Emerging strategies for treating metastasis

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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.

Cancer metastasis, or the systemic spread and growth of tumor cells throughout the body, is the principal cause of cancer-related death1. Despite 70 years of drug development for cancer, survival rates for patients with metastatic disease remain abysmal, with 5-year survival rates ranging from 5–30% across solid tumor types2. This low survival rate has persisted because clinical results have consistently shown that preclinical therapeutic efficacy does not always translate into clinical benefit for patients with metastatic disease3.

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 owing to acquired mechanisms of therapeutic resistance4. On the other hand, the possibility of durable remission after treatment with immunotherapies has begun a paradigm shift for patients with stage IV disease who previously received terminal diagnoses. These advances in immunotherapy have ushered in a new era where patients with advanced cancer can now hope for long-term remission or chronic management of metastases5. Despite this, the majority of metastatic cancers have yet to see therapies with similar efficacy inducing long-term, durable remission.

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, mechanisms of cell plasticity and stress resistance have become key areas of promise for therapies tackling metastasis6. While targeting these interconnected pathways may not show strong efficacy in vitro, these pathways are critical to therapeutic resistance as well as to overcoming metastatic bottlenecks in vivo. In the tumor-extrinsic context, therapies to relieve immune suppression via reprogramming the local immune milieu may open new avenues of metastasis immunotherapy.

In this Review, we aim to (1) rationalize why cancer metastasis should be a principal consideration in future cancer drug development, (2) appraise the therapies that have and have not worked in metastatic settings, and (3) 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 tumors7,8. Although cancer death rates have declined by 29% since their peak in the mid-1990s9, most of this reduction is related to preventative lifestyle changes (smoking cessation10, human papillomavirus (HPV) vaccination11 and hepatitis C treatment), early screening12 and advances in adjuvant therapy for high-risk patients without clinically detectable metastatic disease13. Whereas these interventions have reduced the prevalence of macrometastasis, few of them substantially affect survival in patients with established metastases.

The death toll caused by metastatic cancer is difficult to quantify, as metastatic disease manifests across multiple organs and mortality reporting is inconsistent across healthcare systems and cancer types14. For example, in a case study of patients with breast cancer, 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 metastasis. Only a small minority of the cancer-related deaths could be traced to the primary tumor or drug treatment, and the remainder were attributed to unrelated causes15. 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 spread16.

Comparable mortality burdens due to metastasis are observed across solid tumor types, indicating that preventative and therapeutic approaches to lessen the impact of metastatic cancer must be undertaken to ensure a meaningful change in cancer death rates17.

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 owing to the specific cell of origin and/or mutational lineage18. 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 interventions19. Pancreatic cancer is thought to be slow growing and the formation of distant metastasis does not occur until advanced stages20, although cancer cell dissemination may also occur at earlier stages21. Unlike basal cell carcinoma, the difficulty of early diagnosis in pancreatic cancer means that the majority of clinical patients present with metastasis22. Population-wide measures for early detection of cancers with time-dependent metastasis may...
present an ideal opportunity to reduce the prevalence of metastasis. Common cancers such as breast, colorectal, renal, lung and prostate cancers belong to the early-metastasizing group; while some cases might never spread, around 10–15% of breast cancers develop metastasis within 3 years, and genetic characterization has revealed the existence of primary tumors that disseminate early. This is supported by mouse studies showing that breast cancers can metastasize before the primary tumor is palpable and by patients with prostate cancer showing molecular heterogeneity across bone metastases. 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. Organ transplants that later manifest donor-derived metastases in immune-suppressed recipients further suggest that these tumors metastasize early from undetectable primary cancers. This is supported by the finding that 5–10% of tumor diagnoses are in patients with unknown primary tumors presenting with systemic metastases. For these cancers with parallel progression, early detection may be less effective in preventing the development of metastasis or mortality, as has been witnessed in the controversies surrounding implementation of population-wide prostate-specific antigen (PSA) and mammogram testing. 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 National Cancer Institute (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 (Fig. 1). In contrast, the majority of the more than 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 in surgery, radiation, chemotherapy and targeted therapy, contributed only 4–8% of the decline in cancer mortality from 1991 to 2011 (refs. [34,36]).

Much of the mortality reductions derived from therapeutics are due 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 chemotherapies in breast cancer, apalutamide in prostate cancer, chemotherapy in lung cancer and colon cancers, and imatinib in gastrointestinal stromal tumors. Recent trials have demonstrated adjuvant efficacy for abemaciclib (CDK4/CDK6 inhibitor) in hormone receptor (HR)+ HER2-negative breast cancers, osimertinib (EGFR inhibitor) in early-stage EGFR-mutant lung cancer, dabrafenib and trametinib (BRAF and MEK inhibitors, respectively) in BRAF-mutant melanoma and pembrolizumab (anti-PD-1) in high-risk melanoma.

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 RECIST1 metrics in the advanced or metastatic tumor setting before the expense of adjuvant trials can be justified; thus, metastasis-preventing therapeutics are unlikely to ever reach the adjuvant testing space where they would be most effective. Moreover, identifying responsive sub-populations and predictive pharmacodynamics adds to these challenges. For example, adjuvant treatment with bone metastasis-specific resorption inhibitors is only effective in the postmenopausal subgroup of patients with breast cancer. Finally, long-term adjuvant treatment may be limited by chronic toxicity, such as the cardiotoxicity seen with trastuzumab. 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. This drug development strategy has worked for some exceptionally potent therapies showing dramatic responses in advanced or metastatic settings, such as cabozantinib (VEGFR, AXL and MET inhibitor) in advanced or metastatic renal cell carcinoma, endocrine therapies in HR+ breast cancers and vemurafenib (BRAF inhibitor), which induces potent but short-lived responses in metastatic melanoma.

Newly emerging strategies targeting RET, NTRK and NR1I1 have also recently shown exceptional responses in non-small-cell lung cancer (NSCLC) brain metastases harboring RET fusions (overall response rate (ORR) > 90%), leading to approval of selpercatinib. Meanwhile, many other therapies targeting oncogenic drivers and dependencies, such as regorafenib (VEGFR inhibitor) and cetuximab (EGFR inhibitor) in metastatic colorectal cancer, 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 more than 90% of metastatic cancers will develop resistance to cytotoxic agents, leading to death within 10 years.

ADCs have been enthusiastically pursued for their potential to deliver a one-two punch. An example of this class is ado-trastuzumab emtansine (anti-HER2), which extends 2-year survival in patients with metastatic disease in comparison to lapatinib (HER2 inhibitor) and chemotherapy. In contrast, ADCs targeting non-driver targets such as sacituzumab govitecan-hziy (anti-TROP2 linked to SN-38) in metastatic triple-negative breast cancer (TNNBC) yields only a modest response with short duration (5.5 months) and considerable toxicity. Similarly, ADCs targeting non-driver cell-surface proteins such as folate receptor in ovarian cancer (mirvetuximab soravtansine), DLL3 in lung cancer (rovalpituzumab tesirine) and EpCAM in bladder cancer (oprtuzumab monatox) have met with varying degrees of failure in phase 2 or 3 trials (Table 1).

Perhaps the most notable shortfall for patients with stage IV disease is the numerous targeted therapies that have been approved despite showing only progression-free survival (PFS) benefits and no changes in overall survival (OS). Patients with cancer receiving these therapies may have tumor shrinkage but do not live longer or necessarily have a better quality of life. The discrepancies between PFS and OS are evident in two recent phase 3 trials of nivolumab (anti-PD-1) compared to everolimus (mTOR inhibitor) in advanced renal cell carcinoma and of nivolumab compared to docetaxel in NSCLC: both trials showed compelling increases in OS for nivolumab with nonsignificant changes or even reverse trends in PFS.

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). Experimental models suggested that MMPs were central to cancer metastasis by increasing cell motility and invasion. However, clinical trials of MMP inhibitors showed either no response or worse outcomes. While failure has been chalked up to an incomplete understanding of the specificity of the MMPs, inadequate clinical trial protocols and unintended effects on the immune system, 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 that cancers nonspecifically shed metastasis-competent cells and patients typically harbor metastasis-competent dormant cancer cells before diagnosis and treatment.
Targeting phenotypic plasticity and stress resistance

The concept of dedifferentiation and phenotypic plasticity has been a central theme of metastasis research since pivotal early studies revealed that less differentiated cancers present a higher risk of metastasis and therapeutic resistance[7]. This concept has fluidly evolved over time as tumor cell dedifferentiation, epithelial–mesenchymal transition (EMT) and mesenchymal–epithelial transition (MET), the cancer stem cell (CSC) hypothesis and, more recently, plasticity[44,49]. Controversies have surrounded these theories, with studies claiming that EMT is either essential[40] or dispensable[41] for metastasis 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[42].

Whereas plasticity has recently been linked to metastasis[45], it has long been established that cell state transitions toward stem-like states are central to therapeutic resistance[50]–[58], and this has been validated by discoveries of genes such as MTDH, which has key roles in stemness, EMT–MET, metastasis, stress resistance and therapeutic resistance[41,47]. It is therefore not surprising that all these processes are intrinsically linked (Fig. 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 transforming growth (TGF)–β, Wnt, Hedgehog (SHH) and Notch signaling[49]. These pathways drive broad transcriptional changes and exhibit extensive cross-talk[46,47]. 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 owing to its well-characterized signaling cascade as well as its oncogenic role across tumor types. Notch signaling is initiated by Delta-like (DLL1–DLL4) or Jagged (JAG1 and JAG2) ligands binding to the NOTCH1–NOTCH4 receptors. This causes proteolytic cleavage and translocation of the Notch intracellular domain (NICD) to the nucleus, where it can induce pleiotropic transcriptional activation[4]. Notch signaling has a key role in development, and its dysregulation is central to plasticity in cancer[44]. DLL1 is an important mammary stem cell marker and regulator[45], and forced expression of NOTCH3 expands the CSC-enriched side population in pancreatic cancer[46]. Prostate cancer cells activate Notch to transdifferentiate into osteoblast-like cells that enhance bone metastasis[47], and the interaction of Notch and SHH signaling drives docetaxel resistance in prostate cancer cells[48]. Notch is also critical for tumor-initiating properties downstream of TP53 and RB1 deletion in hepatocellular carcinoma[49]. The JAG1–NOTCH interaction is a bona fide driver in both bone and brain metastasis[50], with both tumor and stromal cells expressing JAG1 and/or Notch receptors to sustain stemness and chemoresistance[51,52]. Treatment of solid tumor patient-derived xenografts (PDXs) with anti-NOTCH2 and NOTCH3 (anti-NOTCH2/3) monoclonal antibody (mAb) in combination with chemotherapy depletes tumor-initiating cells (TICs) and delays recurrence[53]. γ-Secretase inhibitors (GSIs), which prevent Notch processing upon ligand binding, have similar effects in preventing metastasis and MET in hepatocellular carcinoma[54] and in sensitizing prostate CSCs to chemotherapy[55]. JAG1 is critical to the bone metastasis niche[56], and therapeutic inhibition of it synergizes with chemotherapy to slow bone metastasis[57].

Two therapeutic approaches targeting the Notch pathway have progressed into the clinic—GSIs and mAbs against Notch membrane proteins[58]. GSIs exhibited activity in early trials, yet broad gastrointestinal toxicity prevented further development[59]. Thus, other targeted approaches were attempted, including anti-DLL4 (demcizumab), which showed considerable toxicity and low efficacy in pancreatic and lung cancers[60], and the anti-NOTCH2/3 antibody tarextumab, which led to significantly worse survival outcomes in comparison to standard of care (Table 1)[61]. More recent development of an anti-DLL3 ADC, rovalpituzumab tesirine, was recently terminated in NSCLC owing to a lack of survival benefit[62]. Interestingly, one oral GSI (nirogacestat) has progressed into phase 3 trials for desmoid tumors after exhibiting moderate toxicity coupled with very promising results in multiple tumor types[63] (Table 1).

The Wnt pathway has similarly been implicated in metastasis across tumor types[64]. APC mutations in colorectal cancers and amplification of the genes encoding β-catenin, FZD and LRP signaling proteins across solid tumor types implicate Wnt signaling in tumor progression[65]. Wnt also drives the epithelial stem cell state; LGR5, a G-protein-coupled receptor (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[66]. Wnt also maintains plasticity via expression of OCT4 to induce dedifferentiation[67].

Wnt signaling has time-dependent and organ-tropic effects during metastasis[68]. Signaling is induced in the vascular bone metastasis niche by E-selectin, leading to the acquisition of dual epithelial and stem cell properties[69]. Conversely, the Wnt inhibitor DKK1 has
**Table 1 | Target, indication and development stage of therapeutic agents against metastatic cancers**

| Target | Cancer type | Latest stage | Survival benefit or best efficacy\(^\text{a}\) | Safety concerns | Citation or trial number | Status (oncology) |
|--------|-------------|--------------|-----------------------------------------------|-----------------|--------------------------|------------------|
| Zoledronate | Osteoclasts | Phase 3 | Disease-free survival (HR: 0.66) | Osteonecrosis of the jaw | Brufsky et al.\(^\text{2}\) | NDA approval |
| Denosumab | RANKL | Phase 3 | Disease-free survival (HR: 0.82) | Well tolerated | NCT00556374 | BLA approval |
| Tazemetostat | EZH2 | Phase 2\(^\text{a}\) | No OS endpoint | Asthenia, anemia, nausea | NCT02601950 | NDA approval |
| Vantictumab | FZD1/2/5/7 (Wnt) | Phase 2\(^\text{a}\) | No OS endpoint | Myelotoxicity | NCT01631552 | BLA approval |
| Olaparib | PARP | Phase 3 | OS benefit vs. placebo (HR: 0.74) | Fatigue, diarrhea, hypertension | NCT01874353 | NDA approval |
| Vismodegib | SMO | Phase 2\(^\text{a}\) | No OS endpoint | Pneumonia, syncope | NCT00833417 | NDA approval |
| Pembrolizumab | PD-1 | Phase 3 | OS benefit vs. ipilimumab (HR: 0.68) | Colitis, diarrhea, fatigue, hepatotoxicity | NCT01866319 | BLA approval |

**Investigational tumor-intrinsic targets**

| Select therapies approved in the metastatic setting | Target | Cancer type | Latest stage | Survival benefit or best efficacy\(^\text{a}\) | Safety concerns | Citation or trial number | Status (oncology) |
|-----------------|--------|-------------|--------------|-----------------------------------------------|-----------------|--------------------------|------------------|
| Demcizumab | DLL4 (Notch) | Metastatic NSCLC | Phase 2 | 50% ORR | Severe cardiac toxicity | NCT01189968 | Terminated |
| Tarextumab | Notch2/3 (Notch) | Metastatic PDAC | Phase 2 | Reduced OS | Severe GI toxicity | NCT01647828 | Terminated |
| Royalpituzumab tesirine | DLL3 ADC (Notch) | SCLC | Phase 2 | Terminated | Significant grade 3–5 toxicity | NCT02674568 | Terminated |
| Ipafricept | Wnt8 (Wnt) | Ovarian | Phase 1 | Terminated | Significant bone toxicity | NCT02092363 | Terminated |
| Vantictumab | FZD1/2/5/7 (Wnt) | Metastatic PDAC | Phase 1 | Terminated | Significant bone toxicity | NCT02053515 | Terminated |
| Fresolimumab | TGFB | Melanoma and RCC | Phase 1 | <5% ORR | Reversible keratocantheromas | NCT00356460 | Terminated |
| Galunisertib | ALKS (TGF-β) | Metastatic PDAC | Phase 2 | DCR: 25% | Neutropenia, hepatotoxicity | NCT02731460 | Combination trials |
| Decitabine | DNMT1-4 | Metastatic prostate | Phase 2 | SD: 17% | Neutropenia | Thibault et al.\(^\text{2}\) | Combination trials |
| Turcindoostat | HDAC class 1 | Advanced HR+ breast | Phase 3 | PFS (HR: 0.75) | Neutropenia | NCT02482753 | Unknown |
| Metarrestin | Perinucleolar envelope | Advanced solid tumors | Phase 1 | TBD | TBD | NCT04222443 | In development |
| Niragocesat | y-Secretase (Notch) | Desmoid | Phase 3 | TBD | TBD | NCT03785964 | In development |

**Investigational immune-activating strategies**

| Target | Cancer type | Latest stage | Survival benefit or best efficacy\(^\text{a}\) | Safety concerns | Citation or trial number | Status (oncology) |
|--------|-------------|--------------|-----------------------------------------------|-----------------|--------------------------|------------------|
| NC318 | Siglec-15 | Advanced solid tumors | Phase 2 | TBD | TBD | NCT03666285 | In development |
| Efzilagimod alpha | LG3 | Metastatic breast | Phase 1 | 50% ORR with taxol | Asthenia | NCT00349934 | In development |
| Tiragolumab | TIGIT | NSCLC | Phase 2 | TBD | TBD | NCT04294810 | In development |
| Epacadostat | IDO1 | Melanoma | Phase 3 | None | None | NCT02752074 | Terminated |
| MBG453 | TIM3 | Advanced solid tumors | Phase 2 | TBD | Fatigue | NCT02608268 | In development |
| JNI-6105B88 | VISTA | Advanced solid tumors | Phase 1 | Terminated | Terminated | NCT02671955 | Terminated |
| Enoblituzumab | B7-H3 | Advanced solid tumors | Phase 1 | SD reported | Fatigue, infusion reactions | NCT01391413 | In development |
| Hu5F9-G4 | CD47/SIRP1 | Advanced solid tumors | Phase 1 | None | Myelotoxicity | NCT02214049 | In development |
| IPHS201 | CD39/73 | Advanced solid tumors | Phase 1 | TBD | TBD | NCT04261075 | In development |

\(^{a}\)Outcome is shown as survival benefit for select therapies approved in the metastatic setting and best efficacy otherwise. \(^{b}\)Single-arm trial. BLA, biologics license application; DCR, disease control rate; GI, gastrointestinal; HR, hazard ratio; ORR, overall response rate; NDA, new drug application; NSCLC, non-small-cell lung cancer; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; PR, partial response; RCC, renal cell carcinoma; SCLC, small cell lung cancer; SD, stable disease; TBD, to be determined.

pro-metastatic roles by suppressing Wnt activity in dormant bone metastasis cells to evade natural killer (NK) cell-mediated clearance\(^6\) or by enhancing osteolysis in late-stage bone metastasis\(^7\).

Targeting the Wnt pathway is difficult owing 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)\(^8\) or anti-FZD1, FZD2, FZD5 and FZD7 (anti-FZD1/2/5/7; vantictumab) in metastatic cancers identified severe bone toxicities\(^9\). Moreover, research has suggested that targeting the canonical Wnt pathway may induce the pro-metastatic non-canonical pathway\(^8\). Thus, continued exploration of the Wnt pathway and its downstream effectors is required. One potentially interesting route is via LGR5 ADCs, which have shown compelling data across multiple models of tumorigenesis and metastasis. However, LGR5 ADCs have not progressed into the clinic owing to a lack of enthusiasm for CSC-targeted treatments and potential toxicity to normal adult stem cell pools\(^9\). The TGF-β pathway comprises multiple ligands, including the bone morphogenetic proteins (BMPs), activins and TGF-β1–TGF-β3, that, when bound to TGF-β receptors (TGFβRI, TGFβRII, ALK and
BMPR), drive a SMAD-mediated transcriptional program that is highly context dependent\textsuperscript{69}. This signaling ranges from acting as a tumor suppressor in early cancers\textsuperscript{100}, a mediator of immune suppression across diseases and a critical driver of mesenchymal traits, plasticity and EMT in metastasis\textsuperscript{100,105}. TGF-β signaling is responsible for inducing and maintaining the mesenchymal dedifferentiated state in metastatic cancers\textsuperscript{60}, inducing pro-metastatic JAG1 in bone metastasis\textsuperscript{83}, interleukin (IL)-11 in liver metastasis\textsuperscript{102} and ANGPTL4 in lung metastasis\textsuperscript{103} while suppressing Wnt signaling via DKK1 induction\textsuperscript{94}. TGF-β signaling has been shown to be a critical pro-malignancy pathway in late-stage cancers\textsuperscript{104} as well as for the supportive stroma needed for metastasis initiation\textsuperscript{105}.  

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\textsuperscript{106}. Treatment can further deplete metastasis-initiating cells via stromal targeting\textsuperscript{103} or slow the vicious cycle of bone metastasis\textsuperscript{106}. In contrast to the toxicity associated with Wnt and Notch targeting, TGF-β pathway ablation is tolerated, with cardiac toxicity as the major on-target concern\textsuperscript{107}. However, the multifaceted role of TGF-β signaling in both tumor promotion and tumor suppression presents problems for clinical advancement, as one notable side effect of TGF-β inhibition is the transient and reversible onset of various neoplasms, mostly keratoacanthomas\textsuperscript{108}.  

Finally, SHH signaling is also associated with stemness and metastasis\textsuperscript{109}, stromal activation during tumorigenesis\textsuperscript{110} and therapeutic resistance\textsuperscript{106}. The central transcription factors of SHH signaling are GLI1 and GLI2, which show aberrantly high expression in bone metastatic tumor cells, where they enhance PTHRP expression to induce osteolytic metastasis\textsuperscript{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\textsuperscript{+} stem cell pool\textsuperscript{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 for more than 1 year and median survival times nearing 3 years in patients with metastatic disease\textsuperscript{113}. However, SMO inhibitors resulted in worse patient outcomes across pancreatic cancer trials, as SHH inhibition promoted progression by suppressing stromal populations\textsuperscript{114}.  

Epigenetic approaches to restrain tumor plasticity. Tumor cell plasticity often manifests as multiple semi-states that do not coexist in normal physiology. This is best illustrated by the gradient of epithelial–mesenchymal states observed in circulating breast tumor cells that vary over time in response to phosphoinositide 3-kinase (PI3K) targeting or systemic chemotherapy\textsuperscript{116}. This gradient of epithelial–mesenchymal transitions has been observed across tumor types and is ascribed to higher metastatic potential\textsuperscript{115} or increased therapeutic resistance\textsuperscript{116}. Importantly, plasticity presents as phenotypic heterogeneity across the same tumor or metastases, and this plasticity is controlled by the epigenome\textsuperscript{117}. Large-scale genomic studies further support the notion that metastasis is not driven by mutations distinct from those present in the primary tumor\textsuperscript{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\textsuperscript{119}. Indeed, reversible switching between epithelial and mesenchymal states is dependent on concentrations of TGF-β ligand rather than genetic mutations\textsuperscript{119}. This heterogeneity in cell transcription is functional in 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\textsuperscript{120–122}.  

Therapeutic targeting of the epigenome has provided compelling evidence for the feasibility of altering the plastic, dedifferentiated state exhibited during the evolution of metastasis. Early efforts...
in drug development yielded the cytidine analogs (5-aza(deoxy)cytidine) initially developed as anti-neoplastic nucleoside analogs; however, studies identified optimal biological activity at subcytotoxic doses through inhibition of DNA methyltransferases. 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.

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 activity for Polyclomb repressive complex 2 (PRC2). EZH2 activity is responsible for maintaining the dedifferentiated state in Ewing tumors and is an essential driver of melanoma metastasis through suppression of various tumor suppressors. EZH2 activity leads to RB1 and p53 suppression in prostate cancers, and this in turn results in plasticity and metastasis, while EZH2 inhibitors restore sensitivity to anti-androgens. Tazemetostat was recently approved as the first targeted therapy for metastatic epitheloid sarcoma after demonstrating activity in INI1-solid tumors and follicular lymphoma.

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

**Fitness genes and stress resistance.** At the simplest reduction, two types of pathways are responsible for cancer progression: (1) driver genes that cause cells to proliferate unchecked and (2) fitness genes that allow 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* and loss-of-function mutations in tumor suppressors such as *APC*. Stepwise accumulation of driver mutations leads to the linear progression of premalignant clones to clinically detectable tumors. 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. The Cancer Genome Atlas (TCGA) analysis confirms that classic pathways such as P3K signaling, mitogen-activated protein kinase (MAPK) signaling, cell cycle control and receptor tyrosine kinase (RTK) signaling are some of the most highly mutated pathways. Analyzing cancer genomes across patients suggests that most cancers are driven by only two to eight mutational events (Fig. 3). Because of these oncogene-centric discoveries and the impact of these pathways in cancer initiation, most drug development efforts have focused on targeting driver genes, despite the limited efficacy of these drugs in the metastatic setting.

Conversely, metastasis is theorized to be 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 accrued during tumorigenesis. These adaptations enable a discontinuous jump to metastatic competence and survival in the early metastatic niche. The chromosomal instability response is a classic fitness program—metastatic cells exhibiting chromosomal instability activate a non-canonical nuclear factor (NF)-κB response rather than a lethal interferon response to circumvent cytotoxicity. 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.

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. This idea parallels the synthetic lethality space; inhibition of driver pathways such as extracellular signal-regulated kinase (ERK) signaling can be compensated via P13K–mTOR signaling, whereas *BRCA*-mutant cancers have few compensatory mechanisms to resist genotoxicity and poly(ADP-ribose) polymerase (PARP) inhibition results in synthetic lethality. Unfortunately, PARP inhibitors exhibited only incremental improvements in PFS for advanced *BRCA1/BRCA2*-mutant ovarian cancers, 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 therapeutic PD-L1 inhibition has resulted in some of the most durable remission seen to date (Table 1).

Whereas driver mutations offer an experimentally tractable approach for drug discovery, targeting cancer fitness genes requires addition of a further 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, the metabolic stresses of advanced metastatic lesions and therapeutic challenge (Fig. 3). Importantly, the metastatic process is incredibly inefficient, suggesting that exploiting any survival liabilities used 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. Mouse studies have shown that antioxidant supplementation, often with N-acetylcysteine (NAC), is responsible for initiation, progression and reduced survival from lung cancers through a mechanism that limits Trp53 induction. In a landmark study, patient-derived xenografts experienced higher levels of oxidative stress during metastasis, NAC supplementation was sufficient to induce metastasis and NADPH production via folate pathway enzymes ALDH1L2 or MTHFD2 was also necessary for metastasis. Further studies have demonstrated that lactate utilization via the MCT1 transporter increases NADPH levels via the oxidative pentose phosphate pathway to enable melanoma metastasis. Oxidative stress is also the main underlying cytotoxic mechanism of various chemotherapies: NRFe-driven glutathione synthesis enables cisplatin resistance, and antioxidant supplementation prevents the cytotoxic effects of paclitaxel. Oxidative stress appears to be largely metastasis specific; knockout of glucose-6-phosphate dehydrogenase (G6PD) to inhibit the oxidative pentose phosphate pathway does not affect primary tumor initiation or growth in breast, colorectal or lung cancers. 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.

This evidence suggests that oxidative stress is a critical node of synthetic lethality and that pro-oxidant therapy should be considered as a high-priority drug development target. 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. A key node in oxidative stress resistance is NRF2, the master redox transcriptional
regulator whose induction by mutations or oxidative stress increases BACH1-mediated metastasis143 and cisplatin resistance144. NRF2 gain of function is observed across many cancers, and pharmacologic targeting is currently in proof-of-concept studies148. Importantly, NRF2 expression is positively correlated with the stage of many cancers, showing the greatest levels in metastatic disease, and is further responsible for enhancing chemoresistance149.

An alternative 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 outcomes150 and is broadly used as a marker for stemness, metastatic behavior and chemoresistance151. Among the 19 ALDH enzymes, ALDH1a3 has been shown to be the main driver of ALDH activity152 and subsequent metastatic or chemoresistant traits153 via its role in lipid peroxidation or direct drug detoxification154. Serine biosynthesis via the folate pathway is a potential metastatic target as the ALDH1L1 and ALDH1L2 enzymes have been shown to have important roles in metastasis via generation of NADPH155, yet the redundancy of cytosolic and mitochondrial serine biosynthesis makes this metabolism difficult to target in tumors160. Development of drug-like inhibitors of specific ALDH enzymes has not succeeded, despite extensive efforts156.

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 setting162. Ablation of FAO via targeting CPT1a with etomoxir has prevented colorectal cancer metastasis in preclinical models163. Exogenous lipid sourcing is also critical to survival in hypoxic tumors where mTOR activity and TSC2 deficiency create a synthetic dependence on desaturated lipids in hypoxic environments164. The lipid receptor CD36 was demonstrated to be a critical component of metastasis-initiating cells through exogenous palmitate catabolism, and neutralizing antibodies could prevent metastasis165.

In the ever-evolving continuum of stress resistance pathways, endoplasmic reticulum (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 (UPR) and is dependent on IRE1α, GCN2 and PERK166. A partial state of the UPR was responsible for loss of immune surveillance in disseminated tumor cells in pancreatic cancer, allowing long-term persistence of pre-metastatic cells167. PERK signaling is pre-metastatic across solid tumor types, 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 (ref. 168). Desaturated lipids are also critical to survival in hypoxic conditions, where their absence causes UPR-mediated apoptosis169. 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 conflicting170.

Harnessing the immune system to treat metastasis

Given the impact that immune therapies have across tumors previously considered incurable and that the immune tumor microenvironment has also emerged as a key player regulating metastasis, here we review studies focused on the feasibility and efficacy of immune modulation for metastatic disease.

Immune checkpoint blockade. Immune checkpoint-blocking therapies, chiefly targeting PD-1/PD-L1 and CTLA-4, have become the central platform for treating a wide variety of metastatic cancers, and new therapies will be combined with or compared against them. Despite extraordinary responses in a minority of patients, de novo or acquired resistance and nonresponsiveness to the panoply of available PD-1 and CTLA-4 therapies are the rule rather than the exception171. In many carcinomas such as metastatic renal cell carcinoma and hepatocellular carcinoma, PD-1 inhibitors show only moderate activity, while in immunologically suppressed cancers such as breast and pancreatic cancers they have shown only marginal responses160. Whereas the combination of PD-1 inhibitors with various chemotherapies and TKIs has started to show compelling activity in various malignancies172, the discovery of next-generation immune-activating therapies will be essential to expand the rate of durable remission for patients with metastatic disease (Fig. 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 antimetastatic targets in various stages of clinical testing (Table 1)173. 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 efutilagimod alpha, have shown promise in first-line treat-
ment of NSCLC and metastatic HR+ breast cancer. Antibodies against TIGIT, whose blockade leads to dramatic antitumor responses and clearance of chronic viral infections, have shown the most promise for clinical advancement. Recent results with the anti-TIGIT antibody tiragolumab demonstrate compelling efficacy in patients with NSCLC, with phase 3 trials currently underway. Therapies targeting TIM3, VISTA and B7-H3 are also in early clinical testing (Table 1).

While immune checkpoint-blocking therapies have demonstrated the greatest success in metastatic tumors with high mutational burden (melanoma and NSCLC) or harboring exogenous tumor viruses (Merkel cell carcinoma and Epstein–Barr virus (EBV)-positive gastric cancer), less mutationally active tumors may develop alternate means to suppress the antitumor immune response. Chief targets under investigation include immunometabolic targets such as indoleamine 2,3-dioxygenase (IDO1), glutaminase, arginase and the ectonucleotidases CD39 and CD73. IDO1, glutaminase and arginase are each implicated in tumor immunosuppression via depletion of their corresponding amino acid substrates (tryptophan, glutamine and arginine) on which cytotoxic T cells are thought to be dependent. Kynurenine generated via IDO1 and extracellular adenosine generated via the ectonucleotidases CD39 and CD73 are further hypothesized as immune-suppressive factors. Despite considerable commercial activity in advancing these hypotheses, clinical data have not supported any of these immunometabolic 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. Other immunometabolic pathways under investigation include the balance between glycolysis and FAO in T cell subsets: lipid accumulation and resultant lipotoxicity are associated with reduced CD8+ T cell activity and increased regulatory T (Treg) cell survival or differentiation via CD36-mediated lipid scavenging.

Additional mechanisms, including therapies targeting tumor cell phagocytosis by macrophages, antibody-dependent B cell attack and NK 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 show promising efficacy. Chimeric antigen receptor (CAR) T cell therapies have similarly failed to translate from preclinical findings showing the elimination of metastases into a clinically viable solid tumor therapy, likely owing to the same immunosuppressive features of solid tumors that tamp down the natural antitumor immune response.

Depletion of immune-regulatory cells. Broad evidence supports the dominance of suppressor cell populations in the tumor microenvironment. Thus, depletion of Treg cells, 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 complete remission in response to autologous TIL infusion, and this doubled to 22% when TIL infusion was combined with a lymphodepletion regimen. In mouse models, experimental depletion of Treg cells is sufficient to eliminate both primary tumors and distant metastases and Treg cell depletion is further required to enhance antitumor response during anti-CTLA-4 treatment.

Despite the well-established importance of Treg cells in cancer immune tolerance, clinically viable strategies to deplete Treg cells from tumors or metastatic lesions remain scant. Treg cells differentiate from naive 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 Treg maturation. 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. Moreover, small molecule inhibitors of TGF-β have shown only minor effects in hepatocellular carcinoma. Given the role of TGF-β in suppressing T helper type 2 (Th2) 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 nondepleting anti-CD4 antibody has shown substantial efficacy in mouse models, and a ligand trap using the TGFβRII receptor domain fused to an anti-PD-1 antibody showed clinically meaningful responses in HPV-positive cancers.

Modulating IL-2 levels and the abundance of CD25 receptor on T cells has been broadly tested in both autoimmune disease and the metastatic setting. CD25-depleting antibodies show efficient depletion of Treg cells yet also target CD4+ T helper type 1 (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 tumor types.

Retinoid signaling is largely restricted to immune signaling in adults, in contrast to TGF-β signaling. Retinoid signaling is dominant in inhibiting inflammatory T helper type 17 (Th17) cell maturation from naïve T cells, while it is instructive in causing maturation of naïve T cells into FOXP3+ Treg cells. In addition to its role in Treg differentiation, retinoic acid forces differentiation of monocyte populations into immune-suppressive TAMs in sarcomas. 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 therapies157. Given the ability of exogenous retinoids to suppress dysfunctions caused by T cell hyperactivation, such as atopic dermatitis189, therapeutic approaches to deplete this pathway may prove critical to future cancer immunotherapy.

TAM populations complement Tng cells in establishing an immunosuppressive microenvironment that supports metastatic spread. Depletion of TAMs through anti-CSF1R in a Trp53−/− transgenic breast cancer model dramatically extended survival when it was combined with platinum therapies by restoring a type I interferon response199. Whereas numerous CSF1/CSFIR 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 molecules to 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 metastasis200. RON kinase was also recently described as constituting a key immunomodulatory pathway via its role in TAM differentiation, and small molecule inhibitors of RON prevented the outgrowth of micrometastatic colonies201. TAMs enhance Tng cell populations via secretion of TGF-β and IL-10; however, recent studies have shown that they can also promote the invasiveness of cancer cells via stimulation with the CCL8 chemokine of PITPNM3 (ref. 202). Altered lipid catabolism is furthermore important to TAM polarization via oleate-dependent respiration in myeloid cells203.

Future perspectives on treating metastatic disease
Cancer metastasis is a multifactorial process that relies on intrinsic pathways that promote plasticity and stress responses as well as extrinsic pathways to establish an immunosuppressive stromal milieu. While this necessitates far more complex therapeutic strategies compared with 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. 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 depending on the molecular makeup of individual tumors. As evidenced by recent studies, concurrent depletion of immune-suppressive factors and tumor-intrinsic targeting will be a central paradigm to improve overall survival. Combinatorial 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 indicator 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 and OS measures in high-need patients. This is particularly important as patients at high risk often cycle through multiple experimental regimens, making OS a difficult endpoint to assess.

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 recent progress in cancer is the upswinging rates of obesity, which is becoming the greatest etiologic driver of cancer and metastasis initiation204. Other preventable risk factors meanwhile remain a major cause of cancer in much of the world. For those cancers with 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.

Data availability
The human cancer mortality data in Fig. 1 were derived from the SEER database with 2017 as the most recently annotated 5-year survival date: https://seer.cancer.gov/data/. Source data are provided with this paper. All other data are available from the corresponding author on reasonable request.

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