Perspective
An expanded universe of cancer targets

William C. Hahn,[1,*] Joel S. Bader,[2] Theodore P. Braun,[4] Andrea Califano,[3] Paul A. Clemons,[10] Brian J. Druker,[4] Andrew J. Ewald,[16] Haian Fu,[5] Subhashini Jagu,[13] Christopher J. Kemp,[6] William Kim,[11] Calvin J. Kuo,[7] Michael T. McManus,[8] Gordon B. Mills,[9] Xiulei Mo,[5] Nidhi Sahni,[15] Stuart L. Schreiber,[10] Jessica A. Talamas,[1] Pablo Tamayo,[11] Jeffrey W. Tyner,[14] Bridget K. Wagner,[10] William A. Weiss,[12] Daniela S. Gerhard,[13] and the Cancer Target Discovery and Development Network

[1]Dana-Farber Cancer Institute, Department of Medical Oncology, 450 Brookline Avenue, Boston, MA, USA
[2]Department of Biomedical Engineering and Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, USA
[3]Department of Systems Biology, Biomedical Informatics, Biochemistry and Molecular Biophysics, and Medicine, Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY, USA
[4]Knight Cancer Institute and Division of Hematology and Medical Oncology, Oregon Health & Science University, Portland, OR, USA
[5]Department of Pharmacology and Chemical Biology, Emory Chemical Biology Discovery Center, and Winship Cancer Institute, Emory University, Atlanta, GA 30322, USA
[6]Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
[7]Hematology Division, Stanford University School of Medicine, Stanford, CA, USA
[8]Department of Microbiology and Immunology, UCSF Diabetes Center, and Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA, USA
[9]Department of Cell, Development and Cancer Biology, Knight Cancer Institute, Oregon Health and Sciences University, Portland, OR, USA
[10]Broad Institute, 415 Main Street, Cambridge, MA, USA
[11]Moores Cancer Center, Center for Novel Therapeutics and Department of Medicine, UC San Diego, La Jolla, CA, USA
[12]Departments of Neurology, Neurological Surgery, Pediatrics, and Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA, USA
[13]Office of Cancer Genomics, Center for Cancer Genomics, National Cancer Institute, NIH, Bethesda, MD, USA
[14]Knight Cancer Institute, Oregon Health & Science University and Department of Cell, Developmental and Cancer Biology, Oregon Health & Science University, Portland, OR, USA
[15]Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Smithville, TX, USA
[16]Department of Cell Biology, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, USA
*Correspondence: william_hahn@dfci.harvard.edu
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SUMMARY

The characterization of cancer genomes has provided insight into somatically altered genes across tumors, transformed our understanding of cancer biology, and enabled tailoring of therapeutic strategies. However, the function of most cancer alleles remains mysterious, and many cancer features transcend their genomes. Consequently, tumor genomic characterization does not influence therapy for most patients. Approaches to understand the function and circuitry of cancer genes provide complementary approaches to elucidate both oncogene and non-oncogene dependencies. Emerging work indicates that the diversity of therapeutic targets engendered by non-oncogene dependencies is much larger than the list of recurrently mutated genes. Here we describe a framework for this expanded list of cancer targets, providing novel opportunities for clinical translation.

INTRODUCTION

The majority of recently approved molecularly targeted cancer drugs are specific for oncoproteins encoded by somatically mutated genes. These agents include proteins essential for the survival of specific cell lineages (e.g., estrogen receptor and androgen receptor), or proteins that inhibit immune responses such as programmed cell death protein 1 (PD-1). The strong association of these genes with particular tumor subtypes or with immunological phenotypes led to the discovery of small molecules and antibodies specific for these targets. Although large-scale efforts to sequence cancer genomes facilitated the identification of recurrent somatically altered cancer genes, decipher-

ing the deluge of information from these efforts created new challenges for cancer target discovery. In prior work, the Cancer Target Discovery and Development (CTD²) Network defined criteria to assess the strength of evidence supporting a specific target (Cancer Target and Development, 2016), allowing the prioritization of drivers that play a direct role in tumor initiation, maintenance or metastasis. Work from the network using this framework has facilitated the translation of discoveries in experimental models to clinical trials designed to test whether modulating the activity of specific targets leads to clinical responses (Table 1).

In addition, systematic functional studies performed by the CTD² Network and others have identified new categories of

Table 1. List of clinical trials initiated based on the CTD² Network research findings (two different CTD² centers are based at UCSF)

	CTD² center	ClinicalTrials.gov identifier	Cancer type	Intervention/treatment
1	Columbia University	NCT04028245	Clear cell renal cell carcinoma	SPARC1 (Spartalizumab and Canakinumab)
2	Columbia University	NCT03211988	Gastroenteropancreatic neuroendocrine tumors	Entinostat (deacetylase inhibitor)
3	Columbia University	NCT02066532	HR-/HER2+ breast cancer	Ruxolitinib (JAK inhibitor) + trastuzumab (HER2 receptor antagonist)
4	Columbia University	NCT02632071	Inflammatory breast cancer	Ricolinostat (HDAC6 inhibitor) + nab-paclitaxel (mitotic inhibitor)
5	Columbia University	NCT03951831	Metastatic prostate cancer	PRIME-CUT (REGN2810 + Degarelix + Leuprolide Acetate + Docetaxel)
6	Columbia University	NCT04301414	Prostate cancer	NEO-RED-P (BMS-986218 and Degarelix + Degarelix)
7	DFCI	NCT03654716	Rhabdoid tumors	ALRN-6924 (MDM2/MDMX inhibitor)
8	Emory University	NCT04348292	Lung non-small cell carcinoma	(Durvalumab + Sirolimus)
9	FHCRC	NCT02508246	Head and neck squamous cell carcinoma	MK-1775 (WEE1 inhibitor) + docetaxel (anti-microtubule agent) + cisplatin (alkylating agent)
10	OHSU	NCT03557970	Acute myeloid leukemia	JNJ-40346527 (inhibitor of CSF1R)
11	OHSU	NCT03874052	Acute myeloid leukemia	Ruxolitinib (JAK inhibitor) + venetoclax (Bcl2 inhibitor)
12	UCSD	NCT03841110	Solid tumors	FT500 (iPSC-derived natural killer cells)
13	UCSF (1)	NCT01402284	Multiple myeloma	Carfilzomib (proteasome inhibitor) + lenalidomide (angiogenesis inhibitor) + dexamethasone (anti-inflammatory)
14	UCSF (1)	NCT04085315	Non-small cell lung cancer—EGFR mutant	Osimertinib (EGFR inhibitor) + alisertib (aurora kinase inhibitor)
15	UCSF (2)	NCT02947165	Squamous cell carcinomas	NIS 793 (anti-TGF beta antibody) + PDR 001 (anti-PD-1 antibody)

cancer targets beyond those inhibited by approved cancer drugs. Indeed, the number and diversity of these putative cancer targets far exceed oncogenes identified to date. This expanded repertoire of targets can be classified as tumor cell autonomous (*intrinsic*) or microenvironment-mediated (*extrinsic*) (Figure 1). Tumor intrinsic factors include mutated oncogenes, epigenetic changes, transcriptional and signal transduction dysregulation, aberrant pathway and metabolic activity, and changes in DNA damage responses (DDRs). Together the effects of oncogenes as well as cell state change results in stress responses that require mitigation strategies for the tumor to survive (Luo et al., 2009), often eliciting oncogene dependency (Weinstein, 2002). In particular, tumors that harbor these oncogenes often exhibit cell death when signaling induced by these oncogenes is extinguished, a phenomenon referred to as oncogene addiction. These cell state changes may introduce therapeutic vulnerabilities that can be exploited through synthetic lethal strategies as well as emergent therapeutic opportunities due to regulatory, signaling, and metabolic network rewiring. Tumor extrinsic factors include changes in the tumor microenvironment (TME) and alterations in the differentiation state and composition of tumor-infiltrating stromal cells, such as immune cells and fibroblasts. At the organismal level, aberrant endocrine signals and the microbiome impact cancer pathogenesis and response to therapy. Intrinsic and extrinsic factors can affect cancer cell plasticity and their persistence during therapy. In this perspect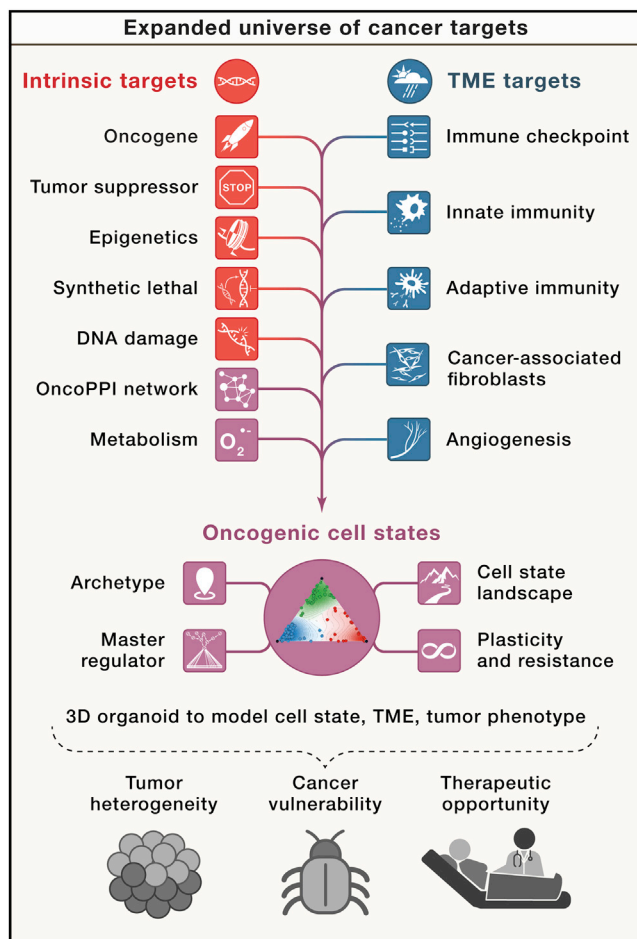ive, we describe a growing repertoire of intrinsic and extrinsic classes of targets as well as therapeutic opportunities.

TUMOR INTRINSIC TARGETS

Oncogene addiction

The initial drugs targeting oncogenic tyrosine kinases showed remarkable efficacy. For example, Imatinib (Gleevec) transformed the treatment of chronic myeloid leukemia (CML) due to the robustness of BCR-ABL as a driver oncogene in CML (Braun et al., 2020). Other success stories include epidermal growth factor receptor (EGFR)-, ALK-, or ROS1-driven lung cancers (Arbour and Riely, 2019); KIT mutant gastrointestinal stromal tumors (Bannon et al., 2017); and ERBB2 in HER2+ breast cancer (Waks and Winer, 2019). However, oncogenic signaling in solid tumors is genetically complex, and empirical experience suggests limited activity for a majority of drugs blocking any given individual oncogene aberrantly expressed in solid tumors.

As an example of genetic complexity driving oncogenic signaling in solid tumors, aberrant tumorigenic protein function arises from deleterious events in coding genes, such as point mutations, fusions, translocations, copy-number changes, methylation, and transcriptional dysregulation. Gene copy-number alterations, among the most prevalent somatic mutational events in human cancers (Beroukhim et al., 2010; Stratton et al., 2009), are fundamental drivers for tumor initiation and evolution. Chromosomal rearrangements produce deleterious

Expanded universe of cancer targets

Intrinsic targets
- Oncogene
- Tumor suppressor
- Epigenetics
- Synthetic lethal
- DNA damage
- OncoPPI network
- Metabolism

TME targets
- Immune checkpoint
- Innate immunity
- Adaptive immunity
- Cancer-associated fibroblasts
- Angiogenesis

Oncogenic cell states
- Archetype
- Master regulator
- Cell state landscape
- Plasticity and resistance

3D organoid to model cell state, TME, tumor phenotype

- Tumor heterogeneity
- Cancer vulnerability
- Therapeutic opportunity

Figure 1. Categories of cancer targets
Recent studies have identified an expanded universe of cancer targets that include both tumor intrinsic targets and extrinsic targets including components of the TME. Among growing number of emerging targets (in purple), the concept of oncogenic cell states captures the multi-dimensional complexity of a tumor with dynamic transition states, underlying regulatory mechanisms, and subpopulation-dependent plasticity that defines cellular phenotype and therapeutic response. These new targets are the consequence of tumor heterogeneity and represent non-oncogene-dependent cancer vulnerabilities, which inform new types of therapeutic opportunities.

events including creation of fusion genes with novel functions, altered gene dosage to levels that drive regulatory pathway activity toward cancer progression (Tsherniak et al., 2017), or translocations that change physiologic regulation of gene expression. Aneuploidy is also frequent, yet it recapitulates similar issues as large-scale paralog formation, making it difficult to unravel bystander passenger events acquired during cancer evolution from bona fide driver events that cause cancer or result in more aggressive tumors.

Dysregulated signaling represents an important conserved oncogenic mechanism. For example, RAS is mutated in ~19% of all human cancers (Prior et al., 2020). In addition, RAS is activated by mutation of tyrosine kinases and RAS regulatory proteins (NF1, SOS1, PTPN11). This pathway is also activated by the mutation of downstream mediators (BRAF, RAF1). Therapeu-

tic inhibition of RAS signaling has proven difficult due to the challenging biochemical properties of RAS itself and redundancies in downstream signaling. However, recent work has renewed enthusiasm for direct RAS inhibition, including approaches that target RAS dimerization or drugs that covalently bind G12C mutant RAS, locking it in a guanosine diphosphate (GDP)-bound inactive state (Moore et al., 2020). In contrast, targeting MEK and other downstream proteins in the RAS signaling cascade has shown limited efficacy in RAS-driven malignancies, likely owing to dose-limiting toxicity and loss of auto-inhibitory feedback. Inhibitors of BRAF have shown marked, albeit temporary, efficacy in *BRAF* mutant melanoma. Both tyrosine kinase and RAS pathway mutations also result in activation of other downstream, druggable signaling pathways such as phosphoinositide-3 kinase (PI3K). PI3K can also be activated by direct mutations of *PIK3CA*, and inhibitors of PI3K or downstream mediators AKT or mTOR have shown variable efficacy against cancers with *PIK3CA* or upstream mutations (Cox et al., 2014; Gysin et al., 2011).

Oncogene amplification has proven challenging to target. The transcription factors MYC and MYCN, for instance, are recurrently amplified or translocated in a subset of cancers, and the MYC network is dysregulated in a significant percentage of human cancers (Dang, 2012; Schaub et al., 2018). Therapeutic targeting of MYC has been challenging due to the lack of enzymatic activity or suitable binding sites for drugs. Indirect targeting of MYC via BRD4 inhibition, regulators of MYC stability, and inhibition of downstream interacting kinases are important areas of investigation. Other approaches to reverse effects of amplified genes, such as targeting proteins for degradation or use of CRISPR, small interfering RNA (siRNA), and antisense therapeutics, remain of clinical and conceptual interest.

Epigenetic regulators are also mutated recurrently in cancer. While challenging to target as a class, an increasing number are becoming tractable. For example, two DNA methyltransferase (DNMT) inhibitors, azacytidine and decitabine, are approved for the treatment of myelodysplastic syndrome. Numerous drug-development efforts are focused on other recurrently mutated epigenetic regulators, either via direct inhibition of enzymatic activity (i.e., EZH2) or immediate downstream dependencies (MLL-Menin or MLL-DOT1L). However, the broad actions of epigenetic regulators on many genes raise questions regarding the therapeutic index for these inhibitors.

Tumor suppressor rescue
Loss of tumor suppressor genes occurs commonly in cancer, and several approaches to target these loss-of-function events are now in development. For example, loss-of-function mutations in the ubiquitin ligase *CBL* result in increased signaling through a number of tyrosine kinases. Similarly, mutations in *PTEN,* which inactivate phosphatase activity, increase PI3K signaling. In these cases, pharmacological targeting of the activated pathway, rather than the aberrant protein itself, holds promise. Targeting mutant TP53 with drugs that stabilize its three-dimensional (3D)-structure and restore its normal function has also emerged as a promising approach (Levine, 2019). More generally, a variant-directed protein-protein interaction inducer screen identified small molecules that restored interactions lost

as a result of tumor suppressor mutations (Tang et al., 2020). This study identified a bisindolylmaleimide derivative that restored interaction between mutated SMAD4 and SMAD3 and reactivated the cell growth suppression function of the SMAD4/SMAD3 complex. Collectively, these approaches offer versatile strategies to target many tumor suppressor mutations.

Synthetic lethal targets

Experimental approaches from yeast genetics (Hartwell et al., 1997) led to screens that identify proteins whose activities are essential for cancers harboring specific oncogenic mutations. Synthetic lethality allows targeting of cancers that harbor mutations in undruggable proteins with potential to improve therapeutic index. The success of poly (ADP-ribose) polymerase (PARP) inhibition in the setting of BRCA1 and BRCA2 deficiency, for instance, provided an important proof of principle (Lord and Ashworth, 2017). Inhibition of PARP capitalized on defects in DNA repair induced by loss of BRCA1/2 and other homologous recombination DNA repair mediators, leading to mitotic catastrophe. One mechanism of resistance to PARP inhibition is the reversion of BRCA1 to produce a wild-type protein, confirming a direct relationship between BRCA1 mutation and PARP inhibitor sensitivity (Lord and Ashworth, 2017). Recent genome-scale genetic and small-molecule screens identified several new synthetic lethal combinations, including the finding that SRC and BCL2 family inhibitors cooperate, the helicase WRN is necessary for the survival of tumors exhibiting microsatellite instability (Chan et al., 2019), and BET inhibition synergizes with PARP inhibitors (Lui et al., 2020).

Recent studies have defined new classes of synthetic lethal interactions. As predicted by Elledge and coworkers (Luo et al., 2009), some of these synthetic lethal genes are required to suppress cell-stress mechanisms induced by particular oncogenes. For example, CSNK1E is required for the survival of MYC-amplified tumors (Toyoshima et al., 2012). Additional synthetic lethal opportunities are created when essential genes are located near tumor suppressors. The copy-number alterations yielding cancer liabilities owing to partial loss (CYCLOPS) (Nijhawan et al., 2012) genes are never deleted homozygously, and gene expression closely matches copy number. Inhibition of CYCLOPS genes induces cell death only in cells that lack the corresponding tumor suppressor gene due to copy-number loss. For example, PRMT5 is required for survival of tumors that lose MTAP, which is frequently lost due to its proximity to the commonly deleted tumor suppressor gene CDKN2A (Mavrakis et al., 2016; Kryukov et al., 2016). CYCLOPS genes are currently the most common copy-number-associated gene dependency (Tsherniak et al., 2017) and likely represent a general paradigm in cancer synthetic dependencies.

Finally, synthetic essentiality also occurs when deletion of one paralog or family member renders the cell dependent on the remaining one. For example, tumors that harbor deletions of ENO1 require expression of ENO2 (Muller et al., 2012). A recent interrogation of cancer genomes identified 87 instances for which functional loss of one paralog was associated with a dependency on another paralog (Tsherniak et al., 2017). Identifying and characterizing paralog dependencies may reveal new genes that are sensitive to expression dosage, uncovering new targetable de-

pendencies. Similarly, in cancer cell lines, mutation of ARID1A confers sensitivity to knockdown of the paralog ARID1B, while superimposed loss of ARID1B in ARID1A mutant cells increases radiosensitivity (Helming et al., 2014; Niedermaier et al., 2019).

DNA damage response

Unrepaired cellular DNA damage results in mutations or altered chromatin structure, events that drive the initiation and progression of tumors. Deleterious events in genes that mediate DDR are frequent across multiple tumor lineages. Thus, understanding DDR and its clinical impact is critical to improving cancer outcomes. Defects in DDR cause genomic instability that promotes oncogenesis, so accurate annotation contributes to early detection and cancer prevention. DDR deficiencies can also sensitize cells to specific therapeutic cancer regimens and are associated with responses to conventional chemo and radiation treatments (Brown et al., 2017). As noted above, mutations in the BRCA1/2 genes result in synthetic lethality for PARP inhibitors.

Despite systematic identification of genes critical for DDR function and drug sensitivity, many observed mutations have not been assessed for functional, therapeutic, and clinical relevance. It is thus difficult to predict whether a mutation highlights a critical dependency or lacks therapeutic relevance. Saturation mutagenesis, an approach to address this challenge, identified BRCA1 and PARP1 mutations as inducing protein function loss (Findlay et al., 2018) or PARP inhibitor resistance (Pettitt et al., 2018), respectively. Identifying downstream effects caused by DDR remains another promising approach.

EMERGING CANCER TARGETS

Protein-protein interactions

Oncogenic missense mutations can alter protein-protein interactions and drive cancer progression. Thus, identifying and characterizing critical nodes and hubs in cancer-related protein-protein interaction networks, which control the output of oncogenic programs, may reveal unique opportunities for therapeutic intervention. In particular, the systematic interrogation of new protein-protein interactions driven by mutant oncoproteins may reveal new types of cancer targets that are cancer specific.

For example, the cancer-associated protein-protein interaction network (OncoPPi) focuses on experimentally generated interactions among proteins with known or likely involvement in cancer (Li et al., 2017b). In contrast to interactomes established by proteomics approaches, such as immunoprecipitation mass spectrometry, OncoPPi presents binary interactions of cancer-related proteins derived from a proximity-based biosensor to reflect direct interactions and, coupled with genomics, clinical, and pharmacological information, facilitates therapeutic targeting. OncoPPi reveals prominent protein-interaction hubs with new partners and suggests novel mechanisms of action for major oncogene drivers, such as MYC with NSD3. Importantly, OncoPPi uncovered interactions for non-enzymatic proteins, offering potential intervention strategies by perturbing their interactions to target those once "undruggable" classes of proteins. Similarly, efforts to comprehensively map protein-protein interactions in human cells (Huttlin et al., 2017) or in specific oncogenic contexts provide new ways to identify protein-protein

interactions and complexes involved in cancer phenotypes. For structure-based prediction of protein-protein interactions, the PrePPI algorithm and database (Zhang et al., 2012) offers a valuable resource for the scientific community.

Metabolic vulnerabilities

The rapid proliferation of cancer cells requires extensive escalation of cellular catabolic and anabolic metabolism to meet energy and structural needs (Li et al., 2019). For example, it has long been recognized that expanded nucleotide synthesis leads to sensitivity to nucleoside analogs including 5-fluorouracil (5-FU), gemcitabine, and cytarabine (Wagner et al., 2007), while elevated glucose uptake (part of the Warburg effect) enables tumor imaging using fluorodeoxyglucose positron emission tomography (FDG-PET) (Zhu et al., 2011). Recently, significant improvements in metabolomics and isotope tracing (Jang et al., 2018) propelled the discovery of many other metabolic alterations in cancer. While most metabolic changes are neutral, or only slightly modify cancer cell fitness under stress (Vander Heiden and DeBerardinis, 2017), certain pathways are essential for the progression of selected cancers and can be exploited therapeutically.

Despite the general increase in glucose uptake and consumption in tumors, direct inhibition of aerobic glycolysis using glucose mimetics or inhibitors of pyruvate kinase has seen limited preclinical and clinical success (Cortés-Cros et al., 2013; Raez et al., 2013). In addition to their dependence on glucose metabolism, cancer cells are also dependent on the uptake or *de novo* biosynthesis of a subset of amino acids, including glutamine (DeBerardinis et al., 2007), glycine (Jain et al., 2012), serine (Kim et al., 2015; Piskounova et al., 2015), and aspartate (Sullivan et al., 2015; Sullivan et al., 2018) in a context-, lineage-, and oncogene-dependent manner. These amino acids not only contribute to protein synthesis, but also to the biosynthesis of other essential metabolites, such as pyrimidines, purines, phospholipids, glutathione, and regeneration of NADPH, as approaches to limit toxicity from reactive oxygen species (ROS) (Luengo et al., 2017).

Certain tumors contain high levels of polyunsaturated lipids. Due to limited *de novo* fatty acid biosynthesis in normal tissues other than the liver, adipose tissue, and lactating breast (Menendez and Lupu, 2006), lipid metabolism in tumors is thus an attractive target for therapeutic intervention because it modulates fatty acid uptake, synthesis, unsaturation, and incorporation into structural lipids (Viswanathan et al., 2017). For example, the tumorigenic potential of ovarian cancer stem cells is dependent on SCD1 (Li et al., 2017a), whereas metastasis-initiating cells are reliant on fatty acid receptor CD36-mediated fatty acid uptake (Pascual et al., 2017). While lipids are often available through regular diet, pharmacological inhibition of fatty acid synthesis may need to be combined with dietary interventions to generate a therapeutic window. One of the major challenges in targeting lipid metabolism is our limited understanding in the plasticity of cancer lipidome.

More recently, mutations in isocitrate dehydrogenase 1 and 2 (*IDH1, IDH2*), which are frequent in myeloid malignancies, glioma, chondrosarcoma, cholangiosarcoma, and hepatocellular carcinoma (Tommasini-Ghelfi et al., 2019), direct production of the oncometabolite 2-hydroxyglutarate (2-HG), which inhibits the demethylation of DNA, leading to gene silencing. This neomorphic enzymatic activity can be blocked by drugs that inhibit 2-HG production.

In addition, it has long been appreciated that some tumors aberrantly express specific metabolites, such as serine. In some tumors, increased expression of the serine synthesis enzyme phosphoglycerate dehydrogenase (PHGDH) results from copy-number gain of a region on chromosome 1p (Beroukhim et al., 2010; Locasale et al., 2011; Possemato et al., 2011), while other tumors exhibit increased serine levels as a consequence of oncogenic signaling, including NRF2 and ATF4 signaling (DeNicola et al., 2015) or hypoxia responses (Samanta et al., 2016). Suppressing serine metabolism in these tumor cells leads to tumor regression.

A class of synthetic lethal genes acts by targeting aberrant metabolism necessary for cancer-cell survival. For example, ODGH is required for the survival of tumors that harbor mutations in *PIK3CA* due to increased reliance on aspartate signaling (Ilic et al., 2017). Although targeting tumor metabolism requires a therapeutic index between tumor and normal tissue, this class of cancer targets remains promising.
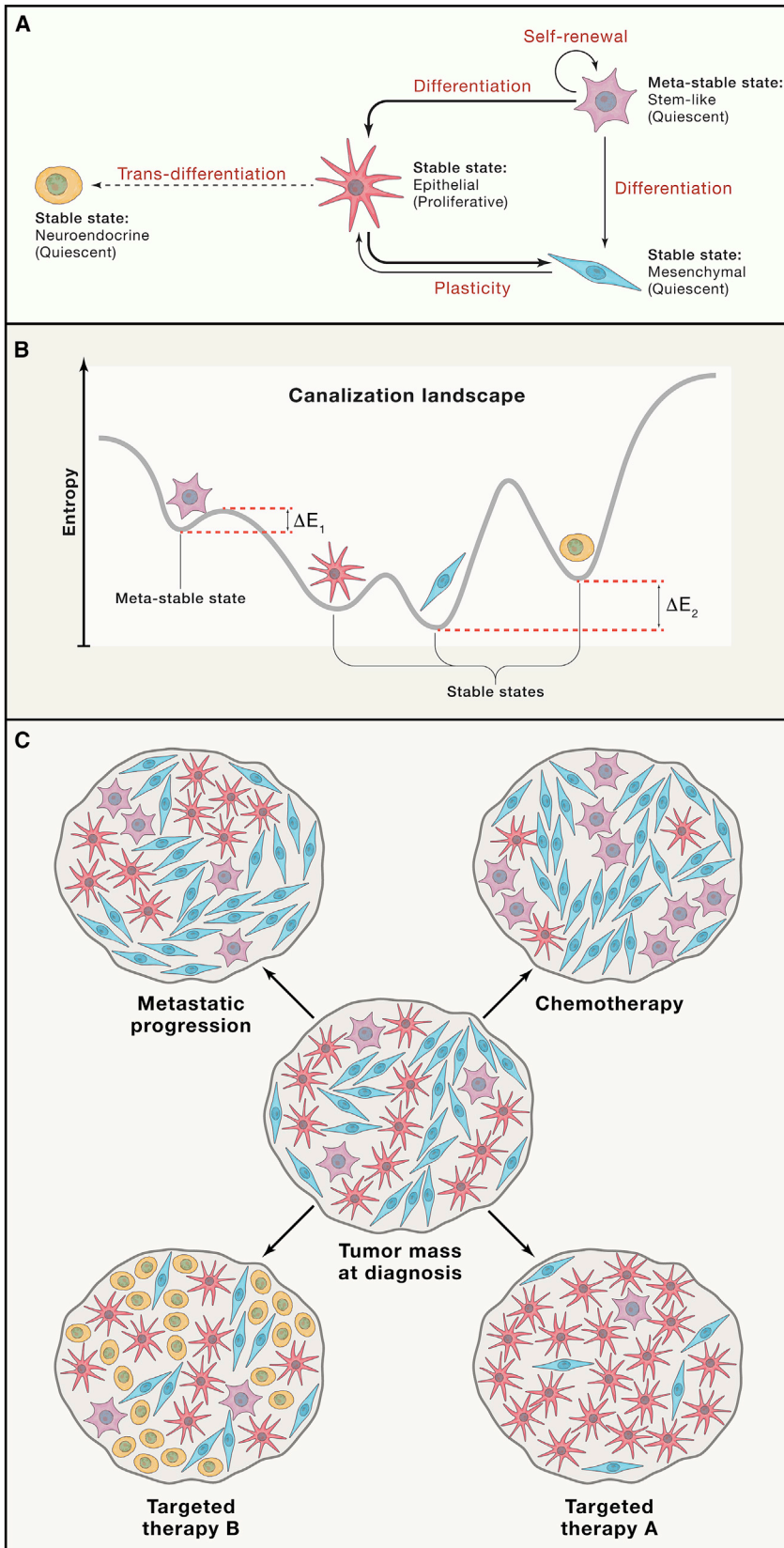
Cell states
Cell states, archetypes, and tumor checkpoints

The enabling technologies that represent the pillars of systems biology, such as molecular interaction networks and multivariate analysis, have led naturally to the concept of "cell states." Cell states represent high-dimensional vectors of variables that uniquely determine a cell's phenotype. For instance, a tumor may comprise multiple subcellular populations with distinct drug sensitivity, metabolism, or stemness characteristics. In that case, the cell state would be a vector that uniquely identifies the cells in each subtype. Thus, this paradigm is especially useful to account for the heterogeneity of primary tumors as *composites* that incorporate multiple, often highly plastic cellular and microenvironment states (Figure 2).

One paradigm to identify oncogenic cell states is to model them as reproducible configurations of pathway activation or repression, defined by transcriptional, dependency, or sensitivity profiles. The most common and representative configurations provide operational "archetypes" or "tumor checkpoint modules" (Califano and Alvarez, 2017) that help categorize cancers into *functional* taxonomies. While archetypes define the cell state itself, tumor checkpoints define regulatory protein modules comprising master regulator (MR) proteins responsible for mechanistically implementing and maintaining cell state. For example, the mesenchymal cell state of glioblastoma, which co-exists with isogenic proneural states at the single cell level (Neftel et al., 2019), was shown to be mechanistically induced by aberrant activation of a tumor checkpoint comprising three synergistic MR proteins—CEBPβ, CEBPδ, and STAT3 (Carro et al., 2010)—whose aberrant activation is induced mechanistically by specific mutations in upstream pathways, including focal *STAT3* amplifications, and homozygous *KLHL9* deletions (Chen et al., 2014).

Cell states can be stable, meta-stable, or transient. Stable states represent regions of the state space where cells are "trapped" for days—such as at the end stages of lineage development

Figure 2. Oncogenic targets and cell states
This schematic represents the coexistence of distinct yet isogenic malignant cell states within a tumor, each one presenting an equally distinct repertoire of non-oncogene dependencies. To avoid cluttering, TME-related cell states are not shown, even though they are involved in critical paracrine interactions with tumor cells.

(A) Schematic representation of isogenic tumor cells presenting with distinct transcriptional states and epigenetic profiles. These include (1) a low-proliferative (i.e., quiescent) meta-stable stem-like progenitor cell state capable of self-renewal and asymmetric differentiation, (2) two stable, differentiated cell states, persisting for long time periods, associated with either an epithelial (proliferative) or a mesenchymal (quiescent) cell phenotype, and (3) an additional neuroendocrine (quiescent) stable state that can only be achieved by drug-induced trans-differentiation. The size of the arrows illustrates the likelihood of transition from one cell state to another. Stem-like progenitors can differentiate into either epithelial or mesenchymal cells, which can plastically reprogram between these states, albeit at different rates, for instance as a result of epithelial mesenchymal transformation processes. The neuroendocrine state is not pathophysiologically accessible but can be reached via drug-mediated transdifferentiation from the epithelial cell state.

(B) Schematic representation of the canalization entropy landscape that underlies the possible cell states. Cells tend to move from a higher to a lower entropy state with a probability that is inversely proportional to the entropic barrier that separates them DE1 (i.e., height of the peak separating two adjacent valleys) and directly proportional to their differential entropy DE2 (i.e., differential depth of two adjacent valleys). For instance, an epithelial state cell can reprogram to a mesenchymal state cell because the entropic barrier between the two is low (plasticity) but the forward direction is more likely than the reverse one because the entropy of the mesenchymal state is lower.

(C) Schematic representation of the tumor composition changes following drug treatment or spontaneous progression. For simplicity, only four transitions are illustrated, including (1) metastatic progression, associated with an increased ratio of mesenchymal to epithelial cells but no change in the fraction of stem-like progenitors, (2) chemotherapy treatment, resulting in ablation of the proliferative epithelial state and increase of the stem-like progenitor and mesenchymal quiescent states, (3) targeted therapy A, inducing reduction of the stem-like progenitor and mesenchymal quiescent states and increase of the epithelial proliferative state, and (4) targeted therapy B, resulting in the emergence of a novel neuroendocrine state resulting from drug-induced transdifferentiation.

(Stadhouders et al., 2019; Arendt et al., 2016). In contrast, meta-stable states correspond to regions where cells are trapped for several hours, rather than days, on their way to a more stable state or following a transient perturbation. Finally, transient states represent the most sparsely populated regions of state space and are populated by cells rapidly transitioning between states. This concept has increased relevance for cancer biology and therapeutics because of the advent of methods allowing the direct observation of tumorigenesis, tumor plasticity, and adaptive resistance at the single-cell level. However, these advances are currently limited by the reliance on measuring gene expression (Grosse-Wilde et al., 2015; Tsoi et al., 2018). It is also important to provide a framework to interpret the results from studies of transitions within a complex "landscape" of states (Janes, 2016; MacLean et al., 2018; Neftel et al., 2019).

Both tumor progression (e.g., to metastasis) and sensitivity to therapeutic agents largely depend on the presence of specific stable and meta-stable cell states—both tumor- and microenvironment-related—rather than on tumor histology, genetics, and natural history of the tumor evolution (Kim et al., 2017). For instance, poor outcomes in breast cancer patients have been linked to a mixed epithelial-mesenchymal chimeric cell state (MacLean et al., 2018). Further, novel states were identified in acute myeloid leukemia (AML) that did not recapitulate traditional cell-surface markers but whose gene-expression signature was predictive of patient survival. Neuroendocrine tumors' cell states were also predictive of response to therapy (Alvarez et al., 2018). Finally, melanoma cell lines and tumors displayed a treatment-induced differentiation trajectory with four phases and an inverse relationship between differentiation progress and sensitivity to iron-dependent compound-induced oxidative stress (Viswanathan et al., 2017).

Cell states and master regulators

MR proteins represent the mechanistic regulators of transcriptional cell-state homeostasis. As such, MR activity analysis permits identification of both stable and meta-stable cancer-related cell states and cell-state transitions (Califano and Alvarez, 2017), as well as of proteins that can reprogram cell state when ectopically expressed. For example, ectopic co-expression of three computationally inferred prostate lineage MRs (AR, FOXA1, and NKX3.1) was sufficient to reprogram fibroblast to a competent prostate epithelium tissue (Talos et al., 2017), while shRNA-mediated co-silencing of computationally-inferred mesenchymal GBM MRs (CEBPβ and STAT3) was sufficient to reprogram mesenchymal cells to a proneural state and abrogate tumorigenesis *in vivo* (Carro et al., 2010). Candidate MR proteins can effectively be identified by network-based analysis of mRNA profiles, e.g., via the VIPER algorithm, which—akin to a multiplexed gene-reporter assay—measures enrichment of particular protein transcriptional targets in genes that are differentially expressed in a specific cell state. MR-based studies have identified novel, experimentally validated drivers and mechanisms in malignancies ranging from lymphoma, neuroblastoma, and glioma to prostate, breast, and gastroenteropancreatic neuroendocrine tumors (Califano and Alvarez, 2017).

Elucidation of the cellular logic responsible for the implementation and maintenance of cancer cell states has identified tightly autoregulated modular structures (tumor checkpoints), which comprise a small number of MR proteins working together to maintain cancer cell-state homeostasis by integrating the effect of genetic and epigenetic alterations in their upstream pathways (Paul et al., 2019). Indeed, genetic or pharmacological inhibition of tumor checkpoint MRs leads to their collapse and loss of cancer viability *in vitro* and *in vivo* (Alvarez et al., 2018), providing a means to abrogate the effects of multiple genetic alterations.

Cell-state plasticity and resistance

Tumor-cell plasticity and adaptive responses to therapeutic stress play key roles in acquired drug resistance. Drug-tolerant cancer persister cells are a prime example. Found within a wide range of tumor types, persister cells are proposed to arise as a result of stochastic processes (Kupiec, 1997), introducing transient phenotypic heterogeneity into tumor-cell populations (Raj and van Oudenaarden, 2008). For example, non-genetic transcriptional variability within cell populations may identify cells likely to resist drug treatment in melanoma (Shaffer et al., 2017). In general, persister cells share common features with the stress mitigation pathways of myofibroblastic non-cancer cells under stress conditions other than those induced by cancer treatments (see Cancer-associated fibroblasts). They possess (1) a distinct chromatin state, (2) increased stemness, and (3) a switch in lipid content from highly saturated and monounsaturated to highly polyunsaturated lipids and phospholipids. Persister cells often implement a more quiescent state to escape chemotherapeutic stress and can in some cases become resensitized to treatment upon drug withdrawal, pointing toward a non-genetic mechanism of drug tolerance. They can maintain this pro-survival state for weeks to months of drug treatment. However, in some patients, a subset of persister cells can re-enter the cell cycle and regrow tumors that are often irreversibly drug resistant due to selection of genetic or epigenetic alterations. Therefore, targeting persister cells holds great promise to prevent or delay acquired drug resistance and tumor relapse (Viswanathan et al., 2017).

Persister cells present specific vulnerabilities. Many have increased levels of polyunsaturated phospholipids, making them exquisitely sensitive to chemical inducers of ferroptosis. Ferroptosis is a caspase-independent non-apoptotic cell death due to excessive polyunsaturated lipid peroxidation within cellular membranes. The antioxidant enzyme glutathione peroxidase 4 (GPX4) catalyzes the conversion of the resulting and otherwise lethal hydroperoxides into their corresponding alcohols (many of which regulate hematopoietic cells in the cancer microenvironment), protecting them from ferroptotic death. Chemical inhibition or genetic ablation of GPX4 selectively and potently induces persister cell ferroptosis in a wide array of solid tumor types (Viswanathan et al., 2017) and prevents melanoma relapse following targeted therapy in mouse xenografts (Hangauer et al., 2017). GPX4 is a vulnerability in epithelial tumors that undergo epithelial-mesenchymal transition (EMT), melanoma cells that transdifferentiate from a high-MITF to a high AXL-cell state in response to targeted therapy and immunotherapy (Tsoi et al., 2018), prostate cancer cells that transdifferentiate into an androgen-indifferent state, and cancers derived from tissues with an intrinsic high-polyunsaturated lipid state such as sarcomas (Viswanathan et al., 2017). Therefore, GPX4 and targets related to this ferroptotic cell circuitry represent promising

non-oncogene therapeutic targets for a spectrum of high polyunsaturated lipid-based tumors.

TUMOR MICROENVIRONMENT

The TME is a rich milieu in which diverse non-neoplastic cell types and extracellular matrix proteins interact to regulate the biology of cancer cells (Binnewies et al., 2018). Deeper mechanistic insights into these dynamic molecular exchanges have enabled therapeutic strategies directly targeting aspects of the TME that are essential for tumor function (Figure 3). Proof-of-principle for TME-directed cancer therapy was achieved by anti-angiogenic treatments targeting vascular endothelial growth factor (VEGF), inhibiting the tumor's recruitment of new blood vessels and exploiting the relative lack of neovascularization in healthy organs (Apte et al., 2019). The intricate cellular complexity of the TME spans immune cells, fibroblasts, extracellular matrix (ECM) (Wong et al., 2017), and even neuronal elements (Monje, 2020; Venkatesh et al., 2019), creating a multitude of potential targets.

Adaptive and innate immune cells

Recent TME-targeting strategies have focused on immune cells, spanning both adaptive and innate immunity, including lymphocytes, macrophages, natural killer (NK) cells, myeloid-derived suppressor cells (MDSCs), dendritic cells (DCs), and others. The immune system is regulated by autocrine and paracrine cell-cell and cytokine interactions and exhibits a dynamic equilibrium between tumor-infiltrating and peripheral pools (Gajewski et al., 2013; Palucka and Coussens, 2016; Quail and Joyce, 2013). Modulation of immune function represents a high priority for cancer target identification and drug development, with strategies either exploiting the immunogenicity of tumor cells, spurring the host immune response, or both. Advances in immunotherapy have transformed the cancer treatment landscape in a number of cancers, embodied by antibody-based inhibitors of immune checkpoints such as PD-1/PD-L1 and CTLA-4, with a progression toward combinations of checkpoint inhibitors with themselves or with targeted therapies, chemotherapy, and radiotherapy (Larkin et al., 2019; Pardoll, 2012; Socinski et al., 2018) as well as new immune populations such as macrophages (Kaneda et al., 2017). In parallel, "adoptive" cellular T cell therapies involve infusion of chimeric antigen receptor T cells (CAR-T) effective for hematologic malignancies or T cell receptor (TCR)-T cells directed against tumor antigen(s), the former having substantial efficacy in hematologic malignancies, or bulk infusion of *ex vivo*-expanded tumor-infiltrating lymphocytes (Hekman et al., 2020) or NK cells (Hammerl et al., 2018; Rosenberg and Restifo, 2015; Tran et al., 2017).

New techniques link high-throughput CRISPR screening to tumor immunology both *in vitro* and *in vivo*. Systematic CRISPR perturbation of cancer cells have revealed PTPN2, ADAR1, and SWI/SNF complex members and other essential modulators of tumor immunotherapeutic action (Ishizuka et al., 2019; Lane-Reticker et al., 2018; Manguso et al., 2017; Pan et al., 2018; Patel et al., 2017). Conversely, CRISPR screens in T cells or DCs have identified potential novel immunoregulatory molecules with relevance to cancer immunotherapy (Dong et al., 2019; Parnas et al., 2015; Shifrut et al., 2018; Ye et al., 2019). Future tumor immunology screens may incorporate organoid approaches that preserve tumor cells along with infiltrating immune components, including T, B, and NK cells and macrophages without reconstitution (Neal et al., 2018) or microfluidic approaches (Jenkins et al., 2018). Alternatively, tumor cell lines or organoids can be reconstituted with peripheral blood lymphocyte populations to identify both tumor cell intrinsic and extrinsic immune response modifiers (Dijkstra et al., 2018; Mo et al., 2019; Yuki et al., 2020).

Similar methods also enable co-culture with NK cells, a population known to be early responders to disseminated cancer cells and which can be directly modulated or used as cell-based therapies. Notably, in one set of studies, cancer cells were found to "co-opt" and convert NK cells to an alternative molecular state that promotes metastatic outgrowth (Chan et al., 2020). Analyses of receptor-ligand pairings between cancer cells and NK cells identified multiple cell surface receptors (e.g., Klgr1 and TIGIT) that can be inhibited with antibodies to block metastatic colonization and may have clinical relevance. Additionally, ligands for NK cell activating receptors are often upregulated on tumor cells or during infection (Miller and Lanier, 2019), and the loss of these ligands reduces NK cell recognition and killing. Notably, the NK cell activating ligands MHC class I polypeptide-related sequence A/B (MICA/B) are often downregulated by diverse tumors including leukemia; prostate cancer; melanoma; and breast, lung, ovarian, and colon carcinomas, leading to decreased engagement of the NK cell activating receptor NKG2D and decreased anti-tumor activity (Lanier, 2019). Additionally, NKG2D ligands can be secreted by tumor cells, leading to impaired NK cell function (Ashiru et al., 2010; Clayton et al., 2008; Hedlund et al., 2011; Lundholm et al., 2014). Therapeutically, a novel antibody that blocks this shedding of NKG2D ligands was found to improve NK cell-mediated anti-tumor activity (Ferrari de Andrade et al., 2018). Expression of NKG2D ligands by non-tumor cells such as endothelial cells can also lead to desensitization of NK cells and reduced anti-tumor activity (Thompson et al., 2017).

Cancer-associated fibroblasts

Fibroblasts are an abundant cell type in normal and malignant tissues and accumulate along with fibrillar collagens in cancers characterized by a fibrotic response in pancreatic cancer. Genetically wild-type cancer-associated fibroblasts (CAFs) may be intrinsically less able to acquire resistance to targeted agents compared to genomically unstable cancer cells. The CAF cell state changes during malignant progression, typically to a contractile myofibroblast or secretory phenotype, and therefore has similarities to the high-polyunsaturated lipid state described above, associated with therapy-resistant persister cancer cells. Single-cell RNA sequencing (scRNAseq) has identified additional phenotypic diversity in this population, including those with contractile versus secretory behaviors. Mouse models of pancreatic cancer revealed context-specific tumor-promoting or tumor-inhibitory CAF functions (Biffi and Tuveson, 2021; Elyada et al., 2019; Hosein et al., 2020; LeBleu and Kalluri, 2018; Sahai et al., 2020). This complexity highlights the importance of robust physiologically relevant model systems that can define the precise function of a specific fibroblastic
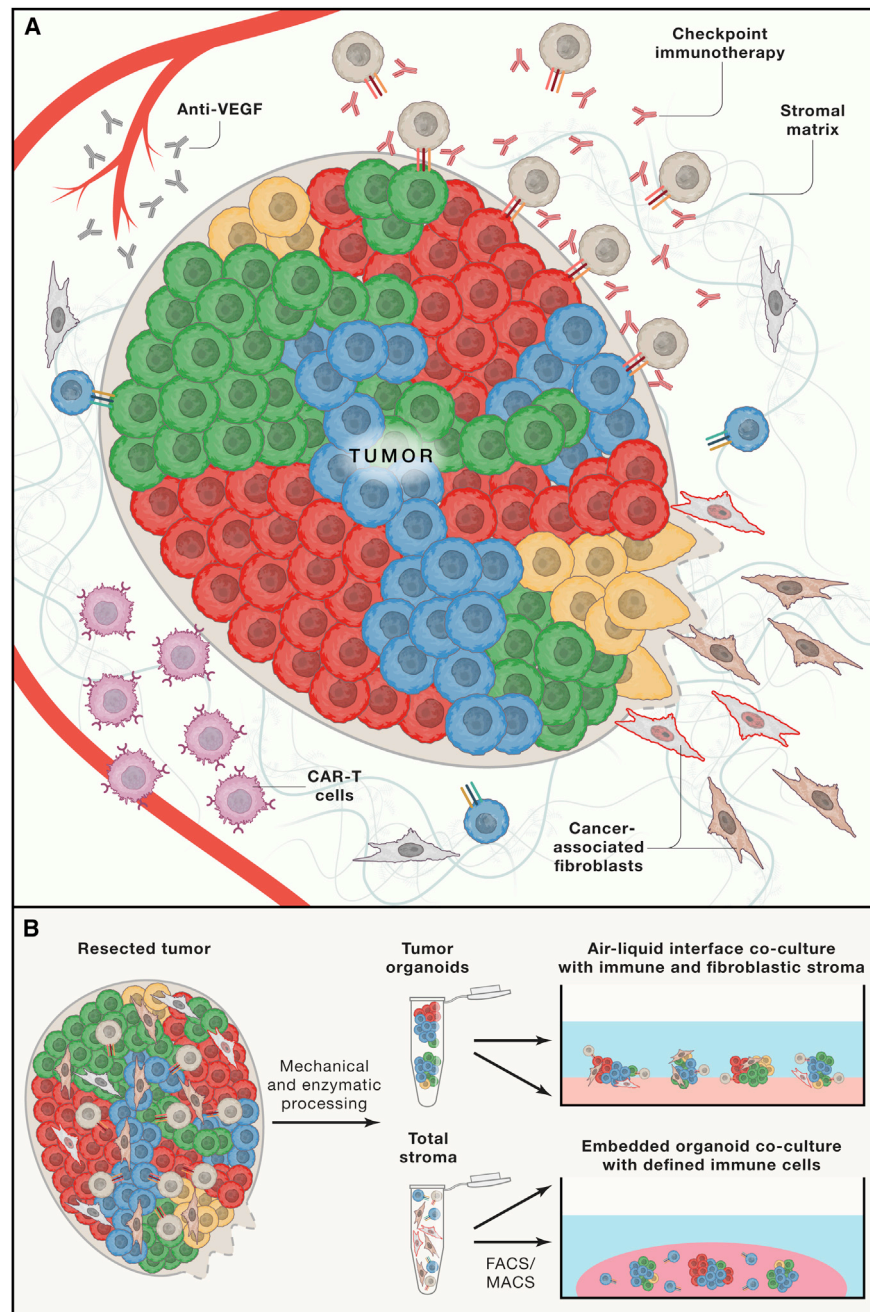
Figure 3. Tumor microenvironment targets
(A) Tumors are composed of a complex mixture of cancer cells in genetically and phenotypically distinct cell states surrounded by a fibrillar extracellular matrix and a diverse fibroblastic and immune stroma. Current therapies target VEGF to inhibit angiogenesis, disrupt T cell immune checkpoints with antibody based immunotherapy, disrupt survival signaling pathways that are mediated by cancer-associated fibroblasts, and deliver engineered immune cells to eliminate cancer cells. Our understanding of the molecular basis of interactions among cell types remains incomplete and therefore new methods are required to understand the individual and collaborative functions of these various cell populations.
(B) Recent technical advances enable primary and metastatic tumors to be deconstructed into their constituent parts and then reassembled in culture with either total immune and fibroblastic stroma or through selective incorporation of molecularly defined stromal cell populations. These assays from a platform to identify new cancer targets and to test candidate therapeutic compounds in a systematic fashion.

phenotype, enabling development of biomarkers to inform the selection of patients for targeted treatments and leading to the direct therapeutic targeting of CAFs for therapeutic purposes.

These studies have greatly benefitted from co-cultures of CAFs with cancer cells or organoids which can then be exposed to candidate therapies (Öhlund et al., 2017). Alternatively,

air-liquid interface tumor organoid cultures incorporate endogenous CAFs without requiring reconstitution (Neal and Kuo, 2016).

FUTURE OPPORTUNITIES

Identification of robust therapeutic targets across cancer types is a high priority. Tremendous efforts have led to the successful targeting of somatically altered oncoproteins. However, many cancers fail to express kinase oncogenes, and resistance to single agent therapy often rapidly occurs. As such, developing new therapeutic agents targeting the emerging classes of cancer targets described in this Perspective may provide the means to use targeted approaches for more patients but also provide complementary agents to create combination regimens. The diversity of new types of cancer targets provides hope that all patients will benefit in the future from precision-medicine-guided therapies.

In parallel, future efforts must consider cell-state heterogeneity in both the cancer cells themselves and the cells of the TME. The development of complex organoid co-cultures that incorporate fibroblastic, immune, and vascular compartments by reconstitution or by intrinsic preservation should further enable modeling of tumor microenvironmental influences on cell state (Neal et al., 2018; Öhlund et al., 2017; Yuki et al., 2020). For example, 3D cultures of primary tumor tissue maintain some of the cellular heterogeneity and cell state transitions observed in vivo (Roerink et al., 2018). 3D cultures also allow analysis of tumor-level phenotypes, like collective invasion, immune surveillance, and organ colonization, which are difficult to model in 2D culture). These types of approaches may also permit the development of more accurate animal models of human cancer.

In addition, scRNAseq analyses have revealed multiple distinct cell states with unequal contributions to tumor relapse. The selective depletion of stem-like progenitor populations or of a subset of persister cells, which may seed tumor relapse, represent a more tractable goal than completely eliminating all residual tumor cells. Furthermore, deeper understanding of the processes by which persister cells gain the ability to survive therapeutic stress and regrow may suggest new therapeutic approaches.

To improve patient outcomes, it is essential to target cancer not only in isolation, but also as part of a larger ecosystem. While deconvolution of the intricate composition and activities of the TME is in its infancy, notable functional demonstrations have included the clinical efficacy of immune- and blood vessel-targeted therapies, raising the potential for further target identification. The ability to effectively disrupt the tumor ecosystem may help address a major unmet clinical challenge of having to target multiple heterogeneous cell populations within tumors and the microenvironment. Further, as TME components fulfill important functions in other contexts, such as in healthy organs, the potential toxicity of therapeutic stromal targeting will require thorough evaluation. The cell state concept could significantly assist this process by collapsing a daunting, essentially infinite universe of possible mutational patterns and TME to a very finite repertoire of distinct tumor cell states.

Although our initial framework to define criteria to assess the strength of evidence supporting a specific target was focused on oncogene targets, the same principles apply to these new categories of cancer drivers. Specifically, experimental evidence in multiple experimental systems from several different laboratories both in vitro and in vivo provides a clear path to prioritizing targets for clinical translation. Since in some cases only a few experimental models exist to study specific categories of the cancer targets described here, a concerted effort to create and characterize more experimental models must remain a priority.

Finally, the discovery and credentialing of non-oncogene and TME targets will be accompanied by a need to define markers or features that permit the stratification of patients who are most likely to benefit from such new classes of cancer therapies (Chari et al., 2019). For some targets, immunohistochemical methods or molecular profiling or sequencing will be sufficient to stratify patients likely to respond to treatment. However, for other targets, defining tumors that are likely or not to respond to particular tumor intrinsic or extrinsic focused therapies will require new tools that can be applied to clinical samples, such as the ability to assess multi-parameter gene expression in tumor tissue. Although a small number of diagnostic tests are based on gene expression, expansion of these efforts into clinical practice will require substantial changes to sample acquisition and testing.

Despite these challenges, defining and credentialing an expanded universe of cancer targets are now possible. In addition, future work will be needed to develop robust methods to test potential combinations, particularly if one is targeting both the tumor and TME. These approaches will require both scale as well as novel endpoints that go beyond cell fitness. Combining approaches to target both tumor intrinsic and extrinsic factors will lead to rational combinations, which promise to provide robust and durable outcomes.

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