Perspective

Targeting pan-essential genes in cancer: Challenges and opportunities

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SUMMARY

Despite remarkable successes in the clinic, cancer targeted therapy development remains challenging and the failure rate is disappointingly high. This problem is partly due to the misapplication of the targeted therapy paradigm to therapeutics targeting pan-essential genes, which can result in therapeutics whereby efficacy is attenuated by dose-limiting toxicity. Here we summarize the key features of successful chemotherapy and targeted therapy agents, and use case studies to outline recurrent challenges to drug development efforts targeting pan-essential genes. Finally, we suggest strategies to avoid previous pitfalls for ongoing and future development of pan-essential therapeutics.

INTRODUCTION

A key component of successful drug development is the assessment of the therapeutic index (TI), the ratio of the dose or exposure of a drug required to elicit the desired therapeutic effect compared with the dose or exposure at which toxicity becomes limiting (Figure 1). While drugs with a high TI effectively kill cancer cells with manageable toxicities, drugs with a low TI or even "inverted" TIs cause significant side effects at or below efficacious doses. Cytotoxic chemotherapies, which typically target proliferating cells, generally have low TIs and thus require dose and schedule optimization and "rescue" interventions to mitigate side effects. The development of targeted therapeutics has provided alternative routes to achieving high TIs by either targeting cancer dysregulated genes with limited requirements for homeostasis in adults (e.g., ABL, KIT, TRK, ALK) or by developing mutation-biased inhibitors (e.g., EGFR, BRAF, IDH1/2, KRAS[G12C]). However, therapeutics targeting of pan-essential genes (e.g., those genes where inactivation leads to loss of fitness in multiple normal human tissues, see later section for details) are often aggregated within this "targeted" paradigm. Yet such therapeutics will often have low TIs and in many ways are more similar to chemotherapy. A lack of consideration for the specific problems of targeting pan-essential genes likely contributes to high clinical failure rates. Here, we focus on small-molecule targeted therapeutics; however, we believe that these principles would apply to antibody-based therapeutics such as antibody-drug conjugates and other newer modalities.

STRATEGIES TO INCREASE THE THERAPEUTIC INDEX FOR CYTOTOXIC CHEMOTHERAPIES

Cytotoxic chemotherapeutics remain pillars of systemic cancer therapy. The majority broadly inhibit proliferating cells by disrupting key mechanisms involved in DNA replication and cell division. As a result, many cancers are sensitive to chemotherapy. Normal proliferating cells are also vulnerable to chemotherapy, thus side effects such as nausea, vomiting, mucositis, anemia, thrombocytopenia, and leukopenia are prevalent (Nurgali et al., 2018). To achieve TIs with chemotherapy, several strategies have been implemented.

Schedule optimization
Intermittent dosing of chemotherapy drugs, rather than the continuous administration, decreases side effects while retaining anti-tumor activity (Kirkwood et al., 1981). The on-off cycles enable sufficient total drug exposure to kill tumor cells while allowing normal cell recovery during "drug holidays" (Foote, 1998).

Side-effect mitigation
Supportive medications have been key to enabling advances in chemotherapy. "Leucovorin rescue" after methotrexate treatment restores folate levels and rescues normal cells (Papac et al., 1973). G-CSF (granulocyte colony-stimulating factor) and GM-CSF (granulocyte-macrophage colony-stimulating factor) accelerate white blood cell recovery (Neidhart et al., 1992; Sheridan et al., 1992) and similarly, bone marrow transplant rescues hematopoiesis from myeloablative doses of chemotherapy (Tallman et al., 1997). Finally, the development of 5-HT$_3$ antagonists dramatically improved the tolerability of highly emetogenic agents such as cisplatin (Gralla et al., 2005).

Formulation optimization
Formulation strategies have been developed to try to shift drug distribution toward the tumor. For example, liposome-encapsulated or nanoparticle formulations are thought to lead to fewer toxicities than standard formulations of doxorubicin and paclitaxel (Leonard et al., 2009; Scripture et al., 2005), although few
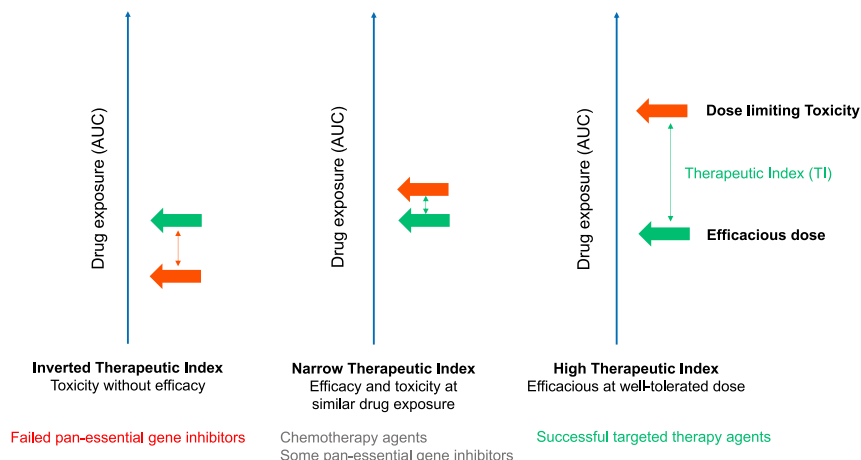
Figure 1. Therapeutic index of cancer therapeutic agents
Therapeutic index (TI) is the ratio of the dose or exposure of a drug required to elicit the desired therapeutic effect (green arrow) compared with the dose or exposure at which toxicity becomes limiting (orange arrow). High-TI drugs (many successful targeted therapy drugs) are efficacious at well-tolerated doses; narrow-TI drugs (chemotherapies, some pan-essential gene inhibitors) often have high-efficacy doses slightly below doses leading to dose-limiting toxicities; the effective doses of inverted-TI drugs (some pan-essential gene inhibitors) are lower than doses that lead to severe toxicities, and these drugs often fail in clinical development.

truly dose- and exposure-equivalent clinical studies of such formulations have been reported.

Personalized dosing
The dose of chemotherapy drugs is generally determined on the basis of each patient's body surface area, weight, and renal function (Van Den Bongard et al., 2000), allowing individualized dosing in each patient to avoid under- or overdosing. This would result in an aggregate improvement of TI in the patient population.

KEY FEATURES OF HIGH-THERAPEUTIC-INDEX TARGETED THERAPIES

Compared with normal cells, cancer cells rely on specific oncogenic pathways for proliferation and survival ("oncogene addiction") or have genetic or phenotypic features that confer vulnerabilities to specific perturbations ("non-oncogene addiction" and "synthetic lethality"), and thus can be specifically targeted by drugs without major adverse effects on normal tissues (Francies et al., 2020; Luo et al., 2009). Successful targeted therapies have achieved high TIs through distinct modalities including the development of the following agents.

Mutant-selective or mutant-biased inhibitors
EGFR, IDH1/2, BRAF, and KRAS[G12C] inhibitors are mutant-biased or mutant-selective either by serendipity or by design (Canon et al., 2019; Chapman et al., 2011; DiNardo et al., 2018; Tsai et al., 2008). Most recently, clinical data from EGFR inhibitor osimertinib and KRAS[G12C] inhibitors have all demonstrated impressive TIs in patients with the corresponding mutations (Hallin et al., 2020; Hong et al., 2020; Mok et al., 2017).

Lineage-restricted inhibitors
BTK and PI3Kδ inhibitors, anti-CD20 antibodies, and CD19-directed CAR-T cells, among others, all effectively treat B cell malignancies despite also killing normal B cells, an important but dispensable lineage (Burger and Wiestner, 2018; Hendriks et al., 2014). Similarly, hormonal therapies for breast and prostate cancers act on mechanisms operant in both tumor and normal tissues, yet the normal tissue side effects are modest at the organismal level.

Synthetic lethal gene inhibitors
PARP inhibitors selectively impair the viability of cells lacking functional BRCA1/2 (Huang et al., 2020), and in a recent study patients with mutations in ATM and CHK2 also conferred sensitivity (Mateo et al., 2015).

Widely differential surface antigen expression (e.g., HER2, EGFR)
Certain therapeutic antibodies take advantage of marked increases in target abundance in cancer by their ability to completely occupy every target and drive enhanced killing through antibody-dependent cellular cytotoxicity, often with threshold effects sparing lower-expressing normal tissues.

Predictive biomarkers
Predictive biomarkers (e.g., HER2 overexpression, EGFR[mut], PI3K[mut], BRAF[mut]), restricting treatment to those with a high probability of response, substantially increases the population benefit (Hyman et al., 2017).

"Pan-ESSENTIAL" CANCER TARGETS: PROBLEMS REVEALED IN CLINICAL DEVELOPMENT

The success of targeted therapies led to a surge in cancer drug development, with more than 3,000 oncology phase 1 trials initiated between 2006 and 2015. Despite new genomic technologies for target validation and patient selection, only 5.1% of oncology drugs entering phase 1 progressed to Food and Drug Administration (FDA) approval—the lowest among 21 major diseases (Thomas et al., 2015). We posit that a contributing factor to this failure rate is the misapplication of the targeted therapy paradigm to the drugging of pan-essential genes. More specifically, the selective targeting of a pan-essential gene might on its surface appear to be "targeted" therapy, but is in fact closer to the chemotherapy paradigm. By not recognizing this distinction, the peril is to ignore the difficulties in empiric drug discovery and the lessons learned from chemotherapeutic development.

Table 1. Representative pan-essential genes as oncology drug targets

Pan-essential genes	Cell function	Representative drug and most recent progress
AURKA	cell-cycle regulation	alisertib (failed in a phase 3 trial in PTCL)
AURKB	cell-cycle regulation	barasertib (failed in a phase 2 trial in AML)
PLK1	cell-cycle regulation	volasertib (failed in a phase 3 trial in AML)
CDC7	cell-cycle regulation	TAK-931 (currently in a phase 2 trial in advanced solid tumors)
CDK4/6	cell-cycle regulation	palbociclib (approved in combination with fulvestrant for treating HR + HER- breast cancer)
CDK1	cell-cycle regulation	dinaciclib (failed in a phase 3 trial in CLL)
CDK9	cell-cycle regulation	dinaciclib (failed in a phase 3 trial in CLL)
BRD4	epigenetic regulation	CPI-0610 (currently in a phase 3 trial in myelofibrosis)
HDAC3	epigenetic regulation	vorinostat (approved for CTCL, failed in 100 + clinical trials for various oncology indications)
DNMT1	epigenetic regulation	azacitidine (approved for treating MDS)
PRMT5	epigenetic regulation	GSK-3326595 (currently in phase 2 trials for various oncology indications)
SF3B1	splicing regulation	H-3B8800 (currently in phase 2 trial in MDS)
NEDD8	protein homeostasis	MLN4924 (currently in a phase 3 trial in AML and MDS)
20S proteasome subunits	protein homeostasis	bortezomib (approved for treating multiple myeloma and MCL)
ATM	DNA-damage response	AZD0156 (currently in a phase 1 clinical trial in solid tumors)
ATR	DNA-damage response	VX-970 (currently in phase 2 trials in multiple solid tumors)
WEE1	DNA-damage response	AZD1775 (multiple phase 2 trials were terminated because of safety concerns)
CHK1	DNA-damage response	MK8776 (failed in a phase 2 trial in AML)
KIF11	microtubule stability	ARRY-520 (development halted after a phase 2 trial in multiple myeloma)
MEK1/2	proliferation	trametinib (approved for treating BRAF-mutant advanced melanoma)
XPO1	nuclear export	selinexor (approved for treating advanced diffuse large B cell lymphoma, and multiple myeloma)

What is a pan-essential gene? The definition of pan-essential can vary in the context of cellular level and organism level, and here we refer to a gene as pan-essential if losing that gene leads to loss of fitness or cell death in multiple normal tissues or cell lineages in humans. Pan-essential genes in single-cell model organisms have been extensively characterized by transposon/chemical-induced mutagenesis and single-gene knockouts (Rancati et al., 2018). Technological advancements, in particular CRISPR knockout (Haley and Roudnicky, 2020), have enabled the robust identification of genes essential for cell growth and fitness in human cancer cell lines (Behan et al., 2019; Hart et al., 2015; Tsherniak et al., 2017; Wang et al., 2015), human haploid cell lines (Blomen et al., 2015), and human pluripotent stem cells (Mair et al., 2019; Yilmaz et al., 2018). The criteria for defining essential genes generally include genes where single guide RNAs (sgRNAs) are strongly depleted during cell propagation in a majority of cell lines (although cutoffs are slightly different across studies). For example, Hart et al. (2015) included genes whose depletion robustly impaired viability effect in at least three out of five screened cell lines, yielding a list of 1,580 genes (at a stringent 5% false discovery rate threshold). Pan-essential genes identified from these different datasets show high degrees of correlation (Chen et al., 2019b). Recent advances in dual CRISPR screens using multiplexed sgRNAs reveals additional pan-essential paralogous gene pairs. Although performed for limited numbers of genes and cell lines, such studies show that paralogous pairs such as *CDK4/6*, *MEK1/2*, and *HDAC1/2* exhibit pan-essential profiles (DeWeirdt et al., 2020; Gonatopoulos-Pournatzis et al., 2020; Han et al., 2017). Additional pan-essential paralogous pairs can also be inferred from the genome-wide single-gene CRISPR and RNAi screens (Viswanathan et al., 2018).

Since the characterization of human pan-essential genes became available only recently, it is likely that many cancer targets initially posited to manifest context-specific dependence are in retrospect pan-essential. We compiled a list of molecular targets of clinical-stage oncology drug candidates from canSAR and the Therapeutic Target Database (Tym et al., 2016; Wang et al., 2020) and compared this with a list of human pan-essential genes in at least two published CRISPR single-gene datasets (Dempster et al., 2019; Hart et al., 2015; Yilmaz et al., 2018) or in one paralog dataset. We identified therapeutics that target ~20 pan-essential genes, including regulators of the cell cycle (*PLK1*, *CDK1*, *CDK7*, *CDK9*, *AURKA*, *AURKB*, *CDK4/6*), epigenetic regulators (*DNMT1*, *BRD4*, *HDAC3*), protein homeostasis regulators (*NEDD8*, 20S proteasome subunits), and DNA-damage response modulators (*ATR*, *WEE1*, *CHK1*) (Table 1). Targeting such pan-essential genes could be associated with limiting on-target toxicities and difficulties in patient stratification. Indeed, such drug candidates have suffered numerous phase 2/3 trial failures. Examination of the cell-line sgRNA knockout vulnerability distributions of genes targeted by inhibitors such as BRAF and ALK (Tsherniak et al., 2017) shows impaired viability in only a small fraction of cell lines. In contrast, sgRNAs against chemotherapy target genes such as *TOP1*, *TOP2*, and *DHFR* show broad viability effects with median CERES score close to −1 (Figures 2A and 2B). Similarly, the knockout phenotype of recent pan-essential targets also shows broadly lethal patterns (Figure 2C). Likewise, many inhibitors of pan-essential
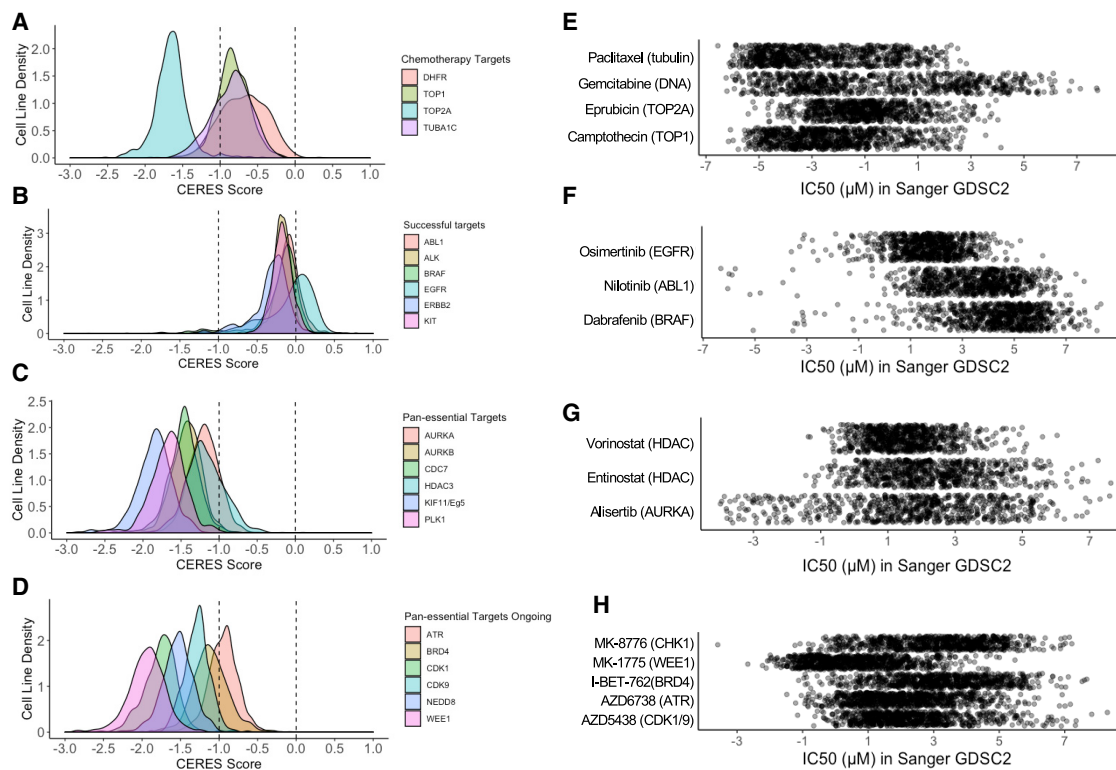
Figure 2. Genome-wide CRISPR knockout and compound profiling in cancer cell lines revealed the pan-essential nature of many cancer drug targets

(A–D) Density plots representing the CERES score distribution of cancer cell lines after the indicated gene knockout. A CERES score of −1 represents the median effect of knocking out essential genes, and a CERES score of 0 represents no growth disadvantage. CRISPR (Avana) Public 20Q2 dataset from Broad Institute was used in this analysis.

(E–H) Scatterplots representing the IC_{50} distributions of the indicated drugs and their molecular targets in ∼800 cancer cell lines. GDSC2 dataset from Sanger Institute was used in this analysis.

targets show broad cytotoxic patterns whereas targeted therapeutics show selective sensitivity in small subsets of cell lines (Figures 2E–2G). Thus, large-scale genetic or compound profiling can unveil broadly cytotoxic profiles of compounds that might have initially appeared to have contextual specificities. For example, BRD4 was initially thought of as a therapeutic target for *MYC*-amplified cancers, and indeed both genetic knockdown and small-molecule inhibition of *BRD4* lead to anti-tumor effect in various relevant preclinical models (Delmore et al., 2011; Zuber et al., 2011). However, in the drug-sensitivity profiles across >700 cell lines for two clinical-stage BRD4 inhibitors, neither *MYC* copy numbers nor BRD4 knockout effects correlate with the sensitivity of either BRD4 inhibitors. In contrast is the high correlations between the sensitivity to a BRAF or ABL inhibitor (dabrafenib or nilotinib, respectively), the viability effects of BRAF or ABL1 knockouts, and the presence of a BRAF mutation or ABL fusion, respectively (Figure 3).

Since targeting pan-essential genes will lead to broad cytotoxic effects, "apparent" validation of a specific anti-cancer effect of interest is essentially guaranteed when performing experiments in only a few cell lines or xenografts. This bias will therefore prioritize pan-essential mechanisms or inhibitors in cancer drug discovery (see below for examples). However, inhibitors of pan-essential targets are likely to manifest difficulty in clinical development with respect to both the identification of re-

sponding patients and an enriched responding patient population, and with respect to tolerability. This divergence has led to costly failures in late clinical development. Here we take a deeper look into inhibitors of several pan-essential cancer targets that have attracted significant interest and investment in drug discovery, but failed in multiple phase 2 and phase 3 clinical trials.

CASE STUDIES OF INHIBITORS TARGETING PAN-ESSENTIAL GENES IN CANCER

Histone deacetylase inhibitors

Hyperpolar compounds such as hexamethylene bisacetamide (HMBA) were observed to induce leukemic cancer cell differentiation. Modifications led to SAHA (vorinostat), which also had pro-differentiation and anti-proliferative effects, and was found to inhibit histone deacetylation (Dokmanovic et al., 2007; Marks et al., 2004). Intriguingly, the differentiation phenotype was also induced by HMBA, which lacked histone deacetylase (HDAC) inhibitory activity, hence the link between the differentiation phenotype and either HDAC inhibition or epigenetic regulation was not established. Nonetheless, the empiric anti-proliferative activity and the relative ease of targeting HDACs led to the widespread pursuit of pharmaceutical HDAC inhibitors.

In this pursuit three challenges emerged. First, HDAC inhibition leads to broad cytotoxic effects in a large selection of cancer
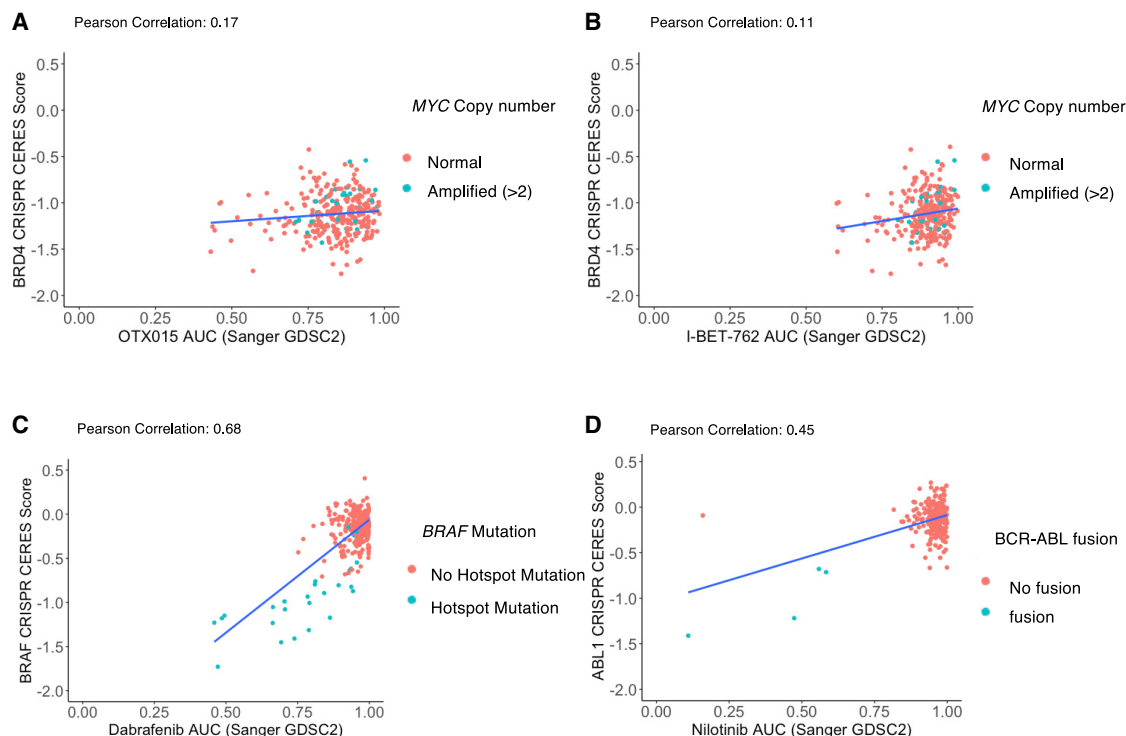
Figure 3. Biomarker correlations with BRD inhibitor, BRAF inhibitor, and ABL inhibitor drug sensitivities

The AUC values of two BET inhibitors, OTX015 (A) and I-BET-762 (B), are plotted against the CERES score of BRD4 knockouts in the same cell line. The AUC values of the BRAF inhibitor dabrafenib (C) and ABL inhibitor nilotinib (D) are plotted against the CERES score of BRAF knockout in the same cell line. Each dot represents a cancer cell line and red colors represent *MYC* amplification (A and B), BRAF hotspot mutation (C), or BCR-ABL fusion (D) in the cell line. Genomic and CRISPR data were obtained from Broad Institute Depmap portal. BET inhibitor AUC data were obtained from Genomics of Drug Sensitivity in Cancer Portal.

preclinical models. Thus, if one experimentally "looks under the lamppost" at a specific cancer lineage or molecular class in isolation, one will by default generate positive supportive data. Second, the majority of HDAC inhibitors in clinical development are broad-spectrum inhibitors targeting multiple isoforms of HDACs (Falkenberg and Johnstone, 2014). Thus, it is challenging to attribute the anti-tumor effects of inhibitors to the specific function of a single HDAC isoform and allow optimization. Third, nearly all HDACs are ubiquitously expressed in essential tissues of humans, and the individual knockouts of *Hdac1*, *Hdac2*, *Hdac3*, *Hdac4*, *Hdac5*, *Hdac7*, and *Hdac8* in mice are either embryonic/perinatal lethal or lead to major organ defects after birth (Falkenberg and Johnstone, 2014). Thus, the broad requirement for HDAC activity in normal human tissues along with inhibitor polypharmacology made it likely that side effects would be limiting.

Four HDAC inhibitors are FDA approved: vorinostat in cutaneous T cell lymphoma (CTCL), belinostat in peripheral T cell lymphoma (PTCL), romidepsin in PTCL and CTCL, and panobinostat in multiple myeloma. While this is a measure of success, the efficacy is modest and the overall clinical benefit is marginal due to the severity of adverse events. In the phase 3 trial of panobinostat in refractory multiple myeloma, patients in the panobinostat treatment group showed a longer median progression-free survival (mPFS) than the control group (11.99 versus 8.08 months) (San-Miguel et al., 2014). However, 96% of patients in the panobinostat-combination group experienced grade

3/4 severe adverse events. The FDA initially rejected the new drug application in refractory multiple myeloma because "the drug's benefits did not outweigh its risks," but later approved it in a narrower indication in myeloma (Jalloh, 2015). A later analysis showed that the overall survival (OS) of patients was not improved (San-Miguel et al., 2016).

The broad cellular activity of HDAC inhibitors and the description of such inhibitors as "epigenetic regulators" concomitantly led to numerous new hypotheses. Again, the pan-lethal nature of the inhibitors guaranteed that every proposed cell-line-based indication when tested in isolation would appear positive (absent robust controls). Such hypotheses lacking more robust validation led to multiple phase 2/3 failures of HDAC inhibitors in non-small cell lung cancer (NSCLC), acute myeloid leukemia (AML), mesothelioma, ovarian cancer, and high-grade gliomas among others. These trials showed low response rates and high grade-3/4 adverse event rates consistently raising the red flag of low TI. Vorinostat itself was studied in 156 phase 1, 139 phase 2, and 9 phase 3 trials with only the phase 2 trial in CTCL leading to FDA approval (Figure 4), thus invalidating most, if not all preclinical HDAC therapeutic hypotheses. Despite these data, HDAC-inhibitor hypotheses continue to emerge with investigators testing entinostat + endocrine therapy in a failed phase 3 trial (Yeruva et al., 2018), entinostat + anti-PD1 antibody in a failed phase 2 trial (O'Shaughnessy et al., 2020), and recent proposals to test vorinostat in pediatric glioma (Hashizume, 2017), despite cellular IC$_{50}$ data in that indication being
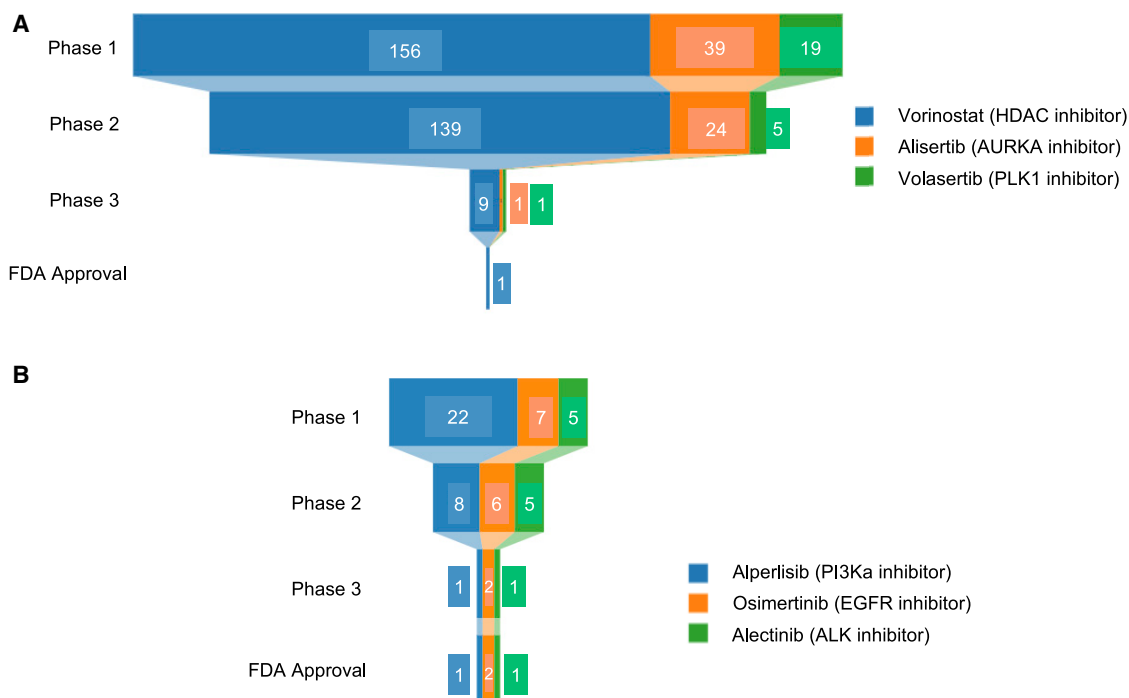
Figure 4. Clinical development trajectories of a selection of pan-essential and highly selective targeted therapy drug candidates
Clinical trial information for three pan-essential drug candidates discussed in case studies (A) and three successful targeted therapy agents (B) were obtained from clinicaltrials.gov. Clinical trials of corresponding drug candidates are included for this analysis if they have the status suspended, terminated, completed, or withdrawn, or have published trial results.

indistinguishable from the IC_{50} distribution of vorinostat in large cell-line panels.

Aurora kinase inhibitors
Aurora kinases regulate entry into mitosis, spindle assembly, and cytokinesis. Aurora kinase A (*AURKA*) localizes to centrosomes and drives centrosome maturation, separation, and spindle assembly while aurora kinase B (*AURKB*) is a chromosomal passenger protein localizing along the chromosome and centromere kinetochore to facilitate mitosis. *AURKA* and *AURKB* are amplified in several types of cancers, leading to the notion that they might make attractive cancer targets (Lens et al., 2010; Mehra et al., 2013). Paradoxically, AURKA and AURKB function as both oncogenes and tumor suppressors. In mice, *Aurka* transgenic overexpression increases mammary and skin carcinomas while *Aurka* heterozygous deletion leads to lymphoma. Similarly, *Aurkb* overexpression increases lymphomagenesis while heterozygous deletion gives rise to multiple cancers (Otto and Sicinski, 2017). While AURKA was thought to be selectively lethal in NMYC-driven neuroblastoma, in CRISPR screening datasets, *AURKA* and *AURKB* are both pan-essential genes (Figure 1C), and inhibition of AURKB impairs cell viability in actively proliferating tumor and normal cells (Mehra et al., 2013). Therefore, we can anticipate a low TI.

Since 2010, more than ten aurora kinase inhibitors have entered clinical development (Otto and Sicinski, 2017), but none have achieved FDA approval. Alisertib is an AURKA inhibitor with >200-fold selectivity over AURKB that was studied in 24 phase 2 trials in breast, bladder, and prostate cancers, SCLC,

NSCLC, multiple myeloma, neuroblastoma, melanoma, and PTCL. From these trials alisertib progressed to phase 3 testing only in PTCL (Figure 4), where alisertib was not superior to standard of care (33% versus 45% overall response rate [ORR]) and had a median PFS of 115 versus 104 days (O'Connor et al., 2019).

Similarly, AURKB inhibitors have not achieved clinical success. To date, only barasertib/AZD1152 has reached phase 2 testing. In a phase 2 trial comparing barasertib with low-dose cytarabine (LDAC) in elderly AML patients, while treatment with barasertib led to higher response rates, serious adverse events were significantly higher in patients treated with barasertib compared with LDAC (stomatitis 29% versus 0%, febrile neutropenia 50% versus 19%, pneumonia 23% versus 8%), leading to its discontinuation (Kantarjian et al., 2013). Similarly, the development of AURKB inhibitor BI811283 was discontinued after a phase 1 trial showed 0% ORR (Mross et al., 2016).

PLK inhibitors
Polo-like kinases (PLKs) regulate cell cycle and mitosis. *PLK1* has an essential role in regulating G_2/M transition, centrosome maturation, sister chromatid separation, mitosis exit, and cytokinesis initiation (Strebhardt and Ullrich, 2006). PLK1 is overexpressed in a series of solid and hematologic malignancies, and is associated with poor prognosis (Strebhardt, 2010). PLK1 is also a negative regulator of tumor suppressor p53 (Ando et al., 2004). Thus, PLK1 was considered an interesting cancer target.

Plk1 knockout or knockdown leads to viability effects in various cancer models; however, whether such effects are

cancer selective remains questionable. While small interfering RNA silencing data in transgenic mice and primary mammalian cell lines shows that toxicity of targeting PLK1 might be tolerable (Raab et al., 2011), Plk1 homozygous knockouts are embryonic lethal at the 4/8-cells stage (Lu et al., 2008) and PLK1 is essential in human pluripotent stem cells. Interestingly, Plk1 heterozygous knockout mice show aneuploidy and increased tumor development at advanced ages (Strebhardt, 2010), and PLK1 overexpression prevents the development of KRAS- and HER2-induced mammary gland tumors in transgenic mouse models (de Cárcer et al., 2018). PLK1 knockdown results in anti-proliferative effects in many cancer cell lines without robust biomarkers for distinguishing sensitive cell lines (Strebhardt and Ullrich, 2006) and in CRISPR screening datasets PLK1 is a pan-essential gene (Figure 2C).

To date, no PLK1 inhibitors have been approved by the FDA. Two inhibitors have progressed to phase 3 testing although both have suffered multiple clinical failures. Volasertib is a selective PLK1 inhibitor with >1,000-fold selectivity against other kinases (Otto and Sicinski, 2017). In a phase 3 trial in previously untreated AML patients, volasertib + LDAC failed to improve either ORR or OS compared with LDAC alone, but produced higher rates of grade 3/4/5 adverse events (AEs), especially higher grade 5 serious AEs and treatment-related deaths (27.9% versus 15.2%) (Döhner et al., 2016). In NSCLC, volasertib alone or in combination with pemetrexed shortened PFS and increased toxicity compared with pemetrexed (Ellis et al., 2015). In platinum-resistant ovarian cancer, volasertib shortened PFS (13.1 versus 20.6 weeks) and increased grade 3/4 AEs compared with chemotherapy (61.1% versus 30.9%) (Pujade-Lauraine et al., 2016). Finally, in a single-arm phase 2 trial in bladder cancer, volasertib failed to meet the prespecified criteria for activity while 62% of patients experienced grade 3/4 AEs (Stadler et al., 2014).

Rigosertib is a multi-kinase inhibitor with modest PLK1 selectivity (Otto and Sicinski, 2017). In a phase 3 trial in high-risk myelodysplastic syndrome (MDS), rigosertib failed to improve OS and increased grade 3/4 AEs, including anemia, thrombocytopenia, and neutropenia (Garcia-Manero et al., 2016). In a phase 2/3 metastatic pancreas cancer trial, adding rigosertib to gemcitabine failed to improve PFS and increased grade 3/4 AEs (O'Neil et al., 2015). Currently, rigosertib is in phase 2 and 3 trials in second-line higher-risk MDS patients, and alone or in combination with azacitidine.

CDC7 inhibitors

CDC7 is a serine-threonine kinase that phosphorylates and activates MCM2 and regulates initiation of DNA synthesis, chromosomal segregation in mitosis, DNA-damage response, response to DNA-replication stress, and entry into mitosis (Montagnoli et al., 2004; Yamada et al., 2014). Although CDC7 is upregulated and associated with poor prognosis in several types of cancer (Kulkarni et al., 2009), there are no data supporting an oncogenic role for CDC7 nor that the overexpression confers sensitivity to CDC7 inhibition. Indeed, CDC7 expression might simply correlate with the portion of cells in S phase (Montagnoli et al., 2010). Cdc7 knockouts are embryonic lethal in mice between embryonic day 3.5 (E3.5) and E6.5 (Kim et al., 2002), and CRISPR screening data show that CDC7 is pan-lethal in human cell lines

(Figure 2C). Thus, CDC7 inhibitors are likely to behave similarly to low-TI chemotherapies.

To date, no CDC7 inhibitors have reached phase 3, and several have failed or were terminated in phase 1/2. BMS-863233/XL413 is a potent and selective oral CDC7 inhibitor with good preclinical pharmacokinetic (PK) properties and in vivo anti-tumor activity in a colorectal cancer xenograft study (Koltun et al., 2012). Two phase 1/2 trials in advanced hematologic and solid cancers (NCT00838890, NCT00886782) were terminated prior to completion due to issues with drug metabolism and lack of efficacy (EU Final Clinical Study Report CA198002). In further characterizing this inhibitor in 64 cancer cell lines neither mutational status, doubling time, nor CDC7, DBF4, or MCM2 mRNA expression was associated with sensitivity. NMS-1116354 is a potent and selective CDC7 inhibitor with in vitro and in vivo anti-tumor activity in colon cancer models (Montagnoli et al., 2008). NMS-1116354 was terminated early after two phase 1 trials in advanced solid tumors (NCT01016327 and NCT01092052). TAK-931 is a potent ($IC_{50} < 0.3$ nM) and selective oral CDC7 inhibitor still in clinical development. In large-panel cell-line data, TAK-931 showed anti-tumor activities in KRAS-mutant cells than KRAS wild-type cells but with questionable statistical significance (Iwai et al., 2019). In a dose-escalation phase 1 trial in advanced solid tumors, the majority of patients experience grade ≥ 3 AEs, including neutropenia, decreased white blood cell count, and leukopenia, although 5 out of 25 patients had partial remission or stable disease (Shimizu et al., 2018). TAK-931 is currently being evaluated in a phase 2 clinical trial of patients with advanced solid tumors.

LESSONS LEARNED

We can observe several recurrent themes worth considering for future development of therapeutics in this space.

Misidentifying pan-essential genes as selective-essential targets based on limited preclinical modeling

Before large-scale characterized cancer cell lines and patient-derived xenografts were available (Barretina et al., 2012; Gao et al., 2015; McDonald et al., 2017), the evaluation of most cancer drug targets was limited to small sets of preclinical models. Even today, academic labs lack the resources required to conduct large-scale cell-line testing and hence preclinical hypothesis validation remains underpowered. In addition, the publication pressure to equate novel cancer biology with a potential therapeutic intervention is also a contributing factor. For example, HDAC and bromodomain inhibitors are frequently used to provide evidence in support of a selective epigenetic hypothesis when in fact these are broadly cytotoxic agents. More importantly, the mistaken identification of a pan-essential target as selectively essential will lead to the clinical testing of erroneous hypotheses, lack of efficacy, and the exposure of patients to unnecessary toxicity.

The inability to stratify and enrich the clinical trial population

When the pan-lethal activity of a therapeutic is recognized, it is not clear that cell-line testing, even when there is a broader range of effect (Figure 2C), will provide clear translatable predictive

biomarkers. It is possible that the ability to define increased tumor sensitivity for cytotoxic targets is impaired by the relative normalized growth rates of most cell lines. In the absence of a predictive genetic feature, overexpression of the target is often invoked as a sensitizing feature. However, as pertains to small-molecule inhibitors, from a kinetic view increased levels of a pan-essential target will create resistance to a therapeutic, not sensitivity. Indeed, *DHFR* amplification results in resistance, not sensitivity, to methotrexate (Trent et al., 1984). Ultimately, clinical development then progresses based on idiosyncratic responses in the clinic and a haphazard empirical development approach.

Inadequate attention to therapeutic pharmacology
Chemotherapeutics were typically developed using intermittent schedules, drugs having shorter half-lives, individualized patient dosing, and often intravenous formulations. This type of pharmacology might be ideal for tuning therapeutics having a narrow range of tolerated doses (narrow TI). Fixed daily oral dosing and longer half-lived therapies emerging from the Gleevec paradigm provide far less flexibility for narrow-TI drugs. It is notable that the CDC7 inhibitors in clinical developments are all oral despite the knowledge that neutropenia and other cytopenia would be the dose-limiting toxicity.

Lack of a therapeutic window due to on-target toxicity
We expect that therapeutics targeting pan-essential genes will exhibit low-TI profiles; however, the inability to predict whether there is an adequate TI or overestimating the TI from preclinical studies remains problematic. First, there are obvious differences between human and rodent and dog toxicology. Second, new pan-essential targets may have poorly understood cytotoxic effects in non-proliferating cells, creating new classes of AEs. For example, patients treated with HDAC inhibitors consistently experienced profound fatigue or asthenia when mitigation strategies were not known (Krug et al., 2015; O'Shaughnessy et al., 2020; San-Miguel et al., 2014). Third, overestimation of efficacy is common. Slowed tumor progression in preclinical models, rather than regression, is often interpreted as efficacy, whereas in humans this constitutes progression. This leads to lower estimated efficacious drug concentrations and therefore an overestimation of the TI (Figure 1). Finally, because cancer drugs are "expected" to have a narrow TI, untoward toxicity in dose-range finding and good-lab-practice toxicology studies is seen as acceptable.

Lack of a therapeutic window due to inhibitor polypharmacology
The problem of polypharmacology is not limited to targeting pan-essential genes; however, as pan-essential targets can have highly related family members (e.g., 18 human HDACs, 9 human cyclin-dependent kinases [CDKs]), fewer selective inhibitors may target multiple pan-essential genes. For example, several CDK inhibitors broadly target CDK1, 2, 7, and 9, which are all pan-essential (Otto and Sicinski, 2017). While selective polypharmacology can enhance the anti-tumor effect of certain drugs (e.g., CDK4/6 inhibitors, MEK1/2 inhibitors), balancing on-target synergies and toxicities of inhibiting multiple pan-essential targets is often challenging.

Therapeutic failure occurs late in drug development rather than during therapeutic optimization
Several factors contribute to this issue: first, pan-lethal targets are often selected because of their "druggable" enzymatic function. Thus, the odds of making a potent drug are high. Second, because the target is pan-lethal, many if not most preclinical models will appear to be responsive. Third, as even the viability responses to pan-lethal inhibitors still follow a distribution (Figure 2), *in vitro* responses to a therapeutic can appear to be differential in small-cell-line sets. Fourth, the broad cellular activity will result in many positive experiments in the academic preclinical setting, generating enthusiasm for the target. In aggregate, these features facilitate and pave the way for preclinical drug development eliminating many typical points of preclinical failure. Thus, HDAC inhibitors were "easy" to make in the preclinical setting, yet were disastrous in clinical development.

FUTURE STRATEGIES TO IMPROVE THE DEVELOPMENT OF "NEXT-GENERATION" PAN-ESSENTIAL TARGET INHIBITORS

The success of chemotherapy and of pan-essential inhibitors such as CDK4/6 and MEK inhibitors suggests that targeting pan-essential genes will remain an important strategy for cancer therapeutics development. It is also clear that many of the above challenges are likely to apply to a "next generation" of inhibitors of pan-essential targets including BRD4, ATR, WEE1, NEDD8, CHK1, and CDK9 inhibitors, among others. Akin to prior pan-essential targets, these genes control key cellular processes such as cell-cycle regulation, DNA-damage response, proteolysis homeostasis, and transcriptional control, and fit the criteria of pan-essential genes (Figure 2D). Indeed, the distribution of cellular sensitivity to many of these next-generation therapeutics including inhibitors of CDK1/2/9 (AZD5438), ATR (AZD6738), WEE1 (MK-1775), CHK1 (MK-8776), and BRD4 (I-BET-762) are similar to the distributions of chemotherapeutics (Figure 2H).

Since it is likely that the development of newer pan-essential therapeutics will face hurdles similar to those outlined above, strategies are needed to prevent an ongoing "death row" of clinical development failures. It is essential that we improve our ability to validate and prosecute pan-essential targets, as well as to separate and optimize those likely to provide patient benefits while deprioritizing and discontinuing those likely to engender toxicity over efficacy.

Target evaluation and validation in multi-omic and functional datasets
Large-scale projects have enabled the characterization of mRNA and protein expression levels in many normal human cell lines and tissues (Ardlie et al., 2015; Consortium et al., 2019; Regev et al., 2017; Uhlén et al., 2015). Simultaneously, profiling of tumors and tumor models has been greatly expanded. The Cancer Cell Line Encyclopedia (CCLE) has generated comprehensive "omic" data in more than 1,000 cancer cells (Barretina et al., 2012; Ghandi et al., 2019). While The Cancer Genome Atlas and related projects have made genetic, mRNA expression, and proteomic data in thousands of patients readily available (Chen et al., 2019a; Gillette et al., 2020), the availability of these

data, must be coupled with far greater sophistication in their use beyond the monocular assumption that overexpression should predict drug sensitivity. While overexpression of cell-surface proteins indeed enhances tumor killing by antibodies through increased antibody recruitment to the tumor, the overexpression of low-molecular-weight inhibitor targets is just as likely to confer resistance by raising the enzymatic target concentration. Moreover, there is now clear evidence that heterozygous loss of a pan-essential gene, or homozygous loss of one gene from a pan-essential paralog pair, confers selective lethality (Nichols et al., 2020; Viswanathan et al., 2018). Therefore, it would be wise to equally consider the possibility that reduced concentrations of pan-essential enzymes could confer increased tumor sensitivity. In addition, mRNA expression analyses often compare a tumor with its "matched" normal. However, for dispensable lineages (e.g., B cells, breast and prostate epithelium) targeting both the tumor and the normal tissue is acceptable. Thus, we should instead consider defining "critical normal" tissues as key comparators for data mining (e.g., heart, brain, liver, gastrointestinal tract, bone marrow, stem cells).

Unbiased target discovery is increasingly enabled through CRISPR knockout screens. Notably, when comparing large-scale short hairpin RNA (shRNA) screens and CRISPR screens, certain pan-essential genes (e.g., PRMT5) are selectively lethal when inactivated by shRNA. This raises the question as to whether genome-wide partial-loss-of-function studies focused on the pan-lethal genes might provide clearer evidence for differential effects across cancer (see further discussion below). Additionally, genome-wide CRISPR screens have so far been performed only in a small number of "normal" cell lines including fibroblast and pluripotent stem cells, thus limiting the ability to discern cancer-specific effects of pan-lethal genes. Future efforts to characterize a panel of normal cell lines from diverse "critical normal" lineages might serve as robust controls for this purpose.

Small-molecule perturbation projects have generated sensitivities of more than 300 cancer cell lines to more than 400 small molecules (Iorio et al., 2016; Seashore-Ludlow et al., 2015). This approach has been extended to a "drug repurposing hub," where more than 4,500 compounds have been tested in more than 500 cancer cell lines (Corsello et al., 2020). These large-scale drug-sensitivity datasets might allow the robust identification of response or resistance biomarkers to certain compounds, and compound mechanism of action (MOA) determination by correlation with CRISPR loss-of-function datasets. Thus, therapeutic hypotheses initially derived from the profiling of small cell-line sets should be immediately assessed in such datasets. Lastly, *in vitro* profiles are nearly always assessed using continuous compound exposure often over 48 to 72 h. In contrast, *in vitro* 10-h pulse dosing of HDM2 inhibitors revealed induction of apoptosis not seen in continuously exposed cells (Jeay et al., 2018). Such data raise the possibility that *in vitro* pulse profiling might result in differential cytotoxicity missed by continuous exposure.

New technologies to enable partial/temporal target inhibition in preclinical models

Since the complete and permanent knockout of pan-essential genes can lead to broad cytotoxicity, partial and temporal loss-of-function perturbations in preclinical models will be pivotal in defining the therapeutic indices of pan-essential inhibitors. Previously, such perturbations required the generation of tool compounds or even optimized drug candidates. Rapid generation of highly effective tool compound remains an indispensable aspect for target validation. However, the degree of optimization required for an informative tool compound can be challenging for many targets. For example, the phenotypic differences mentioned above elicited by pulsatile versus continuous HDM2 inhibition was not recognizable until a potent clinical candidate was developed (Jeay et al., 2018). The discovery of ligand-mediated degrons, including the auxin-inducible degron (Zhang et al., 2015), Small-Molecule-Assisted Shutoff (SMASh) tag, CRBN-recruiting dTAG (Nabet et al., 2018), and VHL-recruiting dTAG (Nabet et al., 2020), has enabled conditional and temporal control of protein degradation and to some degree might bypass the need for tool compounds. Here, the fusion of degrons to an endogenous pan-essential protein of interest by CRISPR-mediated locus-specific knockin will allow conditional controlled perturbation of the target protein in cell lines, xenografts (Nabet et al., 2018, 2020; Natsume et al., 2016), and genetically engineered mice (Banaszynski et al., 2008), and could enable direct assessment of efficacy and toxicity much earlier in the drug-discovery process.

Similarly, using CRISPR technology to induce partial rather than complete loss of function may be advantageous. To this end, engineered panels of mismatch "attenuated" sgRNAs were shown to establish differentially graded gene expression outputs through a dCAS9-based CRISPRi system (Jost et al., 2020). These and other technologies are likely to be of enormous benefit in validating specific hypotheses pertaining to pan-lethal targets.

Biomarker identification through expanded preclinical models

Biomarkers that enable patient stratification can maximize the therapeutic benefit by reducing the non-responder population. For MEK1/2 inhibitors, BRAF mutant cancer cell lines are more sensitive than BRAF wild-type cell lines. For CDK4/6 inhibitors, hormone receptor (HR)-positive breast cancers show increased sensitivity and often have frequent cyclin D1 amplification or CDKN2 inactivation (sensitive biomarkers) but infrequent RB (resistance biomarker) inactivation. These features, at least in part, account for the clinical success of these therapeutics despite their relatively broad requirement for cell viability (Álvarez-Fernández and Malumbres, 2020). For the more potent pan-essential therapeutics, it has been nearly impossible to discover such markers prior to clinical development. The application of novel preclinical models may help with this challenge.

PRISM is a pooled mixture of more than 750 barcoded CCLE cell lines (Yu et al., 2016) enabling efficient compound testing across many preclinical models simultaneously. This approach can be used to identify the anti-tumor MOA of previously reported compounds and confirm the MOA of lead compounds (Corsello et al., 2020; Li et al., 2019; You et al., 2020). Thus, PRISM might help overcome the throughput and cost limitations of large-scale cell-line profiling.

As noted earlier, cell-line models might have a limited ability to discern a range of sensitivities for pan-lethal target inhibitors.

Thus, in addition to cancer cell lines, scalable patient-derived xenografts (PDXs) and organoids might expand the preclinical oncology model repertoire for responder versus non-responder stratifications by enabling testing at tolerated doses (in the PDX setting) and enabling the profiling of tumor types that are under-represented in cell-line space. Large-scale efforts such as the PDX encyclopedia (PDXE) and ProXe, as well as several organoid biobanks, provide both resources and the conceptual framework for future scaling-up and compound screens (Dijkstra et al., 2018; Gao et al., 2015; Townsend et al., 2016). For example, in the PDXE dataset chemotherapy drugs such as paclitaxel and abraxane show differential and limited responses across PDXs of different lineages. Notably, this is substantially different from the pan-lethal profiles of the same drug in *in vitro* cell-line screens. Future expansions of patient-derived cancer models might provide new opportunities for biomarker identification of drug candidates with pan-essential properties.

Dosing and schedule optimization
It is likely that most therapies directed at pan-essential genes will be administered intermittently. Hence, we should not predicate drug-discovery efforts on the default assumptions of continuous and/or oral administration. Instead, exploring different dosing strategies based on PK, pharmacodynamic, and toxicology considerations should precede the commitment to a given PK profile. While rodents and dogs are unlikely to provide an exact guide for the human PK and schedule, the pharmacologic parameters most closely associated with efficacy and toxicity can be elucidated—for example, determining whether efficacy or toxicity is driven by C_{max}, area under the curve (AUC), or the duration of exposure over a C_{min} value.

For certain targeted therapies whereby toxicity was dose limiting, alternative dosing strategies or PK profiles have also improved the TIs. Palbociclib and ribociclib adopted 3-week-on/1-week-off dosing to mitigate myelotoxicity (Klein et al., 2018) while the flat PK profile of trametinib with a peak-to-trough ratio of 1.8 resulted in exposures falling within a narrow tolerated range of MEK inhibition (Infante et al., 2012). Unfortunately, insufficient attention is paid to the preclinical testing of these parameters, and instead trial and error typically takes place during clinical development, ultimately delaying the discovery of an optimal regimen or leading to clinical failure due to intolerability.

Formulation optimization or enhanced tumor delivery
Nanoparticle formulations for chemotherapy agents have been actively explored to increase the TI of chemotherapeutic agents, and several have gained FDA approval (Shi et al., 2017). In general, the goal is to enhance tumor exposure over normal tissue. However, it should be noted that simply modulating tissue exposure away from the most sensitive normal tissue might be sufficient to substantially improve the TI of a drug (e.g., bone marrow sparing). Interestingly, a nanoparticle formulation of AURKB inhibitor barasertib (AZD1152) is being tested in phase 1 (NCT03217838) based on preclinical data showing improved anti-tumor activity and reduced bone marrow toxicities in preclinical AML models (Ashton et al., 2016). Despite the opportunities to improve TI, many chemotherapy nanoparticle formulations have failed to improve clinical outcomes (Shi et al., 2017). The preclinical testing of such formulations remains largely fixated on bulky tumor xenografts that are unlikely to model the human tumor microenvironment. In this setting, formulations with low volumes of distribution (a characteristic of formulations confined to the vascular space) are likely to simply leak into the xenograft and appear to have dramatically improved TIs, which then fail to translate to the clinical setting. Therefore, investigators should consider formulation testing in lower volume dispersed or orthotopic models. While tail vein and portal vein injections are not true models for lung or liver metastasis, they nonetheless result in multiple small-volume tumors and are likely better for detecting meaningful improvements in drug distribution.

Recent developments in novel therapeutic modalities have also enabled the selective delivery of low-TI therapeutics (cytotoxic drugs or immune cells) to tumor cells. Antibody-drug conjugates (ADCs) combine antibodies targeting antigens abundant on tumor cell surfaces with highly potent cytotoxic drugs, allowing the tumor-specific delivery of cytotoxic drug and minimizing toxicities to normal cells. Four ADCs have obtained FDA approval, followed by more than 60 ongoing clinical trials of ADCs in many different tumor types (Khongorzul et al., 2020). In the ADC space, dose-limiting toxicities are still driven by off-target side effects, thus antibody-based delivery of cytotoxic therapeutics requires further optimization. In the immune oncology space, the tumor-specific delivery of cytotoxic T cells and natural killer cells by engineered chimeric antigen receptors (CARs) or bispecific antibodies similarly exploits surface antigens that are selectively abundant on tumor cells, sometimes at the expense of dispensable lineage normal cells (e.g., CD19 and CD20 on B cell acute lymphoblastic leukemia cells and normal B cells, B cell maturation antigen on multiple myeloma cells and normal plasma cells). These new modality therapeutics provide exciting future directions to increase the TIs of therapeutics often considered too toxic to administer systematically.

Combinations with highly selective therapeutics
Combination strategies might ultimately provide a path to significantly improving the TI of therapeutics targeting pan-essential genes. A common pitfall in the development of HDAC and PLK1 inhibitors was the combination with other low-TI drugs leading to marked increases in serious AEs (e.g., HDAC inhibitors with bortezimib). An alternative approach for low-TI therapies would be to prioritize combinations with high-TI drugs. Three CDK4/6 inhibitors are approved in metastatic ER$^+$/HER2$^-$ breast cancer in combination with endocrine therapy whereby the pivotal trials consistently showed >60% improvement in the median PFS (Klein et al., 2018). Similarly, three MEK1/2 inhibitors (trametinib, cobimetinib, and binimetinib) are approved in combination with BRAF inhibitors to treat BRAF-mutant melanoma, again delivering significant clinical benefits (Yaeger and Corcoran, 2019). In these examples, although CDK4/6 and MEK1/2 double knockouts show a pan-lethal effect and CDK4/6 and MEK1/2 inhibitors are broadly acting, when the inhibitors are combined with either tissue-specific therapeutics (e.g., fulvestrant) or mutant-selective agents (BRAF inhibitors) (Im et al., 2019; Long et al., 2015; Robert et al., 2019) the additive or synergistic activity is confined to the tumor, thus resulting in a better than expected TI for the combination. This strategy could also be beneficial in creating barriers to the development of

acquired resistance. For example, AURKA and AURKB can be resistance nodes to third-generation EGFR inhibitors in NSCLC models (Bertran-Alamillo et al., 2019; Shah et al., 2019), and AURKA appears to be a node for KRAS^{G12C}-specific inhibitor resistance (Xue et al., 2020). Thus, the combination of aurora kinase inhibitors with EGFR or KRAS^{G12C} inhibitors in patients with such mutations could provide clinical benefit in the future. Lastly, in indications such as microsatellite instability cancers where PD-1/PD-L1 antagonists have biomarker-driven anti-cancer activity, combinations with pan-essential inhibitors again might shift the TI in a more favorable direction.

CONCLUSION

The high failure rate in oncology drug development has impeded our efforts to efficiently bring new therapeutics to cancer patients. The 5.1% success rate from phase 1 to FDA approval is far from optimal, yet we can learn from these prior experiences. From the case studies in this review, it is apparent that not all cancer targets are created equal. Developing therapeutics that target pan-essential genes requires careful target prioritization and validation, biomarker identification, pharmacokinetic optimization, and combination strategies to increase the TI. While it is obvious that testing another pan-HDAC inhibitor monotherapy in another non-stratified solid tumor indication should be avoided, implementing new strategies to improve our current drug development steps is not trivial. Hence, in addition to the mindset shift, future research innovations including extending genomic and functional datasets to normal lineages, new genetic technologies for partial loss-of-function experiments, advances in cancer preclinical models, and comprehensive drug combination assessment in preclinical settings will be pivotal to further optimizing drug-discovery efforts to benefit cancer patients. Finally, future basic science efforts to thoroughly understand the fundamental biology of pan-essential genes, and their specific involvements in different cancer types, will also benefit the discovery of the next generation of cancer therapeutics.

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