts the normal homeostatic mechanisms that maintain signaling balance. For example, receptor tyrosine kinases (RTKs) activate both the PI3K/AKT and RAS/ERK pathways that co-regulate pro-survival activity, at least in part, through converging on mTOR signaling[32,33]. As described in Figure 2, inhibition of either PI3K/AKT or the RAS/ERK pathway can lead to compensatory activation of the other pathway with subsequent maintenance of mTOR signaling and cell survival[34]. RTK signaling can be upregulated transcriptionally[35], or post-translationally by decreased internalization and degradation[36], decreased proteolytic shedding of receptors[37], formation of novel RTK complexes[38] and increased production of receptor ligands[39,40]. Together these processes engage a suite of mechanisms that could be targeted to interfere with bypass mechanisms and thus increase utility of signal transduction inhibitors.

Section 1.2: Adaptive responses that ameliorate cell death pathway activity

Dysregulated cell death programs including apoptosis, ferroptosis, pyroptosis, and necroptosis play critical roles in therapeutic resistance[41]. Many cellular compensatory mechanisms and stress mitigation pathways shift the balance of cell death pathways towards cell survival. In the case of apoptosis for example, these programs involve induction of anti-apoptotic proteins (e.g. BCL2 family proteins such as BCL2, BCLxL, MCL1) and inactivation or downregulation of pro-apoptotic proteins (e.g. p53, BH3-only BCL2 family proteins). Inhibitors of PI3K and MEK and other cancer therapies can induce stabilization and transcription of BIM and other BH3 family proteins[42–45], thus promoting a pro-apoptotic state. However, this is often balanced by upregulation of anti-apoptotic BCL2-family proteins (Table 1). These BCL2-family proteins can be induced by multiple programs

at the level of transcription, alternate IRES-dependent translational[46,47], or post-translational stabilization[48].

Section 1.3: Adaptive responses induced by DNA damage and replication stress

Under conditions where DNA integrity is compromised (e.g. mutation, metabolic stress or environmental toxins), a suite of cell cycle arrest and DNA repair pathways are activated to mitigate effects of the DNA damage[49–51]. In the presence of DNA damage in normal cells, DNA integrity sensors such as ATM or ATR proteins are recruited to sites of DNA damage where they activate p53 and induce DNA damage repair pathways and cell cycle arrest[52] (Figure 2B). p53 is maintained at low levels in unstressed cells by the E3 ubiquitin ligase MDM2 that targets p53 for degradation. In normal cells, multiple stress signals, including oncogenic stress and DNA damage, stabilize p53 with subsequent activation of stress mitigation pathways. Thus, p53 serves as a major cellular stress sensor. Loss or mutation of p53, the most common genomic aberration in cancer, compromises the G1 checkpoint allowing stressed or metabolically compromised cells with decreased dNTPs available for accurate DNA synthesis and repair to enter S phase. Loss of p53 function also allows accumulation of DNA damage by suppressing DNA damaged-induced apoptotic response[53]. Furthermore, p53 targets multiple genes involved in mismatch repair, nucleotide-excision repair and base-excision repair resulting in dysfunctional DNA repair[53]. Taken together, loss or mutation of p53 compromises multiple cellular stress mitigation processes allowing accumulation of DNA damage.

Cancer therapies can directly induce DNA damage resulting in activation of the DNA damage response (DDR) pushing tumor cells with high levels of DNA damage over the threshold into cell death. Further a number of chemotherapies decrease the availability of dNTPs required to repair DNA damage, such as gemcitabine that targets RRM2 (Table 1). Several targeted therapies decrease the levels of members of key DNA damage pathways[54,55] such as MEK inhibitors compromising homologous recombination in RAS mutant tumors. Tumor initiation and progression is also associated with replication stress (RS) which is defined as failure to protect the replication fork in S phase resulting in replication fork stalling or collapse with subsequent accumulation of DNA damage (Figure 2B). Activation of oncogenes and/or loss of tumor suppressors can elevate RS through: 1) premature S phase entry and a resultant dNTP shortage, 2) stalled replication forks due to replication and transcriptional imbalance, and 3) replication fork collapse[56]. Interestingly, oncogenes that induce RS and or DNA damage frequently engage stress mitigation pathways[56]; for example, KRAS mutations can increase HR competence[54,57] and MYC activation can resolve transcription-replication conflicts, upregulate dNTP synthesis and increase HR competence[58,59].

A number of chemotherapy and targeted agents increase RS by depleting dNTPS or by inducing DNA damage including crosslinks and adducts that result in stalled polymerase[60–63] (Table 1, Figure 2B). Drugs that induce replication fork collapse and subsequent double strand DNA breaks (DSBs), such as poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi)[64], lead to activation of the S and G2 cell cycle DNA damage checkpoints to allow resolution of the stress (S phase checkpoint) or repair of the damage

(G2 phase checkpoint) to occur through HR and other repair pathways prior to entry into M phase. Unresolved RS results in phospho-RPA deletion and further accumulation of DSBs as well as dsDNA in micronuclei and the cytosol[65]. Cytosolic DNA can lead to activation of the STING pathway, with resultant interferon induction and engagement of the innate and adaptive immune system[66–69].

Section 1.4: Adaptive responses and stress mitigation pathways involving metabolic, endoplasmic reticulum and oxidative stress

Metabolic stress: In normal cells, metabolic plasticity allows cells to quickly adapt to energy and metabolite requirements during conditions where nutrients are limiting. While oxidative phosphorylation (OXPHOS) is the dominant source of energy for slow-growing or stem cells, glycolysis is the preferred energy source for highly proliferative cells. The ability to switch between glycolysis and OXPHOS is controlled by master regulators such as AMP-activated protein kinase (AMPK) and HIF-1, which act as perturbation sensors[70,71]. Many different cancer therapies induce metabolic stress including PI3K pathway inhibitors that decrease glucose and amino acid uptake[72] or BRAF inhibitors that impact glycolysis[73,74] (Table 1). For example, BRAF mutant melanoma cells that are reliant on glycolysis are more sensitive to the BRAF inhibitor vemurafemib[74], while resistant cells display upregulation of the mitochondrial biogenesis coactivator PGC1a, via the melanocyte master regulator MITF, leading to increased mitochondrial respiration, adaptive resistance to therapy, and sensitivity to OXPHOS inhibition[73]. Cellular adaptation to BRAF inhibition in melanoma cells depends on the general control nonderepressible 2 (GCN2) kinase, an eIF2α kinase that senses amino acid levels[75] (Figure 2C).

Oxidative stress: Tumor cells often exhibit high oxidative stress as a consequence of unbalanced generation and elimination of reactive oxygen species (ROS) and reactive nitrogen species (RNS) resulting from internal and external metabolic stresses as well as from reduced cellular anti-oxidant levels[76–78]. Further, aberrant tumor cell metabolism can elevate ROS and RNS[77]. Most chemotherapies (anthracyclines, platinum coordination complexes, topoisomerase inhibitors, etc.) and many targeted therapies (Figure 2C) that alter nutrient uptake and oxidative phosphorylation (e.g. inhibitors of EGFR, BRAF, HER2, and others) increase ROS/RNS through alterations in mitochondrial electron transport and reduction in anti-oxidant systems like glutathione, peroxidases, superoxide dismutases, and others[79] (Table 1). The two broadest-acting ROS-mitigation programs are regulated by NRF2 and NFκB[80]; these transcription factors induce expression of genes that serve as ROS scavengers and prevent apoptosis associated with high ROS[81].

Endoplasmic reticulum (ER) stress: ER stress response is triggered by various stressors such as hypoxia, metabolic stress or misfolded proteins[82,83]. Although a strong activation of the ER stress response can lead to cell death, a moderate level of activity can be protective. Thus ER stress response can support both survival and therapy resistance of tumor cells. For example, RTK-targeted therapy in endometrial and renal cancers activates a PERK-driven ER stress response program, resulting in activation of autophagy and NF-κB signaling stress mitigation pathways that promote cell survival[84,85] (Table 1, Figure 2C).

Section 1.5: Multiple stress converge on the “integrated stress response” program.

Many of the stress response pathways described above feed into one more kinases (e.g. PERK, GCN2) that phosphorylate eIF2α. Phosphorylation of eIF2α promotes formation of an eIF2-eIF2B inhibitory complex that suppresses global cap-dependent translation, while at the same time promoting cap-independent translation of a subset of mRNAs that regulate homeostasis[86,87]. Together, these eIF2α kinases integrate signals from many different stresses to induce a common set of stress mitigation programs referred to as the ‘Integrated Stress Response’ (Figure 2C). Therapy-induced stress can overwhelm the integrated cell stress response in cancer cells, thus resulting in cell death[88]. Further, the switch from cap-dependent to cap-independent protein translation including the anti-apoptotic factor MCL1 can expose novel therapeutic opportunities such as targeting the apoptotic pathway. While this manuscript focusses on a number of independent stress pathways and mitigation process, it is important to acknowledge that they converge, in many cases, on the integrated stress pathway as a final downstream effector.

Section 2: Adaptive responses in tumor stromal cells

Adaptive responses to therapy can also be mediated by tumor-host interactions, where cell types in the tumor ecosystem, produce signals that engage stress mitigation pathways in tumor cells. In addition, therapy-induced alterations in the tumor ecosystem can reprogram stromal cells toward either a pro- or anti-tumor phenotype. Importantly, therapy-induced alterations in tumor-host interactions can reactivate signaling pathways and establish emergent therapeutic opportunities.

Section 2.1 Tumor rewiring during adaptive responses and stress mitigation alters immune contexture

Therapy-induced alterations in the tumor ecosystem can create additional vulnerabilities that can be exploited to enhance therapy efficacy. In order to mediate immunoevasion, tumors produce soluble factors that program both innate and adaptive immune cells in the tumor microenvironment to allow tumor progression[89]. For example, in melanoma BRAF inhibition can recruit and reprogram macrophages into a protumorigenic M2 phenotype and increase macrophage-derived secretion of VEGF that reactivates ERK in melanoma cells leading to therapeutic resistance[90] (Figure 2D). Further, targeted therapy designed to inhibit tumor cell survival can also directly impact immune cell function. The multi-RTK inhibitor sorafenib impairs dendritic cell function and induction of antigen-specific T-cells[91], while MEK inhibition can increase anti-tumor activity of CD8+ T cells[92]. Thus, therapeutic targeting of tumor cells can impact immune population composition, exacerbate tumor supportive immune mechanisms through multiple different mechanisms[93], or alternatively can induce an anti-tumor immune context. However, it is clear that the effects of targeting signaling pathways on immune cell phenotype and functional outcomes is nuanced and requires further exploration in model systems and clinical trials to develop approaches that shift the multiple types of immune cells present from an immune evasive microenvironment to a tumor suppressive immune contexture[94]. Hence, further investigation of therapy-induced adaptation in the complete tumor ecosystem of samples from patients on therapy[95,96] will be necessary to inform effective clinical translation.

Section 2.2: Interactions with cancer associated fibroblasts and extracellular matrix

Drug resistance can be sustained by a plethora of growth factors and cytokines released by the neighboring tumor microenvironment (TME), composed by adipocytes, cancer-associated fibroblasts (CAFs), immune and endothelial cells[97]. Compared to normal fibroblasts, CAFs release elevated levels of pro-inflammatory cytokines and growth factors[98]. Each of these TME cell types can be influenced by juxtacrine and paracrine interactions with tumor cells. For example, CAFs associated with different tumor types undergo distinct state changes[99]. Secreted factors from CAFs can in turn influence tumor cells states, including reactivation of signaling pathways that limit the effectiveness of kinase inhibitors in multiple solid tumors[100–103] and these pathways can be modulated by both cytotoxic and targeted therapies[100,104,105]. Extracellular matrix (ECM) remodeling driven by CAFs treated with BRAF inhibitors[106] promotes cell survival (Figure 2D). Furthermore, cell-ECM interactions can mediate resistance to PI3K/mTOR and HER2-targeted therapy by adaptive responses involving upregulation of anti-apoptotic Bcl-2 family signaling in ovarian and breast cancer[46,107,108]. For a comprehensive discussion of effects of cancer therapies on the diverse cell types in the tumor ecosystem, we refer the reader to other review articles[16,109].

Section 3: Strategies to exploit tumor cell vulnerabilities associated with adaptive responses to therapies

Tumor intrinsic therapeutic stress and stress mitigation processes engaged by the tumor ecosystem provide three independent therapeutic strategies (Figure 1B) to enhance efficacy of cancer therapies a) block the stress mitigation pathways that allow tumor cells to survive ongoing stress to induce death, b) accentuate existing stress and thus overwhelm mitigation strategies resulting in stress-induced catastrophe and c) exploit emergent vulnerabilities in the tumor ecosystem. In this section, we provide examples of each of these strategies (Figure 3 and Table 2), describe the challenges in deploying these strategies due to tumor heterogeneity (intratumoral and interlesional) (Box 1).

3.1. Strategies to block stress mitigation pathways that allow tumor cells to survive ongoing stress

Blocking bypass of signal transduction pathway activation: Cell survival resulting from adaptive signaling rewiring can be targeted by blocking bypass pathways or inhibiting redundant kinase members in the same family (Table 2, Figure 3A). For example, blocking multiple PI3K family members using PI3K-α/β inhibitors enhances antitumor efficacy in combination with multiple therapies in preclinical studies[110]. Targeting bypass pathways involving the PI3K/AKT and RAS/MAPK signaling axes can be accomplished by inhibiting receptor tyrosine kinases that are activated by negative feedback loops. For example, reactivation of AKT in breast cancer cells treated with mTOR inhibitors can be blocked using IGF-1R inhibitors[111]. To combat the heterogeneity and redundancy of receptor tyrosine kinase adaptive responses, pathway reactivation can be targeted by inhibiting downstream kinases. Blocking focal adhesion kinase which is downstream of multiple RTKs can effectively induce cancer cell apoptosis following treatment with HER2-targeted agents in breast cancer[112] and with BRAF inhibitors in melanoma[106]. RAS/MAPK

therapy-induced activation of the PI3K/AKT/mTOR stress mitigation pathway in pancreatic cancer[113] and melanoma[114] has been prevented using AKT and mTOR inhibitors. These examples provide evidence that coordinated inhibition of a target along with the adaptive responses used as a mitigation strategy can prevent bypass and convert cell survival mediated by adaptive responses to cell death. Although this predicts that coordinate targeting of the PI3K/AKT and RAS/ERK pathways would be highly effective, this combination therapy is associated with marked toxicity and is not tolerated in patients[115].

Blocking apoptotic stress mitigation: Multiple targeted therapies (RTK, PI3K/mTOR, PARP and RAS/MAPKi inhibitors) have been shown to result in coordinate upregulation of pro-apoptotic and anti-apoptotic proteins that prime tumors cells for induction of apoptosis by BCL2-family inhibitors. Specifically, blocking BCL-2, BCL-X_L or MCL-1 activity has been shown to synergize with RTK, PI3K/AKT and RAS/MAPK pathway blockade in multiple tumor lineages[116–120] (Figure 3A, Table 2). Thus, targeting anti-programmed cell death proteins that are downstream of stress mitigation pathways represents an attractive therapeutic approach to increase the efficacy of many different targeted and chemotherapies. Notably, since multiple stress mitigation programs lead to elevation of anti-apoptotic proteins, targeting these proteins represents a potentially more broadly effective therapeutic approach than others that are specifically associated with a single stress mitigation program.

Blocking epigenetic factors: Targeting epigenetic regulators of cellular programs also represents a candidate approach to inhibit multiple adaptive responses. In melanoma and other cancer types, histone marks alterations, such as the loss of H3K4me3, H3K27me3 and gain of H3K9me3 in response to therapeutic stress has been linked to the development of multidrug resistance[121,122]. Importantly, silencing methyltransferases can reverse the resistant phenotype. In another study, a BET (BRD4) inhibitor (JQ1) blocked the adaptive stress mitigation response involving upregulation of multiple receptor and non-receptor kinases following HER2 targeted-therapy in breast cancer cells[123] (Figure 3A, Table 2). In addition, novel approaches to inhibit formation of active enhancer complexes with a CDK7/12 inhibitor blocked the transcriptional activation of survival programs in response to multiple RTK inhibitors and resulted in enhanced tumor cell killing *in vitro* and tumor regression *in vivo*[124]. Thus, combination therapies that target a key tumor driver while concurrently targeting transcriptional regulators that mediate stress mitigation pathways can increase the activity of a broad suite of drugs and block multiple stress mitigation pathways. Although inhibition of epigenetic regulators that mediate stress mitigation programs engaged downstream of a wide range drugs can reverse adaptive resistance; these combinations have been plagued by toxicity in solid tumors[125].

Blocking stress responses associated with DNA damage or RS: As described above, therapy-induced DNA damage and RS stress mitigation pathways serve as targets to enhance drug efficacy. For example, because PARPi engage the S and G2 DNA damage stress mitigation checkpoints, PARPi combined with inhibition of the ATR/CHK1/WEE1 cascade, which is responsible for activation of the S phase and G2 damage checkpoints, induces synergistic cell death through mitotic catastrophe, and tumor regression *in vivo* in ovarian and lung cancer models[126–128]. A number of studies, albeit controversial,

suggest that inhibitors of ATR/CHK1/WEE1 cascade are more active in cells that lack p53 due to an increased dependence on the S phase and G2 damage checkpoints[129]. In addition to engaging DNA damage checkpoints, PARP inhibition can increase homologous recombination (HR) repair competency via RAS/MAPK pathway activation that can be interdicted using MEK inhibitors to sensitize cells to the effects of PARPi[130] (Table 2, Figure 3A). Combinations of PARPi with ATR/CHK1/WEE1 or MEK inhibitors have been translated to clinical trials with promising early activity. Importantly, the optimal combination partner for effective PARPi therapy depends on which DNA repair pathways are activated in each tumor. For example, ATR inhibition is most effective in ATM-deficient models[127], gemcitabine in cells with premature cell cycle entry and limited dNTP stores [131], and WEE1 inhibition in tumors with high endogenous RS[132,133].

Blocking metabolic stress mitigation programs: Several different approaches to target metabolic adaptations to therapies have also been described, e.g. increasing therapy-induced metabolic stress by coordinate inhibition of the pentose phosphate shunt and glutaminolysis to limit generation of anti-oxidant mediators and render cells sensitive to reactive oxygen species (ROS) generated by OXPHOS[134,135]. The effects of decreasing or increasing ROS, as with many other targets, is likely to be complex as ROS not only have effects on tumor viability but can influence metastatic potential as well as immune activity[136,137]. In addition, treatment of breast and colorectal tumors with CDK4/6 inhibitors results in metabolic reprogramming with a shift to utilization of glutamate as an energy source that can be exploited by targeting glutaminase[138] (Table 2). While it is clear that adaptation to metabolic stress is a mitigation strategy utilized by both tumor and normal cells, currently there are no facile clinically applicable approaches to identify and target the mitigation processes engaged by the different cell populations in individual tumors.

Blocking oxidative stress mitigation programs: Development of effective targeted inhibitors of anti-oxidant stress mitigation programs presents challenges in specificity, pharmacodynamics, efficacy and safety[139,140]. However, inhibition of targets of NRF2 and NFκB such thioredoxin or glutathione generating enzymes can enhance drug sensitivity[141]. For example, the elevated oxidative stress induced by PARPi that contributes to the induction of DNA damage and cell death[142], can be exploited by inhibiting the anti-oxidant enzyme thioredoxin reductase to induce tumor regression in prostate cancer xenografts[128]. Further work is required to develop optimal anti-oxidant dosing strategies and delivery to avoid toxicities and develop a therapeutic index in cancer patients.

Section 3.2: Strategies to increase ongoing and therapy-induced stress to induce cellular catastrophe

As indicated above, sustained therapeutic stress in tumor cells results in the engagement of stress mitigation programs. If the stress can be increased to a level that overwhelms the stress mitigation programs, this ultimately results in tumor cell death. Given that cancer cells, as opposed to normal cells, are subject to ongoing stress, combination therapies that exploit this differential stress level could provide an adequate therapeutic index. Previous studies have shown that normal cells compared to tumor cells exhibit lower baseline levels of stress[143,144]. To explain the increased sensitivity of tumor cells compared to normal

cells to a number of stress-inducing therapies, it has been proposed that stress-induced catastrophe when cell pass a common stress threshold[145,146]. This is particularly evident in cases such as RS or apoptosis where catastrophe or cell death occurs above a stress threshold[65,147]. Several approaches have been used to increase RS or to target pathways that mitigate the effects of RS[60,62]. Addition of gemcitabine or PI3K inhibitors to PARPi can significantly increase RS by depleting dNTPs thus overloading RS mitigation pathways resulting in accumulation of DNA damage, S phase and mitotic catastrophe and tumor growth inhibition[61,148]. Overloading the DDR pathways and the DNA damage checkpoint mitigation strategy can be induced by combining PARPi with radiotherapy (Figure 3A) resulting in increased double strand breaks, apoptosis and slower tumor growth in Ewing's sarcoma models[149]. We have demonstrated that an equal increase in ongoing RS in tumor cells induced by sequential therapy with PARP and WEE1 inhibitors induces death of tumor cells while concurrently sparing normal cells and mitigation of toxicity[132]. The concept that ongoing RS in tumor cells with low RS in normal cells could result in a therapeutic index is being tested in an ongoing clinical trial (NCT0419773).

Exacerbating the metabolic, ER and oxidative stress pathways described above can also overwhelm mitigation pathways and result in cell death through apoptosis or other cell death mechanisms such as necrosis, ferroptosis and autophagic cell death[150]. For example, EGFR inhibition has also been shown to elevate ER stress (Figure 3A) and in combination with PDK1 inhibitors increase ER stress to a level that overwhelmed the mitigation pathway and resulted in cell death and improved survival in lung cancer xenografts[151]. Thus, combination therapies that effectively increase therapy-induced stress levels in tumor cells provide a promising strategy to block cell survival by inducing stress overload that can overwhelm stress mitigation processes and this approach could lead to a therapeutic index.

Section 3.3: Targeting stroma cell adaptive responses

Immune cell responses: Both the pro and anti-tumor immune effects induced by therapeutic targeting provide novel opportunities for intervention. For example, in melanoma targeting CSF1R on M2 macrophages reduced their recruitment and reduced tumor growth rates in xenografts[90]. PARPi can induce both anti- and protumorigenic macrophages through metabolic reprograming in BRCA1-deficient breast cancer syngeneic models[152]. The protumorigenic effects mediated by PARPi-induced CSF1 production by tumor cells can be reversed by targeting CSF1R on macrophages.

Conversely, activation of the STING pathway by PARPi in lung, colorectal, breast and ovarian tumors leads to production of interferon that can activate an innate immune response that, in turn, induces mobilization of CD4+ and CD8+ T cells[68,153] (Figure 3B, Table 2). Treatment with immune checkpoint inhibitors (ICI) against PD-1 or PD-L1 capitalized on this therapy-induced alteration in the immune tumor ecosystem and induced complete regression and durable responses in both BRCA-proficient and BRCA-deficient tumor models, highlighting the critical role of therapy-induced immune cell recruitment for therapeutic efficacy[154]. Indeed, multiple PARPi and ICI combination trials are underway[95,155]. Oncogenic activation can induce an immunosuppressive microenvironment with targeting of oncogene signaling in tumor cells both demonstrating tumorintrinsic

activity and altering the tumor microenvironment. For example, mutant *KRAS* is associated with the production of multiple cytokines that recruit myeloid derived suppressor cells (MDSCs). The recruitment of MDSCs can be reversed by MEK inhibitors with the inhibition of the tumor intrinsic effects increasing tumor cell sensitivity to ICI[156]. Thus, it is possible to capitalize on new vulnerabilities in the tumor ecosystem resulting from adaptive responses induced by therapeutic targeting that are not directly a consequence of the agent on tumor cells. To translate these findings to the clinic, it is critical to identify recurrent mechanisms of tumor-immune and immune-immune interactions directly in samples from patients on therapy, while considering both the pro- and anti-tumor effects of the tumor microenvironment. Unfortunately, many of our anti-tumor therapies mediate deleterious effects on the immune system (e.g. impaired dendritic cell function by RTK inhibition[91]). Thus it will be critical to determine the effects of targeting adaptive responses in tumor and the microenvironment on tumor cells as well as on components of the immune system to identify approaches that compromise tumor cell viability while at the same time engaging an effective anti-tumor immune response.

Targeting CAF responses and the vascular barrier: Tumors adapt to limited nutrient and O_2 availability by inducing a neoangiogenic program[157]. Notably, strategies aimed at targeting CAF-mediated ECM remodeling using angiotensin blockers (Figure 3B) have also been shown to normalize blood vessels leading to enhanced sensitivity to chemotherapy in ovarian cancer xenografts[158] and the recruitment of immune cells and improved response to immunotherapy in a syngeneic breast model[158], as well as the response to chemotherapy in human pancreatic cancer patients[159]. Therapies directed towards the vascular barrier (e.g. via the VEGFC/VEGFR3 axis[160]) represent a stroma-targeted approach (Figure 3B) that has been FDA-approved in combination with cytotoxic chemotherapy or immunotherapy[160–162] for a subset of solid tumors (lung, ovary and breast). Taken together, effects of therapies designed to target tumor cells can rewire the extracellular stromal environment providing opportunities to develop an anti-tumor environment.

Section 4. Translation to the clinic

The concept that tumors and the tumor ecosystem are under ongoing stress that is accentuated by therapy underlies the rationale for translating the three proposed approaches (Figure 1B, Table 1) to exploit adaptive responses and resultant mitigation processes to the clinic. Compared to empirical combination therapy design, this concept provides a framework to rationalize choice of drug pairs and biomarkers of therapeutic response. Translating these opportunities to the clinic with subsequent patient benefit requires deploying a complex suite of combination therapies able to: a) target adaptive responses in the clinic by interdicting stress mitigation resulting in cell death, b) increase stress in tumor cells resulting in cellular catastrophe or c) exploit the network rewiring that occurs as cells explore different states and mitigation strategies through combination therapies targeting tumor or stromal cells in the tumor ecosystem. Importantly, the drug combinations able to exploit each of these alternatives are markedly different and need to be deployed in the appropriate manner (potentially with different scheduling and dosing strategies) and in patients most likely to benefit (Figure 4). The combination therapies must secondly exhibit a

therapeutic index so that efficacy is displayed at tolerable doses. Indeed, as therapeutic index represents the rate limiting step for utility of any therapy, combination therapies including those targeting adaptive responses are all too often limited by toxicity rather than by lack of efficacy (Box 2)[163]. The underlying precept we have espoused wherein tumors, in contrast to normal cells, are under chronic stress and that this stress state can be accentuated by therapy provides the potential for a therapeutic index[1–3,132].

Importantly, a suite of cost-effective CLIA compliant assays that can select combinations for specific patients based either on the pre-therapy state or on the changes that are induced by therapy stress must be deployed. The overarching goal is to develop assays that facilitate selection of therapies targeting adaptive responses and determine when changes in therapies are necessary at a pace that is faster than the tumor can evolve to utilize alternative mitigation pathways. Importantly, the information content in widely used DNA sequence-based analyses is inadequate as the mitigation processes described herein are primarily mediated by non-genomic mechanisms[10–16]. Assessment of transcriptional changes that mediate adaptive responses or more direct measurement of the protein and metabolic events that mediate the mitigation strategies will be required. The analytic platforms and computational biology pipelines must be able to deliver results to the physician in near real time. The suite of potential approaches to deliver on this opportunity include molecular imaging[164–166] or analysis of tissue or liquid biopsies[167–169] (Figure 4). Other surrogates such as analyzing immune state in peripheral blood to predict immune contexture in the tumor also hold promise [170,171]. Optimally, the CLIA compliant assays should capture the state of tumor cells and the tumor ecosystem across the set of metastatic lesions present in the patient. Liquid biopsies as they integrate information across all of the tumor sites in the body are thus attractive. To fulfill this goal, analysis of circulating tumor cells[169], circulating hybrid cells[172], RNA[173] and miRNA[174] and proteins in exosomes[175] as they change with therapeutic stress has the potential to alleviate the need for tumor biopsies, allow assessment of multiple time points, make personalized therapy approaches available to a wider set of patients and help to democratize the concept of targeting adaptive responses.

Currently we do not have approved combination therapies that can exploit the broad suite of mitigation strategies engaged by tumors. Fortunately, the repertoire of available drugs is increasing rapidly. Considering both on-target and known off-target activities covers an impressive set of potential targets and is beginning to make targeting adaptive responses and stress mitigation pathways practical. Furthermore, drugging what was previously thought to be undruggable targets by selectively degrading proteins or decreasing protein production through antisense or miRNA therapies is changing the landscape of potentially targetable molecules[176–178]. Whether CRISPR will prove applicable to cancer therapy and further increase the target repertoire is only beginning to be explored[179]. Most current targeted therapies target both the wild type and mutated molecule with attendant challenges with toxicity. The emerging ability to target mutant molecules such as $KRAS^{G12C}$ while sparing the wild type molecule has resulted in high activity with limited toxicity[180,181].

To fully exploit opportunities to target mitigation strategies, novel trial designs with the flexibility to both measure adaptive responses and to rapidly change therapy as the mitigation strategies used by the tumor evolve under therapeutic stress will be

needed. Further, as the mitigation strategies engaged by tumors are likely to be patient- or tumor-specific, the trials must allow selection of patient-specific approaches. Novel trials design such as monotherapy run-ins followed by on-therapy biopsies and subsequent combination therapy selection provide the opportunity to identify patient-specific combination therapies designed to exploit adaptive responses[182]. However, the monotherapy run-in with subsequent combination therapy selection comes with challenges of requiring multiple biopsies (pre- and on-therapy) and also of deploying effective analytics in real time. Longitudinal profiling of adaptive responses coupled with genomic analysis of mutant clone evolution (e.g. estrogen receptor in luminal breast cancer[23–25], PI3K in HER2 breast cancer[183], MET in lung cancer[184] and KRAS in melanoma[185]) will inform the design of therapies that capitalize on vulnerabilities of the heterogeneous tumor clones with the ultimate goal to prevent the emergence of new resistant clones.

The amount of information that will be available on each patient based on emerging technologies that assess mitigation strategies, the broad array of available drugs particularly considering both on and off target activity, the challenge of predicting toxicity of combination therapies and the need to have up-to-date results from clinical trials to guide patient management are rapidly emerging challenges. Physician support approaches such as highly curated databases linking molecular markers to therapeutic options are sorely needed. Even more importantly, these databases must be easily accessed and readily understood. Artificial intelligence approaches linked to natural language processing have the ability to overcome some of the challenges. However, even the best current approaches require additional validation and curation. Data management and visualization tools will be required for tumor boards to engage the “wisdom of the crowd” that could aid in selecting patient specific combination therapies that effectively take advantage of adaptive responses.

Section 5 Conclusions & Perspectives

Resistance to cancer therapy remains a major challenge. To improve patient outcomes, we need to advance our understanding of the mechanisms that tumor ecosystems employ to survive both ongoing and therapy-induced stress. Single-cell RNA-seq or single-cell proteomics approaches that separately analyze the heterogeneity of tumor, stroma and immune cell states will aid in the discovery of new therapeutic opportunities that can exploit vulnerabilities induced by adaptive responses to therapy at the tumor-stroma interface[186–188]. Profiling of the complete tumor ecosystem will also advance understanding of tumor microenvironment adaptation to therapeutic stress, given the intense focus on immunotherapy combinations[189]. The efficacy of combination therapies on tumor cell intrinsic states will need to be balanced with their potentially deleterious effects on the immune system[190]. Importantly, the effects of stress, stress mitigation pathways and attempts at targeting are likely to be more nuanced than presented herein and will require a much deeper analysis of underlying mechanisms and importantly of the effects of perturbations on tumor, stromal and immune cell states with both temporal and spatial resolution in the clinic and in model systems. Indeed, while this review has focused on using examples to elucidate critical concepts, the effects of therapeutic targeting is likely to be dependent on the particular tumor context, treatment history and also on the complete tumor ecosystem. Further, the complex network of stress mitigation pathways and utilization

of different stress mitigation pathways in different tissues and different individuals and the likelihood that multiple different mitigation pathways are initiated in individual tumors will require detailed analysis of the effects of therapies on stress mitigation pathways in patient samples and in particular samples from patients on therapy. The challenges behind obtaining and analyzing samples in depth will require the development of national and international consortia as well as mechanisms to share data in a more efficient manner.

Objective analysis of therapy-induced stress mitigation responses in the clinic will be necessary to develop therapeutic strategies that prevent the emergence of drug resistance. While current analytic approaches are dependent on tumor biopsies, surrogate assays with imaging modalities[164–166] or liquid biopsies[167–169] to alleviate the cost, technical challenges and morbidity associated with repeat tumor biopsies would be preferable. Molecular imaging-based approaches (e.g. reporters of specific signaling pathways[106,191]) offer the ability to capture adaptive responses in multiple metastatic sites; an area of critical importance to combat metastatic cancers. Furthermore, longitudinal monitoring will help address the challenge of designing optimal treatment schedules (sequential administration and the order of each inhibitor[192] versus simultaneous delivery) to balance on-target toxicity with tumor cell eradication. As the spectrum of clinically-relevant adaptive responses employed in tumors are revealed, high fidelity, spatially resolved tumor models[34,193,194 195–197] will be needed to allow longitudinal analysis of tumor cell intrinsic and extrinsic adaptive responses. Importantly, barcoding technologies[198] have the potential to allow better characterization of the plasticity of the stress mitigation pathways. Systems-level profiling[199,200] of therapy-induced cellular stress homeostasis in diverse tumors will address the open questions of whether different tumor genotypes share a finite suite of adaptive responses and approaches to identify optimal nodes for combination therapy.

Despite the challenges, the opportunity to target adaptive responses and stress mitigation pathways provides a potential to not only directly impact tumor cell survival but also to prevent the emergence of genomic mutation driven resistant clones that are notoriously hard to target while at the same time to engage an effective immune response. Thus, exploiting stress and stress mitigation pathways has the potential to provide major benefits to cancer patients.

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Box 1:

Heterogeneity of adaptive responses and mitigation pathways:

Cancer cells can adopt a broad range of adaptive responses to survive therapeutic stress. For example, the adaptive rewiring of RTK activity in response to the EGFR/HER2 inhibitor lapatinib was heterogenous across a suite of HER2-positive breast cancer cell lines[123]. Interestingly, although adaptive responses are heterogeneous across cancer cells, convergence into downstream pathways have been observed. For example, although different RTKs have been shown to be induced across tumor cell lines treated with HER2 inhibitors, bromodomain inhibition blocked the induction of the majority of kinases, suggesting that this epigenetic regulator mediated a common mitigation mechanism[123]. Further, profiling the proteomic response of ovarian cancer patient derived xenografts treated with PI3K/AKT/mTOR and MAPK pathway inhibitors also revealed heterogeneous patterns of induced RTK expression, but a common coordinated induction of anti-apoptotic proteins[116,225]. The heterogeneity of adaptive responses engaged by cancer therapies engenders a critical question relating to the extent to which specific stress mitigation pathways adopted by a tumor to bypass therapy stress are driven by underlying genomic aberrations or epigenetic cell states. As a corollary question, can cells be steered with therapy to a particular state with limited mitigation pathways being available to escape therapeutic stress? For example, BRD4 inhibitors have been shown to block expression of multiple kinases precluding the upregulation of RTK activation as a mitigation strategy potentially representing a more therapeutically tractable state[226,227].

Importantly, while the impact of intratumoral and interlesion genetic heterogeneity on drug response has been well documented in various cancer types, there is currently little information about the impact of that heterogeneity on adaptive responses to anticancer therapy[228]. Many genomic alterations converge into a limited set of functional events and protein networks[229]. Thus, it is possible that a tumor underlying molecular features dictate the possible adaptive resistance mechanisms and that increased tumoral heterogeneity has limited effect on the availability of those adaptive responses. Clearly, further investigation with single-cell analyses will be needed in order to determine the heterogeneity of adaptive responses across cells from a single tumor and across multiple metastatic sites. It will also be important to better determine the impact of intratumoral and interlesion genetic heterogeneity on the variety of available stress mitigation pathways for a given tumor.

Our team has shown that adaptive responses to PARP inhibition can vary greatly across different cell lines as well as patient tumor samples. Adaptive responses included the induction of the DNA damage checkpoint and the PI3K-AKT-mTOR and RAS-MAPK viability pathways[126]. Importantly, differences were observed in the directionality (e.g. more or less DNA damage), magnitude (e.g. extent of RAS/MAPK activation) and selection of mitigation pathways both within a cell line panel and across patient samples[126]. The lack of uniformity in mitigation strategies engaged by different tumors highlights the challenge of developing therapies designed to interdict adaptive responses to targeted therapies. Despite the heterogeneity, there appear to be a limited number of programs able to mitigate the therapeutic stress induced by PARPi suggesting that if

the mitigation strategy engaged by a particular tumor could be predicted or measured following initiation of therapy, it would be possible to select drug combinations likely to provide patient-specific benefit. Interestingly, although high-grade ovarian cancer tumors have high inter-lesion heterogeneity[230,231], our study revealed conserved adaptive responses in different lesions within the same patient[126], suggesting that targeting patient-specific mitigation strategies could be effective across different metastatic sites.

Box 2.

Limitation of drug combinations targeting stress mitigation pathways

Based on the current literature, translation of the three proposed approaches (Figure 1B) to exploit adaptive responses and resultant mitigation processes to the clinic has the potential to improve patients care. However, there are still several questions that grants further exploration. For example, it is unclear how different cells in the tumor ecosystem will respond to the therapy. Also, where multiple stress mitigation pathways are activated simultaneously, targeting one might not be sufficient. The heterogeneity in mitigation processes between and within tumors are still poorly described. Other challenges that might be encountered comes from the complexity of some networks and the effects of perturbing them, as well as the impact of the presence of pre-existing resistant clones that could be selected by the proposed approaches.

Although many drug combinations have demonstrated promising results in pre-clinical studies, the additive or synergistic activity often comes at the cost of increased toxicity in patients. This can be associated specifically with acute tumor cell cytotoxicity such as tumor lysis syndromes[232] or with more direct effects of the drugs on host organs that are dependent on the targeted pathways. One potential advantage of targeting stress mitigation pathways is that these programs are generally not required for normal tissue homeostasis. Several strategies can be used to improve the therapeutic index. With therapeutic option ‘a’ (Figure 1B), the second drug that inhibits the stress mitigation pathway can be delivered sequentially to reduce toxicity. For option ‘b’ that involves use of drugs that enhance stress, it is important to specifically design combinations that target tumor specific stress programs to avoid systemic toxicities. Therapies that target molecules preferentially expressed in cancer cells, as well as immunotherapies tends to present with a better therapeutic index than cytotoxic agents. For drugs that have similar toxicity profiles (e.g. selumetinib and erlotinib[233]) dose-escalation to the recommended phase II monotherapy doses is unlikely. Thus, exploring different treatment schedule such as sequential drug administration or periodic drug holiday (intermittent treatment) can be considered[132]. Finally, delivering or trapping the drugs at the desirable site using nanocarriers or anti-body drug conjugates for example, can improve drug bioavailability selectively in tumor and decrease adverse events affecting normal cells[234].

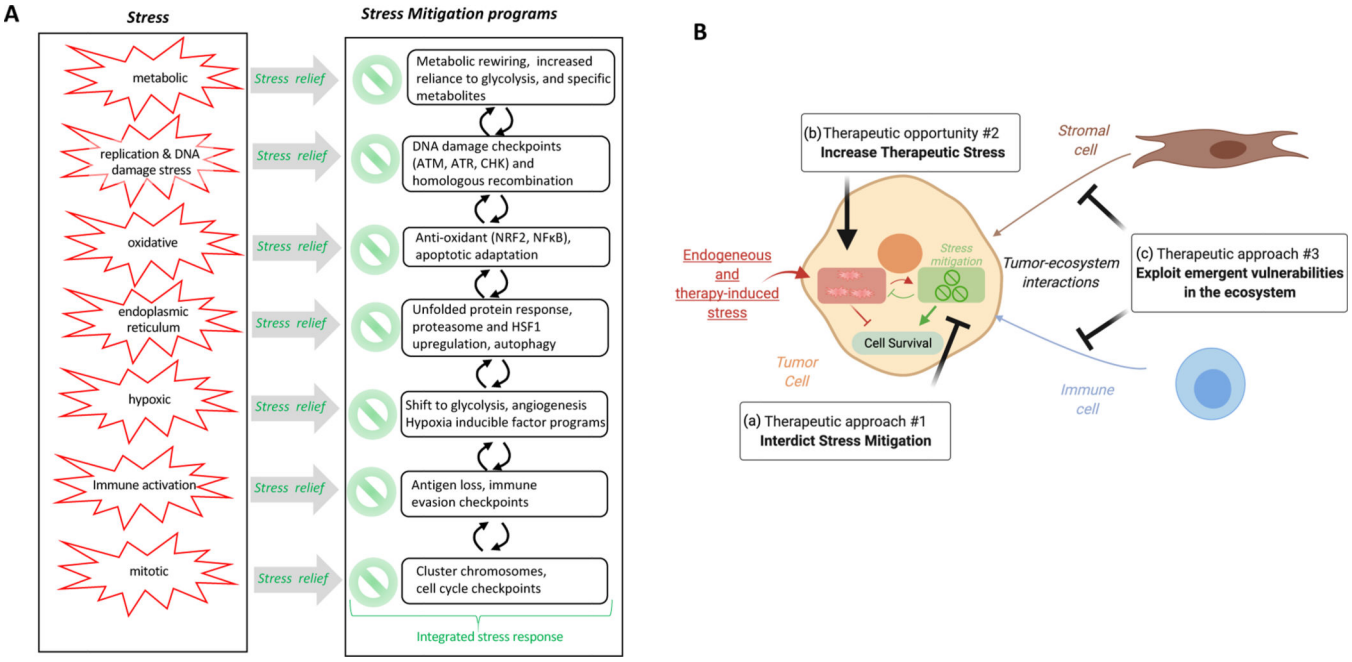

Figure 1: Therapeutic opportunities created by adaptive responses due to tumor cell endogenous stress, engagement of stress mitigation pathways and interactions in the tumor ecosystem.

(A) Stresses associated with tumor initiation and stress mitigation programs that are engaged to allow cancer cells to survive and proliferate. (B) Adaptation to therapeutic stress and engagement of stress mitigation pathways in the tumor ecosystem (tumor, stromal and immune cells) provide three therapeutic options: a) induce tumor cell death by interdicting stress mitigation pathways that allow tumor cell survival, b) overwhelm the mitigation pathways by increasing existing stress that results in stress-induced catastrophe, c) exploit emergent vulnerabilities in the tumor ecosystem by targeting tumor-host interactions. The difference in stress levels and stress mitigation programs between normal and tumor cells provides an opportunity for increased therapeutic index.

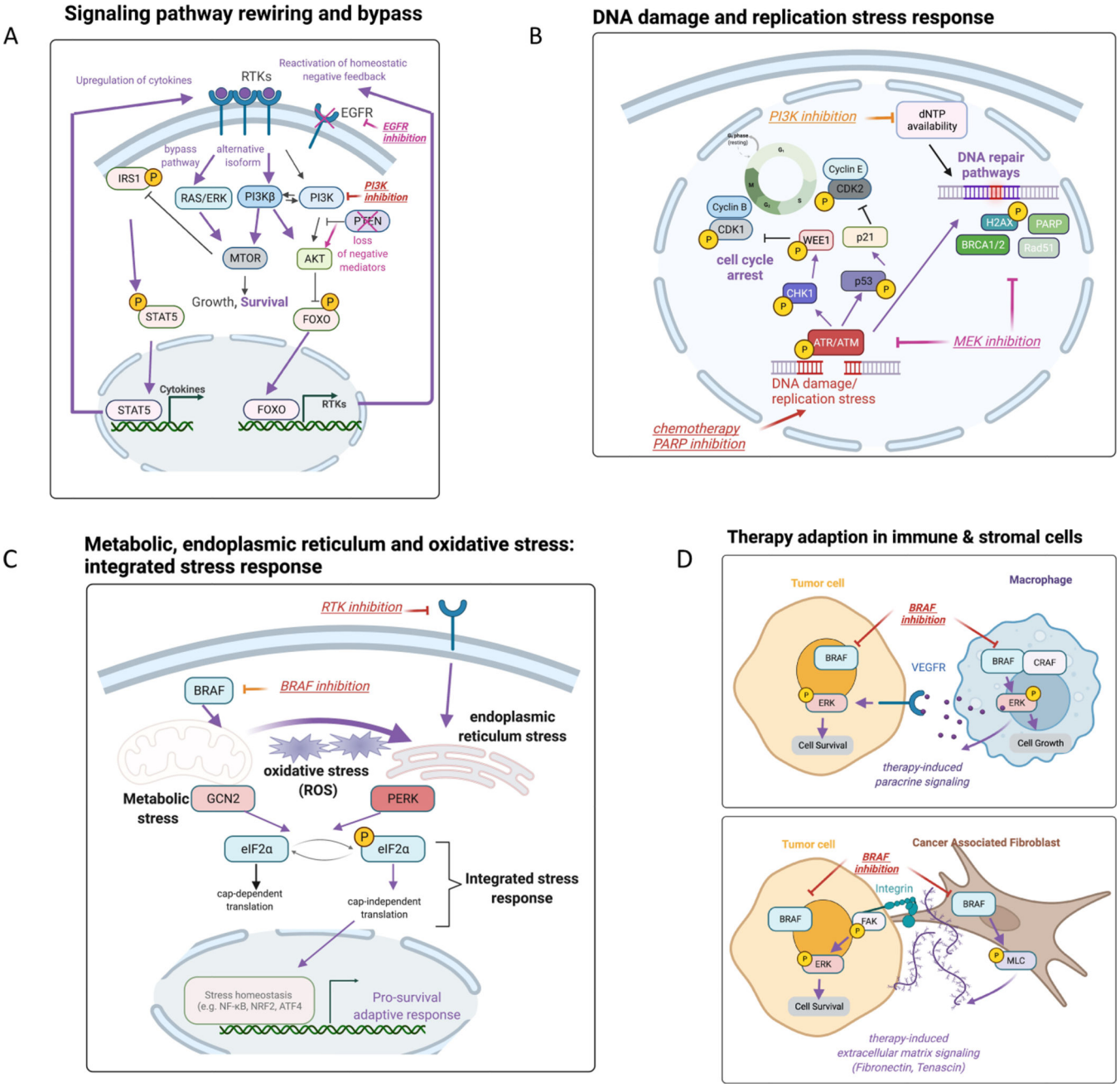

Figure 2: Adaptive response programs in tumor cells and in the tumor ecosystem.

(A) Examples of adaptive responses that bypass inhibition of the PI3K/mTOR pathway: 1) Transcriptional upregulation of RTKs via FOXO transcription factors, 2) secretion of autocrine growth factors via release of the mTOR-driven IRS1 negative feedback loop and 3) utilization of a non-targeted isoform of PI3K. We also highlight another example of signaling rewiring following EGFR inhibition due to downregulation of negative mediators in the PI3K pathway such as PTEN. Purple bolded arrows indicate adaptive responses following each targeted therapy (e.g. EGFRi, PI3Ki, PARPi, MEKi). (B) Engagement of DNA repair pathways to mitigate DNA damage and replication stress.

DNA damaging anti-cancer therapies (examples of chemotherapy and PARP inhibition) induce activation of DNA integrity sensors ATM/ATR that induce cell cycle arrest and active DNA repair pathways. PI3K inhibition promotes DNA damage by depletion of nucleoside availability that is required for DNA repair. MEK inhibition represents another example that decreases the levels of DNA repair pathway members resulting in elevated DNA damage. (C) Stress response pathways feed into kinases that phosphorylate eIF2α to induce cap-independent translation of transcription factors that regulate stress homeostasis (integrated stress response). For example, BRAF inhibition mediates metabolic stress via upregulating the upstream kinase GCN2, while RTK inhibition results in PERK upregulation and endoplasmic reticulum (ER) stress. Targeted therapies (e.g. BRAFi) can also result in unbalanced generation of reactive oxygen species (ROS) that via the same eIF2α kinases engage ROS-mitigation programs that are regulated by transcription factors such as NRF2 and NFkB. (D) Tumor-immune paracrine signaling following BRAF inhibition that mediates immunoevasion (proliferation of pro-tumor M2 macrophages) and reactivation of ERK in tumor cells via macrophage-derived VEGF. BRAF inhibition has also been shown to reactivate focal adhesion kinase-driven ERK in melanoma cells via cancer associated fibroblast-mediated extracellular matrix remodeling. Purple bolded arrows indicate adaptive responses resulting from cell-cell communication.

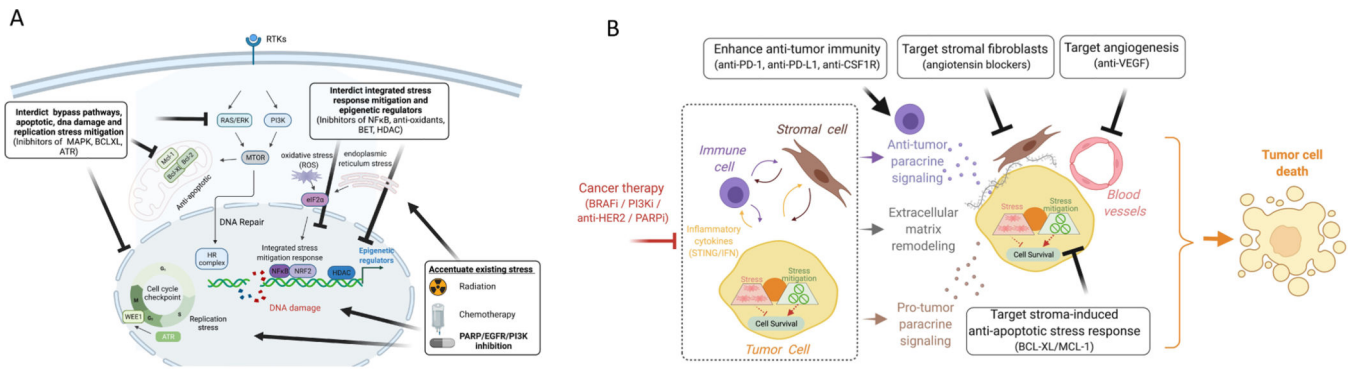

Figure 3. Strategies to induce tumor cell death by exploiting stress adaptation, accentuating existing stress and targeting emergent vulnerabilities in the tumor ecosystem

(A) Example of therapeutic opportunities emerging from therapy-induced stress. PARP inhibitors induce DNA damage and replication stress that results in cell cycle arrest and engagement of DNA repair pathways. This diagram show strategies involving combination therapies to enhance the efficacy of PARP inhibition that increase stress via radiation or chemotherapy. In addition, there are multiple approaches to interdict the stress mitigation process via 1) blocking anti-apoptotic survival proteins such as BCL-XL, 2) inhibiting upstream mediators of DNA repair (RAS/ERK inhibition) or cell cycle checkpoints (ATR), 3) blocking epigenetic reprograming (HDAC/BET) and 4) stress mitigation programs (e.g. anti-oxidant NRF2, NFkB). (B) Targeting emergent opportunities in the tumor ecosystem represents another approach to develop rational combination therapies. Tumor cells in therapy-resistant tumor ecosystems can be targeted by 1) inhibiting immune checkpoints to augment anti-tumor immunity that is mediated via tumor-immune paracrine signaling, 2) blocking therapy-induced extracellular matrix remodeling that enhances recruitment of cytotoxic immune cells, 3) targeting anti-apoptotic signaling that is induced via stromal-derived cytokines and extracellular matrix remodeling, and 4) blocking angiogenesis to normalize blood vessels and enhance immune cell recruitment.

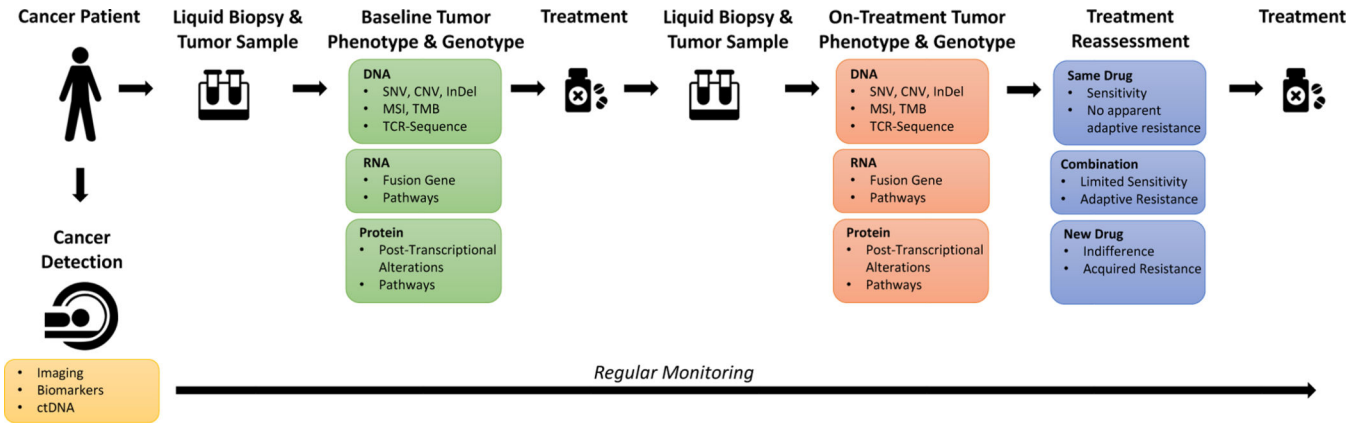

Figure 4. Potential approach to deliver personalized combination therapies in real time.

Liquid biopsy and tumor samples are collected at diagnosis. A suite of CLIA assays are performed to establish the baseline tumor phenotype and genotype of the tumor and select an initial therapy. After a short treatment period, a second liquid biopsy and tumor samples are collected and re-analyzed. The on-treatment tumor phenotype and genotype is established and the therapy is reassessed for one of three scenarios. (1) If sensitivity to therapy is suspected, no changes are made in terms of treatment. (2) If targetable adaptive responses are induced, a second drug is added to generate a patient-specific combination therapy. (3) If the tumor is indifferent to therapy or displays acquired resistance, a new treatment will be selected. Importantly, during the whole process, the progression of the disease is monitored regularly. In case of progression, new samples are acquired and analyzed for therapy reassessment.

Table 1.

Stresses and stress mitigation pathways that can limit therapeutic activity.

Endogenous and therapeutic stresses	Master regulators of stress mitigation pathways	Stress mitigation pathways	Examples of therapeutic response affected by stress mitigation pathways	Ref
Metabolic	AMPK, SIRT1, mTOR, PHD/HIF system	Oxphos/Glycolysis balance, autophagy, mitophagy, mitochondial metabolism, lipid metabolism	Metformin, BRAFi, phenformin	73,201,202
Oxidative	NRF2, NF-kB, HIF1	Antioxidant programs, metabolic reprograming	Radiation therapy, temozolomide, gemcitabine	77,203–205
Apoptosis	p53, TRAIL	BCL2 family balance, epithelial to mesenchymal transition, senescence phenotype	Doxorubicin, paclitaxel, BCL2i, BCL2/XLi, radiation therapy	206–209
Endoplasmic reticulum/ proteostatic	GRP78, PERK, IRE1a, ATF6	Unfolded protein response, E-Rassociated degradation, heat shock response, proteasome upregulation, autophagy	Chemotherapy, tamoxifen, multi-RTKi	210
Hypoxia	HIFs	Hypoxic response, angiogenesis, metabolic reprograming, autophagy, extracellular matrix remodelling, immune suppression	anti-VEGFR, multi-RTKi	211–213
DNA damage/ replication stress	ATM, ATR, CHK1, CHK2, RPA, H2AX	Cell cycle checkpoints, DNA repair, anti-apoptotic response, replication stress response	Gemcitabine, PARPi, doxorubicin, cisplatin, radiation therapy,	214–216
Immune activation	Sting, STAT3, TGFb	Reduced antigen presentation, immune checkpoint activation	Radiation therapy, PARPi, anti-PD1, anti-PD-L1	68,217,218

Table 2 –
Therapeutic opportunities created by adaptive responses to cancer therapies.

Combination therapies are organized based on therapeutic approach and the primary therapy that induced the adaptive response.

Therapeutic strategy	Primary therapy	Combination therapy partner	Cancer Type	Ref
Exploit tumor rewiring: block bypass pathway reactivation				
Blocking signaling reactivation via upstream target inhibition	MTORi	IGF-1Ri	breast	111 *
Blocking multiple members of the same kinase family	PI3Kαi	PI3Kβi	breast	110
Blocking pathway activation via downstream target inhibition	BRAFi	FAKi	melanoma	106, 219 *
Blocking pathway activation via downstream target inhibition	MTORi	MAPKi	breast	220 *
Interdict stress mitigation programs				
Targeting DNA damage repair checkpoints	PARPi	ATRi or WEE1i	ovarian	126 *
Blocking homologous recombination repair competency	PARPi	MEKi	ovarian	54,130
Targeting anti-oxidant to prevent adaptation to elevated oxidative stress	PARPi	TrxR1i	prostate	142
Inhibiting autophagy to prevent adaption to endoplasmic reticulum stress	multi-RTKi	chloroquine	endometrial	84
Blocking metabolic reprograming	CDK4/6i	GLS-i	breast& colorectal	138
Inhibiting anti-apoptotic proteins to target multiple stress mitigation pathways	MAPKi, PI3Ki, HER2i	BCL-XLi, MCL-1i	multiple	46,116–120,221 *
Preventing upregulation of pathway reactivation by targeting transcriptional reprograming	HER2i	BETi	breast	123
Blocking prosurvival reprogramming by targeting transcriptional activity	PI3Ki	anti-estrogen Receptor	breast	222 *
Increase therapy-induced stress				
Overloading DNA damage repair pathways via chemotherapy-induced stalled replication forks	gemcitabine	PARPi	lung	148
Overloading DNA damage repair pathways via irradiation	radiotherapy	PARPi	Ewing sarcoma	149
Replication stress increased due to decreased nucleotide synthesis	PI3Ki	PARPi	breast cancer	61
Synergistic cell death via increased reactive oxygen species generation	EGFRi	HDACi	lung cancer	223
Elevated endoplasmic reticulum stress overwhelms unfolded protein response pathway	EGFRi	PDK1i	lung cancer	151
Exploit emergent vulnerabilities in the tumor ecosystem				
Reactivation of CD8+ T cells that were recruited via PARPi-induced STING upregulation	PARPi	anti-PD-L1	multiple	69, 95 *
Targeting macrophages that reactivate ERK in melanoma cells via VEGF secretion	BRAFi	anti-CSF1R	melanoma	90 *
Enhancing recruitment of cytotoxic T cells by normalized blood vessels	anti-PD-1, anti-CTLA4	AGTR1i, AGTR2i	breast cancer	158, 224 *

*References with clinical evidence of adaptive responses to the primary therapy.