Published in final edited form as:

Nat Cancer. 2021 March ; 2(3): 258–270. doi:10.1038/s43018-021-00181-0.

Emerging strategies for treating metastasis

Mark Esposito[1], **Shridar Ganesan**[2,3,4], **Yibin Kang**[1,2,5,#]

[1]Department of Molecular Biology, Princeton University, Princeton, New Jersey, USA

[2]Rutgers Cancer Institute of New Jersey, New Brunswick, New Jersey, USA

[3]Center for Systems and Computational Biology, Rutgers Cancer Institute of New Jersey, Rutgers University, New Brunswick, New Jersey, USA

[4]Department of Medicine, Rutgers Robert Wood Johnson Medical School, Rutgers University, New Brunswick, New Jersey, USA

[5]Ludwig Institute for Cancer Research, Princeton University, Princeton, USA

Abstract

The systemic spread of tumor cells is the ultimate cause of the majority of deaths from cancer, yet few successful therapeutic strategies have emerged to specifically target metastasis. Here we discuss recent advances in our understanding of tumor-intrinsic pathways driving metastatic colonization and therapeutic resistance, as well as immune activating strategies to target metastatic disease. We focus on therapeutically exploitable mechanisms, promising strategies in preclinical and clinical development, and emerging areas with potential to become innovative treatments.

Introduction

Cancer metastasis, or the systemic spread and growth of tumor cells throughout the body, is the principal cause of cancer-related deaths[1]. Despite seventy years of drug development for cancer, survival rates for patients with metastatic disease remain abysmal, with five-year survival rates ranging from 5–30% across solid tumors[2]. This low survival rate has persisted because clinical results have consistently shown that preclinical therapeutic efficacy does not always translate to clinical benefit for patients suffering from metastatic disease[3].

[#]**Correspondence to:** Yibin Kang, Ph.D., Department of Molecular Biology, LTL255, Washington Road, Princeton University, Princeton, NJ 08544, USA, Phone: 609-258-8834, Fax: 609-258-2340, ykang@princeton.edu.

Conflicts of interest

ME holds equity interest and a management position in KayoThera, a company developing cancer therapeutics. YK holds equity interest in KayoThera and Firebrand Therapeutics, and is a member of Scientific Advisory Boards of Kayothera, Firebrand Therapeutics, and Cytocares. YK has consulted for Merck, Amgen, Ono Pharma and has previously received funding support from Merck, Amgen, Johnson & Johnson, Janssen, Glycomimetics, and Ono Pharma. SG has consulted for Merck, Roche, Foundation Medicine, Foghorn Therapeutics, Novartis, Silagene, EQRX and Inspirata, has received research funding from M2GEN, and has equity interest in Inspirata and Silagene.

Data Availability

The human cancer mortality data in Figure 1 were derived from the SEER database with 2017 as the most recently annotated 5-year survival date: https://seer.cancer.gov/data/

The raw data are provided as a supplementary file.

The majority of approved cancer drugs, including tyrosine kinase inhibitors (TKIs), systemic cytotoxic therapies (chemotherapy), and antibody-drug conjugates (ADCs), do not have a durable impact in the setting of established metastatic disease - often due to acquired mechanisms of therapeutic resistance[3]. On the other hand, the possibility of durable remissions after treatment with immunotherapies has begun a paradigm shift in Stage IV patients who previously received terminal diagnoses. These advances in immunotherapy have ushered in a new era where advanced cancer patients can now hope for long term remissions or chronic management of metastases[4, 5]. Despite this momentous advance, the majority of metastatic cancers have yet to see therapies with similar efficacy in inducing long-term, durable remissions.

Given this era of rapid progress in novel therapeutic modalities, multiple opportunities are under investigation that may have a transformative impact on cancer death rates. Rather than following the conventional development pipeline of prioritizing therapeutic leads by *in vitro* cytotoxicity, focusing on the mechanisms of cell plasticity and stress resistance have become key areas of promise for therapies tackling metastasis[6]. While targeting these interconnected pathways may not show strong efficacy *in vitro*, they are critical to therapeutic resistance as well as overcoming metastatic bottlenecks *in vivo*. In the tumor-extrinsic context, therapies to relieve immune suppression via reprogramming the local immune milieu may open a new avenues of metastasis immunotherapy.

In this Review we aim to i) Rationalize why cancer metastasis should be a principal consideration in future cancer drug development, ii) Appraise the therapies that have and have not worked in metastatic settings, and iii) Discuss emerging strategies of promise in treating metastatic disease.

Justifying a shift in drug development

The survival of patients diagnosed with localized or regional cancers has increased dramatically in the last decades, yet metrics for metastatic disease have remained constant for many solid tumors[7–9]. Although cancer death rates have declined by 29% since the mid-1990 peak[2], most of this reduction is related to preventative lifestyle changes (smoking cessation[10], HPV vaccination[11], and Hepatitis C treatment), early screening[12] and advances in adjuvant therapy for high-risk patients without clinically detectable metastatic disease[13]. Whereas these interventions have reduced the prevalence of macro-metastasis, few of them significantly affect survival in patients with established metastases.

The death toll caused by metastatic cancer is difficult to quantify as it manifests across multiple organs and mortality reporting is inconsistent across healthcare systems and cancer types[14]. For example, in a case study of breast cancer patients, 45% of deaths were attributed directly to metastatic disease that manifested as pulmonary insufficiency, central nervous system failures, hepatic failure and hypercalcemia. An additional 24% of deaths were caused by pneumonia or sepsis subsequent to extensive pulmonary metastases. Only a small minority of the cancer-related deaths could be traced to the primary tumor or drug treatment, and the remainder of deaths were attributed to unrelated causes[15]. Similar patterns were found in lung cancer, with metastasis as the direct cause of 44% of deaths, and an additional

32% of deaths attributed to pneumonia, sepsis and hemorrhaging subsequent to extensive tumor spread[16]. Comparable mortality burdens due to metastasis are observed across solid tumors, indicating that preventative and therapeutic approaches to lessen the impact of metastatic cancer must be undertaken to ensure a meaningful change in cancer death rates[17].

Two generalized models of metastatic progression have emerged: 1) cancers that metastasize as a function of time and/or tumor size and 2) those that metastasize due to the specific cell of origin and/or mutational lineage[18]. Cancers with time-dependent metastasis include basal cell carcinoma, which is the most common cancer, yet fewer than 0.55% of cases develop metastatic disease. The low rate of metastasis in basal cell carcinoma reflects the ease of diagnosis and effective surgical interventions[19]. Pancreatic cancer is thought to be slow-growing and the formation of distant metastasis does not occur until advanced stages[20], although cancer cell dissemination may also occur at earlier stages[21]. Unlike basal cell carcinoma, the difficulty of early diagnosis in pancreatic cancer means that the majority of clinical patients present with metastasis[20]. Population-wide measures for early detection of time-dependent cancers may present an ideal opportunity to reduce prevalence of metastasis. Common cancers such as breast, colorectal, renal, lung and prostate cancer belong to the early metastasizing group; while some cases might never spread, around 10–15% of breast cancers develop metastasis within three years, and genetic characterization reveals the existence of primary tumors that disseminate early[22]. This is supported by mouse studies showing that breast cancers can metastasize before the primary tumor is palpable[18] or by prostate cancer patients showing molecular heterogeneity across bone metastases[23]. Mutational profiles of brain and liver metastases across cancer types also support a parallel progression model where metastasis can occur early and distinct metastatic clones convergently evolve[24, 25]. Organ transplants that later manifested donor-derived metastases in immune-suppressed recipients further suggest that these tumors metastasize early from undetectable primary cancers[26]. This is supported by the finding that 5–10% of tumor diagnoses are patients with unknown primary tumors presenting with systemic metastases[27]. For these parallel progression cancers, early detection may be less effective in preventing the development of metastasis or mortality, which has been witnessed by the controversies of implementing population-wide PSA and mammogram testing[28, 29]. Thus, while early screening and diagnosis may help to improve survival for some cancer types, effective therapies targeting metastatic disease will always be needed in the medical repertoire.

Limitations of targeted therapies

The 2020 NCI Surveillance, Epidemiology and End Results Program (SEER) report highlighted that some of the reduction in cancer mortality could be traced to key advances in targeted therapies and immunotherapies that are highly effective in treating metastatic melanoma and lung cancers (Figure 1)[2]. In contrast, the majority of the >200 drugs approved to treat cancer have done little to reduce mortality, revealing a glaring disconnect between approval and patient benefit. Improvements in cancer treatment, including surgery, radiation, chemotherapy and targeted therapy, contributed to only 4%−8% of the cancer mortality declines from 1991 to 2011[30, 31].

Much of the mortality reductions derived from therapeutics owe to advances in adjuvant treatment, which prevents metastatic relapse by eliminating disseminated tumor cells. Numerous conventional treatments have shown the ability to extend survival in the adjuvant setting, such as trastuzumab (anti-HER2) and chemotherapy in breast cancer[32], apalutamide in prostate cancer[33], chemotherapy in lung[34] and colon cancers[35], and imatinib in gastrointestinal stromal tumors. Recent trials demonstrate adjuvant efficacy for abemaciclib (CDK4/6) in HR+/Her2- breast cancers, osimertinib (EGFR) in early stage EGFR-mutant lung cancer, dabrafenib/trametinib (BRAF/MEK) in BRAF-mutant melanoma, and pembrolizumab (PD-1) in high-risk melanoma[36].

While adjuvant therapy to prevent recurrence is currently the most effective strategy to reduce post-diagnosis cancer mortality, approval in this setting requires exceptionally high-powered clinical trials of long duration that are usually not feasible for studies attempting a first approval. A typical clinical development plan requires demonstration of efficacy according to RECIST.1 metrics in the advanced/metastatic tumor setting before justifying the expense of adjuvant trials, thus metastasis-preventing therapeutics are unlikely to ever reach the adjuvant testing space where they would be most effective. Moreover, identifying responsive sub-groups and predictive pharmacodynamics adds to these challenges. For example, adjuvant treatment with bone metastasis-specific resorption inhibitors is only effective in the post-menopausal subgroup of breast cancer patients[37]. Finally, long-term adjuvant treatment may be limited by chronic toxicity, such as cardiotoxicity from trastuzumab[38]. Thus, the current regulatory and financial framework for cancer drug development does not facilitate the development of metastasis-specific therapies.

Many treatment limitations in metastasis trace back to the shortcomings of the classical discovery of cancer therapeutics, which requires cytotoxicity *in vitro*, primary tumor shrinkage in preclinical models, and approval based on RECIST criteria[39]. This drug development strategy has worked for some exceptionally potent therapies showing dramatic responses in advanced or metastatic settings, such as cabozantinib (VEGFR, AXL, MET) in advanced/metastatic RCC[40], endocrine therapies in HR+ breast cancers[41] or vemurafenib (BRAF), inducing potent but short-lived responses in metastatic melanoma[42]. Newly emerging strategies targeting RET, NTRK and NRG1 have also recently demonstrated exceptional responses in NSCLC brain metastases harboring RET fusions (ORR >90%), leading to approval of selpercatinib[43]. Meanwhile, many other therapies targeting oncogenic drivers and dependencies, such as regorafenib (VEGFR)[44] or cetuximab (anti-EGF)[45] in metastatic CRC, show modest short-term responses but no impact on 5-year survival. Chemotherapy trials in metastatic breast cancer have similarly been futile; these cancers respond acutely to treatment, but >90% of metastatic cancers will develop resistance to cytotoxics, leading to death within 10 years[46].

Antibody-drug conjugates (ADCs) have been enthusiastically pursued as potential one-two punch approaches. Examples of this class include ado-trastuzumab emtansine (anti-HER2), which extends 2-year survival in metastatic patients compared to lapatinib (HER2 inhibitor) and chemotherapy[47]. Alternately, ADCs targeting non-driver targets such as sacituzumab govitecan-hziy (anti-TROP2 linked SN-38) in metastatic TNBC yield only a modest response with short duration (5.5 months) and considerable toxicity[48]. Similarly, ADCs

against non-driver surface proteins such as folate receptor in ovarian cancer (mirvetuximab soravtansine), DLL3 in lung cancer (rovalpituzumab tesirine) and EpCAM in bladder cancer (oportuzumab monatox) have met varying degrees of failure in phase II/III trials (Table 1)[49].

Perhaps the most notable shortfall for Stage IV patients are the numerous targeted therapies that have been approved despite showing only progression-free survival (PFS) benefits with no changes in overall survival[50]. Cancer patients receiving these therapies may have tumor shrinkage, but do not live longer or necessarily with a better quality of life. The discrepancies between PFS and OS are evident in two recent Phase III trials of nivolumab (anti PD-1) compared to everolimus (mTOR inhibitor) in advanced RCC or nivolumab compared to docetaxel in NSCLC: both trials showed compelling increases in overall survival for nivolumab with non-significant changes or even reverse trends in PFS[51, 52].

Conversely, attempts to specifically target metastatic pathways have met with near-universal failure. At the forefront of this effort are the numerous trials targeting matrix metalloproteases (MMPs)[53]. Experimental models suggested MMPs were central to cancer metastasis by increasing motility and invasion. However, clinical trials of MMP inhibitors showed either no response or worse outcomes[53]. While failure has been chalked to an incomplete understanding of the specificity of the MMPs, inadequate clinical trial protocols and unintended effects on the immune system[53], the fundamental reason is likely that therapeutics targeting invasion, migration and extravasation may not be effective in treating established metastatic disease. A myriad of evidence shows cancers non-specifically shed metastasis-competent cells[54] and patients typically harbor metastasis-competent dormant cancer cells before diagnosis and treatment[55, 56].

Targeting phenotypic plasticity and stress resistance

The concept of de-differentiation and phenotypic plasticity has been a central theme of metastasis research since pivotal early studies revealed that less differentiated cancers presented a higher risk of metastasis and therapeutic resistance[57]. This concept has fluidly evolved throughout time as tumor cell dedifferentiation, epithelial-mesenchymal transition (EMT)/mesenchymal-epithelial transition (MET), the cancer stem cell (CSC) hypothesis, and more recently, plasticity[6, 58, 59]. Controversies have surrounded these theories with studies claiming that EMT is either essential[60] or dispensable for metastasis[61], or that CSCs are only important to some cancer types[62], are not present at all in others[63], or are not yet defined well enough to be understood[64].

Whereas plasticity has been recently linked to metastasis[59], research has long established that cell state transitions toward stem-like states are central to therapeutic resistance[65–70] and have been validated by discoveries of genes such as MTDH, which plays key roles in stemness, EMT-MET, metastasis, stress resistance and therapeutic resistance[71, 72]. It is therefore unsurprising that all these processes are intrinsically linked (Figure 2). These states describe the same underlying phenomenon of transcriptional and epigenetic plasticity, and while the phenotypic outputs are diverse, targeting the underlying regulators of plasticity has emerged as a critical therapeutic strategy.

Developmental pathways in plasticity and therapy resistance

Several key developmental pathways driving metastasis, often through plasticity and stress resistance, are TGF-β, Wnt, Hedgehog and Notch signaling[73]. These pathways drive broad transcriptional changes and exhibit extensive cross-talk[69, 73]. Because of their central importance in human physiology, targeting these pathways has been limited by narrow therapeutic indices, but continued validation of pathway targets may yield success in treating metastatic disease.

Translational efforts have most extensively targeted the Notch signaling pathway due to its well characterized signaling cascade as well as its oncogenic role across tumor types. Notch signaling is initiated by Delta-like (DLL1–4) or Jagged ligands (JAG1–2) binding to the NOTCH1–4 receptors. This causes proteolytic cleavage and nuclear translocation of the Notch intracellular domain (NICD) where it can induce pleiotropic transcriptional activation[73]. Notch signaling plays a key role in development and its dysregulation is central to plasticity in cancer[74]. DLL1 is an important mammary stem cell marker[75], and forced expression of NOTCH3 expands the CSC side population in pancreatic cancer[76]. Prostate cancer cells activate Notch to transdifferentiate into osteoblast-like cells that enhance bone metastasis[77], and the interaction of Notch and Shh signaling drives docetaxel resistance in prostate cancer cells[69]. Notch is also critical for tumor-initiating properties downstream of p53- and RB1 deletion in HCC[78]. The JAG1-NOTCH interaction is a bona-fide driver in both bone and brain metastasis[79], with both tumor and stromal cells expressing JAG1 and/or Notch receptors to sustain stemness and chemoresistance[79, 80]. Treatment of solid tumor PDXs with anti-NOTCH2/3 mAb plus chemotherapy depletes tumor-initiating cells and delays recurrence[81]. Gamma secretase inhibitors (GSI), which prevent Notch processing upon ligand binding, have similar effects in preventing HCC metastasis and MET[82] or in sensitizing prostate CSCs to chemotherapy[69]. JAG1 is critical to the bone metastasis niche[80] and whose therapeutic inhibition synergizes with chemotherapy to slow bone metastasis[83].

Two therapeutic approaches targeting the Notch pathway have progressed into the clinic – GSI and mAbs against Notch membrane proteins[73]. GSI exhibited activity in early trials yet broad gastrointestinal toxicity prevented further development[84]. Thus other targeted approaches were attempted, including anti-DLL4 (demcizumab), which showed considerable toxicity and low efficacy in pancreatic and lung cancers[85], or the NOTCH2/3 antibody tarextumab, which led to significantly worse survival outcomes compared to standard of care (Table 1)[86]. More recent development of an anti-DLL3 ADC, rovalpituzumab tesirine, was recently terminated in NSCLC due to a lack of survival benefit[87]. Interestingly, one oral GSI (nirogacestat) has progressed into Phase III trials for desmoid tumors after exhibiting moderate toxicity coupled with very promising results in multiple tumor types[88] (Table 1).

The Wnt pathway has similarly been implicated in metastasis across tumor types[89]. APC mutations in colorectal cancers and amplification of multiple signaling proteins such as CTNNB1, FZD or LRP across solid tumors implicate Wnt signaling in tumor progression[89]. Wnt also drives the epithelial stem cell state; LGR5, a GPCR involved in Wnt signaling, has become a critical marker for colon crypt stem cells and has been similarly shown to enrich

for and functionally induce EpCAM^{+} colorectal CSCs[90]. Wnt also maintains plasticity via expression of OCT4 to induce dedifferentiation[91].

Wnt signaling has time-dependent and organ-tropic effects during metastasis[92]. Wnt signaling is induced in the vascular bone metastasis niche by E-selectin, leading to the acquisition of dual epithelial and stem cell properties[93]. Conversely, the Wnt inhibitor DKK1 has pro-metastatic roles by suppressing Wnt activity in dormant bone metastasis cells in order to evade NK cell-mediated clearance[94] or by enhancing osteolysis in late-stage bone metastasis[95].

Targeting the Wnt pathway is difficult due to the complexity of the signaling cascade as well as its central role in bone homeostasis. Initial safety data with a decoy receptor sponge for WNT8 (ipafricept)[96] or anti-FZD1, 2, 5, 7 (vantictumab) in metastatic cancers identified severe bone toxicities[97]. Moreover, research has suggested that targeting the canonical Wnt pathway may induce the pro-metastatic non-canonical pathway[98]. Thus, continued exploration of the Wnt pathway or its downstream effectors is required. One potentially interesting route is via LGR5-ADCs that have shown compelling data across multiple models of tumorigenesis and metastasis. However, LGR5-ADCs have not progressed into the clinic due to a lack of enthusiasm for CSC-targeted treatments and potential toxicity to normal adult stem cell pools[87].

The TGF-β pathway comprises multiple ligands, including the bone morphogenic proteins (BMPs), activins, and TGF-β1–3, that, when bound to TGF-β receptors (TGFβRI-II, ALK and BMPR), drives a SMAD-mediated transcriptional program that is highly context-dependent[99]. This ranges from acting as a tumor suppressor in early cancers[100], a mediator of immune suppression across diseases, and a critical driver of mesenchymal traits, plasticity, and EMT in metastasis[60, 99]. TGF-β signaling is responsible for inducing and maintaining the mesenchymal de-differentiated state in metastatic cancers[101], inducing pro-metastatic Jagged1 in bone metastasis[83], IL11 in liver metastasis[102], ANGPTL4 in lung metastasis[103], and suppressing Wnt signaling via DKK1 induction[94]. TGF-β has been shown to be a critical pro-malignant pathway in late-stage cancers[104] or for the supportive stroma for metastasis initiation[102].

Preclinical testing of TGF-β inhibitors resulted in compelling evidence supporting the development of this class of therapies. For instance, inhibition of TGF-β in xenograft models prevents bone and lung metastasis while primary tumor growth remains unchanged[105]. Treatment can further deplete metastasis-initiating cells via stromal targeting[102] or slow the vicious cycle of bone metastasis[106]. In contrast to toxicity associated with Wnt and Notch targeting, TGF-β pathway ablation is tolerated, with cardiac toxicity as the major on-target concern[107]. However, the multi-faceted role of TGF-β signaling in both tumor promotion and tumor suppression presents problems with clinical advancement, as one notable side effect of TGF-β inhibition is the transient and reversible onset of various neoplasms, mostly keratoacanthomas[108].

Finally, Hedgehog signaling (SHH) is also associated with stemness and metastasis[109], stromal activation during tumorigenesis[110], and therapeutic resistance[69]. The central

transcription factors of Shh signaling are GLI1/2, which show aberrantly high expression in bone metastatic tumor cells where it enhances PTHRP expression to induce osteolytic metastasis[111]. Multiple studies have explored the importance of SHH in metastasis. For instance, cyclopamine (SMO inhibitor) derivatives inhibited pancreatic cancer metastasis by depleting the ALDH-positive stem cell pool[112]. Importantly, long-term follow-up studies of vismodegib, an approved SMO inhibitor, yielded exceptional response rates in metastatic basal cell carcinoma, a cancer characterized by loss of function mutations in PTCH1. Vismodegib treatment results in durable responses exceeding one year and median survival nearing three years in metastatic patients[113]. However, SMO inhibitors resulted in worse patient outcomes across pancreatic cancer trials as SHH inhibition promoted progression via suppressing stromal populations[114].

Epigenetic approaches to restrain tumor plasticity

Tumor cell plasticity often manifests as multiple hemi-states that do not co-exist in normal physiology. This is best illustrated by the gradient of epithelial-mesenchymal (E-M) states observed in circulating breast tumor cells that vary over time in response to PI3K targeting or systemic chemotherapy[68]. This gradient of E-M transitions has been observed across tumor types, and is ascribed to higher metastatic potential[115] or increased therapeutic resistance[116]. Importantly, plasticity presents as phenotypic heterogeneity across the same tumor or metastases, and that this plasticity is controlled by the epigenome[117]. Large scale genomic studies further support the notion that metastasis is not driven by mutations distinct from the primary tumor[118], while *in vivo* selection models suggest that metastasis may enrich for certain mutational combinations already present in the primary tumor without a requirement for *de novo* metastatic mutations[119]. Indeed, reversible switching between E-M states is dependent on concentrations of TGF-β ligand rather than genetic mutations[101]. This heterogeneity in cell transcription is functional to the metastatic process; epithelial to mesenchymal transition is observed from the core to the invasive edge of the primary tumor and then a reversion is observed in the metastatic site, with both phases deemed critical for metastatic progression[93, 120–122].

Therapeutic targeting of the epigenome provides compelling evidence for the feasibility of altering the plastic, dedifferentiated state exhibited during the evolution of metastasis. Early efforts of drug development yielded the cytidine analogs (5-aza(deoxy)cytidine) initially developed as anti-neoplastic nucleoside analogs, however, studies revealed optimal biological activity at sub-cytotoxic doses through inhibition of DNA-methyltransferases[123]. Early trials in solid tumor metastasis revealed minor activity that was curtailed by myelosuppression. This limitation proved to be a clinical asset as these drugs were eventually approved to treat Myelodysplastic Syndromes[123].

Owing to the toxicological limitations of hypomethylating therapies, yet stimulated by promising signs of efficacy, epigenetic therapies have slowly progressed through clinical trials. A key promising therapy is tazemetostat, an inhibitor of EZH2, the H3K27 methyltransferase that defines PRC2 complex activity. EZH2 activity is responsible for maintaining the dedifferentiated state in Ewing tumors[124] and is an essential driver of melanoma metastasis through suppression of various tumor suppressors[125]. EZH2 activity

leads to RB1 and p53 suppression in prostate cancers, this results in plasticity and metastasis while EZH2 inhibitors restore sensitivity to anti-androgens[126]. Tazemetostat was recently approved as the first targeted therapy for metastatic epitheloid sarcoma after demonstrating activity in INI1-negative solid tumors and follicular lymphoma[127].

Another promising epigenetic strategy is targeting histone deacetylases (HDAC), as loss of H4K16 acetylation is a common hallmark of cancer[128]. Pan-HDAC inhibitors such as romidepsin, vorinostat and belinostat are approved for the treatment of cutaneous T-cell lymphomas[128]. Class-specific HDAC-inhibitors such as entinostat and tucidinostat have demonstrated modest efficacy in endocrine therapy-resistant breast cancer patients by increasing PFS[129, 130], and class I HDAC inhibition suppresses cancer stem cell activity and metastasis initiating properties in TNBC[131].

Fitness genes and stress resistance

At the simplest reduction, two types of pathways are responsible for cancer progression: 1) Driver genes that cause the cells to proliferate unchecked, and 2) Fitness genes that allow the cells to survive intrinsic and exogenous stressors. Alterations of driver genes are responsible for the competitive advantage of premalignant cells, including activating mutations in oncogenes such as BRAF, or loss-of-function mutations in tumor suppressors such as APC[118]. Stepwise accumulation of driver mutations leads to the linear progression of premalignant clones to clinically detectable tumors[132]. Only a small set of driver events are required for primary tumors to form, for example, *APC* deletion in colorectal cancer or BRAF and KRAS mutations in melanoma and pancreatic cancer, respectively[118]. TCGA analysis confirms that classic pathways such as PI3K signaling, MAPK signaling, cell cycle control and RTK signaling were some of the most highly mutated pathways[133]. Analyzing cancer genomes across patients suggests that most cancers are driven by only two to eight mutational events[118] (Figure 3). Due to these oncogene-centric discoveries and the impact of these pathways in cancer initiation, most drug development efforts have focused on targeting driver genes despite limited their efficacy in the metastatic setting.

Conversely, metastasis is theorized as a discrete step in tumor evolution that may be independent of specific oncogene pathways or mutations, and instead co-opts cellular traits that mitigate immunologic, genotoxic and therapeutic stressors accreted during tumorigenesis. These adaptations enable a discontinuous jump to metastatic competence and survival in the early metastatic niche[132] (Figure 3). The chromosomal instability response is a classic fitness program – metastatic cells exhibiting chromosomal instability activate a noncanonical NF-κB response rather than a lethal interferon response to circumvent cytotoxicity[134]. Supporting this discovery, screening for molecules that disassemble the tumor-specific perinucleolar compartment yielded Metarrestin, a molecule in early clinical testing to prevent or reverse metastasis[135].

It has long been appreciated that oncogenes are not sufficient for metastasis, and furthermore, the genotoxic and metabolic stresses resulting from unchecked proliferation require specific compensatory pathways[136]. This idea parallels the synthetic lethality space; driver pathways such as ERK inhibition can be compensated via PI3K/mTOR signaling[137], whereas BRCA-mutant cancers have few compensatory mechanisms to resist genotoxicity

and PARP inhibition results in synthetic lethality. Unfortunately, PARP inhibitors only exhibited incremental improvements in PFS for advanced BRCA1/2-mutant ovarian cancers[138] with resistance arising in most patients. Targeting survival adaptations in the metastatic niche could lead to effective treatments that are independent of specific genomic alterations in the cancer. For example, PD-L1 expression on tumor cells is not a driver mutation, but rather a stress resistance mechanism, and its therapeutic inhibition resulted in some of the most durable remissions seen to date (Table 1).

Whereas driver mutations offer an experimentally tractable approach for drug discovery, targeting cancer fitness genes requires the addition of an additional element – stress. By developing multiple stress resistance mechanisms, metastatic cells are able to overcome the oxidative and shear stresses of circulation, immune surveillance in the early metastatic niche, metabolic stresses of advanced metastatic lesions, and therapeutic challenge[139] (Figure 3). Importantly, the metastatic process is incredibly inefficient[132], suggesting that exploiting any survival liabilities utilized by these cells may have outsized effects on the early metastatic process.

Oxidative stress has emerged as a ubiquitous challenge to metastasizing cells. Whereas early studies assumed that oxidative stress led to cancer development, multiple clinical studies have shown that patients treated with antioxidants fare worse, particularly when treated concurrently with chemotherapy and/or radiotherapy[140–142]. Mouse studies have shown that antioxidant supplementation, often with N-acetylcysteine (NAC), is responsible for the initiation[143], progression, and reduced survival from lung cancers by a mechanism that limits p53 induction[144].

A landmark study demonstrated that patient-derived melanomas experience higher levels of oxidative stress during metastasis, that NAC supplementation was sufficient for inducing metastasis, and that NAPDH production via folate pathway enzymes ALDH1L2 or MTHFD2 was also necessary for metastasis[145]. Further studies have demonstrated that lactate utilization via the MCT1 transporter increases NADPH via the oxidative pentose phosphate pathway to enable melanoma metastasis[146]. Oxidative stress is also the main underlying cytotoxic mechanism of various chemo- and radiotherapies: NRF2-driven glutathione synthesis enables cisplatin resistance[147] and antioxidant supplementation prevents the cytotoxic effects of paclitaxel[148]. Oxidative stress appears to be largely metastasis-specific; G6PD knockout to inhibit the oxidative pentose pathway does not affect primary tumor initiation or growth in breast, colorectal or lung cancers[149]. Cystine uptake via SLC7a11 provides cysteine for glutathione biosynthesis in an NADPH-dependent process, and some studies have identified this transporter as an essential component of redox homeostasis in tumors[150].

This evidence suggests that oxidative stress is a critical node of synthetic lethality[151] and that pro-oxidant therapy should be considered as a high priority drug development target[141]. Multiple enzymes have been validated as potential targets to induce oxidative stress such as superoxide dismutases, glutathione peroxidases, thioredoxins, catalases and others. These have shown some promise as cancer targets, yet are often redundant[152]. A key node in oxidative stress resistance is NRF2, the master redox transcriptional regulator whose

induction by mutations or oxidative stress increases BACH1-mediated metastasis[153] and cisplatin resistance[147]. NRF2 gain-of-function is observed across many cancers and pharmacologic targeting is currently in proof-of-concept[154]. Importantly, NRF2 expression is positively correlated to the stage of many cancers, showing the greatest levels in metastatic disease, and is further responsible for enhancing chemoresistance[155].

An alternate pro-oxidant therapy is via targeting of metabolic processes that eliminate oxidative stressors and concomitantly generate reduced NAD^+ or $NADP^+$; one such strategy is via Aldehyde dehydrogenase (ALDH) activity. ALDH activity predicts tumorigenicity and worse clinical outcomes[156] and is broadly used as a marker for stemness, metastatic behavior, and chemoresistance[157]. Among the 19 ALDH enzymes, ALDH1a3 has been shown as the main driver of ALDH activity[158], and subsequent metastatic or chemoresistant traits[159] via its role in lipid peroxidation or direct drug detoxification[160]. Serine biosynthesis via the folate pathway is a potential metastatic target as the ALDH1L1/1L2 enzymes have shown importance in metastasis via the generation of NADPH[145], yet the redundancy of cytosolic and mitochondrial serine biosynthesis makes this metabolism difficult to target in tumors[161]. However, development of drug-like inhibitors of specific ALDH enzymes has not succeeded despite extensive efforts.[157]

Fatty acid oxidation (FAO) has received comparatively little interest in cancer drug development despite the numerous therapeutics available from cardiometabolic research. Increased FAO is a direct response to the Warburg effect to sustain ATP and NADH levels, particularly in the metastatic setting[162]. Ablation of FAO via targeting CPT1a with etomoxir prevents CRC metastasis in preclinical models[163]. Exogenous lipid sourcing is also critical to survival in hypoxic tumors where mTOR activity and TSC2 deficiency creates a synthetic dependence on desaturated lipids in hypoxic environments[164]. The lipid receptor CD36 was demonstrated as a critical component of metastasis-initiating cells through exogenous palmitate catabolism, and neutralizing antibodies could prevent metastasis[165].

In the ever-evolving continuum of stress resistance pathways, ER stress and autophagy have similarly emerged as promising targets in the face of metabolic, genotoxic, therapeutic, and immune stresses. This intrinsic stress response is activated by nutrient limitation or the unfolded protein response, and is dependent on IRE1α, GCN2 and PERK[166]. A partial state of unfolded protein response was responsible for loss of immune surveillance in pancreatic cancer DTCs, allowing long-term persistence of pre-metastatic cells[167]. PERK signaling is pro-metastatic across solid tumors, yet clinical application of PERK inhibitors is limited by on-target toxicity. Recent studies have shed light on potential downstream targets of PERK such as CREB3L1[168]. Desaturated lipids are also critical to survival in hypoxic conditions where their absence causes UPR-mediated apoptosis[164]. ER stress and autophagy are linked, yet autophagy is not a selective cancer pathway and reports of its directionality of impact as well as therapeutic potential are conflicting[169].

Immune checkpoint blockade

Immune checkpoint blocking therapies, chiefly PD-1/PD-L1 and CTLA-4, have become the central platform for treating a wide variety of metastatic cancers from which new therapies will be combined with or compared against. Despite extraordinary responses in a minority of

patients, *de novo* or acquired resistance and non-responsiveness to the panoply of available PD-1 and CTLA4 therapies is the rule rather than exception[170]. In many carcinomas such as metastatic RCC and HCC, PD-1 inhibitors show only moderate activity, while immunologically suppressed cancers such as breast and pancreatic cancer have shown only marginal responses[50]. Where the combination of PD-1 inhibitors with various chemotherapies and TKIs has started to show compelling activity in various malignancies[171], the discovery of next-generation immune activating therapies will be essential to expand the rate of durable remissions for metastatic patients (Figure 4).

The most notable second generation of immune checkpoint receptors include TIM3, LAG3, TIGIT, B7-H3, Siglec-15, and VISTA, all of which have shown promise as anti-metastatic targets in various stages of clinical testing (Table 1)[171]. Early results with Siglec-15 therapies such as NC318 have shown little promise in expansion cohorts. On the other hand, therapeutics against LAG3, such as eftilagimod alpha, have shown promise in 1st-line NSCLC and metastatic HR^{+} breast cancer. Antibodies against TIGIT, whose blockade leads to dramatic anti-tumor responses and clearance of chronic viral infections[172], have shown the most promise for clinical advancement. Recent results with anti-TIGIT tiragolumab demonstrate compelling efficacy in NSCLC patients, with Phase 3 trials currently underway. Therapies targeting TIM-3, VISTA and B7-H3 are also in early clinical testing (Table 1).

While immune checkpoint blocking therapies have demonstrated the most potent successes in metastatic tumors with high mutational burdens (melanoma, NSCLC) or harboring exogenous tumor viruses (Merkel cell carcinoma, EBV+ gastric cancer)[173], less mutationally active tumors may develop alternate means to suppress the anti-tumor immune response. Chief targets under investigation have included immunometabolic targets such as indoleamine 2,3 dioxygenase (IDO1), glutaminase, arginase, and the ectonucleotidases CD39/73. IDO1, glutaminase, and arginase are each implicated in tumor immunosuppression via the depletion of their corresponding amino acid substrates (W, Q, R) on which cytotoxic T cells are thought to be dependent[174]. Generation of kynurenine via IDO1 or extracellular adenosine via the ectonucelotidases CD39/CD73 are further hypothesized as immune-suppressive factors[174, 175]. Despite considerable commercial activity in advancing these hypotheses, clinical data has not supported any of these immune-metabolic targets. In retrospect, preclinical data may have amplified the reported specificity of these pathways while clinical experience has highlighted the broad activity of each target across adult physiology[176]. Other immunometabolic pathways under investigation include the balance between glycolysis and FAO in T cell subsets: Lipid accumulation and resultant lipotoxicity is associated with reduced CD8^{+} T cell activity[177] and increased T_{reg} survival or differentiation via CD36-mediated lipid scavenging[178].

Additional mechanisms, including therapies targeting tumor cell phagocytosis by macrophages, antibody-dependent B cell attack, and natural killer cell-mediated destruction, have shown little activity in the clinic despite preclinical support. For example, the CD47-SIRP1 complex is an important “don’t eat me signal” to prevent phagocytosis, yet early results in clinical trials do not support promising efficacy[179]. Chimeric antigen receptor T cell therapies (CAR-T) have similarly failed to translate from preclinical findings showing the elimination of metastases[180] into a clinically viable solid tumor therapy, likely due to the

same immunosuppressive features of solid tumors that tamp the natural anti-tumor immune response[181].

Depletion of immune-regulatory cells (MDSCs, TAMs, Tregs)

Broad evidence supports the dominance of suppressor cell populations in the tumor microenvironment. Thus, depletion of regulatory T cells (T_{reg}), myeloid-derived suppressor cells (MDSCs) or tumor-associated macrophages (TAMs) may be central to success in harnessing the immune system against metastasis. For example, 11% of patients with metastatic melanoma achieved a complete remission in response to autologous TIL infusion, this doubled to 22% when combined with a lymphodepletion regimen[182]. In mouse models, experimental depletion of T_{regs} is sufficient to eliminate both primary tumors and distant metastases[183–185], and T_{reg} depletion is further required to enhance anti-tumor response during anti-CTLA-4 treatment[186].

Despite the well-established significance of T_{regs} in cancer immune tolerance, clinically viable strategies to deplete T_{regs} from tumors or metastatic lesions remain scant. T_{regs} differentiate from naïve $CD4^+$ T cells via paracrine signaling of immunoregulatory factors from dendritic cells (DCs). Signaling via IL-2, TGF-β and all-trans retinoic acid (ATRA) secreted from these DCs is required for T_{reg} maturation[187–189]. However, the anti-TGF-β antibody fresolimumab showed no objective response when combined with radiotherapy in advanced breast cancer, and dysfunctional $CD8^+$ T cell signaling was not affected by TGF-β blockade[190]. Moreover, small molecule inhibitors of TGF-β have shown only minor effects in HCC[191]. Given the role of TGF-β in suppressing T helper 2 cell-mediated cancer immunity, bifunctional approaches depleting TGF-β signaling toward T cells provided promising preclinical and clinical activity: a TGF-β trap linked to a non-depleting anti-CD4 antibody has shown significant efficacy in mouse models[192], and a ligand trap using the TGF-βII receptor domain fused to an anti-PD-1 antibody showed clinically meaningful responses in HPV^+ cancers[193].

Modulating IL-2 levels or the CD25 receptor on T cells has been broadly tested in both autoimmune disease and the metastatic setting. CD25-depleting antibodies show efficient depletion of T_{regs}[194], yet also target $CD4^+$ Th1 cells and NK cells, thus causing undesired immune suppression. This dual activity led to early abandonment of anti-CD25 approaches in metastatic cancer, however, an alternative approach maximizing the immune-stimulating effects of IL-2 while avoiding its immunosuppressive activity is via synthetic amino acid incorporation to tune receptor affinity. Promising therapies such as Synthorin IL-2 are under investigation in multiple solid tumors[195].

Retinoid signaling is largely restricted to immune signaling in adults, as compared to TGF-β[196]. Retinoid signaling is dominant in inhibiting inflammatory T_h17 maturation from naïve T cells while it is instructive in causing T_n maturation to FOXP3+, CTLA-4-expressing T_{reg} cells[197]. In addition to its role in T_{reg} differentiation, retinoic acid forces differentiation of monocyte populations into immune-suppressive TAMs in sarcoma[198]. Despite emerging support for inhibiting retinoid signaling to deplete immunosuppressive subsets, inhibitors of the RAR nuclear receptors exhibit broad toxicity and upstream targeting of the ALDH1 enzymes that catalyze the oxidation of retinal into bioactive retinoic acid has not progressed

into viable therapies[157]. Given the ability of exogenous retinoids to suppress dysfunctions caused by T cell hyperactivation, such as atopic dermatitis[199], therapeutic approaches to deplete this pathway may prove critical to future cancer immunotherapy.

TAM populations complement T_{regs} in establishing an immunosuppressive microenvironment that supports metastatic spread. Depletion of TAMs through anti-CSF1R in a $p53^{-/-}$ transgenic breast cancer model dramatically extends survival when combined with platinum therapies by restoring a type I interferon response[200]. Whereas numerous CSF1/CSF1R inhibitors and mAbs, such as pexidartinib, have failed as monotherapies, rational combinations based on new preclinical research may provide a powerful means to deplete regulatory immune populations and enhance responses to immune checkpoint blockade. Numerous studies have sought additional targetable molecules involved in TAM function, including those that deplete TAM populations or abrogate the downstream effects of TAMs. MARCO was identified as a pattern-recognition receptor whose targeting reprograms TAM populations to an inflammatory phenotype and could be combined with checkpoint inhibitors to block metastasis[201]. RON kinase was also recently described as a key immunomodulatory pathway via its role in TAM differentiation, and small molecule inhibitors of Ron prevented the outgrowth of micrometastatic colonies[86]. TAMs enhance regulatory T cell populations via TGF-β and IL-10 secretion, however recent studies show they can also promote the invasiveness of cancer cells via CCL8 stimulation of PITPNM3[202]. Altered lipid catabolism is furthermore important to TAM polarization via oleate-dependent respiration in myeloid cells[203].

Future perspectives on treating metastatic disease

Cancer metastasis is a multifactorial process that relies both on intrinsic pathways that promote plasticity and stress responses as well as extrinsic pathways to establish the immunosuppressive stromal milieu. While this creates far more complex treatment demands compared to the driver-centric approaches to treating primary tumors, convergent evolution of distinct tumor types during the metastatic process offers compelling potential for tumor type-agnostic treatments. Despite the complexity and diversity in the pathways and mechanisms driving metastasis, some common themes emerge pointing to the importance of cellular plasticity and stress-relieving pathways for metastasis competency. Notably, a few candidate targets have been validated as key nodes of regulation and are shared across cancer types. Therefore, metastatic cancers should be approached through a biomarker-driven strategy that validates single agent or combinatorial strategies dependent upon the molecular make-up of individual tumors. As evidenced by recent studies, dual depletion of immune suppressive factors and tumor-intrinsic targeting will be a central paradigm to improve overall survival. Combinational therapies with distinct mechanisms of action will also be critical to preventing metastasis-associated resistance and promoting long term responses.

Relatively little emphasis has been placed on discovery of therapeutics enforcing metastatic regression in the preclinical setting. While this is the most difficult threshold to achieve in preclinical models, these proof-of-concept measures should become a central measure of efficacy to inform early drug development. Additionally, regulatory flexibility will be

required to advance these novel classes of therapeutics in a resource-effective manner. For example, predictive pharmacodynamic markers may be used as key secondary outcome measures in addition to 1-year PFS/OS measures in high-need patients. This is particularly important as high-risk patients often cycle through multiple experimental regimens, making overall survival a difficult endpoint.

In the search for new therapeutics, the importance of lifestyle and diagnostic factors in reducing cancer mortality must also be prioritized. The greatest threat to our recent progress in cancer is the upswing in obesity rates becoming the greatest etiologic driver of cancer and metastasis initiation[204]. Other preventable risks meanwhile remain a major cause of cancer in much of the world. For those cancers with a slow onset of metastasis, improved diagnostic and early detection efforts will substantially improve survival. Ultimately, prevention, early diagnosis and treatment approaches must all be comprehensively optimized to further reduce cancer mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the members of our laboratory for helpful discussions. We also apologize to the many investigators whose important studies could not be cited directly here owing to space limitations. The work in the authors' laboratories is supported by grants from the Brewster Foundation, American Cancer Society, Susan G. Komen Foundation, Breast Cancer Research Foundation, the NIH and the U. S. Department of Defense to YK. SG is supported by grants from the NCI, US Department of Defense, Breast Cancer Research Foundation, Hugs for Brady, AHEPA, Val Skinner Foundation and Gertrude Fogarty Trust. Illustrations created with Biorender.com.

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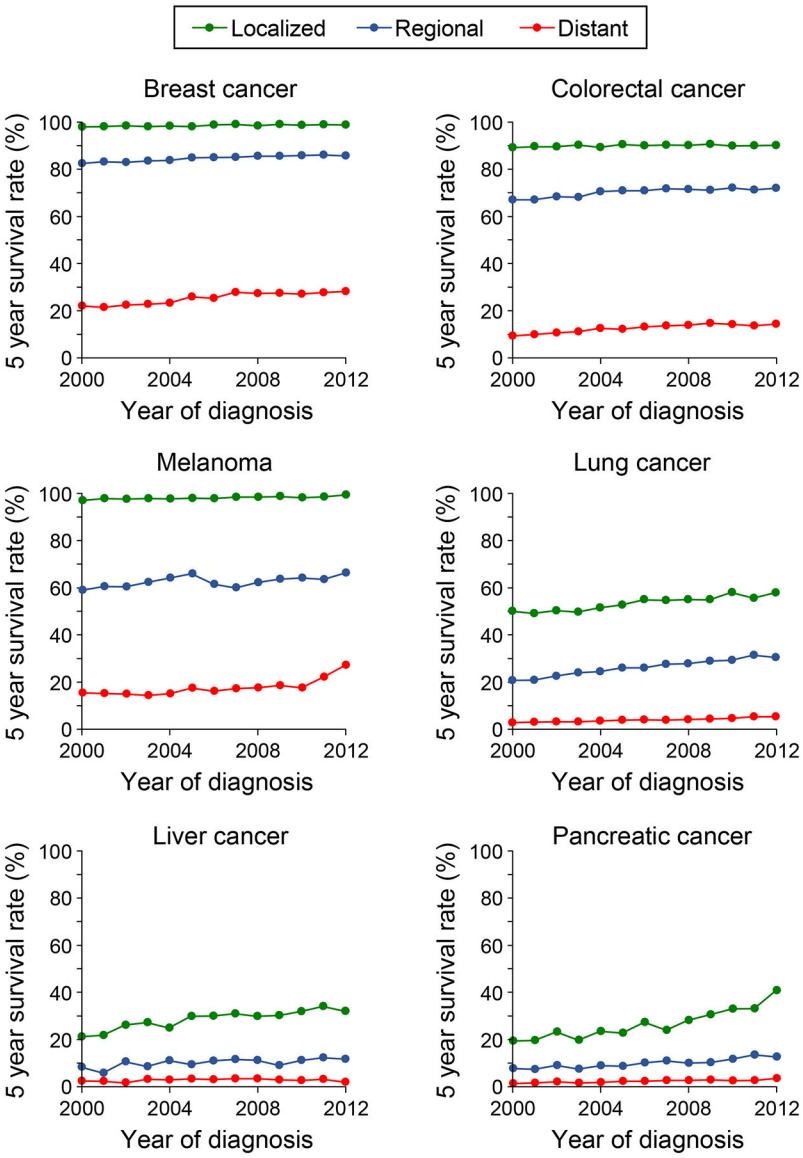

Figure 1. 5-Year survival rates of patients diagnosed with select cancer types over the time period from 2000–2017.

Patients were stratified into three groups according to the extent of invasion or metastasis upon the original diagnosis. The year in the x-axis indicates the year of diagnosis and the first year of the 5-year monitoring period with data last reported in 2017. Data adapted from the NIH-NCI SEER database[2].

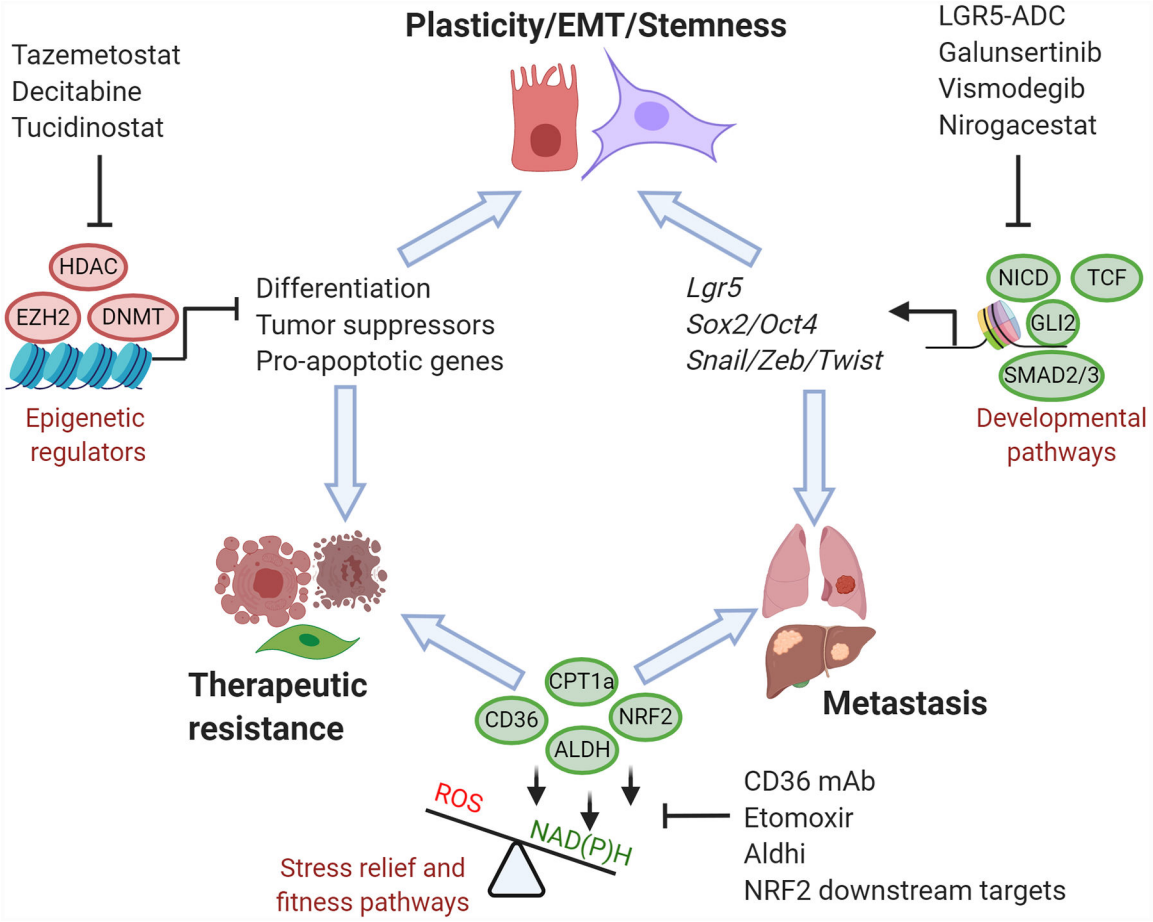

Figure 2. Metastatic ability, therapeutic resistance and plasticity are intrinsically linked by genetic, epigenetic and metabolic pathways that offer promising therapeutic nodes for drug development.

Epigenetic modifications created by DNA methyltransferases (DNMT1–4), histone methyltransferases (EZH2), and histone deacetylases (HDAC) repress tumor suppressors while promoting dedifferentiation to enhance both plasticity and therapeutic resistance. Metastatic ability and therapeutic resistance are linked by metabolic adaptations that enhance the reductive capacity of tumor cells via NAD(P)H generating pathways, such as lipid catabolism and antioxidant pathways. Cellular plasticity, epithelial-mesenchymal transition (EMT), stemness and metastasis are commonly driven by aberrant activities of key developmental pathways (TGF-β, Wnt, Notch and SHH). Therapeutic strategies to target shared mechanisms between these three hallmark characteristics of metastatic cancers may yield durable remissions either as monotherapies or when combined with classic cytotoxic therapies.

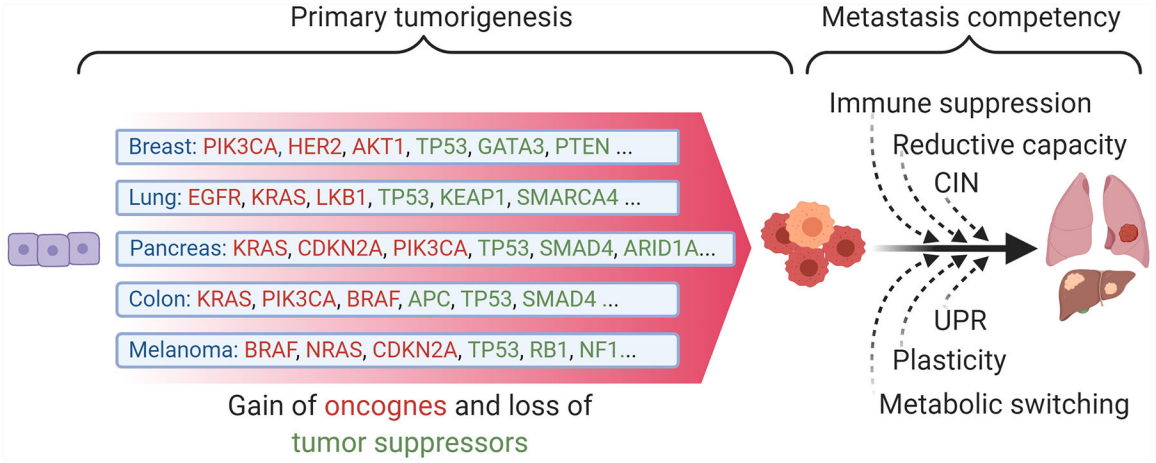

Figure 3. Development of metastasis competency requires additional malignant properties beyond primary tumorigenesis.

Distinct primary tumor types evolve in a stepwise fashion with each additional mutation in a tumor suppressor or oncogene leading to incremental increases in competitive advantage. Key oncogene and suppressor mutations driving common cancers are often distinct from other cancer types and relatively few mutations are required for tumor formation (estimated at 2–8 driver mutations). In comparison, mutations in common oncogenes are not sufficient for metastatic competence, which instead requires the acquisition of multiple cellular programs that must align in order for the cells to withstand the stresses imposed by the metastatic process. Distinct tumor types therefore convergently evolve to acquire these pro-metastatic programs, which in turn offer the opportunity for biomarker-driven tumor type-agnostic therapies in metastatic cancer patients. CIN: Chromosomal instability UPR: Unfolded protein response.

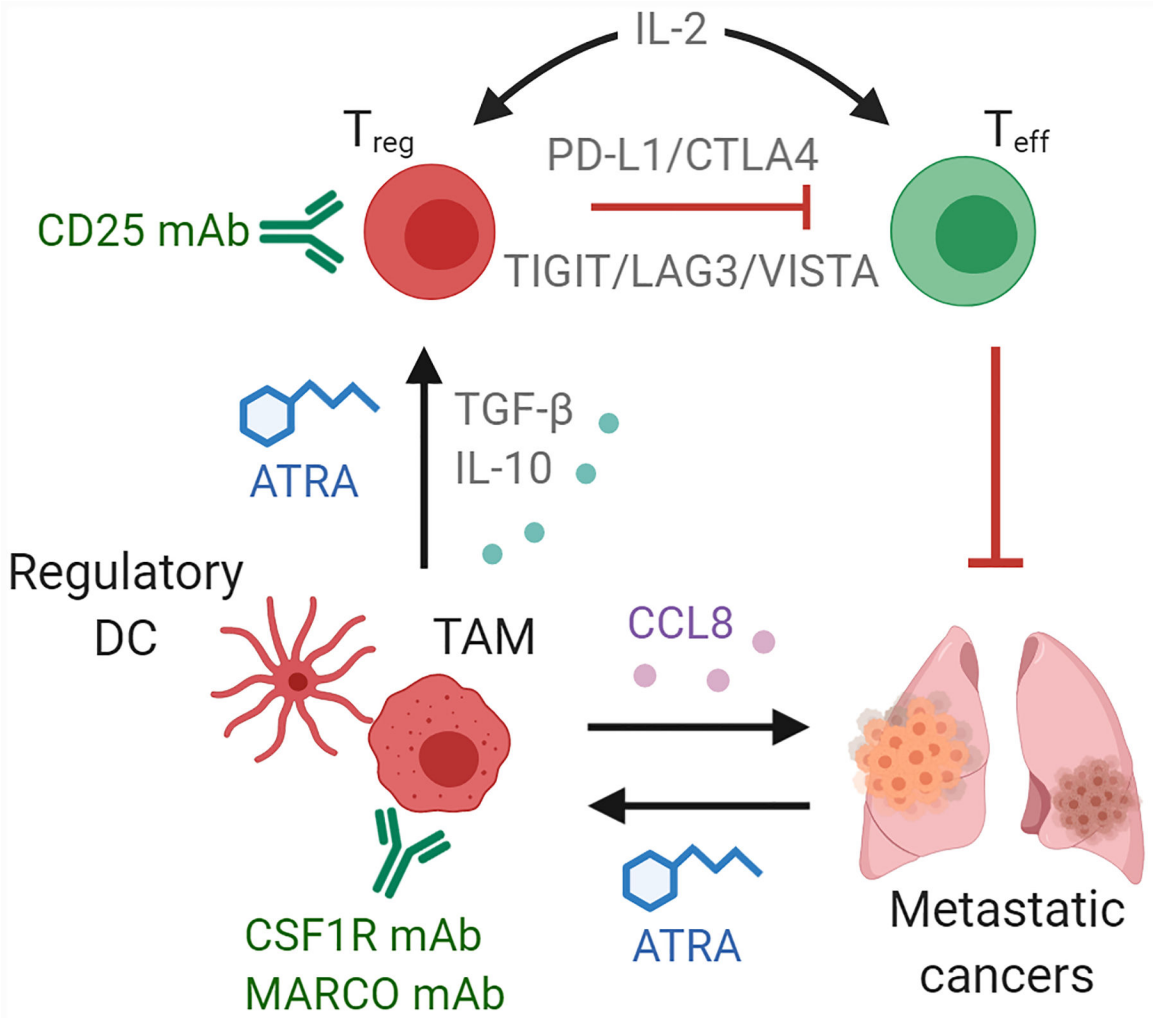

Figure 4. Targeting the immune microenvironment of metastatic cancers.

Metastatic lesions enforce immune suppression through the stimulation of key immunoregulatory cell types, including tumor-associated macrophages (TAM), regulatory dendritic cells (DC), regulatory T cells (T_{reg}) and other myeloid-derived suppressive cell populations, that prevent effector T cells (T_{eff}) from recognizing and destroying tumor cells. PD-1/PD-L1 inhibitors have already shown strong efficacy in alleviating T_{reg}-imposed immune suppression at the metastatic site of immunologically-active tumors and additional immune checkpoint blocking therapies are in development (TIGIT, LAG3, VISTA). For immunologically-cold tumors, depletion of immunosuppressive cells through antibody treatment (CSF1R, MARCO, CD25) or inhibition of various differentiation/growth pathways (retinoid and cytokine) may enforce durable remissions of metastatic disease.

Table 1.

Target, indication, and development stage of therapeutic agents against metastatic cancers

	Target	Cancer type	Latest stage	Survival benefit (i) or best efficacy (ii and iii)	Safety concerns	Citation or Trial	Status (Oncology)
i. Select therapies approved in the metastatic setting							
zoledronate	Osteoclasts	Breast	Phase III	Disease-free survival (HR: 0.66)	Osteonecrosis of the jaw	Brufsky et al. 2015	NDA approval
denosumab	RANKL	Breast	Phase III	Disease-free survival (HR: 0.82)	Well-tolerated	NCT00556374	BLA approval
tazemetostat	EZH2	Epitheloid sarcoma (INI1-)	Phase II *	No OS endpoint	Asthenia/Anemia/Nausea	NCT02601950	NDA approval
sacituzumab govitecan-hziy	TROP2 ADC	Breast (TNBC)	Phase II *	No OS endpoint	Myelotoxicity	NCT01631552	BLA approval
ado-trastuzumab emtansine	HER2 ADC	Breast	Phase III	OS benefit vs Her2 mAb + Chemo (HR: 0.68)	Thrombocytopenia and Hepatotoxicity	NCT00829166	BLA approval
olaparib	PARP	Ovarian	Phase III	OS benefit vs placebo (0.74)	Fatigue, Diarrhea and hypertension	NCT01874353	NDA approval
vismodegib	SMO	Basal cell carcinoma	Phase II *	No OS endpoint	Pneumonia and syncope	NCT00833417	NDA approval
pembrolizumab	PD-1	Melanoma	Phase III	OS benefit vs Ipilimumab (HR:0.68)	Colitis, diarrhea, fatigue, hepatotox	NCT01866319	BLA approval
ii. Investigational tumor-intrinsic targets							
demcizumab	DLL4 (Notch)	Metastatic NSCLC	Phase II	50% ORR	Severe cardiac toxicity	NCT01189968	Terminated
tarextumab	Notch2/3 (Notch)	Metastatic PDAC	Phase II	Reduced OS	Severe GI toxicity	NCT01647828	Terminated
rovalpituzumab tesirine	DLL3-ADC (Notch)	SCLC	Phase II	Terminated	Significant Grade 3–5 toxicity	NCT02674568	Terminated
ipafricept	Wnt8 (Wnt)	Ovarian	Phase I	Terminated	Significant bone toxicity	NCT02092363	Terminated
vantictumab	FZD1,2,5,7 (Wnt)	Metastatic PDAC	Phase I	Terminated	Significant bone toxicity	NCT02005315	Terminated
fresolimumab	TGF-β	Melanoma and RCC	Phase I	<5% ORR	Reversible keratoacanthomas	NCT00356460	Terminated
galunisertib	ALK5 (TGF-β)	metastatic PDAC	Phase II	DCR: 25%	Neutropenia and Hepatotoxicity	NCT02734160	Combination trials
decitabine	DNMT1–4	Metastatic prostate	Phase II	PR: 17%	Neutropenia	Thibault et al 1998	Combination trials
tucidinostat	HDAC class 1	Advanced HR+ breast	Phase III	PFS (HR: 0.75)	Neutropenia	NCT02482753	Unknown
metarrestin	Perinucleolar envelope	Advanced solid tumors	Phase I	TBD	TBD	NCT04222413	In development

	Target	Cancer type	Latest stage	Survival benefit (i) or best efficacy (ii and iii)	Safety concerns	Citation or Trial	Status (Oncology)
nirogacestat	γ-secretase (Notch)	Desmoid	Phase III	TBD	TBD	NCT03785964	In development
iii. Investigational immune activating strategies							
NC318	Siglec-15	Advanced solid tumors	Phase II	TBD	TBD	NCT03665285	In development
eftilagimod alpha	LAG3	metastatic breast	Phase I	50% ORR w/taxol	Asthenia	NCT00349934	In development
tiragolumab	TIGIT	NSCLC	Phase II	TBD	TBD	NCT04294810	In development
epacadostat	IDO1	Melanoma	Phase III	None	None	NCT02752074	Terminated
MBG453	TIM3	Advanced solid tumors	Phase II	TBD	Fatigue	NCT02608268	In development
JNJ-61610588	VISTA	Advanced solid tumors	Phase I	Terminated	Terminated	NCT02671955	Terminated
Enoblituzumab	B7-H3	Advanced solid tumors	Phase I	SD reported	Fatigue and infusion reactions	NCT01391143	In development
Hu5F9-G4	CD47/SIRP1	Advanced solid tumors	Phase I	None	Myelotoxicity	NCT02216409	In development
IPH5201	CD39/73	Advanced solid tumors	Phase I	TBD	TBD	NCT04261075	In development

* single arm; TBD: to be determined