Cancer Stem Cells as a Therapeutic Target: Current Clinical Development and Future Prospective

Philchenkov, A.; Dubrovska, A.;

Originally published:

December 2023

Stem Cells 42(2024)3, 173-199

DOI: https://doi.org/10.1093/stmcls/sxad092

Stem Cells, 2023, **XX**, 1–27
https://doi.org/10.1093/stmcls/sxad092
Advance access publication XX XX XXXX
Concise Review

Cancer Stem Cells as a Therapeutic Target: Current Clinical Development and Future Prospective

Alex Philchenkov[*,1], **Anna Dubrovska**[*,2,3,4,5,6,7,8]

[1]RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kyiv, Ukraine
[2]OncoRay-National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden and Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany
[3]Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiooncology-OncoRay, Dresden, Germany
[4]German Cancer Consortium (DKTK), Partner Site Dresden and German Cancer Research Center (DKFZ), Heidelberg, Germany
[5]National Center for Tumor Diseases (NCT), Dresden, Germany
[6]German Cancer Research Center (DKFZ), Heidelberg, Germany
[7]Faculty of Medicine, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany
[8]Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Dresden, Germany

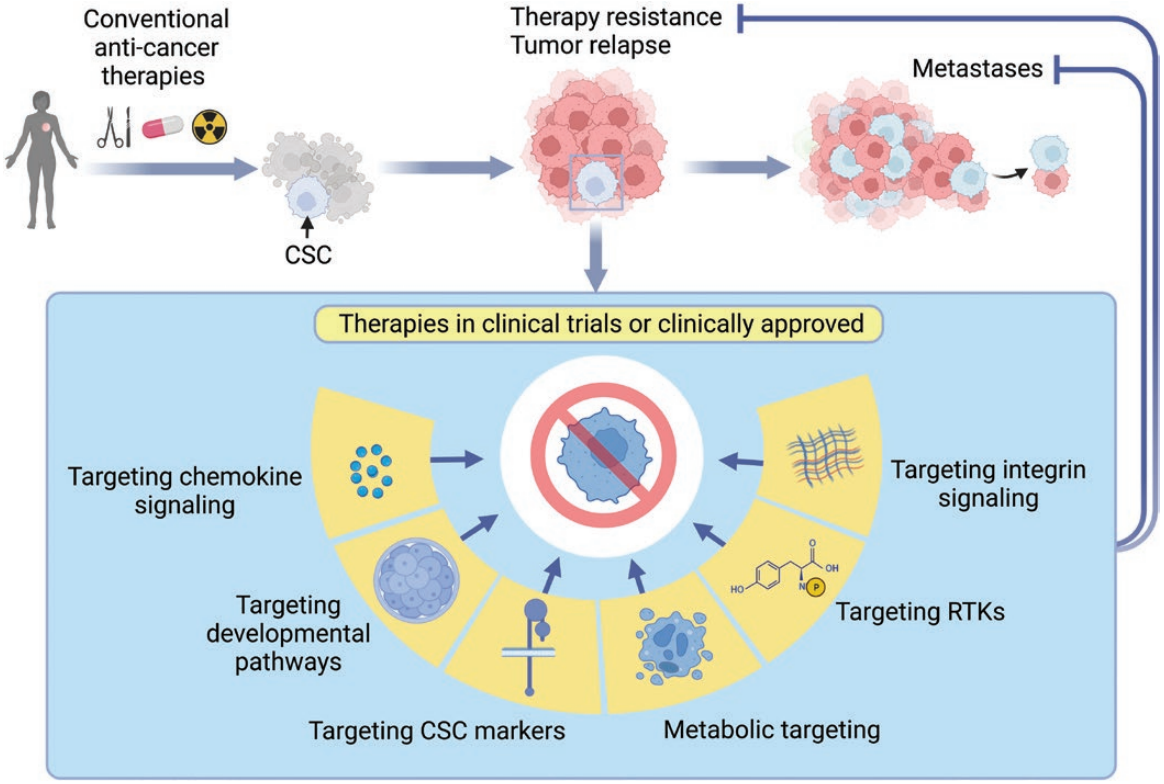
[*]Corresponding author: Anna Dubrovska. Email: a.dubrovska@hzdr.de; or, Alex Philchenkov. Email: apoclub@i.ua

Abstract

The key role of cancer stem cells (CSCs) in tumor development and therapy resistance makes them essential biomarkers and therapeutic targets. Numerous agents targeting CSCs, either as monotherapy or as part of combination therapy, are currently being tested in clinical trials to treat solid tumors and hematologic malignancies. Data from ongoing and additional clinical trials testing novel approaches to target tumor stemness-related biomarkers and pathways may pave the way for further clinical development of CSC-targeted treatments and CSC-guided selection of therapeutic regimens. In this concise review, we discuss recent progress in developing CSC-directed treatment approaches, focusing on clinical trials testing CSC-directed therapies. We also consider the further development of CSC-assay-guided patient stratification and treatment personalization.

Key words: cancer stem cells; leukemia stem cells; patient stratification; clinical trials; treatment personalization.

Graphical Abstract



Received: 4 September 2023; Accepted: 21 November 2023.

Significance Statement

Like their healthy tissue counterparts, CSCs possess 2 fundamental features: self-renewal capacity and differentiation potential. Tumor heterogeneity depends on the heterogeneity of CSCs and their evolution during tumor treatment and progression. Although CSC populations are highly dynamic and tumor stemness is not constant but a transient state, eradicating the entire CSC population is a prerequisite for efficient tumor cure. This concise review is focused on the recent progress in the clinical translation of CSC-targeting therapies and discusses their further clinical development.

Introduction

The tumors of various histogenesis consist of heterogeneous cell clones, which differ by their mutational and epigenetic landscape, morphological features and metabolic phenotype, and survival and proliferation properties. Intratumoral heterogeneity depends on the number of clonogenic cancer stem cells (CSC) contributing to the tumor growth but also on the evolution of CSCs during tumor treatment and progression.[1,2] Similar to their counterparts in healthy tissues, CSCs possess 2 fundamental features: self-renewal capacity and differentiation potential. That means that upon asymmetric division typical for stem cells, CSC is capable of making an identical CSC and giving rise to more differentiated progenitor cell that could further be differentiated into the bulk tumor cells. The maintenance, survival, self-renewal, and differentiation of CSCs are tightly controlled by the cellular and non-cellular components of the tumor microenvironment (TME) within the specific anatomical location called the CSC niche.[3] Different microenvironmental stimuli such as hypoxia, inflammation, epithelial-mesenchymal transition, and anti-cancer therapy might induce tumor cell plasticity when non-CSCs could be epigenetically converted to CSC populations.[4-6] Although CSC populations are highly dynamic and tumor stemness is not constant but a transient state, eradicating the entire CSC population is a prerequisite for efficient tumor cure. Tumor recurrence after the course of treatment occurs if not all CSCs are eradicated.[7,8] Furthermore, some CSC populations are more therapy-resistant than non-CSC counterparts due to intrinsic factors such as activation of the pro-survival signaling pathways, activation of the DNA repair, efficient defense against therapy-induced oxidative stress, and extrinsic mechanisms such as hypoxia, matrix stiffness, and TME acidification.[7,9-12] CSC populations are often slow-proliferating or quiescent, which makes CSC a hard-to-treat target for conventional treatments, mainly targeting rapidly dividing tumor cells.[13-15] A pretreatment load of CSCs was related to tumor curability in different types of cancer.[7,16,17] The key role of CSC in tumor development and therapy resistance makes them important biomarkers and therapeutic targets. Indeed, there is an accumulating preclinical and clinical effort to improve the efficacy of anti-cancer therapy by targeting CSC populations.[7,17-20] Standard approaches for anti-CSC treatment include inhibition of the CSC-related signaling pathways, tumor environment, and targeting surface markers. Several cell surface proteins, including CD133, CD44v6, the mesenchymal-epithelial transition factor receptor (c-MET, also called hepatocyte growth factor receptor, HGFR), epithelial cell adhesion molecule (EpCAM), CD47, and others, have been described as CSC-related markers in different tumor entities.[21,22] The vast majority of studies analyzing CSC-targeting treatment employ in vitro analyses such as spherogenicity assays, CSC-related markers, and gene expression analyses that do not always indicate CSC properties in vivo.[23] A tumor serial transplantation assay is a "gold standard" approach for the analysis of CSC populations.[24] Using CSC-based analysis of the patient-derived specimens is a critical prerequisite for the clinical development of CSC research. As for now, numerous agents targeting CSCs either as monotherapy or as a part of combination therapy are being tested in clinical trials for the treatment of both solid tumors and hematological malignancies. The data obtained from the current and further clinical trials testing novel approaches to target CSC-related biomarkers and signaling pathways might prove their clinical importance and open the way for further clinical development of the CSC-directed treatments and CSC-assay-based selection of the therapeutic regimens (Fig. 1). In this concise review, we discuss the recent progress in the development of CSC-directed treatment approaches for improving the clinical outcome of patients with cancer with a focus on clinical studies testing CSC-targeting therapies.

Targeting CSC in Solid Tumors

Targeting CSC Antigens and Receptor Tyrosine Kinases

Eradicating CSCs in hematopoietic and solid tumors is a prerequisite for long-term treatment success. Therefore, many efforts have been made across solid tumor entities to identify CSC-specific targets. Several CSC-related molecules were validated as promising target candidates in clinical studies, including cell surface molecules CD44v6, c-MET, EpCAM, and epidermal growth factor receptor (EGFR)[21,22] (Fig. 2). These proteins are targeted in several ways, including antibody-drug conjugates, bispecific antibodies, chimeric antigen receptor (CAR) T cells, and chemical inhibitors for the targets with RTK activity, such as c-MET and EGFR (Table 1).

The first antibody-based approach for targeting tumor cells was developed by Seiter and team in 1993 in the experimental tumor models with monoclonal antibody (mAb) against CD44v6, an isoform variant of CD44 protein. CD44v6 is a transmembrane adhesion molecule serving as a co-receptor for c-MET and vascular endothelial growth factor receptor 2 RTK, and potentiating their activation and signaling.[55] CD44v6 has been characterized as a marker and regulator of CSCs in colon and gastric tumors and is associated with tumor progression and worse clinical outcomes.[56-58] By now, several immune-based anti-CD44v6 therapies have entered clinical trials for advanced solid malignancies, as recently reviewed by Köseer et al[59] In brief, these CD44v6-specific immune approaches include mAb RG7356, ^{186}Re-labeled mAb bivatuzumab, and bivatuzumab conjugated with a microtubulin inhibitor mertansine (Table 1). The development of ADC bivatuzumab mertansine was terminated due to severe skin toxicity in a clinical trial. The emerging cell-based immunotargeting approaches, such as engineered T

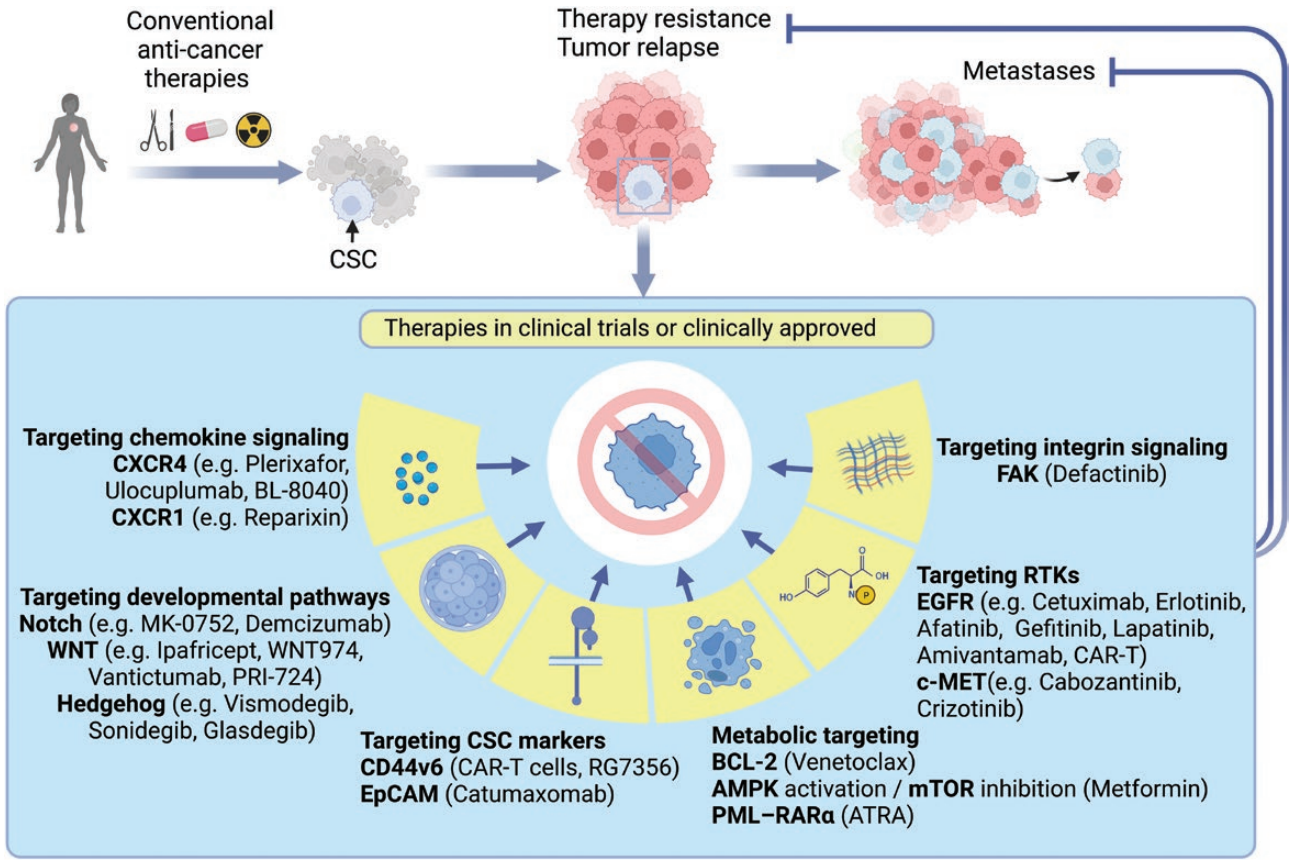


Figure 1. CSC-targeting strategies by therapies in clinical testing or clinically approved. *Source*: Created with BioRender.com.Abbreviations: AMPK, AMP-activated protein kinase; ATRA, all-trans retinoic acid; BCL-2, B-cell lymphoma 2; CD44v6, splice variant 6 of CD44; cMET, MET proto-oncogene, receptor tyrosine kinase; CSC, cancer stem cells; CXCR1, C-X-C chemokine receptor type 1; CXCR4, C-X-C chemokine receptor type 4; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; FAK, focal adhesion kinase; mTOR, mammalian target of rapamycin; PML, promyelocytic leukemia; RARα, retinoic acid receptor α.

cells expressing CAR specific to CD44v6 as a target antigen, demonstrated high toxicity for tumor cells in vitro and potent anti-tumor activity in vivo in solid and hematological malignancies.[59-62] The current clinical trials are evaluating the efficacy of CAR T-cell therapy against CD44v6 for targeting breast cancer and other tumors[59] (Table 1).

c-MET/HGFR is an RTK activating several downstream effectors, including phosphoinositide 3-kinase (PI3K)/Akt, the Janus kinase (JAK)/signal transducer, and activator of transcription (STAT), mitogen-activated protein kinase, and crosstalking with other surface molecules such as CD44, integrins, and EGFR.[63] The c-MET-driven signaling cascades regulate the maintenance and therapeutic resistance of CSC in the colon, prostate, breast cancer, glioblastoma, and other tumor types.[64-67] More than a dozen c-Met inhibitors have been tested in clinical trials up to now, and 2 of them, cabozantinib and crizotinib have been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency for clinical use; however, they are multi-target drugs and not specific for c-MET (Table 1).[68] Cabozantinib is a small molecule multi-tyrosine kinase inhibitor approved for the treatment of advanced renal cell carcinoma, metastatic medullary thyroid cancer, and hepatocellular carcinoma.[26] Crizotinib is also a multi-target inhibitor approved for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) having *ROS1* gene alteration and patients with inflammatory myofibroblastic tumors with anaplastic lymphoma kinase (*ALK*) gene alterations.[27-29] Beyond approved indications, these inhibitors are tested for other tumor entities as single agents or in combination therapy in several other cancer types. Preclinical analyses demonstrated that cabozantinib induced apoptosis of patient-derived and CSC-enriched pancreatic cancer cell cultures,[69] and crizotinib reduced CSC-like properties in the echinoderm microtubule-associated protein-like 4 (EML4)-ALK^{+} NSCLC cells.[70]

EGFR is highly expressed in cancer cells and is a key regulator of tumor cell proliferation, apoptosis, survival, and metastatic dissemination.[71] Although EGFR expression is not specific for CSCs, EGFR^{high} tumor cells are enriched for tumor-propagating cells,[72,73] and the inhibition of EGFR has a pronounced effect on the maintenance and therapy response of the CSC-like populations in prostate, breast, and NSCLC cancer models.[74-76] Several EGFR small molecule inhibitors have been clinically approved for the treatment of advanced or metastatic NSCLC, colorectal cancer (CRC), pancreatic cancer, HER2-positive breast cancer, and head and neck squamous cell carcinoma (HNSCC), as reviewed elsewhere.[77] EGFR is highly expressed in high-grade gliomas (HGG) and contributes to tumor progression.[78] Inhibition of EGFR in adult patients with progressive GBM with chemical inhibitors such as erlotinib, gefitinib, afatinib, and lapatinib or blocking antibodies such as cetuximab or nimotuzumab alone or in combination with conventional or targeted treatments was not associated with anti-tumor therapy efficacy.[78] EGFR

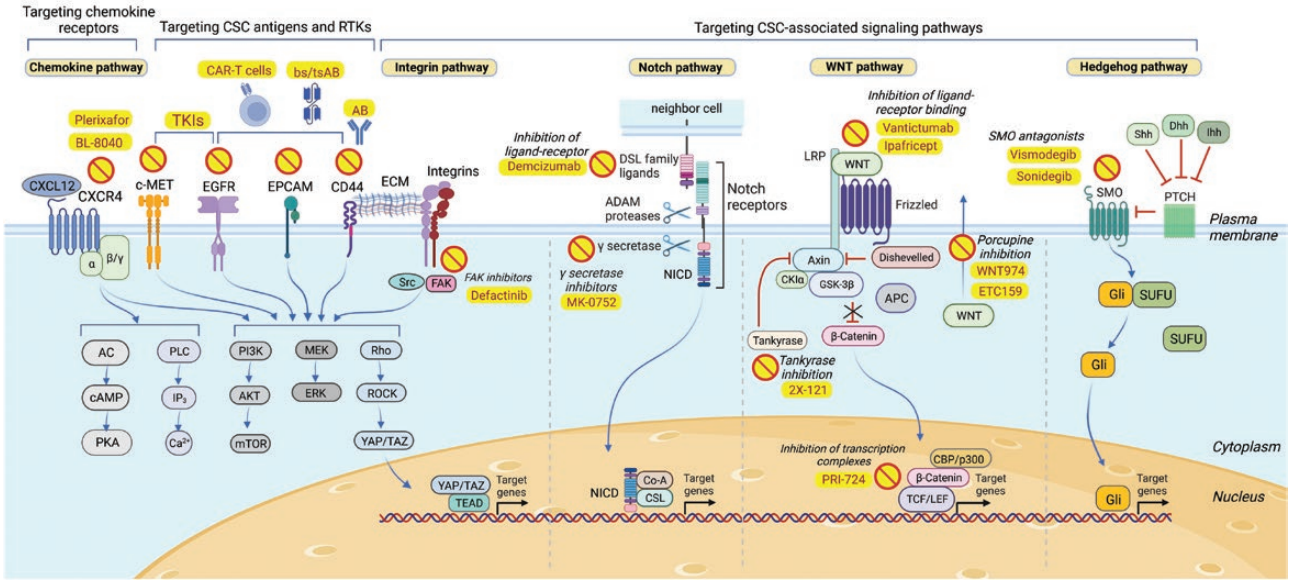


Figure 2. CSC-related signaling pathways targeted in the clinical trials. *Source:* Created with BioRender.com. Abbreviations: AB, antibodies; AC, adenylyl cyclase; ADAM, a disintegrin and metalloprotease; AKT, protein kinase B; APC, adenomatous polyposis coli; bs/tsAB, bispecific/trispecific antibodies; cAMP, cyclic adenosine monophosphate; CBP/p300, CREB binding protein/histone acetyltransferase p300; CD44, cluster of differentiation 44; CKIα, casein kinase 1 α; cMET, MET proto-oncogene, receptor tyrosine kinase; Co-A, coactivators; CSL, CBF1, suppressor of hairless, Lag-1; CXCL12, C-X-C motif chemokine ligand 12; CXCR4, C-X-C chemokine receptor Type 4; Dhh, Desert Hh ligand; DSL, delta/serrate/lag-2; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; Gli, glioma-associated oncogene homolog; GSK-3β, glycogen synthase kinase 3β; Ihh, Indian Hh ligand; IP3, inositol 1,4,5-t; LRP, lipoprotein receptor-related protein; MEK, mitogen activated protein kinase kinase; mTOR, mammalian target of rapamycin; NICD, Notch intracellular domain; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PLC, phospholipase C; PTCH, receptor patched; Rho, small GTPase; ROCK, rho-associated protein kinase; RTK, receptor tyrosine kinase; Shh, sonic Hh ligand; SMO, GPCR-like protein smoothened; Src, proto-oncogene tyrosine-protein kinase; SUFU, suppressor of Fu; TCF/LEF, T-cell factor/lymphoid enhancer factor; TEAD, transcriptional enhanced associate domain; TKIs, tyrosine kinase inhibitors; YAP/TAZ, yes-associated protein/transcriptional coactivator with PDZ-binding motif.

inhibition by erlotinib in combination with local radiotherapy was well tolerated in patients in pediatric care with central nervous system (CNS) malignancies such as HGG, recurrent medulloblastoma, and ependymoma. However, it was also not associated with improved clinical outcomes.[79,80] A lack of clinical efficacy of the EGFR inhibition could be explained by the activation of the alternative tumor pro-survival pathways.[78] Of importance, acquired resistance to EGFR inhibitors erlotinib, afatinib, and gefitinib in *EGFR*-mutant NSCLC was associated with the selection of refractory tumor cells with CSC phenotype.[81-84] EGFR promotes CD44-mediated breast CSC aggregation, and EGFR inhibition blocks the circulating CSC clustering associated with increased tumor-initiating and metastatic potential.[85] A combination of EGFR inhibition with treatments targeting CSC populations such as inhibitory antibody targeting Notch2/3 receptors,[76] small molecule pictilisib (GDC-0941) inhibiting PI3K-Akt pathway,[75] or inhibition of Hsp90 combined with irradiation[84] was suggested as a treatment alternative for tumor with acquired resistance to EGFR inhibitors. The EGFR signaling could be efficiently targeted using immunological approaches. A bispecific (bsAB) antibody amivantamab (rybrevant), binding EGFR and cMet and thereby inhibiting their interaction with ligands, was clinically approved by FDA in 2021 for the treatment of patients with advanced or metastatic NSCLC with *EGFR* ex20ins mutations and with cancer progression after platinum-based chemotherapy.[30-32] The recent early-stage clinical trials for the anti-CD3 × anti-EGFR bispecific antibody-armed activated T cells (BATs) in patients with pancreatic cancer and EGFR-specific CAR T cells in patients with NSCLC provided encouraging results on the safety and efficacy of these therapeutic approaches.[33,34]

EpCAM, also known as CD326, was first discovered in 1979 as a colorectal carcinoma-specific antigen.[86] EpCAM-dependent signaling activates target gene transcription through a protein complex including FHL2, β-catenin, and Lef-1. In addition, EpCAM has a crosstalk with mTOR/PI3K/Akt,[87,88] EGFR,[89] and transforming growth factor β1 (TGF-β1)[90] signaling axes and is critical for the regulation of tumor cell adhesion, proliferation, and migration.[91,92] Since the initial identification of CSCs with $EpCAM^{+}/CD44^{+}/CD24^{-}$ phenotype in human breast tumors,[93] EpCAM was characterized as one of the CSC markers in the colorectal,[94] hepatocellular,[95] nasopharyngeal carcinoma,[96] and some other tumor types. EpCAM is highly expressed in a subpopulation of circulating tumor cells (CTC), which are assumed to be a source for metastatic dissemination, and the EpCAM-based CellSearch system became a clinically approved method for CTC enumeration in the liquid biopsies.[97] However, like many other above-mentioned markers, EpCAM expression is not restricted to CSCs.[98] Nevertheless, EpCAM-directed therapy such as EpCAM/CD3 bispecific T-cell engager (BiTE) has been proven to be efficient against CSC in pancreatic carcinoma.[99] An autologous dendritic cell vaccine specific to CD44 and EpCAM showed cytotoxic efficacy against prostate CSC-like cells in vitro and in xenograft tumor models.[100] The EpCAM/CD3-targeted trifunctional antibody catumaxomab has been clinically approved for the treatment of patients with EpCAM-positive cancer with malignant ascites,[35] and more EpCAM-targeting immunotherapeutic approaches have

Table 1. Overview of selected clinical trials for CSC-directed therapies in solid tumors (as of August 30, 2023; https://clinicaltrials.gov).

Specificity/generic name	Description	Tumor entity tested	Study ID (ClinicalTrials.gov) and outcome	References
Targeting CSC antigens and receptor tyrosine kinases (RTK)				
CD44v6/RG7356	Anti-CD44v6 humanized monoclonal antibodies	Advanced solid tumors expressing CD44v6	NCT01358903; phase I study; $n = 65$; safety: treatment-related AEs are mostly mild to moderate; grades 3 and 4 AEs were observed in 25% and 5% of pts, respectively. Clinical efficacy is modest; 21% of pts had SD; no specific trend in immune cell proliferation or infiltration; no dose-dependent biological activity was observed	25
CD44v6	Fourth generation CAR-T cells	CD44v6 positive solid tumors (including stomach cancer, breast cancer, prostate cancer), MM and lymphoma	NCT04427449; phase I/II; no results posted	
Her2, GD2, and CD44v6	Fourth generation CAR-T cells	Stage III, IV, or relapsed breast cancer	NCT04430595; phase I/II; no results posted	
c-Met	Cabozantinib, multi-target inhibitor	Advanced RCC, metastatic MTC, and HC	Clinically approved by FDA and European Medicines Agency	26
c-Met	Crizotinib, multi-target inhibitor	Metastatic NSCLC having *ROS1* gene alteration and pts with IMT having *ALK* gene alterations	Clinically approved by FDA and European Medicines Agency	27-29
EGFR and c-MET/ amivantamab/rybrevant	bsAB antibody binding EGFR and cMet, thereby inhibiting their interaction with ligands	Advanced or metastatic solid tumors including *EGFR*-mutated NSCLC	Clinically approved by FDA in 2021 for the treatment of adult pts with advanced or metastatic NSCLC with EGFR ex20ins mutations and with disease progression after platinum-based chemotherapy	30-32
EGFR	CAR T cells generated by the piggyback transposon system	Advanced relapsed/refractory NSCLC after chemotherapy	NCT03182816; phase I study; $n = 9$; safety: treatment was tolerable, there were no pts with grade 4 AEs; grades 1 to 3 AEs were observed in 7 pts (77.8%). Clinical efficacy: 6 pts (66,7%) had a SD; 1 patient (11,1%) had a PR, 2 pts (22,2%) had a PD	33
EGFR and CD3	bsAB armed activated T cells (BATs)	Advanced and metastatic colorectal and pancreatic cancer after chemotherapy	NCT01420874; NCT02620865; phase Ib/II study; $n = 5$ (phase I trial); $n = 2$ (phase II); safety: there were no dose-limiting toxicities or cytokine storm; treatment was tolerable; there were no pts with persistent grades 3 and 4 AEs. Clinical efficacy: 2 pts out of 7 had CR; and 2 pts out of 7 had a SD.	34
EpCAM and CD3 Catumaxomab/Removab	trAb targeting EpCAM, CD3 and accessory immune cells	EpCAM-positive carcinomas with malignant ascites	Clinically approved by European Medicines Agency	35
EpCAM and TM4SF1	CAR-T cells	Advanced solid tumors (refractory/recurrent pancreatic cancer, colorectal cancer, gastric cancer, lung cancer)	NCT04151186; phase: n/a; no results posted	
Focal adhesion kinase inhibition				
FAK kinase/defactinib	RAF/MEK inhibitor avutometinib (VS-6766) alone and avutometinib (VS-6766) + defactinib	Recurrent LGSOC with and without a *KRAS* mutation	NCT04625270; phase II; no results posted	36

Table 1. Continued

Specificity/generic name	Description	Tumor entity tested	Study ID (ClinicalTrials.gov) and outcome	References
FAK kinase/defactinib	RAF/MEK inhibitor avutometinib (VS-6766) alone and avutometinib (VS-6766) + defactinib	Recurrent NSCLC with *KRAS* and *BRAF* mutations	NCT04620330; phase II; no results posted	[36]
FAK kinase/defactinib	RAF/MEK inhibitor avutometinib (VS-6766) in combination with defactinib	Advanced solid tumors (*KRAS* mutated NSCLC, LGSOC, CRC, pancreatic cancer, gynaecological cancers	NCT03875820; phase II; $n = 42$; safety: there were AEs of grades 1-2. All changes were reversible. Clinical efficacy: the response rate was 67% (4/6) for *KRAS* mutated LGSOC and 50% (4/8) for all LGSOC pts	[37]
Notch pathway inhibition				
γ-secretase/MK-0752	GSI in combination with docetaxel	Locally advanced or metastatic breast cancer	NCT00645333; phase Ib; $n = 30$; safety: 12 different toxicities of grade 3 were observed in 30 treated pts; there were 1 AEs of grade 5; clinical efficacy: a PR was observed in 46% of pts (11/24), a SD was observed in 38% of pts (9/24), and 3 pts had PD. Anti-CSC-related efficacy: multiple rounds of treatment reduced $ALDH^+$ and $CD44^+/CD24^-$ cell populations in some pts as measured by consequent biopsies	[38]
γ-secretase/MK-0752	GSI in combination with mTOR inhibitor ridaforolimus	Metastatic or locally advanced solid tumor that has failed to respond to standard therapy	NCT01295632; phase I; $n = 28$; safety: AEs of grade 3 was observed in 46% of pts (13/28), 85.7% of pts experienced ≥ 1 drug-related AE. Clinical efficacy: 1 of 18 evaluated pts had a CR; 1 patient had a PR; 1 patient had a SD	[39]
γ-secretase/MK-0752	GSI in combination with chemotherapeutic drug gemcitabine	Stage III (inoperable) and IV PDAC	NCT01098344; phase I; $n = 44$; safety: AEs of grade ≥ 3 was observed in 68% of pts (30/44). Clinical efficacy: 1 of 19 evaluated pts had a PR; 13 pts had a SD	[40]
DLL4/Demcizumab	Demcizumab in combination with chemotherapeutic drugs pemetrexed and carboplatin	Metastatic non-squamous NSCLC	NCT01189968; phase Ib; $n = 46$; safety: AEs of grade ≥ 3 was observed in 91% of pts (42/46). Clinical efficacy: 1 of 46 evaluated pts had a CR; 19 pts (48%) had a PR; 15 pts (38%) had a SD. Anti-CSC-related efficacy: gene expression was analyzed in the pretreated and post-treated white blood cells from the pts. A decrease in the levels of Notch signaling genes *NOTCH1*, *NOTCH2*, *MAML1*, *MAML3* and CSC marker *CD44*	[41]
Wnt pathway inhibition				
Ipafricept (OMP-54F28)/a truncated Fzd8 receptor with IgG1 Fc region	Recombinant fusion protein binding Wnt ligands and blocking Wnt signaling	Previously treated metastatic or unresectable solid tumors for whom no remaining standard curative therapy is available	NCT01608867; phase I; $n = 26$; safety: all treatment-related AEs were of grade 1 or 2 except 2 AEs of grade 3; clinical efficacy: 7 of 26 evaluated pts (27%) had a SD; partial or complete responses were not observed. Anti-CSC-related efficacy: gene expression was analyzed in the pretreated and post-treated hair follicles from the pts and healthy volunteers. A decrease in the levels of Wnt signaling-regulated genes *LGR6* and *DKK1* and stem cell marker *CXCR4* as well as an increase in the expression level of differentiation marker *NRCAM* were observed	[42]

Table 1. Continued

Specificity/generic name	Description	Tumor entity tested	Study ID (ClinicalTrials.gov) and outcome	References
Ipafricept (OMP-54F28)/a truncated Fzd8 receptor with IgG1 Fc region	Blocking Wnt signaling with Ipafricept in combination with chemotherapeutic drugs gemcitabine and nab-paclitaxel	Previously untreated stage IV pancreatic cancer	NCT02050178; phase Ib; n = 26; safety: AEs of grade ≥ 3 were observed in 69% of pts (18/26). Clinical efficacy: 9 out of 26 pts (35%) had a PR; 12 of 26 pts (46%) had a SD; complete responses were not observed. Anti-CSC-related efficacy: gene expression was analyzed in the pretreated and post-treated hair follicles from the pts and healthy volunteers. A decrease in the levels of Wnt signaling-regulated genes for example, *FZD10*, *LEF1*, *AXIN2*, and stem cell marker *ALDH1A1* were observed	[43]
Porcupine/WNT974	Porcupine inhibition	Advanced solid tumors dependent on Wnt ligands	NCT01351103; phase I; n = 94; safety: AEs of grade ≥ 3 were observed in 70% of pts (66/94). Clinical efficacy: 15 of 94 pts (16%) had a SD; one patient had a BOR of non-CR/non-PD; no CR or partial response was observed.	[44]
β-catenin/PRI-724	Inhibition of the CBP/β-catenin interaction	Advanced solid tumors	NCT01302405; phase Ia; n = 18; safety: AEs of grade ≥ 3 were observed in AEs of grade 3 were observed in 2 pts; acceptable toxicity profile. CTC-related biomarkers: survivin/*BIRC5* expression in CTCs was measured by immunomagnetic RT-PCR and was found to be decreased in up to 72% of pts	[45]
Hedgehog pathway inhibition				
Vismodegib (GDC-0449, Erivedge)	Inhibiting Hh signaling by binding to SMO	Adult pts with locally advanced and metastatic BCC	Clinically approved by FDA and European Medicines Agency	[46,47]
Vismodegib (GDC-0449, Erivedge)	Inhibiting Hh signaling in combination with chemotherapeutic drug gemcitabine	Metastatic pancreatic adenocarcinoma	NCT01195415; phase II; n = 25; safety: AEs of grade ≥ 3 were noted in 56% of pts (14/25). Clinical efficacy (responses on GDC-0449 alone, after 4 week): 14 of 23 pts (60.9%) had a SD; no CR or PR was observed. Clinical efficacy (best overall): 5 of 23 pts (21.7%) had a PR; 10 of 23 pts (43.5%) had a SD; no CR was observed. The median PFS and OS were 2.8 and 5.3 months. CSC-related readout: The percentage of $CD44^+CD24^+ESA^+$ cells from needle biopsy was calculated at baseline and at 3 weeks using flow cytometry. There was no significant correlation between the changes in CSC populations and OS. Inhibition of the Hh pathway was analyzed by the mRNA levels of *GLI1* and *PTCH1* measured by qRT-PCR analysis of pts biopsies. On day 22, GDC-0449 treatment decreased the levels of GLI1 and PTCH1 in 22 (95.6%) and 19 (82.6%) pts, correspondingly. The levels of *GLI1* or *PTCH1* did not correlate with OS.	[48]

Table 1. Continued

Specificity/generic name	Description	Tumor entity tested	Study ID (ClinicalTrials.gov) and outcome	References
Vismodegib (GDC-0449, Erivedge)	Inhibiting Hh signaling in combination with chemotherapeutic drug gemcitabine and nab-paclitaxel	Untreated metastatic pancreatic adenocarcinoma	NCT01088815; phase II; $n = 71$; safety: 18 treatment-related AEs among 8 individuals were observed. Clinical efficacy: median PFS and overall survival (OS) were 5.42 months; 26 of 67 pts (38.8%) had PR and 1 patient had CR. CSC-related readout: CSC marker ALDH and marker of the Hh pathway activation, Gli1 were analyzed by IHC in tumor biopsies from the primary tumors and metastatic lesions. Inhibition of the Hh pathway was additionally analyzed by the mRNA levels of *GLI1* and *PTCH1* measured by qRT-PCR analysis of pts biopsies. PBMC from pts were stained with EpCAM antibody and ALDEFLUOR reagent for analysis of the ALDH activity. There were no significant changes in $ALDH^+$ CSCs following treatment and no significant association with PFS or OS. Baseline IHC levels of GLI-1 were not significantly associated with PFS or OS. In contrast to the previous study,[48] qRT-PCR analysis revealed a statistically significant upregulation of Hh signaling. The study demonstrated that circulating CSCs can be measured in pts with PDAC during the treatment	[49]
Sonidegib (LDE225, Odomzo)	Inhibiting Hh signaling by binding to SMO	Locally advanced and metastatic BCC	Clinically approved by FDA and European Medicines Agency	[50]
Chemokine pathway inhibition				
BL-8040	CXCR4 inhibition by antagonistic synthetic peptide BL-8040 in combination with pembrolizumab or pembrolizumab and chemotherapy liposomal irinotecan (Onivyde)/5-fluorouracil/leucovorin (5-FU/LV)	Metastatic pancreatic adenocarcinoma	NCT02826486; phase II; $n = 37$; safety: AEs of grade ≥ 3 were noted in 40.9% of pts (9/22) treated by a triple combination of BL-8040, pembrolizumab and chemotherapy and in 18.9% of pts (7/37) treated with BL-8040 in combination with pembrolizumab. Clinical efficacy: mOS was 3.3 months in the ITT population; mOS was 7.5 months in pts receiving study treatment as second-line therapy; 9 of 29 pts (31%) had SD, and 1 patient had PR. CSC-related readout: analysis of the paired screening and on-treatment biopsies was performed by IHC. Treatment with BL-8040 led to a marked decrease in the density of $PanCK^+$ and $CXCR4^+$ tumor cells. Anti-tumor immune response mobilization of WBCs and T-cells in the peripheral blood was analyzed by flow cytometry. Treatment with BL-8040-induced WBC- and T-cell mobilization in the peripheral blood, decreased the intratumoral density of MDSCs and increased tumor infiltration of activated $CD8^+$ granzyme B^+ cytotoxic T cells.	[51,52]

Table 1. Continued

Specificity/generic name	Description	Tumor entity tested	Study ID (ClinicalTrials.gov) and outcome	References
Reparixin	CXCR1/2 inhibition	Primary operable HER-2 negative breast cancer	NCT01861054; phase II; $n = 20$; safety: treatment-related AEs of grade ≥ 1 were noted in 50% of pts (10/20), all AEs were of grade ≤ 2. CSC-related readout: $ALDH^+$ and $CD44^+/CD24^-$ CSC populations were analyzed by flow cytometry in biopsies obtained before and after treatment. A reduction of ≥20% in $ALDH^+$ or $CD44^+/CD24^-$ cell populations were observed in 4 (23.5%) and 9 pts (52.9%) out of 17 pts whose biopsies were analyzed. A portion of $CD44^+/CD24^-$ cells were stained positive for CXCR1. $CXCR1^+$ cell population was decreased in 7 of 13 evaluated pts (53.8%).	[53]
Conventional chemotherapy				
Standard-of-care chemotherapy drugs or combinations: Carboplatin; Irinotecan; Etoposide; BCNU; CCNU; Temozolomide; Procarbazine; Vincristine; Imatinib; Procarbazine + CCNU + Vincristine; Carboplatin + Irinotecan; Carboplatin + Etoposide; Temozolomide + Etoposide; Temozolomide + Imatinib.	ChemoID CSC assay-guided treatment compared to standard therapy chosen by the physician	Recurrent glioblastoma	NCT03632135; $n = 123$; clinical efficacy: the pts whose treatment was guided by the ChemoID assay, had a median survival of 12.5 months compared to 9 months for the pts whose treatment was selected by physicians. ChemoID CSC assay: patient-derived CSC were grown under 3D conditions in growth factor-depleted medium. CSC enrichment was confirmed by flow cytometry for CD133, CD24, CD44 markers, and by transplanting CSCs in immune-deficient mice in a limiting dilution assay. Tumor cells were treated with conventional chemotherapeutic drugs, and their combination, and viability of the non-CSCs and CSCs was analyzed by cell counting.	[54]

Abbreviations: AEs, adverse events; ALDH, aldehyde dehydrogenase; ALK, anaplastic lymphoma kinase; BAT, bsAB armed activated T cells; BCC, basal cell carcinoma; BCNU, carmustine; BIRC5, baculoviral inhibitor of apoptosis repeat-containing 5; BOR, best overall response; bsAB, bispecific antibodies; CCNU, lomustine; CAR T, chimeric antigen receptor T cells; CBP, CREB-binding protein; CR, complete response; CRC, colorectal carcinoma; CSC, cancer stem cells; DKK1, dickkopf WNT signaling pathway inhibitor 1; CTC, circulating tumor cells; DLL4, delta-like ligand 4-Notch; EGFR, epidermal growth factor receptor; EGFR ex20ins, EGFR exon 20 insertion mutations; EpCAM, epithelial cell adhesion molecule; FAK, focal adhesion kinase; FDA, US Food and Drug Administration; FOLFIRI, 5-fluorouracil (5FU)/leucovorin with irinotecan; FOLFOX, 5-fluorouracil (5FU)/leucovorin with oxaliplatin; GBM, glioblastoma; GD2, disialoganglioside; GSI, γ-secretase inhibitor; HC, hepatocellular carcinoma; Hh, Hedgehog pathway; IHC, immunohistochemical tissue staining; IMT, inflammatory myofibroblastic tumors; ITT, intention to treat; LGR6, leucine rich repeat containing G protein-coupled receptor 6; LGSOC, low-grade serous ovarian cancer; MDSC, myeloid-derived suppressor cells; MEK, mitogen-activated protein kinase kinase; mOS, median overall survival; MTC, medullary thyroid cancer; mTOR, mammalian target of rapamycin; NSCLC, non-small cell lung cancer; SMO, Smoothened; OS, overall survival; PD, progressive disease; PBMC, peripheral blood mononuclear cells; PD, progressive disease; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; PR, partial response; pts, patients; RCC, renal cell carcinoma; SD, stable disease; TM4SF1, transmembrane 4 l six family member 1; trAB, trifunctional antibody; VEGF, vascular endothelial growth factor; WBCs, whole blood cells.

entered the clinical trials and showed promising therapeutic efficacy (Table 1).

Targeting CSC-Associated Signaling Pathways and TME

The properties of CSCs, such as self-renewal and differentiation, metastatic dissemination and quiescence, tumor propagation, and therapy resistance, are dictated by their anatomic location.[3] This place-to-be of CSCs called niche and is made by microenvironmental factors, including cellular components (such as cancer-associated fibroblasts, mesenchymal stem cells, immune cells (eg, T cells, natural killer (NK) cells, tumor-associated macrophages, (TAM), tumor-associated neutrophils (TAN)), endothelial cells, tumor non-CSCs, secreted factors (growth factors, chemokines, hormones, metabolites), extracellular matrix (ECM) components (fibronectin, collagen, vitronectin), as well as chemical factors (supplementation of oxygen, non-organic ions, glucose, and other nutrients) and physical forces.[3] CSC niches within a tumor are highly heterogeneous and dynamic structures affected by environmental stimuli such as treatment, inflammatory stress, and aging, but also by the signals released by CSCs.[4,5,101-103] Indeed, stem cells in hematological and solid tumors might shape their microenvironment and convert it to a more pro-tumorigenic, angiogenic, and immunosuppressive niche.[103,104] Targeting CSC niche components and signaling pathways mediating crosstalk between CSCs and TME holds great therapeutic potential.

Focal Adhesion Kinase

In particular, focal adhesion kinase (FAK) is one of the promising targets to eradicate CSC populations. FAK mediates intracellular signaling from the growth factor receptors and integrin dimers upon their binding to the ECM molecules[105] (Fig.2). A FAK/Yes-associated protein (YAP)/mammalian target of rapamycin (mTOR) signaling is essential for the expansion of the undifferentiated transit-amplifying (TA) cells and tissue renewal.[106] Furthermore, accumulating data suggest that FAK activity and its interplay with other signaling pathway, such as WNT and YAP axes, are critical for the maintenance of CSCs in breast,[107-110] colon,[111,112] pancreatic[113] cancer, HNSCC, acute myeloid leukemia (AML),[114] and other types of solid tumors and hematological malignancies. Inhibition of FAK activity in pancreatic ductal adenocarcinoma (PDAC) CSCs makes these cells more susceptible to anti-tumor T-cell activity by inducing expression of MHC-I essential for antigen presentation.[113] A stem cell-related marker CD133 promotes FAK phosphorylation and activation in a Scr-dependent manner, promoting migration of $CD133^+$ cell populations.[115] Furthermore, chemotherapy activates FAK-YAP signaling in dormant colon CSC cells, stimulating them to enter the cell cycle and induce tumor regrowth.[111] Inhibition of FAK synergizes with inhibition of the RAS/RAF/MEK signaling in the repressing of the YAP pathway.[116] Furthermore, FAK signaling is augmented in response to MEK inhibition, serving as a tumor adaptive mechanism that has an important clinical application.[37] The ongoing clinical trials evaluate a combination of FAK inhibitors with inhibitors of the RAS/RAF/MEK axis in patients with solid tumors with and without KRAS mutations.[36] In particular, the phase I/II clinical study is investigating the safety, tolerability, and efficacy of defactinib, FAK inhibitor in combination with VS-6766, RAF/MEK inhibitor in patients with advanced solid tumors, including low-grade serous ovarian cancer, NSCLC, or colorectal cancer with KRAS mutations.[36] The biopsy analysis revealed increased p-FAK levels after the administration of avutometinib (VS-6766) as a single agent that was reduced after the combination therapy,[37] therefore justifying a combination approach. This treatment is showing encouraging clinical activity and a manageable safety profile (Table 1).

Notch Pathway

The developmental signaling pathways such as Notch, Hedgehog (Hh), Wnt, and Hippo mediate tumor-stroma interaction and play a critical role in the regulation of the normal and CSC maintenance and therapy resistance[117-119] (Fig. 2). In particular, the Notch pathway is activated in a juxtacrine or paracrine manner by the Notch ligands, the proteins of the DSL (Delta/Serrate/LAG-2) family, including Delta-like (Dll)1, Dll3, Dll4, Jagged (Jag)1 and Jag2. These ligands bind to the Notch 1-4 receptors on the neighbor cells, inducing 2 consequent cleavages of Notch first mediated by ADAM protease and then by γ-secretase complex, leading to the release of the Notch intracellular domain (NICD) and its consequent nuclear translocation and regulation of the gene expression in association with transcriptional factor CBF1/RBP-jk/Su(H)/Lag1 (CSL) and co-activators such as mastermind-like (MAML).[117] Inhibition of the Notch pathway resulted in the depletion of CSCs in breast,[120,121] ovarian,[121] colon,[122] and pancreatic[121] preclinical tumor models. The main strategies to target Notch signaling in clinical trials include antibodies preventing receptor-ligand binding and γ-secretase inhibitors (GSIs) alone or in combination with other drugs. Several clinical trials were conducted to evaluate the clinical efficacy and safety of Notch inhibition in combination with other drugs in patients with solid and hematopoietic malignancies (Table 1). However, only some of these studies included measurement of the CSC-related parameters. In particular, the phase I/II clinical trial determined the safety of MK-0752 GSI in combination with docetaxel for the treatment of patients with metastatic breast cancer. This study evaluated breast CSC markers and self-renewal capacity in serial tumor biopsies and demonstrated that GSI decreased $CD44^+/CD24^-$ and $ALDH^+$ CSC populations and sphere-forming efficiency indicative of the self-renewal properties. This clinical trial suggested that a combination of MK-0752 with docetaxel has manageable toxicity and evidence of clinical efficacy associated with an effect on CSC populations.[38] Notch signaling is highly activated in several types of pediatric CNS malignancies, including medulloblastoma,[123,124] astrocytoma,[125] ependymoma,[124] and diffuse intrinsic pontine glioma (DIPG).[126] GSI inhibition with MK-0752 was well tolerated in patients in pediatric care with recurrent CNS tumors with evidenced inhibition of Notch 1 cleavage, although no objective responses were reported for these early-phase clinical studies.[127,128] Another early-stage clinical trial used DLL4 inhibitor Demcizumab in patients with metastatic non-squamous NSCLC. Gene expression analysis in the pretreated and post-treated white blood cells from the patients revealed a decrease in the levels of Notch signaling genes and stem cell marker CD44[41] (Table 1). However, the observations for the anti-CSC activity of the Notch signaling inhibitors require further clinical testing, including normal stem cell toxicity analyses.

Wnt Pathway

Wnt signaling is another developmental pathway playing a critical role in the maintenance of normal stem cells and CSC populations and mediating their interplay with TME. A canonical Wnt signaling pathway is activated by the binding of Wnt ligands to Frizzled receptors (FZDs) and associated with a cytoplasmic stabilization of β-catenin, its nuclear translocation, binding to the T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors and activation of the target gene expression (Fig. 2). The Wnt target gene Leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), also known as a positive regulator of Wnt/β-catenin signaling, is one of the most investigated markers of normal stem cells and CSCs in the intestinal epithelium and other tissues.[129-131] A non-canonical Wnt pathway does not depend on β-catenin stabilization and includes, that is, planar cell polarity and the Wnt-calcium signaling as reviewed elsewhere.[132] Wnt ligands are secreted by tumor and tumor stroma cells, playing a role in tumor initiation, growth, and therapy resistance in paracrine and autocrine mechanisms.[133-137] Recent studies demonstrated that Wnt ligands released by M2 macrophages induced ALDH$^+$ CSC-like population in the coculture of macrophages and ovarian cancer cells.[138] The activity of porcupine, a membrane-bound O-acyltransferase, is essential for the palmitoylation of the Wnt ligands, their secretion, and activity.[139] Inhibition of porcupine was associated with a prolongation of metastatic free survival in different in vivo tumor models, including breast cancer,[140] HNSCC,[141] and Ewing sarcoma.[142] The liposomal delivery of porcupine inhibitor CGX1321 resulted in the apoptosis of LGR5$^+$ CSCs in the patient-derived gastric tumor xenografts.[143] A few porcupine inhibitors, including WNT974 and ETC159, entered clinical trials for patients with advanced or metastatic solid tumors[144] (Table 1, Fig. 2). Recent clinical studies for the blocking of Wnt signaling with Ipafricept, a Fzd8 decoy receptor, in advanced solid tumors demonstrated a decrease in the levels of Wnt signaling-regulated genes and stem cell markers, that is, CXCR4 and ALDH1A1 in response to the treatment[42,43] (Table 1). Ipafricept sensitizes CSCs to paclitaxel in ovarian cancer xenograft models. A depletion of CSCs in tumor xenografts was confirmed by limiting dilution analysis.[145] Tankyrase catalyzes poly-ADP-ribosylation of tumor suppressor axin, a part of the protein complex mediating β-catenin degradation.[146] Therefore, tankyrase inhibitors stabilize axin and negatively impact Wnt/β-catenin signaling. Tankyrase inhibitors negatively regulate ALDH$^+$ CSCs in breast and prostate cancer models.[147-149] The results of the early phase clinical study of the PARP/tankyrase inhibitor 2X-121 (E7449) suggested that it is well tolerated and has promising anti-tumor activity. This study developed a gene signature predictive of the therapeutic response and consisting of the Wnt/β-catenin signaling and DNA damage response.[150] cAMP-responsive element-binding-binding protein (CBP) is a critical co-activator of the β-catenin/TCF/LEF dependent transcription. Preclinical studies demonstrated that inhibition of the β-catenin transcription complexes inhibits CSC populations and metastatic growth in renal,[151] breast,[152] liver cancer,[153] and chronic myelogenous leukemia.[154] PRI-724 is a small molecule inhibitor disrupting CBP/β-catenin interaction. PRI-724 was tested in early clinical trials for advanced solid tumors and demonstrated an acceptable toxicity profile and modest clinical activity.[155] Thus, the most clinically advanced Wnt inhibitors are designed to inhibit palmitoylation of Wnt ligands (porcupine inhibitors), prevent the binding of Fzd receptor with Wnt ligands, induce β-catenin degradation, and block its interaction with transcriptional co-activators[44,144] (Table 1, Fig. 2). Most of these inhibitors demonstrated anti-CSC activity in preclinical studies; however, only a few clinical trials included CSC-related parameters in the outcome measures (Table 1).

Hedgehog Pathway

A Hedgehog (Hh) pathway is an evolutionally conserved signaling mechanism essential for embryogenesis and regulation of niche signaling for normal stem cells in adult tissues. Hh pathway is highly activated in tumor tissues and controls the maintenance and survival of CSCs in basal cell carcinoma (BCC), medulloblastoma, CRC, glioma, AML, pancreatic, breast cancer, and other tumor entities.[156-160] The binding of Hh ligands Sonic (Shh), Desert (Dhh), and Indian (Ihh) to the canonical receptor patched (PTCH1) leads to the activation of the smoothened (SMO), a G protein-coupled receptor (GPCR)-like protein, nuclear translocation of the Gli transactional factors followed by the activation of the target gene expression (Fig. 2). Several SMO antagonists are currently in clinical development for the treatment of different types of malignancies, and 2 SMO inhibitors, sonidegib (LDE225, Odomzo) and vismodegib (GDC-0449, Erivedge) were clinically approved for the treatment of locally advanced and metastatic BCC.[50,161-163] However, a significant portion of the patients with BCC who demonstrated initial therapeutic response to SMO inhibitors are developing therapy resistance. Hh pathway is highly activated in SHH-subtype medulloblastoma (MB), which accounts for approximately 30% of all MB cases.[164] Despite the initial promising results of the clinical studies, the treatment of MB with SMO inhibitors was not the same effective as for BCC and was associated with early drug resistance.[164] The use of SMO inhibitors sonidegib and vismodegib for patients in pediatric care was associated with severe growth impairment.[165] SHH-MB is a molecularly diverse type of tumor, and additional patient stratification based on the genetic and epigenetic features would be necessary for the appropriate therapy selection.[166] The establishment and analysis of the patient-derived organoids and animal models with acquired resistance to SMO inhibitors could be another promising strategy for optimal treatment selection.[167,168] The Hh pathway is one of the driving forces of CRC metastatic dissemination and therapy resistance, and Hh signaling activation is essential to maintain the self-renewal properties of the CRC stem cells.[169-172] Nevertheless, an early-phase clinical study for patients with metastatic CRC has shown that a combination of vismodegib with standard therapy for metastatic CRC (5-fluorouracil (5FU)/leucovorin with oxaliplatin (FOLFOX) and bevacizumab or 5-fluorouracil (5FU)/leucovorin with irinotecan (FOLFIRI) with bevacizumab) was not associated with improved therapeutic efficacy.[173] The implementation of CSC-specific analysis would be important in such clinical studies to directly assess the therapy efficacy for CSC populations.

Analyses of the genetically engineered murine models, where BCC was induced by Ptch1 knockout or Smo knockin, revealed that vismodegib induces BCC regression by promoting tumor cell differentiation.[15] Furthermore, preclinical studies demonstrated that SMO inhibitors sensitize CSC to conventional therapy in AML.[174] In contrast, early-stage clinical studies found that treatment of the patients with metastatic

PDAC with Vismodegib is not associated with changes in marker-defined CSC populations in tumor biopsies, and there is no significant correlation between the changes in CSC populations and patient survival.[48,49] However, a drug-tolerant and quiescent CSC population might survive SMO inhibitors and induce tumor relapse, as was shown for LGR5$^+$ tumor-propagating cells in patients with BCC.[15] A combination of vismodegib with Wnt/β-catenin inhibitor LGK-974 targeting this Lgr5$^+$ population led to complete BCC eradication in the experimental models.[15] A combination of Wnt and Hh pathway inhibitors could also be effective for the treatment of other malignancies where these pathways are simultaneously activated in CSC populations, for example, small cell lung cancer,[175] prostate cancer,[176] and multiple myeloma (MM).[177] On the other hand, dormant CSCs entering the proliferative pool could be potentially targeted with conventional therapy efficient against rapidly dividing cells, and a combination of SMO inhibitors with chemo- and radiotherapy was associated with promising therapeutic effects for the patients with AML and BCC[178] (Tables 1 and 2). In particular, a combination of SMO inhibitor glasdegib with chemotherapeutic drug cytarabine led to superior overall survival in patients with de novo and secondary AML.[200] Recent studies suggested the importance of the interaction between the developmental pathways, for example, Noth-Hh, Hh-Wnt, for CSC therapy resistance and immune evasion.[201,202] A combination therapy targeting this interplay constitutes a clinically relevant strategy to eliminate therapy-resistant CSC populations.

Chemokines

Chemokines are a critical component of TME, regulating tumor growth, survival, invasion, and stemness. The C-X-C motif chemokine 12 (CXCL12)/C-X-C motif chemokine receptor 4 (CXCR4) axis was originally discovered as a regulator of immune cell trafficking and hematopoiesis. However, now it has become clear that this signaling has pleiotropic functions and plays a role in tumorigenesis. Activation of the CXCL12/CXCR4 signaling is a hallmark of CSC in prostate, colon, and pancreatic cancer and is a prognostic marker for unfavorable clinical outcomes in patients with different types of malignant diseases. The isoforms of CXCL12 are generated by alternative splicing and have different biochemical and physiological properties and association with clinical outcomes.[203] In the last decades, different approaches have been developed to target CXCR4, including small molecule compounds, peptide inhibitors, and antibody-mediated immunotargeting.[204] Plerixafor (AMD3100), a small molecule CXCR4 antagonist clinically approved for the mobilization of hematopoietic stem cells from bone marrow to peripheral blood for their subsequent collection and use for autologous transplantation.[205] CXCR4 small molecule antagonists such as plerixafor and mavorixafor (X4P-001, AMD11070), as well as CXCR4-targeting antibodies demonstrated potent anti-tumor effects in preclinical animal models for solid and hematological malignancies and a remarkable potential to sensitize experimental tumors, including CSCs, to conventional therapies.[206-212] Several early-phase clinical studies for combining CXCR4 inhibition and conventional therapy or chemotherapy were completed for different types of malignancies. In particular, patients with metastatic pancreatic cancer were treated with plerixafor in combination with PD-1 inhibitor cemiplimab (NCT04177810), patients with relapsed/ refractory (R/R) MM received a humanized anti-CXCR4 antibody ulocuplumab (BMS-936564) in combination with standard therapy (NCT01359657), patients with high glioma were treated with plerixafor after radiation therapy and temozolomide (NCT01977677), and patients with R/R AML received plerixafor in combination with conventional chemotherapeutic drugs mitoxantrone, etoposide, and cytarabine (MEC) (NCT00512252). The published results of several completed studies documented the acceptable safety profile and efficacy of these therapeutic combinations. For example, a phase I/II clinical trial indicated that treatment with plerixator in combination with a standard drug bortezomib in patients R/R MM was well tolerated and resulted in a high objective response rate[193] (NCT00903968). However, all the above-mentioned clinical studies are still in the early stage, and the effect of this therapeutic combination on CSC populations were not monitored. CXCR4 inhibition with plerixafor followed by treatment with chemotherapeutic drugs cytarabine and etoposide was also clinically tested in patients in pediatric care with relapsed or refractory acute lymphoblastic leukemia (ALL), AML or myelodysplastic syndrome (MDS). This treatment was well tolerated; however, the overall anti-tumor activity was modest. The patients with ALL and MDS have shown no response to the therapy. However, 2 of 12 patients with AML achieved complete remission (CR), and one patient with AML achieved complete remission with incomplete count recovery (CRi).[213] Of note, inhibition of CXCR4 was also associated with the upregulation of immune cell infiltration and an increase in the activity of immune checkpoint inhibitors (ICIs),[204,214,215] suggesting that a combination of CXCR4 and ICIs could be a promising anti-cancer approach. In NSCLC, CD133$^+$CXCR4$^+$ metastasis-inducing cells (MIC) possess high levels of CD39 and CD73 ectoenzymes, which mediate the production of the immunosuppressive extracellular adenosine affecting different immune cell populations.[216,217] CD133$^+$CXCR4$^+$ lung cancer spheroids also secrete cytokine IL-10 suppressing antigen-presenting cells.[216,218] Inhibition of CXCR4 with a new antagonist peptide R (Pep R) impaired tumor cell migration and invasion, decreased the levels of extracellular adenosine and IL-10, and partially prevented the inducing of TAM polarization and T-cell suppression by MICs.[216] Treatment of the metastatic PDAC by BL-8040, a peptide CXCR4 antagonist, in combination with pembrolizumab or pembrolizumab and chemotherapy decreased the intratumoral density of myeloid-derived suppressor cells (MDSC) and increased tumor infiltration of activated CD8$^+$ granzyme B$^+$ cytotoxic T cells[51,52] (Table 1). This study also demonstrated that treatment with BL-8040 led to a marked decrease in the density of PanCK$^+$ and CXCR4$^+$ tumor cells.[51]

A chemokine receptor CXCR1 is another promising drug candidate and a marker of breast CSCs.[219] A pilot "window of opportunity" clinical study used a single agent reparixin, an inhibitor of CXCR1 and CXCR2, for the treatment of patients with operable HER-2-negative breast cancer. The primary objectives of this clinical trial included the safety of the treatment and the effects of the drug on CSCs analyzed in tumor biopsies taken at baseline and after treatment completion. Analysis of ALDH$^+$ and CD44$^+$/CD24$^-$ CSCs by flow cytometry demonstrated that these populations are reduced by ≥20% in several evaluated patients (Table 1). Similar to CXCR4, CXCR1 mediates tumor growth through different mechanisms, including immune evasion. In tumor tissues, CXCR1 and its ligand IL8 attract neutrophils and

Table 2. Overview of selected clinical trials for LSC-directed therapies in AML and MM treatment (as of March 25, 2023; https://clinicaltrials.gov)

Agent	Description	Study short title	Phase	Combination	Study identifier	Refs.
Cell-surface targets						
BI 836858	Anti-CD33 mAb	BI 836858 in R/R AML and AML in complete remission with high relapse risk	I	—	NCT01690624	[179]
BI 836858	Anti-CD33 mAb	F16IL2 and BI 836858 in posttransplant AML relapse	I	F16IL2	EudraCT 2015-004763-37	[180]
BI 836858	Anti-CD33 mAb	Biomarker-based treatment of AML	Ib/II	AZA	NCT03013998	
BI 836858	Anti-CD33 mAb	BI 836858 in combination with DEC in AML	I/II	DEC	NCT02632721	
Magrolimab	Anti-CD47 mAb	Anti-CD47 mAb therapy for haematological malignancies	I	–	NCT02678338	[181]
Magrolimab	Anti-CD47 mAb	Sabatolimab and magrolimab-based therapy for AML or higher risk MDS	I/II	Sabatolimab and AZA	NCT05367401	
Magrolimab	Anti-CD47 mAb	Magrolimab in combination with VEN and AZA in AML	III	VEN, AZA	NCT05079230	[182]
Sabatolimab/ MBG453	Anti-CD366 mAb	Sabatolimab in combination with hypomethylating agents in AML and high-risk MDS	Ib	DEC or AZA	N/R	[183]
Sabatolimab/ MBG453	Anti-CD366 mAb	Sabatolimab in AML and MRD after aHSCT	I/II	AZA	NCT04623216	
Gemtuzumab-ozogamicin (GO)	Anti-CD33 ADC	Pracinostat and GO in R/R AML	I	Pracinostat	NCT03848754	
Gemtuzumab-ozogamicin (GO)	Anti-CD33 ADC	CPX-351 and GO in R/R AML	I	CPX-351	NCT03904251	
Gemtuzumab-ozogamicin (GO)	Anti-CD33 ADC	DNR and AraC combined to fractionated GO in first relapse of AML	I/II	Daunorubicin, cytarabine	NCT02182596	
Gemtuzumab-ozogamicin (GO)	Anti-CD33 ADC	Combination AZA and GO for treatment of relapsed AML	I/II	AZA	NCT00766116	
Gemtuzumab-ozogamicin (GO)	Anti-CD33 ADC	CLAG-GO in persistent R/R AML	II	Cladribine, cytarabine, and G-CSF	NCT04050280	
Gemtuzumab-ozogamicin (GO)	Anti-CD33 ADC	Fludarabine, cytarabine, filgrastim, GO and idarubicin in ND CBF AML	II	Fludarabine, cytarabine, filgrastim, and idarubicin	NCT00801489	
Anti-CD33 CAR T cells	Anti-CD33 CAR expressing T cells	CD33-CAR T-cell therapy for R/R AML	I	—	NCT05672147	
CD33CART	Anti-CD33 CAR expressing T cells	CD33CART in children and young adults with R/R AML	I/II	—	NCT03971799	
PRGN-3006 T cells	Anti-CD33 CAR expressing T cells	PRGN-3006 adoptive cell therapy for R/R AML or higher risk MDS	I/Ib	—	NCT03927261	[184]
Chimeric antigen receptor T cell	Anti-CD33 CAR expressing T cells	Enhanced CD33 CAR T-cell therapy for R/R AML	I/II	—	NCT04835519	
Anti-CD33 CAR NK cells	Anti-CD33 CAR expressing NK cells	Anti-CD33 CAR NK cell therapy for R/R AML	I	Precondition with fludarabine and cytoxan	NCT05008575	[185]
UniCAR02-T	Anti-CD123 CAR expressing T cells	UniCAR02-T cells and CD123 target module in pts with hematologic and lymphatic malignancies	I	—	NCT04230265	

Table 2. Continued

Agent	Description	Study short title	Phase	Combination	Study identifier	Refs.
CD123-CAR T	Anti-CD123 CAR expressing T cells	CD123-directed autologous T-cell therapy for AML	I	—	NCT04318678	
CART123 cells	Anti-CD123 CAR expressing T cells	Lentivirally redirected CD123 autologous T-cell therapy for AML	I	Cyclophosphamide, fludarabine	NCT03766126	
CART123 cells	Anti-CD123 CAR expressing T cells	CD123 redirected T-cell therapy of AML in pediatric pts	I	—	NCT04678336	
CD123 CAR-T cells	Anti-CD123 CAR expressing T cells	CD123-targeted CAR-T-cell therapy for R/R AML	II	—	NCT04265963	
CLL-1 CAR T cells	Anti-CD371 CAR expressing T cells	CAR T-cell therapy of CLL-1$^+$ AML	I	—	NCT04219163	
KITE-222	Anti-CD371 CAR expressing T cells	KITE-222 in pts with R/R AML	I	—	NCT04789408	
Anti-CLL1 CAR T cells	Anti-CD371 CAR expressing T cells	CAR T-cell therapy of myeloid leukemia	I	—	NCT04923919	
CLL1 CAR T cells	Anti-CD371 CAR expressing T cells	CLL1 CAR-T-cell therapy of hematological malignancies	I	—	NCT05252572	
Anti-CLL1 CAR T cells	Anti-CD371 CAR expressing T cells	Anti-CLL1 CAR T-cell therapy in CLL1$^+$ R/R AML	I/II	—	NCT04884984	
Intracellular targets						
CWP232291/ CWP291	Wnt pathway inhibitor	Clinical study of CWP232291 in AML	I	—	NCT01398462	[186]
PRI-724	Wnt pathway inhibitor	PRI-724 in pts with advanced myeloid malignancies	I/II	Low-dose cytarabine	NCT01606579	
Glasdegib/ PF-04449913	Hh pathway inhibitor	PF-04449913 and AZA in untreated MDS, AML and CMML	Ib	AZA	NCT02367456	[187]
Glasdegib/ PF-04449913	Hh pathway inhibitor	PF-04449913 in combination with chemotherapy in AML or high-risk MDS	II	Low-dose cytarabine	NCT01546038	
Glasdegib/ PF-04449913	Hh pathway inhibitor	AZA with glasdegib in untreated AML, MDS or CMML	III	AZA	NCT04842604	
Sonidegib/ LDE225	Hh pathway inhibitor	AZA and sonidegib or DEC in myeloid malignancies	I/Ib	AZA or DEC	NCT02129101	[188]
Sonidegib/ LDE225	Hh pathway inhibitor	LDE225 in adult pts with R/R acute leukemias	II		NCT01826214	
Ruxolitinib/ INCB018424	JAK2 inhibitor	Ruxolitinib and VEN in R/R AML	I	VEN	NCT03874052	
Ruxolitinib/ INCB018424	JAK2 inhibitor	Ruxolitinib plus DEC in post myeloproliferative neoplasm	I/II	DEC	NCT02257138	
Ruxolitinib/ INCB018424	JAK2 inhibitor	INCB018424 in advanced hematologic malignancies	II	—	NCT00674479	[189]
Pacritinib	JAK2/FLT3 inhibitor	Pacritinib and chemotherapy in AML with *FLT3* mutations	I	Cytarabine, daunorubicin or DEC	NCT02323607	[190]
Components of bone marrow niche-LSC interaction						

Table 2. Continued

Agent	Description	Study short title	Phase	Combination	Study identifier	Refs.
Ulocuplumab/BMS-936564/MDX-1338	Anti-CXCR4 mAb	Mobilization of blasts and LSCs in R/R AML	I	MEC	N/R	[191]
Plerixafor/AMD3100	CXCR4 inhibitor	G-CSF and plerixafor plus sorafenib for AML with *FLT3* mutations	I	Sorafenib, G-CSF	NCT00943943	[192]
Plerixafor/AMD3100	CXCR4 inhibitor	Plerixafor (AMD3100) and bortezomib in relapsed/refractory MM	I/II	Bortezomib	NCT00903968	[193]
Plerixafor/AMD3100	CXCR4 inhibitor	Treatment of young pts with R/R AML	I/II	FLAG-Ida	NCT01435343	[194]
Plerixafor/AMD3100	CXCR4 inhibitor	The safety and efficacy of subcutaneous plerixafor	II	–	NCT01696461	
Plerixafor (AMD3100)	CXCR4 inhibition	Combination plerixafor and bortezomib in relapsed or relapsed/refractory MM	I/II	Bortezomib	NCT00903968	[193]
BL-8040	CXCR4 inhibitor	Combination BL-8040 with cytarabine for the treatment of R/R AML	IIa	HiDAC	NCT01838395	[195]
LY2510924	CXCR4 inhibitor	LY2510924, idarubicin and cytarabine in R/R AML	I	Idarubicin, cytarabine	NCT02652871	[196]
CX-01/DSTAT	CXCR4 inhibitor	CX-01 combined with AZA in the treatment of R/R MDS and AML	I	AZA	NCT02995655	[197]
CX-01/DSTAT	CXCR4 inhibitor	CX-01 with standard therapy in elderly pts with AML	II	Idarubicin, cytarabine	NCT02873338	[198]
CX-01/DSTAT	CXCR4 inhibitor	DSTAT in combination with chemotherapy in ND AML	III	Standard intensive chemo-therapy	NCT04571645	
RG7356/RO5429083	Anti-CD44 mAb	RO5429083 in combination with cytarabine in AML	I	Cytarabine	NCT01641250	[199]
MLM-CAR44.1	CD44v6 directed CAR-T cells	Study of CAR T-cell therapy in AML and MM	I/IIa		NCT04097301	
Uproleselan/GMI-1271	E-selectin antagonist	Uproleselan in combination with chemotherapy to treat R/R AML	III	MEC or FAI	NCT03616470	
Uproleselan/GMI-1271	E-selectin antagonist	Uproleselan in combination with chemotherapy in Chinese pts with R/R AML	III	MEC or HiDAC/IDAC	NCT05054543	

Abbreviations: ADC, antibody-drug conjugate; AML, acute myeloid leukemia; aHSCT, allogeneic haematopoietic stem cell transplantation; AZA, azacitidine; CAR, chimeric antigen receptor; CBF, core binding factor; CMML, chronic myelomonocytic leukemia; DEC, decitabine; FAI, fludarabine, cytarabine and idarubicin; FLAG-Ida, fludarabine, idarubicin, cytarabine, and G-CSF; G-CSF, granulocyte-colony stimulating factor; HiDAC, high-dose cytarabine; IDAC, intermediate doses of cytarabine; MDS, myelodysplastic syndrome; MEC, mitoxantrone, etoposide, cytarabine; MM, multiple myeloma; MRD, measurable residual disease; N/R, not reported; ND, newly diagnosed; pts, patients; R/R, relapsed or refractory; VEN, venetoclax.

myeloid-derived suppressor cells, promote M2 TAM accumulation, and inhibit T cells.[220] CXCR1/2 inhibitor ladarexin not only reduces tumor burden but also activates anti-tumor immune response through inhibiting tumor cell-induced M2 TAM conversion and increasing the therapeutic effect of anti-PD-1 antibody in preclinical murine models of PDAC.[221] Thus, a combination of CXCR4 and CXCR1/2 inhibitors with ICIs might represent an efficient strategy for the treatment of immune refractory malignancies like PDAC, HPV-negative HNSCC, and *RAS*^{MUT} NSCLC tumors.[204,214,216,221]

Targeting Leukemia Stem Cells (LSCs)

AML is the most common form of acute leukemia in adults.[222] The high risk of failure and relapse of AML treatment with standard therapies is considered to be attributable to the persistence of drug-resistant LSC populations (reviewed by Stelmach and Trumpp[223]), and, therefore, the elimination of these LSCs may provide a promising treatment approach. In this section, we will further review the recent progress in the field of LSCs targeting with a particular focus only on the substances that have progressed to clinical testing for AML. However, many of the strategies discussed here are likely to be relevant to other leukemias/lymphomas as well as various solid tumors.

In the recent decade, numerous LSC-directed therapeutics have been designed and extensively investigated worldwide. As a result, at least 3 such drugs are already in use for AML treatment. In particular, in 2017, gemtuzumab ozogamicin (GO; Mylotarg) in combination with daunorubicin and cytarabine or as monotherapy received FDA approval for the treatment of adult patients with newly diagnosed CD33-positive AML.[224] GO was also approved for the treatment of R/R CD33-positive AML in patients aged 2 years and older.[224] GO is a CD33-directed ADC linked to the cytotoxic antibiotic calicheamicin. Two more small molecules, namely glasdegib (Hedgehog pathway inhibitor) and venetoclax (BCL-2-selective inhibitor), are also approved by the FDA for use as a part of combination therapy for the treatment of newly diagnosed AML in adults ≥75 years or those with comorbidities that preclude intensive induction chemotherapy.[225]

Besides already approved drugs, many other LSC-directed investigational agents are currently under clinical development as candidates for AML treatment. The selected clinical trials for some classes of these agents are summarized in Table 2. It should be noted that most of these trials are phase I or II testing predominantly in the R/R setting.

In addition to surface antigen-based immunotherapies listed in Table 2, synthetic antibodies with multiple binding sites such as BiTEs, dual-affinity retargeting (DART) antibodies, and bispecific/trispecific killer engagers have recently developed. These innovative antibodies are typically designed to recruit T or NK cells and to recognize surface target antigens on leukemia cells and LSCs, thereby harnessing host immunity to kill these cells selectively.[226] The clinical trials with encouraging results are underway or have already been performed in AML patients with such drug candidates as anti-CD33/CD3 bivalent, bispecific antibody AMV564 (NCT03144245), anti-CD123/CD3 DART MGD024 (NCT05362773), and anti-CD123/CD3 BiTE Vibecotamab (NCT02730312, NCT05285813). However, a few phase I studies on other bispecific antibodies targeting LSC-related surface markers, namely AMG330 (NCT02520427, NCT04478695), GEM333 (NCT03516760), and half-life extended anti-CD33/CD3 BiTE AMG673 (NCT03224819) for the treatment of patients with AML have been terminated.

Adoptive cell therapy using CAR T cells directed at CD33, CD123, CD47, and CD371 is another promising approach for treating AML in a HLA-independent manner.[227] Dozens of CAR-modified T-cell products alone and in combination with different therapeutic agents are in clinical trials (Table 2). Moreover, several multitargeting CAR-based immunotherapies that harness T (or NK) cells against AML bulk cells and AML LSCs are currently undergoing clinical evaluation.

These targeted drug candidates include c-type lectin-like molecule 1 (CLL1)/CD33 CAR T cells expressing 2 CAR molecules, CLL1 and CD33 (NCT03795779, NCT05248685), anti-CD33/CLL1 CAR-NK cells (NCT05215015), and CAR T-cell therapy combining CAR T cells against CLL-1 with CAR T cells targeting CD123 or CD33 (NCT04010877). Multiple CAR T/NK-cell therapy is believed to increase the specificity of treatment and limit off-target toxicities, especially myelosuppression.

CD47, or integrin-associated protein, is another promising antigen for LSC eradication. It is highly expressed in tumor cells and contributes to their evasion of innate immunity by activating "do not eat me" signal via signal regulatory protein α (SIRPα).[228] Several anti-CD47-SIRPα antibodies are currently being tested in early-stage clinical trials for hematological malignancies and solid tumors (Table 2).

The improved combination regimens for the treatment of AML remain the focus of attention. The results of the clinical trials will be critical to define the best combination of targeted agents with chemotherapy. For instance, a phase I/II, open-label multicenter trial of uproleselan (GMI-1271; a rationally designed E-selectin antagonist that disrupts cell survival pathways) found that uproleselan in combination with conventional chemotherapy provides higher remission rates and lower induction mortality in patients with R/R AML than chemotherapy alone.[229] Other clinical trials involving different molecular-targeting agents in combination with chemotherapeutic drugs in AML are presented in Table 2.

Furthermore, the combinations of multiple drugs targeting the parallel cell pathways or providing synergistic death of AML blasts and AML LSCs are currently being designed to improve therapy efficacy. There are ongoing trials combining azacitidine and venetoclax with eprenetapopt (APR-246), a small-molecule p53 reactivator,[230] with tagraxofusp (SL-401), a diphtheria toxin IL-3 fusion protein,[231] with uproleselan (NCT04964505), with IMGN632, a CD123-targeting ADC[232] or sabatolimab, a humanized anti-CD366 mAb.[232] It remains to be seen whether these investigational therapeutic triplets will be superior to the approved treatments.

As with any targeted treatment, LSC-based therapies have to be applied based on the predictive indicators to identify the patients who may benefit most from such a cure. One approach to improve patient selection is the use of predictive biomarkers specific to LSC populations. Recently, flow cytometry-based direct quantification of the BCL-2 family proteins (BCL-2, BCL-x_L, and MCL-1) in AML LSCs has been proposed as a novel strategy to predict patient response to azacitidine/venetoclax combination treatment with high accuracy.[233] The turnaround time of testing is about several

hours, and the assay can be applicable for cases with contradictory genetic prognosticators.

Further Clinical Development

Thus, many current translational studies aim to shed light on the efficacy of CSC-directed therapies. As for now, none of the CSC-specific therapies has been clinically approved, and CSC eradication for the clinically approved treatment needs to be sufficiently clinically validated. Several approaches targeting CSC-related signaling mechanisms, such as SMO inhibitors sonidegib, which has been approved for the treatment of locally advanced BCC,[50,178] and glasdegib approved for the treatment of AML in combination with low-dose cytarabine[234] or CD123-targeting immunotoxin tagraxofusp approved for blastic plasmacytoid dendritic cell neoplasm,[235] were not yet analyzed for CSC-specific outcomes in clinical trials. Nevertheless, numerous agents targeting CSCs either as monotherapy or as a part of combination therapy are currently being tested in clinical studies for the treatment of both solid tumors and hematological malignancies. The further clinical development of the CSC-targeted therapies requires the implementation of CSC-specific assays to directly measure CSC response in patient-derived specimens (eg, by using organoid formation assays, in vivo limiting dilution and transplantation assays, in vivo analysis of metastatic burden) and CSC-relevant clinical outcome measures (eg, locoregional control, metastasis-free survival). Furthermore, invasive tissue sampling, such as tumor biopsies or surgery material, gives a static snapshot of cancer. Also, for many translational studies, consequent tissue biopsies before, during, and after the course of treatment are not possible. These analyses are critical to understanding CSC dynamics and plasticity and their relation to clinical outcomes. One of the widely used approaches for the study of temporal tumor heterogeneity and plasticity is non-invasive tumor sampling of body fluids, which is called "liquid biopsy." The plasma analytes such as CTCs, circulating tumor DNA (ctDNA), miRNA, tumor-derived metabolites, and exosomes are now broadly investigated as biomarkers for the detection of early tumor development and residual disease and longitudinal patient monitoring.[97,127,236] The enumeration of CTCs in the blood of cancer patients was clinically approved for prognostic tests in patients with breast, prostate, and colorectal cancer.[236] The recent studies demonstrated striking similarities between the phenotypes of CSCs, CTCs, and disseminated tumor cells at the metastatic sites, suggesting that the process of metastatic dissemination can reflect CSC evolution during disease.[127,237] Therefore, the enumeration and molecular characterization of CTCs through single-cell analysis might be beneficial for developing personalized CSC-targeted therapy (Fig. 3).

All above-mentioned CSC-related antigens are not CSC-specific, and their therapeutic efficacy might be compromised by toxicity for non-malignant cells. Therefore, safety assessment plays a critical role in the clinical development of CSC-targeting drugs. CSCs are often quiescent cells, and they are less affected by conventional therapy and immunotherapy than rapidly proliferating tumor cells.[13-15] Together with additional mechanisms such as increased DNA repair, activated anti-apoptotic signaling, scavenging of reactive oxygen species induced by radiotherapy and some types of chemotherapy, and anti-cancer drug export mediated by ABC transporters, this makes CSC resistant to the different types of conventional treatments.[7,9] However, recently reported tumor cell plasticity when non-CSCs can be converted back to the CSC state upon different microenvironmental stimuli motivates the development of combination treatment approaches when CSC-specific therapy is combined with conventional treatment such as radio- and chemotherapy targeting the tumor bulk.[4,7,149,239] This strategy targeting both CSC and non-CSC subsets could potentially prevent CSC replenishment through the reprogramming of the non-CSC populations. Repurposing clinically approved chemical drugs for targeting CSCs could be a potential way to reduce the cost of drug discovery and development. Furthermore, for these drugs, the safety profiles have already been characterized.[240] For example, a potential repurposable drug candidate can be a metformin used to treat type 2 diabetes. Metformin was demonstrated to eradicate CSC populations in different tumor entities in preclinical studies[241] and is reported to improve clinical outcomes and treatment response in clinical trials for prostate,[242] ovarian,[243] and endometrioid endometrial cancer.[244] A therapeutic effect of metformin is believed to be attributed to the inhibition of oxidative phosphorylation (OXPHOS) as the primary energy production mechanism for CSCs in different tumor entities, including ovarian, prostate CSCs, and leukemia stem cells.[23,241,245] All-trans-retinoic acid (ATRA), a vitamin A metabolic derivative, was first used for the treatment of patients with acute promyelocytic leukemia (APL) in 1988.[246] APL is an AML subtype associated with abnormal myelopoiesis and proliferation of blast cells, in which differentiation is arrested at the promyelocytic stage. At the mechanistic level, the blast differentiation process is blocked by PML-RARA chimeric protein present in more than 95% of APL patients as a result of the t(15;17) translocation. PML-RARA protein binds to the retinoic acid responsive elements in the gene promoters and interferes with transcriptional activation. The curative potential of ATRA is based on the induction of the APL blast differentiation through disruption of the repressor complexes and reactivation of the RARA-dependent gene transcription. Treatment with ATRA in combination with arsenic trioxide (ATO) has improved the long-term survival of patients with APL by up to 90%.[247] In the preclinical studies, ATRA therapy inhibited CSC cells in other tumor entities, as reviewed elsewhere.[248] Furthermore, ATRA is also tested as a potential anti-cancer differentiating agent in clinical trials for patients with breast cancer (NCT04113863), NSCLC (NCT01041833), and patients in pediatric care with recurrent neuroblastoma or Wilms' tumor (NCT00001509). An interesting example of a potential CSC targeting drug is a paracetamol bioactive metabolite AM404, which has recently been shown to prevent CRC stem cell features through inhibition of the F-box and leucine-rich repeat protein 5 (FBXL5) E3-ligase.[249] Another example of the standard drug repurposing for CSC treatment is the recent multicenter study that used CSC-assay-guided chemotherapy for patients with recurrent glioblastoma (GBM). This study tested and selected standard first- and second-line cytotoxic chemotherapies based on their therapeutic efficacy in patient-derived CSC cultures. The results of this study demonstrated that CSC assay-guided chemotherapy selection is associated with significantly better overall survival and progression-free survival as compared to the chemotherapy regimens selected by physicians.[54]

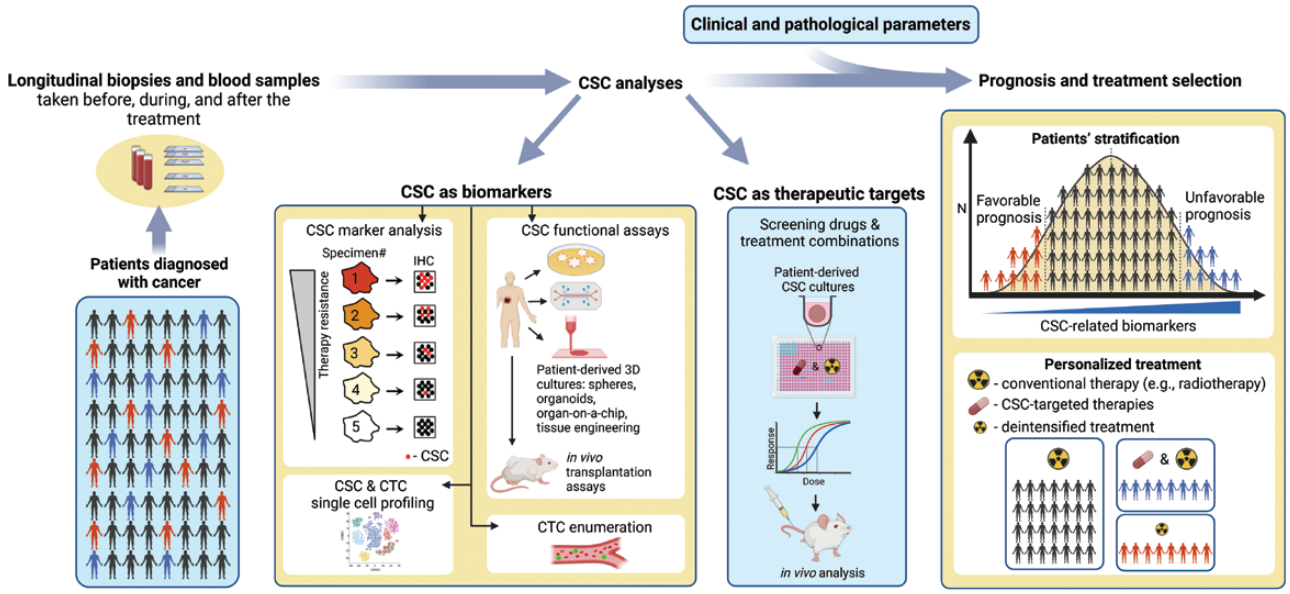


Figure 3. CSC assay-guided patients' stratification and treatment personalization. The longitudinal biopsies and blood sample collection before, during, and after the end of the treatment are used for CSC analysis. Assessment of the CSC load includes analysis of CSC-related biomarkers, CSC functional assays using patient-derived 3D cultured (eg, spheres, organoids, organ-on-a-chip cultures, and engineered tissues), and in vivo transplantation assays. CTC enumeration in the blood of patients with cancer is a clinically approved prognostic test for patients with breast, prostate, and colorectal cancer. Single-cell profiling of CSCs and CTCs might help to identify cell subpopulations with potentially aggressive and therapy-resistant phenotypes.[97,127,236,238] Patient-derived CSC cultures are being used to screen experimental and clinical drugs and different treatment combinations, which can efficiently eradicate CSC populations.[54] Xenotransplantation assay may be used as read-outs of the CSC functions in vivo.[21] CSC-related biomarkers can be used in combination with conventional clinical and pathological parameters (eg, tumor size, grade, location, molecular subtypes, disease stage, and patient's age) for disease prognosis and personalized treatment selection.Abbreviations: CSC: cancer stem cells; CTC: circulating tumor cells; IHC: immunohistochemical analyses.Source: Created with BioRender.com

CSCs possess multiple mechanisms to escape anti-tumor immune response, as reviewed recently.[104] For example, a high expression of the "don't eat me" signals, such as CD47/SIRPα by CSCs and their interplay with the TME triggers immunosuppressive signals, leading to the CSC immune evasion and tumor regrowth.[250] These fundings suggest that the combination of CSC-targeted therapy with immune checkpoint inhibitors boosting immune response[251] might have a synergetic anti-tumor effect. In addition to the CSC immunotargeting with CARs and bsAbs, the novel forms of immune targeting of CSC, such as tumor vaccines and oncolytic viral therapy, are promising therapy approaches, as discussed elsewhere.[59,252] Recent studies identified epigenetic regulation of CSC immune evasion in melanoma by activating the SET Domain Bifurcated Histone Lysine Methyltransferase 1 (SETDB1). SETDB1 is associated with tumor resistance to ICI-based immunotherapy and is upregulated in different human tumors. These studies highlighted an epigenetic mechanism that may be targeted to potentiate the immunogenicity of CSCs in melanoma and, likely, in some other tumor entities.[253,254] Reprogramming of CSC energy, amino acid, and lipid metabolism also contributes to immune evasion and therapy resistance. It opens a possibility for targeting metabolic vulnerabilities of CSC by inhibiting OXPHOS, glutaminolysis, amino acid uptake, or fatty acid synthesis, as discussed previously.[245,255-257]

A well-documented CSC plasticity and associated antigen escape requires the development of multi-target therapy that might decrease the risk of tumor resistance. Furthermore, the interplay between CSC-associated signaling pathways, including EpCAM, EGFR, c-MET, and CD44, suggests that combination therapy for simultaneous targeting of several antigens might be more efficient for CSC and tumor eradication.[59] Targeting of the key reprogramming transcriptional factors (TF) maintaining the self-renewal program in CSCs such as Oct4, Myc, Nanog, and Sox2 by using small interfering RNA, antisense oligonucleotides, by blocking TF binding their target gene promoters or by inhibiting synthetic lethal genes necessary for cell survival is also a very attractive, yet still preclinical approach.[258,259]

The development of reliable CSC analyses using patient-derived samples, which enable the analysis of the fundamental properties of CSC, such as self-renewal and differentiation, is essential to evaluate the efficacy of CSC-directed therapy.[20] The CSC fate is tightly controlled by the cues coming from their niche. Therefore, establishing preclinical ex vivo and in vivo models that recapitulate TME is of high importance for the development of efficient anti-CSC therapies. For example, using humanized[260] or naturalized[261] mice as recipients of the human tumor xenograft can better recapitulate tumor interaction with the human immune system than in conventional immuno-deficient xenograft models. In addition, ex vivo cultures providing physiologically relevant in vivo microenvironment such as organs-on-a-chip and 3D engineered tissues provide additional opportunities for analysis of CSC and efficacy of the CSC-targeted therapy in the physiological conditions.[262,263]

The way of tumor sampling could be another challenge for efficient monitoring of CSC dynamics during the course of treatment. The conventional tumor biopsy analysis provides a tumor snapshot, and longitudinal tumor biopsies are often not possible. Nevertheless, these samples are an important source for the analysis of temporal CSC heterogeneity that can be investigated, for example, by single-cell RNA sequencing

approach (scRNA seq) enabling analysis of the CSC subsets and surrounding non-CSC and stromal cells creating CSC niche and by spatial transcriptomics assessing RNA expression directly in the histological samples. Furthermore, implementation of the enumeration and characterization of the molecular and functional properties of CTCs in the current clinical trials might help to detect metastasis-initiating CSCs at the early dissemination stage, uncover tumor resistance mechanisms, and identify new therapeutic targets for metastasis prevention.[127,238]

Concluding Remarks

CSC populations maintain tumor growth, propagate metastases, and provide tumor recurrence if not eradicated. CSC plasticity and lack of CSC-specific markers remain the significant hurdles to developing efficient CSC-targeted therapy. Furthermore, a reliable preclinical assessment of the CSC response to the treatment requires suitable models that recapitulate the natural CSC microenvironment. Further clinical studies of the CSC-targeted therapies are expected to implement direct analyses of the CSC properties in response to the treatment. The longitudinal biopsy and blood sample collection and molecular characterization of CSCs and CTCs during the treatment would be essential for the CSC-guided treatment selection and improved outcome prognostication.

Acknowledgments

We thank the Armed Forces of Ukraine and the allies for the opportunity to work on this manuscript in Kyiv. Together, we win. This work is dedicated in memoriam of Ukrainian oncohematologist Prof. Daniil Gluzman who passed away in 2022.

Funding

The research in AD group is supported by German Cancer Aid (Deutsche Krebshilfe), project ID: 70114659 and Deutsche Forschungsgemeinschaft (DFG) #416001651 and DFG SPP 2084: µBONE, #401326337 and #491692296.

Conflict of Interest

The authors declared no potential conflicts of interest.

Author Contributions

A.P., A.D.: collected and interpreted the literature data, wrote, edited, and approved the final manuscript.

Data Availability

No new data were generated or analyzed in support of this research.

References

1. Meacham CE, Morrison SJ. Tumour heterogeneity and cancer cell plasticity. *Nature*. 2013;501(7467):328-337. https://doi.org/10.1038/nature12624
2. Kreso A, Dick JE. Evolution of the cancer stem cell model. *Cell Stem Cell*. 2014;14(3):275-291. https://doi.org/10.1016/j.stem.2014.02.006
3. Plaks V, Kong N, Werb Z. The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? *Cell Stem Cell*. 2015;16(3):225-238. https://doi.org/10.1016/j.stem.2015.02.015
4. Tang DG. Understanding cancer stem cell heterogeneity and plasticity. *Cell Res*. 2012;22(3):457-472. https://doi.org/10.1038/cr.2012.13
5. Tang DG. Understanding and targeting prostate cancer cell heterogeneity and plasticity. *Semin Cancer Biol*. 2022;82:68-93. https://doi.org/10.1016/j.semcancer.2021.11.001
6. Lauko A, Lo A, Ahluwalia MS, Lathia JD. Cancer cell heterogeneity and plasticity in glioblastoma and brain tumors. *Semin Cancer Biol*. 2022;82:162-175. https://doi.org/10.1016/j.semcancer.2021.02.014
7. Krause M, Dubrovska A, Linge A, Baumann M. Cancer stem cells: radioresistance, prediction of radiotherapy outcome and specific targets for combined treatments. *Adv Drug Deliv Rev*. 2017;109:63-73. https://doi.org/10.1016/j.addr.2016.02.002
8. Baumann M, Krause M, Hill R. Exploring the role of cancer stem cells in radioresistance. *Nat Rev Cancer*. 2008;8(7):545-554. https://doi.org/10.1038/nrc2419
9. Cojoc M, Mabert K, Muders MH, Dubrovska A. A role for cancer stem cells in therapy resistance: cellular and molecular mechanisms. *Semin Cancer Biol*. 2015;31:16-27. https://doi.org/10.1016/j.semcancer.2014.06.004
10. Peitzsch C, Perrin R, Hill RP, Dubrovska A, Kurth I. Hypoxia as a biomarker for radioresistant cancer stem cells. *Int J Radiat Biol*. 2014;90(8):636-652. https://doi.org/10.3109/09553002.2014.916841
11. Barnes JM, Kaushik S, Bainer RO, et al. A tension-mediated glycocalyx-integrin feedback loop promotes mesenchymal-like glioblastoma. *Nat Cell Biol*. 2018;20(10):1203-1214. https://doi.org/10.1038/s41556-018-0183-3
12. Drain AP, Zahir N, Northey JJ, et al. Matrix compliance permits NF-kappaB activation to drive therapy resistance in breast cancer. *J Exp Med*. 2021;218(5):e20191360. https://doi.org/10.1084/jem.20191360
13. Cheung TH, Rando TA. Molecular regulation of stem cell quiescence. *Nat Rev Mol Cell Biol*. 2013;14(6):329-340. https://doi.org/10.1038/nrm3591
14. Chen K, Zhang C, Ling S, et al. The metabolic flexibility of quiescent CSC: implications for chemotherapy resistance. *Cell Death Dis*. 2021;12(9):835. https://doi.org/10.1038/s41419-021-04116-6
15. Sanchez-Danes A, Larsimont JC, Liagre M, et al. A slow-cycling LGR5 tumour population mediates basal cell carcinoma relapse after therapy. *Nature*. 2018;562(7727):434-438. https://doi.org/10.1038/s41586-018-0603-3
16. Coleman RE, Croucher PI, Padhani AR, et al. Bone metastases. *Nat Rev Dis Primers*. 2020;6(1):83. https://doi.org/10.1038/s41572-020-00216-3
17. Butof R, Dubrovska A, Baumann M. Clinical perspectives of cancer stem cell research in radiation oncology. *Radiother Oncol*. 2013;108(3):388-396. https://doi.org/10.1016/j.radonc.2013.06.002
18. Coppes RP, Dubrovska A. Targeting stem cells in radiation oncology. *Clin Oncol (R Coll Radiol)*. 2017;29(6):329-334. https://doi.org/10.1016/j.clon.2017.03.005
19. Yang L, Shi P, Zhao G, et al. Targeting cancer stem cell pathways for cancer therapy. *Signal Transduct Target Ther*. 2020;5(1):8. https://doi.org/10.1038/s41392-020-0110-5
20. Saygin C, Matei D, Majeti R, Reizes O, Lathia JD. Targeting cancer stemness in the clinic: from hype to hope. *Cell Stem Cell*. 2019;24(1):25-40. https://doi.org/10.1016/j.stem.2018.11.017
21. Baccelli I, Trumpp A. The evolving concept of cancer and metastasis stem cells. *J Cell Biol*. 2012;198(3):281-293. https://doi.org/10.1083/jcb.201202014

22. Walcher L, Kistenmacher AK, Suo H, et al. Cancer stem cells-origins and biomarkers: perspectives for targeted personalized therapies. *Front Immunol*. 2020;11:1280. https://doi.org/10.3389/fimmu.2020.01280
23. Skvortsov S, Skvortsova II, Tang DG, Dubrovska A. Concise review: prostate cancer stem cells: current understanding. *Stem Cells*. 2018;36(10):1457-1474. https://doi.org/10.1002/stem.2859
24. Rycaj K, Tang DG. Cell-of-origin of cancer versus cancer stem cells: assays and interpretations. *Cancer Res*. 2015;75(19):4003-4011. https://doi.org/10.1158/0008-5472.CAN-15-0798
25. Menke-van der Houven van Oordt CW, Gomez-Roca C, van Herpen C, et al. First-in-human phase I clinical trial of RG7356, an anti-CD44 humanized antibody, in patients with advanced, CD44-expressing solid tumors. *Oncotarget*. 2016;7(48):80046-80058. https://doi.org/10.18632/oncotarget.11098
26. Maroto P, Porta C, Capdevila J, et al. Cabozantinib for the treatment of solid tumors: a systematic review. *Ther Adv Med Oncol*. 2022;14:17588359221107112. https://doi.org/10.1177/17588359221107112
27. Sahu A, Prabhash K, Noronha V, Joshi A, Desai S. Crizotinib: a comprehensive review. *South Asian J Cancer*. 2013;2(2):91-97. https://doi.org/10.4103/2278-330X.110506
28. Kazandjian D, Blumenthal GM, Chen HY, et al. FDA approval summary: crizotinib for the treatment of metastatic non-small cell lung cancer with anaplastic lymphoma kinase rearrangements. *Oncologist*. 2014;19(10):e5-11. https://doi.org/10.1634/theoncologist.2014-0241
29. Aschenbrenner DS. New indication for cancer drug crizotinib. *Am J Nurs*. 2022;122(11):20. https://doi.org/10.1097/01.NAJ.0000897112.29413.1a
30. Moores SL, Chiu ML, Bushey BS, et al. A novel bispecific antibody targeting EGFR and cMet is effective against EGFR inhibitor-resistant lung tumors. *Cancer Res*. 2016;76(13):3942-3953. https://doi.org/10.1158/0008-5472.CAN-15-2833
31. Parums DV. Editorial: current status of two adjuvanted malaria vaccines and the World Health Organization (WHO) strategy to eradicate malaria by 2030. *Med Sci Monit*. 2023;29:e939357. https://doi.org/10.12659/MSM.939357
32. Syed YY. Amivantamab: first approval. *Drugs*. 2021;81(11):1349-1353. https://doi.org/10.1007/s40265-021-01561-7
33. Zhang Y, Zhang Z, Ding Y, et al. Phase I clinical trial of EGFR-specific CAR-T cells generated by the piggyBac transposon system in advanced relapsed/refractory non-small cell lung cancer patients. *J Cancer Res Clin Oncol*. 2021;147(12):3725-3734. https://doi.org/10.1007/s00432-021-03613-7
34. Lum LG, Thakur A, Choi M, et al. Clinical and immune responses to anti-CD3 x anti-EGFR bispecific antibody armed activated T cells (EGFR BATs) in pancreatic cancer patients. *Oncoimmunology*. 2020;9(1):1773201. https://doi.org/10.1080/2162402X.2020.1773201
35. Linke R, Klein A, Seimetz D. Catumaxomab: clinical development and future directions. *MAbs*. 2010;2(2):129-136. https://doi.org/10.4161/mabs.2.2.11221
36. Coma S, Paradis JS, Gutkind JS, Pachter JA. Abstract 1425: synergistic antitumor efficacy of the dual RAF/MEK inhibitor VS-6766 with FAK inhibition for treatment of RAS-dependent solid tumors. *Cancer Res*. 2021;81(13_Supplement):1425-1425. https://doi.org/10.1158/1538-7445.am2021-1425
37. Shinde R, Terbuch A, Little M, et al. Abstract CT143: phase I study of the combination of a RAF-MEK inhibitor CH5126766 and FAK inhibitor defactinib in an intermittent dosing schedule with expansions in KRAS mutant cancers. *Cancer Res*. 2020;80(16_Supplement):CT143-CT143.
38. Schott AF, Landis MD, Dontu G, et al. Preclinical and clinical studies of gamma secretase inhibitors with docetaxel on human breast tumors. *Clin Cancer Res*. 2013;19(6):1512-1524. https://doi.org/10.1158/1078-0432.CCR-11-3326
39. Piha-Paul SA, Munster PN, Hollebecque A, et al. Results of a phase 1 trial combining ridaforolimus and MK-0752 in patients with advanced solid tumours. *Eur J Cancer*. 2015;51(14):1865-1873. https://doi.org/10.1016/j.ejca.2015.06.115
40. Cook N, Basu B, Smith DM, et al. A phase I trial of the gamma-secretase inhibitor MK-0752 in combination with gemcitabine in patients with pancreatic ductal adenocarcinoma. *Br J Cancer*. 2018;118(6):793-801. https://doi.org/10.1038/bjc.2017.495
41. McKeage MJ, Kotasek D, Markman B, et al. Phase IB trial of the anti-cancer stem cell DLL4-binding agent demcizumab with pemetrexed and carboplatin as first-line treatment of metastatic non-squamous NSCLC. *Target Oncol*. 2018;13(1):89-98. https://doi.org/10.1007/s11523-017-0543-0
42. Jimeno A, Gordon M, Chugh R, et al. A first-in-human phase I study of the anticancer stem cell agent ipafricept (OMP-54F28), a decoy receptor for Wnt ligands, in patients with advanced solid tumors. *Clin Cancer Res*. 2017;23(24):7490-7497. https://doi.org/10.1158/1078-0432.CCR-17-2157
43. Dotan E, Cardin DB, Lenz HJ, et al. Phase Ib study of Wnt inhibitor ipafricept with gemcitabine and nab-paclitaxel in patients with previously untreated stage IV pancreatic cancer. *Clin Cancer Res*. 2020;26(20):5348-5357. https://doi.org/10.1158/1078-0432.CCR-20-0489
44. Rodon J, Argiles G, Connolly RM, et al. Phase 1 study of single-agent WNT974, a first-in-class porcupine inhibitor, in patients with advanced solid tumours. *Br J Cancer*. 2021;125(1):28-37. https://doi.org/10.1038/s41416-021-01389-8
45. El-Khoueiry AB, Ning Y, Yang D, et al. A phase I first-in-human study of PRI-724 in patients (pts) with advanced solid tumors. *J Clin Oncol*. 2013;31(15_suppl):2501-2501. https://doi.org/10.1200/jco.2013.31.15_suppl.2501
46. Sekulic A, Migden MR, Oro AE, et al. Efficacy and safety of vismodegib in advanced basal-cell carcinoma. *N Engl J Med*. 2012;366(23):2171-2179. https://doi.org/10.1056/NEJMoa1113713
47. Kaatz M, Mohr P, Livingstone E, et al. Effectiveness, safety and utilization of vismodegib for locally advanced basal cell carcinoma under real-world conditions: non-interventional cohort study JONAS. *Acta Derm Venereol*. 2022;102:adv00695. https://doi.org/10.2340/actadv.v102.293
48. Kim EJ, Sahai V, Abel EV, et al. Pilot clinical trial of hedgehog pathway inhibitor GDC-0449 (vismodegib) in combination with gemcitabine in patients with metastatic pancreatic adenocarcinoma. *Clin Cancer Res*. 2014;20(23):5937-5945. https://doi.org/10.1158/1078-0432.CCR-14-1269
49. De Jesus-Acosta A, Sugar EA, O'Dwyer PJ, et al. Phase 2 study of vismodegib, a hedgehog inhibitor, combined with gemcitabine and nab-paclitaxel in patients with untreated metastatic pancreatic adenocarcinoma. *Br J Cancer*. 2020;122(4):498-505. https://doi.org/10.1038/s41416-019-0683-3
50. Migden M, Farberg AS, Dummer R, Squittieri N, Hanke CW. A review of hedgehog inhibitors sonidegib and vismodegib for treatment of advanced basal cell carcinoma. *J Drugs Dermatol*. 2021;20(2):156-165. https://doi.org/10.36849/JDD.5657
51. Bockorny B, Semenisty V, Macarulla T, et al. BL-8040, a CXCR4 antagonist, in combination with pembrolizumab and chemotherapy for pancreatic cancer: the COMBAT trial. *Nat Med*. 2020;26(6):878-885. https://doi.org/10.1038/s41591-020-0880-x
52. Bockorny B, Macarulla T, Semenisty V, et al. Motixafortide and pembrolizumab combined to nanoliposomal irinotecan, fluorouracil, and folinic acid in metastatic pancreatic cancer: the COMBAT/KEYNOTE-202 trial. *Clin Cancer Res*. 2021;27(18):5020-5027. https://doi.org/10.1158/1078-0432.CCR-21-0929
53. Goldstein LJ, Perez RP, Yardley D, et al. A window-of-opportunity trial of the CXCR1/2 inhibitor reparixin in operable HER-2-negative breast cancer. *Breast Cancer Res*. 2020;22(1):4. https://doi.org/10.1186/s13058-019-1243-8
54. Ranjan T, Sengupta S, Glantz MJ, et al. Cancer stem cell assay-guided chemotherapy improves survival of patients with

recurrent glioblastoma in a randomized trial. *Cell Rep Med*. 2023;4(5):101025. https://doi.org/10.1016/j.xcrm.2023.101025
55. Volz Y, Koschut D, Matzke-Ogi A, et al. Direct binding of hepatocyte growth factor and vascular endothelial growth factor to CD44v6. *Biosci Rep*. 2015;35(4):e00236. https://doi.org/10.1042/BSR20150093
56. Min J, Vega PN, Engevik AC, et al. Heterogeneity and dynamics of active Kras-induced dysplastic lineages from mouse corpus stomach. *Nat Commun*. 2019;10(1):5549. https://doi.org/10.1038/s41467-019-13479-6
57. Todaro M, Gaggianesi M, Catalano V, et al. CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. *Cell Stem Cell*. 2014;14(3):342-356. https://doi.org/10.1016/j.stem.2014.01.009
58. Ma L, Dong L, Chang P. CD44v6 engages in colorectal cancer progression. *Cell Death Dis*. 2019;10(1):30. https://doi.org/10.1038/s41419-018-1265-7
59. Koseer AS, Di Gaetano S, Arndt C, Bachmann M, Dubrovska A. Immunotargeting of cancer stem cells. *Cancers (Basel)*. 2023;15(5):1608. https://doi.org/10.3390/cancers15051608
60. Haist C, Schulte E, Bartels N, et al. CD44v6-targeted CAR T-cells specifically eliminate CD44 isoform 6 expressing head/neck squamous cell carcinoma cells. *Oral Oncol*. 2021;116:105259. https://doi.org/10.1016/j.oraloncology.2021.105259
61. Stornaiuolo A, Valentinis B, Sirini C, et al. Characterization and functional analysis of CD44v6CAR T cells endowed with a new low-affinity nerve growth factor receptor-based spacer. *Hum Gene Ther*. 2021;32(13-14):744-760. https://doi.org/10.1089/hum.2020.216
62. Porcellini S, Asperti C, Corna S, et al. CAR T cells redirected to CD44v6 control tumor growth in lung and ovary adenocarcinoma bearing mice. *Front Immunol*. 2020;11:99. https://doi.org/10.3389/fimmu.2020.00099
63. Fu J, Su X, Li Z, et al. HGF/c-MET pathway in cancer: from molecular characterization to clinical evidence. *Oncogene*. 2021;40(28):4625-4651. https://doi.org/10.1038/s41388-021-01863-w
64. Luraghi P, Reato G, Cipriano E, et al. MET signaling in colon cancer stem-like cells blunts the therapeutic response to EGFR inhibitors. *Cancer Res*. 2014;74(6):1857-1869. https://doi.org/10.1158/0008-5472.CAN-13-2340-T
65. van Leenders GJ, Sookhlall R, Teubel WJ, et al. Activation of c-MET induces a stem-like phenotype in human prostate cancer. *PLoS One*. 2011;6(11):e26753. https://doi.org/10.1371/journal.pone.0026753
66. Lau D, Wadhwa H, Sudhir S, et al. Role of c-Met/beta1 integrin complex in the metastatic cascade in breast cancer. *JCI Insight*. 2021;6(12):e138928. https://doi.org/10.1172/jci.insight.138928
67. Li Y, Li A, Glas M, et al. c-Met signaling induces a reprogramming network and supports the glioblastoma stem-like phenotype. *Proc Natl Acad Sci USA*. 2011;108(24):9951-9956. https://doi.org/10.1073/pnas.1016912108
68. Hughes VS, Siemann DW. Have clinical trials properly assessed c-Met inhibitors? *Trends Cancer*. 2018;4(2):94-97. https://doi.org/10.1016/j.trecan.2017.11.009
69. Hage C, Rausch V, Giese N, et al. The novel c-Met inhibitor cabozantinib overcomes gemcitabine resistance and stem cell signaling in pancreatic cancer. *Cell Death Dis*. 2013;4(5):e627. https://doi.org/10.1038/cddis.2013.158
70. Oh SJ, Noh KH, Lee YH, et al. Targeting stemness is an effective strategy to control EML4-ALK+ non-small cell lung cancer cells. *Oncotarget*. 2015;6(37):40255-40267. https://doi.org/10.18632/oncotarget.5434
71. Mitsudomi T, Yatabe Y. Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. *FEBS J*. 2010;277(2):301-308. https://doi.org/10.1111/j.1742-4658.2009.07448.x
72. Savage P, Blanchet-Cohen A, Revil T, et al. A targetable EGFR-dependent tumor-initiating program in breast cancer. *Cell Rep*. 2017;21(5):1140-1149. https://doi.org/10.1016/j.celrep.2017.10.015
73. Zhao L, Qiu T, Jiang D, et al. SGCE promotes breast cancer stem cells by stabilizing EGFR. *Adv Sci (Weinh)*. 2020;7(14):1903700. https://doi.org/10.1002/advs.201903700
74. Rossini A, Giussani M, Ripamonti F, et al. Combined targeting of EGFR and HER2 against prostate cancer stem cells. *Cancer Biol Ther*. 2020;21(5):463-475. https://doi.org/10.1080/15384047.2020.1727702
75. Fu W, Lei C, Yu Y, et al. EGFR/notch antagonists enhance the response to inhibitors of the PI3K-Akt pathway by decreasing tumor-initiating cell frequency. *Clin Cancer Res*. 2019;25(9):2835-2847. https://doi.org/10.1158/1078-0432.CCR-18-2732
76. Hu S, Fu W, Li T, et al. Antagonism of EGFR and Notch limits resistance to EGFR inhibitors and radiation by decreasing tumor-initiating cell frequency. *Sci Transl Med*. 2017;9(380).
77. Zubair T, Bandyopadhyay D. Small molecule EGFR inhibitors as anti-cancer agents: discovery, mechanisms of action, and opportunities. *Int J Mol Sci*. 2023;24(3):2651. https://doi.org/10.3390/ijms24032651
78. Westphal M, Maire CL, Lamszus K. EGFR as a target for glioblastoma treatment: an unfulfilled promise. *CNS Drugs*. 2017;31(9):723-735. https://doi.org/10.1007/s40263-017-0456-6
79. Qaddoumi I, Kocak M, Pai Panandiker AS, et al. Phase II trial of erlotinib during and after radiotherapy in children with newly diagnosed high-grade gliomas. *Front Oncol*. 2014;4:67. https://doi.org/10.3389/fonc.2014.00067
80. Fouladi M, Stewart CF, Blaney SM, et al. A molecular biology and phase II trial of lapatinib in children with refractory CNS malignancies: a pediatric brain tumor consortium study. *J Neurooncol*. 2013;114(2):173-179. https://doi.org/10.1007/s11060-013-1166-7
81. Corominas-Faja B, Oliveras-Ferraros C, Cuyas E, et al. Stem cell-like ALDH(bright) cellular states in EGFR-mutant non-small cell lung cancer: a novel mechanism of acquired resistance to erlotinib targetable with the natural polyphenol silibinin. *Cell Cycle*. 2013;12(21):3390-3404. https://doi.org/10.4161/cc.26417
82. Shien K, Toyooka S, Yamamoto H, et al. Acquired resistance to EGFR inhibitors is associated with a manifestation of stem cell-like properties in cancer cells. *Cancer Res*. 2013;73(10):3051-3061. https://doi.org/10.1158/0008-5472.CAN-12-4136
83. Hashida S, Yamamoto H, Shien K, et al. Acquisition of cancer stem cell-like properties in non-small cell lung cancer with acquired resistance to afatinib. *Cancer Sci*. 2015;106(10):1377-1384. https://doi.org/10.1111/cas.12749
84. Hashida S, Yamamoto H, Shien K, et al. Hsp90 inhibitor NVP-AUY922 enhances the radiation sensitivity of lung cancer cell lines with acquired resistance to EGFR-tyrosine kinase inhibitors. *Oncol Rep*. 2015;33(3):1499-1504. https://doi.org/10.3892/or.2015.3735
85. Liu X, Adorno-Cruz V, Chang YF, et al. EGFR inhibition blocks cancer stem cell clustering and lung metastasis of triple negative breast cancer. *Theranostics*. 2021;11(13):6632-6643. https://doi.org/10.7150/thno.57706
86. Herlyn M, Steplewski Z, Herlyn D, Koprowski H. Colorectal carcinoma-specific antigen: detection by means of monoclonal antibodies. *Proc Natl Acad Sci USA*. 1979;76(3):1438-1442. https://doi.org/10.1073/pnas.76.3.1438
87. Yang L, Wang Q, Zhao Q, et al. Deglycosylated EpCAM regulates proliferation by enhancing autophagy of breast cancer cells via PI3K/Akt/mTOR pathway. *Aging (Albany NY)*. 2022;14(1):316-329. https://doi.org/10.18632/aging.203795
88. Ni J, Cozzi P, Hao J, et al. Epithelial cell adhesion molecule (EpCAM) is associated with prostate cancer metastasis and chemo/radioresistance via the PI3K/Akt/mTOR signaling pathway. *Int J Biochem Cell Biol*. 2013;45(12):2736-2748. https://doi.org/10.1016/j.biocel.2013.09.008
89. Liang KH, Tso HC, Hung SH, et al. Extracellular domain of EpCAM enhances tumor progression through EGFR signaling in

colon cancer cells. *Cancer Lett*. 2018;433:165-175. https://doi.org/10.1016/j.canlet.2018.06.040
90. Gao J, Yan Q, Wang J, Liu S, Yang X. Epithelial-to-mesenchymal transition induced by TGF-beta1 is mediated by AP1-dependent EpCAM expression in MCF-7 cells. *J Cell Physiol*. 2015;230(4):775-782. https://doi.org/10.1002/jcp.24802
91. Munz M, Baeuerle PA, Gires O. The emerging role of EpCAM in cancer and stem cell signaling. *Cancer Res*. 2009;69(14):5627-5629. https://doi.org/10.1158/0008-5472.CAN-09-0654
92. Gires O, Pan M, Schinke H, Canis M, Baeuerle PA. Expression and function of epithelial cell adhesion molecule EpCAM: where are we after 40 years? *Cancer Metastasis Rev*. 2020;39(3):969-987. https://doi.org/10.1007/s10555-020-09898-3
93. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA*. 2003;100(7):3983-3988. https://doi.org/10.1073/pnas.0530291100
94. Dalerba P, Dylla SJ, Park IK, et al. Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci USA*. 2007;104(24):10158-10163. https://doi.org/10.1073/pnas.0703478104
95. Yamashita T, Ji J, Budhu A, et al. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. *Gastroenterology*. 2009;136(3):1012-1024. https://doi.org/10.1053/j.gastro.2008.12.004
96. Hoe SLL, Tan LP, Abdul Aziz N, et al. CD24, CD44 and EpCAM enrich for tumour-initiating cells in a newly established patient-derived xenograft of nasopharyngeal carcinoma. *Sci Rep*. 2017;7(1):12372. https://doi.org/10.1038/s41598-017-12045-8
97. Pantel K, Alix-Panabieres C. Circulating tumour cells in cancer patients: challenges and perspectives. *Trends Mol Med*. 2010;16(9):398-406. https://doi.org/10.1016/j.molmed.2010.07.001
98. Imrich S, Hachmeister M, Gires O. EpCAM and its potential role in tumor-initiating cells. *Cell Adh Migr*. 2012;6(1):30-38. https://doi.org/10.4161/cam.18953
99. Cioffi M, Dorado J, Baeuerle PA, Heeschen C. EpCAM/CD3-bispecific T-cell engaging antibody MT110 eliminates primary human pancreatic cancer stem cells. *Clin Cancer Res*. 2012;18(2):465-474. https://doi.org/10.1158/1078-0432.CCR-11-1270
100. Wang Z, Li Y, Wang Y, et al. Targeting prostate cancer stem-like cells by an immunotherapeutic platform based on immunogenic peptide-sensitized dendritic cells-cytokine-induced killer cells. *Stem Cell Res Ther*. 2020;11(1):123. https://doi.org/10.1186/s13287-020-01634-6
101. Lagadec C, Vlashi E, Della Donna L, Dekmezian C, Pajonk F. Radiation-induced reprogramming of breast cancer cells. *Stem Cells*. 2012;30(5):833-844. https://doi.org/10.1002/stem.1058
102. Bhat K, Saki M, Vlashi E, et al. The dopamine receptor antagonist trifluoperazine prevents phenotype conversion and improves survival in mouse models of glioblastoma. *Proc Natl Acad Sci USA*. 2020;117(20):11085-11096. https://doi.org/10.1073/pnas.1920154117
103. Prieto-Vila M, Takahashi RU, Usuba W, Kohama I, Ochiya T. Drug resistance driven by cancer stem cells and their niche. *Int J Mol Sci*. 2017;18(12):2574. https://doi.org/10.3390/ijms18122574
104. Bayik D, Lathia JD. Cancer stem cell-immune cell crosstalk in tumour progression. *Nat Rev Cancer*. 2021;21(8):526-536. https://doi.org/10.1038/s41568-021-00366-w
105. McLean GW, Carragher NO, Avizienyte E, et al. The role of focal-adhesion kinase in cancer – a new therapeutic opportunity. *Nat Rev Cancer*. 2005;5(7):505-515. https://doi.org/10.1038/nrc1647
106. Hu JK, Du W, Shelton SJ, et al. An FAK-YAP-mTOR signaling axis regulates stem cell-based tissue renewal in mice. *Cell Stem Cell*. 2017;21(1):91-106.e6. https://doi.org/10.1016/j.stem.2017.03.023
107. Williams KE, Bundred NJ, Landberg G, Clarke RB, Farnie G. Focal adhesion kinase and Wnt signaling regulate human ductal carcinoma in situ stem cell activity and response to radiotherapy. *Stem Cells*. 2015;33(2):327-341. https://doi.org/10.1002/stem.1843
108. Timbrell S, Aglan H, Cramer A, et al. FAK inhibition alone or in combination with adjuvant therapies reduces cancer stem cell activity. *npj Breast Cancer*. 2021;7(1):65. https://doi.org/10.1038/s41523-021-00263-3
109. Kolev VN, Tam WF, Wright QG, et al. Inhibition of FAK kinase activity preferentially targets cancer stem cells. *Oncotarget*. 2017;8(31):51733-51747. https://doi.org/10.18632/oncotarget.18517
110. Luo M, Fan H, Nagy T, et al. Mammary epithelial-specific ablation of the focal adhesion kinase suppresses mammary tumorigenesis by affecting mammary cancer stem/progenitor cells. *Cancer Res*. 2009;69(2):466-474. https://doi.org/10.1158/0008-5472.CAN-08-3078
111. Ohta Y, Fujii M, Takahashi S, et al. Cell-matrix interface regulates dormancy in human colon cancer stem cells. *Nature*. 2022;608(7924):784-794. https://doi.org/10.1038/s41586-022-05043-y
112. Xu C, Zhang W, Liu C. FAK downregulation suppresses stem-like properties and migration of human colorectal cancer cells. *PLoS One*. 2023;18(4):e0284871. https://doi.org/10.1371/journal.pone.0284871
113. Zhu Y, Sandow L, Matsui W. Abstract PR-004: inhibition of focal adhesion kinase (FAK) improves pancreatic ductal adenocarcinoma's response to immunotherapy by targeting cancer stem cells (CSCs). *Cancer Res*. 2021;81(22_Supplement):PR-004-PR-004.
114. Despeaux M, Chicanne G, Rouer E, et al. Focal adhesion kinase splice variants maintain primitive acute myeloid leukemia cells through altered Wnt signaling. *Stem Cells*. 2012;30(8):1597-1610. https://doi.org/10.1002/stem.1157
115. Liu C, Li Y, Xing Y, et al. The interaction between cancer stem cell marker CD133 and Src protein promotes focal adhesion kinase (FAK) phosphorylation and cell migration. *J Biol Chem*. 2016;291(30):15540-15550. https://doi.org/10.1074/jbc.M115.712976
116. Zhang B, Zhang Y, Zhang J, et al. Focal adhesion kinase (FAK) inhibition synergizes with KRAS G12C inhibitors in treating cancer through the regulation of the FAK-YAP signaling. *Adv Sci (Weinh)*. 2021;8(16):e2100250. https://doi.org/10.1002/advs.202100250
117. Meurette O, Mehlen P. Notch signaling in the tumor microenvironment. *Cancer Cell*. 2018;34(4):536-548. https://doi.org/10.1016/j.ccell.2018.07.009
118. Capodanno Y, Buishand FO, Pang LY, et al. Notch pathway inhibition targets chemoresistant insulinoma cancer stem cells. *Endocr Relat Cancer*. 2018;25(2):131-144. https://doi.org/10.1530/ERC-17-0415
119. Liu G, Zhang P, Chen S, et al. FAM129A promotes self-renewal and maintains invasive status via stabilizing the Notch intracellular domain in glioma stem cells. *Neuro Oncol*. 2023;25(10).
120. Mamaeva V, Niemi R, Beck M, et al. Inhibiting notch activity in breast cancer stem cells by glucose functionalized nanoparticles carrying gamma-secretase inhibitors. *Mol Ther*. 2016;24(5):926-936. https://doi.org/10.1038/mt.2016.42
121. Yen WC, Fischer MM, Axelrod F, et al. Targeting Notch signaling with a Notch2/Notch3 antagonist (tarextumab) inhibits tumor growth and decreases tumor-initiating cell frequency. *Clin Cancer Res*. 2015;21(9):2084-2095. https://doi.org/10.1158/1078-0432.CCR-14-2808
122. Fischer M, Yen WC, Kapoun AM, et al. Anti-DLL4 inhibits growth and reduces tumor-initiating cell frequency in colorectal tumors with oncogenic KRAS mutations. *Cancer Res*. 2011;71(5):1520-1525. https://doi.org/10.1158/0008-5472.CAN-10-2817
123. Liang KH, Chang CC, Wu KS, et al. Notch signaling and natural killer cell infiltration in tumor tissues underlie medulloblastoma prognosis. *Sci Rep*. 2021;11(1):23282. https://doi.org/10.1038/s41598-021-02651-y

124. de Bont JM, Packer RJ, Michiels EM, den Boer ML, Pieters R. Biological background of pediatric medulloblastoma and ependymoma: a review from a translational research perspective. *Neuro Oncol*. 2008;10(6):1040-1060. https://doi.org/10.1215/15228517-2008-059
125. Brandt WD, Schreck KC, Bar EE, et al. Notch signaling activation in pediatric low-grade astrocytoma. *J Neuropathol Exp Neurol*. 2015;74(2):121-131. https://doi.org/10.1097/NEN.0000000000000155
126. Taylor IC, Hutt-Cabezas M, Brandt WD, et al. Disrupting NOTCH slows diffuse intrinsic pontine glioma growth, enhances radiation sensitivity, and shows combinatorial efficacy with bromodomain inhibition. *J Neuropathol Exp Neurol*. 2015;74(8):778-790. https://doi.org/10.1097/NEN.0000000000000216
127. Hofbauer LC, Bozec A, Rauner M, et al. Novel approaches to target the microenvironment of bone metastasis. *Nat Rev Clin Oncol*. 2021;18(8).
128. Fouladi M, Stewart CF, Olson J, et al. Phase I trial of MK-0752 in children with refractory CNS malignancies: a pediatric brain tumor consortium study. *J Clin Oncol*. 2011;29(26):3529-3534. https://doi.org/10.1200/JCO.2011.35.7806
129. Leung C, Tan SH, Barker N. Recent advances in Lgr5(+) stem cell research. *Trends Cell Biol*. 2018;28(5):380-391. https://doi.org/10.1016/j.tcb.2018.01.010
130. Carmon KS, Lin Q, Gong X, Thomas A, Liu Q. LGR5 interacts and cointernalizes with Wnt receptors to modulate Wnt/beta-catenin signaling. *Mol Cell Biol*. 2012;32(11):2054-2064. https://doi.org/10.1128/MCB.00272-12
131. Kemper K, Prasetyanti PR, De Lau W, et al. Monoclonal antibodies against Lgr5 identify human colorectal cancer stem cells. *Stem Cells*. 2012;30(11):2378-2386. https://doi.org/10.1002/stem.1233
132. Corda G, Sala A. Non-canonical WNT/PCP signalling in cancer: Fzd6 takes centre stage. *Oncogenesis*. 2017;6(7):e364. https://doi.org/10.1038/oncsis.2017.69
133. Le PN, Keysar SB, Miller B, et al. Wnt signaling dynamics in head and neck squamous cell cancer tumor-stroma interactions. *Mol Carcinog*. 2019;58(3):398-410. https://doi.org/10.1002/mc.22937
134. Wei X, Roudier MP, Kwon OJ, et al. Paracrine Wnt signaling is necessary for prostate epithelial proliferation. *Prostate*. 2022;82(5):517-530. https://doi.org/10.1002/pros.24298
135. Geleta B, Tout FS, Lim SC, et al. Targeting Wnt/tenascin C-mediated cross talk between pancreatic cancer cells and stellate cells via activation of the metastasis suppressor NDRG1. *J Biol Chem*. 2022;298(3):101608. https://doi.org/10.1016/j.jbc.2022.101608
136. Kramer N, Schmollerl J, Unger C, et al. Autocrine WNT2 signaling in fibroblasts promotes colorectal cancer progression. *Oncogene*. 2017;36(39):5460-5472. https://doi.org/10.1038/onc.2017.144
137. Fuertes G, Del Valle-Perez B, Pastor J, et al. Noncanonical Wnt signaling promotes colon tumor growth, chemoresistance and tumor fibroblast activation. *EMBO Rep*. 2023;24(4):e54895. https://doi.org/10.15252/embr.202254895
138. Raghavan S, Mehta P, Xie Y, Lei YL, Mehta G. Ovarian cancer stem cells and macrophages reciprocally interact through the WNT pathway to promote pro-tumoral and malignant phenotypes in 3D engineered microenvironments. *J ImmunoTher Cancer*. 2019;7(1):190. https://doi.org/10.1186/s40425-019-0666-1
139. Proffitt KD, Virshup DM. Precise regulation of porcupine activity is required for physiological Wnt signaling. *J Biol Chem*. 2012;287(41):34167-34178. https://doi.org/10.1074/jbc.M112.381970
140. Wellenstein MD, Coffelt SB, Duits DEM, et al. Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. *Nature*. 2019;572(7770):538-542. https://doi.org/10.1038/s41586-019-1450-6
141. Rudy SF, Brenner JC, Harris JL, et al. In vivo Wnt pathway inhibition of human squamous cell carcinoma growth and metastasis in the chick chorioallantoic model. *J Otolaryngol Head Neck Surg*. 2016;45:26. https://doi.org/10.1186/s40463-016-0140-8
142. Hayashi M, Baker A, Goldstein SD, et al. Inhibition of porcupine prolongs metastasis free survival in a mouse xenograft model of Ewing sarcoma. *Oncotarget*. 2017;8(45):78265-78276. https://doi.org/10.18632/oncotarget.19432
143. Li C, Liang Y, Cao J, et al. The delivery of a Wnt pathway inhibitor toward CSCs requires stable liposome encapsulation and delayed drug release in tumor tissues. *Mol Ther*. 2019;27(9):1558-1567. https://doi.org/10.1016/j.ymthe.2019.06.013
144. Shah K, Panchal S, Patel B. Porcupine inhibitors: novel and emerging anti-cancer therapeutics targeting the Wnt signaling pathway. *Pharmacol Res*. 2021;167:105532. https://doi.org/10.1016/j.phrs.2021.105532
145. Fischer MM, Cancilla B, Yeung VP, et al. WNT antagonists exhibit unique combinatorial antitumor activity with taxanes by potentiating mitotic cell death. *Sci Adv*. 2017;3(6):e1700090. https://doi.org/10.1126/sciadv.1700090
146. Stamos JL, Weis WI. The beta-catenin destruction complex. *Cold Spring Harb Perspect Biol*. 2013;5(1):a007898. https://doi.org/10.1101/cshperspect.a007898
147. Cojoc M, Peitzsch C, Kurth I, et al. Aldehyde dehydrogenase is regulated by beta-catenin/TCF and promotes radioresistance in prostate cancer progenitor cells. *Cancer Res*. 2015;75(7):1482-1494. https://doi.org/10.1158/0008-5472.CAN-14-1924
148. Shen W, Zhang X, Tang J, et al. CCL16 maintains stem cell-like properties in breast cancer by activating CCR2/GSK3beta/beta-catenin/OCT4 axis. *Theranostics*. 2021;11(5):2297-2317. https://doi.org/10.7150/thno.51000
149. Peitzsch C, Cojoc M, Hein L, et al. An epigenetic reprogramming strategy to resensitize radioresistant prostate cancer cells. *Cancer Res*. 2016;76(9):2637-2651. https://doi.org/10.1158/0008-5472.CAN-15-2116
150. Plummer R, Dua D, Cresti N, et al. First-in-human study of the PARP/tankyrase inhibitor E7449 in patients with advanced solid tumours and evaluation of a novel drug-response predictor. *Br J Cancer*. 2020;123(4):525-533. https://doi.org/10.1038/s41416-020-0916-5
151. Fendler A, Bauer D, Busch J, et al. Inhibiting WNT and NOTCH in renal cancer stem cells and the implications for human patients. *Nat Commun*. 2020;11(1):929. https://doi.org/10.1038/s41467-020-14700-7
152. Jang GB, Hong IS, Kim RJ, et al. Wnt/beta-catenin small-molecule inhibitor CWP232228 preferentially inhibits the growth of breast cancer stem-like cells. *Cancer Res*. 2015;75(8):1691-1702. https://doi.org/10.1158/0008-5472.CAN-14-2041
153. Kim JY, Lee HY, Park KK, et al. CWP232228 targets liver cancer stem cells through Wnt/beta-catenin signaling: a novel therapeutic approach for liver cancer treatment. *Oncotarget*. 2016;7(15):20395-20409. https://doi.org/10.18632/oncotarget.7954
154. Zhao Y, Masiello D, McMillian M, et al. CBP/catenin antagonist safely eliminates drug-resistant leukemia-initiating cells. *Oncogene*. 2016;35(28):3705-3717. https://doi.org/10.1038/onc.2015.438
155. Ko AH, Chiorean EG, Kwak EL, et al. Final results of a phase Ib dose-escalation study of PRI-724, a CBP/beta-catenin modulator, plus gemcitabine (GEM) in patients with advanced pancreatic adenocarcinoma (APC) as second-line therapy after FOLFIRINOX or FOLFOX. *J Clin Oncol*. 2016;34(15_suppl):e15721-e15721. https://doi.org/10.1200/jco.2016.34.15_suppl.e15721
156. Jamieson C, Martinelli G, Papayannidis C, Cortes JE. Hedgehog pathway inhibitors: a new therapeutic class for the treatment of acute myeloid leukemia. *Blood Cancer Discov*. 2020;1(2):134-145. https://doi.org/10.1158/2643-3230.BCD-20-0007
157. Miyazaki Y, Matsubara S, Ding Q, et al. Efficient elimination of pancreatic cancer stem cells by hedgehog/GLI inhibitor GANT61 in combination with mTOR inhibition. *Mol Cancer*. 2016;15(1):49. https://doi.org/10.1186/s12943-016-0534-2

158. Memmi EM, Sanarico AG, Giacobbe A, et al. p63 Sustains self-renewal of mammary cancer stem cells through regulation of Sonic Hedgehog signaling. *Proc Natl Acad Sci USA*. 2015;112(11):3499-3504. https://doi.org/10.1073/pnas.1500762112
159. Lee D, Gimple RC, Wu X, et al. Superenhancer activation of KLHDC8A drives glioma ciliation and hedgehog signaling. *J Clin Invest*. 2023;133(2).
160. Cancer M, Hutter S, Holmberg KO, et al. Humanized stem cell models of pediatric medulloblastoma reveal an Oct4/mTOR axis that promotes malignancy. *Cell Stem Cell*. 2019;25(6):855-870.e11. https://doi.org/10.1016/j.stem.2019.10.005
161. Nguyen NM, Cho J. Hedgehog pathway inhibitors as targeted cancer therapy and strategies to overcome drug resistance. *Int J Mol Sci* . 2022;23(3).
162. Gurbuz M, Dogan I, Akkus E, et al. Efficacy and tolerability of vismodegib treatment in locally advanced and metastatic basal cell carcinoma: retrospective real-life data. *Dermatol Ther*. 2021;34(6):e15122. https://doi.org/10.1111/dth.15122
163. Dlugosz A, Agrawal S, Kirkpatrick P. Vismodegib. *Nat Rev Drug Discov*. 2012;11(6):437-438. https://doi.org/10.1038/nrd3753
164. Gatto L, Franceschi E, Tosoni A, et al. Molecular targeted therapies: time for a paradigm shift in medulloblastoma treatment? *Cancers (Basel)*. 2022;14(2).
165. Pereira V, Torrejon J, Kariyawasam D, et al. Clinical and molecular analysis of smoothened inhibitors in Sonic Hedgehog medulloblastoma. *Neurooncol Adv*. 2021;3(1):vdab097. https://doi.org/10.1093/noajnl/vdab097
166. Skowron P, Farooq H, Cavalli FMG, et al. The transcriptional landscape of Shh medulloblastoma. *Nat Commun*. 2021;12(1):1749. https://doi.org/10.1038/s41467-021-21883-0
167. Krausert S, Brabetz S, Mack NL, et al. Predictive modeling of resistance to SMO inhibition in a patient-derived orthotopic xenograft model of SHH medulloblastoma. *Neurooncol Adv*. 2022;4(1):vdac026. https://doi.org/10.1093/noajnl/vdac026
168. Lago C, Gianesello M, Santomaso L, et al. Medulloblastoma and high-grade glioma organoids for drug screening, lineage tracing, co-culture and in vivo assay. *Nat Protoc*. 2023;18(7):2143-2180. https://doi.org/10.1038/s41596-023-00839-2
169. Zhang J, Fan J, Zeng X, et al. Hedgehog signaling in gastrointestinal carcinogenesis and the gastrointestinal tumor microenvironment. *Acta Pharm Sin B*. 2021;11(3):609-620. https://doi.org/10.1016/j.apsb.2020.10.022
170. Varnat F, Duquet A, Malerba M, et al. Human colon cancer epithelial cells harbour active HEDGEHOG-GLI signalling that is essential for tumour growth, recurrence, metastasis and stem cell survival and expansion. *EMBO Mol Med*. 2009;1(6-7):338-351. https://doi.org/10.1002/emmm.200900039
171. Varnat F, Siegl-Cachedenier I, Malerba M, Gervaz P, Ruiz i Altaba A. Loss of WNT-TCF addiction and enhancement of HH-GLI1 signalling define the metastatic transition of human colon carcinomas. *EMBO Mol Med*. 2010;2(11):440-457. https://doi.org/10.1002/emmm.201000098
172. Po A, Citarella A, Catanzaro G, et al. Hedgehog-GLI signalling promotes chemoresistance through the regulation of ABC transporters in colorectal cancer cells. *Sci Rep*. 2020;10(1):13988. https://doi.org/10.1038/s41598-020-70871-9
173. Berlin J, Bendell JC, Hart LL, et al. A randomized phase II trial of vismodegib versus placebo with FOLFOX or FOLFIRI and bevacizumab in patients with previously untreated metastatic colorectal cancer. *Clin Cancer Res*. 2013;19(1):258-267. https://doi.org/10.1158/1078-0432.CCR-12-1800
174. Kobune M, Takimoto R, Murase K, et al. Drug resistance is dramatically restored by hedgehog inhibitors in CD34+ leukemic cells. *Cancer Sci*. 2009;100(5):948-955. https://doi.org/10.1111/j.1349-7006.2009.01111.x
175. Cheng LH, Hsu CC, Tsai HW, et al. ASPM activates hedgehog and Wnt signaling to promote small cell lung cancer stemness and progression. *Cancer Res*. 2023;83(6):830-844. https://doi.org/10.1158/0008-5472.CAN-22-2496
176. Luo W, Rodriguez M, Valdez JM, et al. Lgr4 is a key regulator of prostate development and prostate stem cell differentiation. *Stem Cells*. 2013;31(11):2492-2505. https://doi.org/10.1002/stem.1484
177. Tricot GJY, Xu C, Xu H, Zeng M, Zangari M, Zhan F. Targeting myeloma stem cells through simultaneous inhibition of Wnt and hedgehog (Hh) signaling pathways. *Blood*. 2010;116(21):615.
178. Brancaccio G, Pea F, Moscarella E, Argenziano G. Sonidegib for the treatment of advanced basal cell carcinoma. *Front Oncol*. 2020;10:582866. https://doi.org/10.3389/fonc.2020.582866
179. Vasu S, Altman JK, Uy GL, et al. A phase I study of the fully human, fragment crystallizable-engineered, anti-CD-33 monoclonal antibody BI 836858 in patients with previously-treated acute myeloid leukemia. *Haematologica*. 2022;107(3):770-773. https://doi.org/10.3324/haematol.2020.274118
180. Berdel AF, Ruhnke L, Angenendt L, et al. Using stroma-anchoring cytokines to augment ADCC: a phase 1 trial of F16IL2 and BI 836858 for posttransplant AML relapse. *Blood Adv*. 2022;6(12):3684-3696. https://doi.org/10.1182/bloodadvances.2021006909
181. Brierley CK, Staves J, Roberts C, et al. The effects of monoclonal anti-CD47 on RBCs, compatibility testing, and transfusion requirements in refractory acute myeloid leukemia. *Transfusion*. 2019;59(7):2248-2254. https://doi.org/10.1111/trf.15397
182. Daver N, Senapati J, Maiti A, et al. Phase I/II study of azacitidine (AZA) with venetoclax (VEN) and magrolimab (Magro) in patients (pts) with newly diagnosed (ND) older/unfit or high-risk acute myeloid leukemia (AML) and relapsed/refractory (R/R) AML. *Blood*. 2022;140(Supplement 1):141-144. https://doi.org/10.1182/blood-2022-170188
183. Brunner AM, Esteve J, Porkka K, et al. Efficacy and safety of sabatolimab (MBG453) in combination with hypomethylating agents (HMAs) in patients with acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome (HR-MDS): updated results from a phase 1b study. *Blood*. 2020;136(Supplement 1):1-2. https://doi.org/10.1182/blood-2020-136855
184. Sallman DA, Elmariah H, Sweet K, et al. Phase 1/1b safety study of Prgn-3006 ultracar-T in patients with relapsed or refractory CD33-positive acute myeloid leukemia and higher risk myelodysplastic syndromes. *Blood*. 2021;140(Supplement 1):10313-10315. https://doi.org/10.1182/blood-2022-169142
185. Huang R, Wen Q, Wang X, et al. Off-the-shelf CD33 CAR-NK cell therapy for relapse/refractory AML: first-in-human, phase I trial. *Blood*. 2022;140(Supplement 1):7450-7451. https://doi.org/10.1182/blood-2022-170712
186. Lee JH, Faderl S, Pagel JM, et al. Phase 1 study of CWP232291 in patients with relapsed or refractory acute myeloid leukemia and myelodysplastic syndrome. *Blood Adv*. 2020;4(9):2032-2043. https://doi.org/10.1182/bloodadvances.2019000757
187. Sekeres MA, Schuster M, Joris M, et al. A phase 1b study of glasdegib + azacitidine in patients with untreated acute myeloid leukemia and higher-risk myelodysplastic syndromes. *Ann Hematol*. 2022;101(8):1689-1701. https://doi.org/10.1007/s00277-022-04853-4
188. Tibes R, Kosiorek HE, Dueck A, et al. Phase I/IB study of azacitidine and hedgehog pathway inhibition with sonidegib (LDE225) in myeloid malignancies. *Blood*. 2017;130(Supplement 1):2629.
189. Eghtedar A, Verstovsek S, Estrov Z, et al. Phase 2 study of the JAK kinase inhibitor ruxolitinib in patients with refractory leukemias, including postmyeloproliferative neoplasm acute myeloid leukemia. *Blood*. 2012;119(20):4614-4618. https://doi.org/10.1182/blood-2011-12-400051
190. Jeon JY, Zhao Q, Buelow DR, et al. Preclinical activity and a pilot phase I study of pacritinib, an oral JAK2/FLT3 inhibitor, and chemotherapy in FLT3-ITD-positive AML. *Invest New Drugs*. 2020;38(2):340-349. https://doi.org/10.1007/s10637-019-00786-4

191. Chien S, Beyerle LE, Wood BL, et al. Mobilization of blasts and leukemia stem cells by anti-CXCR4 antibody BMS-936564 (MDX 1338) in patients with relapsed/refractory acute myeloid leukemia. *Blood*. 2013;122(21):2882.
192. Borthakur G, Zeng Z, Cortes JE, et al. Phase 1 study of combinatorial sorafenib, G-CSF, and plerixafor treatment in relapsed/refractory, FLT3-ITD-mutated acute myelogenous leukemia patients. *Am J Hematol*. 2020;95(11):1296-1303. https://doi.org/10.1002/ajh.25943
193. Ghobrial IM, Liu CJ, Zavidij O, et al. Phase I/II trial of the CXCR4 inhibitor plerixafor in combination with bortezomib as a chemosensitization strategy in relapsed/refractory multiple myeloma. *Am J Hematol*. 2019;94(11):1244-1253. https://doi.org/10.1002/ajh.25627
194. Martinez-Cuadron D, Boluda B, Martinez P, et al; CETLAM and PETHEMA groups. A phase I-II study of plerixafor in combination with fludarabine, idarubicin, cytarabine, and G-CSF (PLERIFLAG regimen) for the treatment of patients with the first early-relapsed or refractory acute myeloid leukemia. *Ann Hematol*. 2018;97(5):763-772. https://doi.org/10.1007/s00277-018-3229-5
195. Borthakur G, Ofran Y, Tallman MS, et al. BL-8040 CXCR4 antagonist is safe and demonstrates antileukemic activity in combination with cytarabine for the treatment of relapsed/refractory acute myelogenous leukemia: an open-label safety and efficacy phase 2a study. *Cancer*. 2021;127(8):1246-1259. https://doi.org/10.1002/cncr.33338
196. Boddu P, Borthakur G, Koneru M, et al. Initial report of a phase I study of LY2510924, idarubicin, and cytarabine in relapsed/refractory acute myeloid leukemia. *Front Oncol*. 2018;8:369. https://doi.org/10.3389/fonc.2018.00369
197. Huselton E, Rettig MP, Campbell K, et al. Combination of dociparstat sodium (DSTAT), a CXCL12/CXCR4 inhibitor, with azacitidine for the treatment of hypomethylating agent refractory AML and MDS. *Leuk Res*. 2021;110:106713. https://doi.org/10.1016/j.leukres.2021.106713
198. Kovacsovics T, Levy MY, Cook RJ, et al. A randomized phase II trial of CX-01 with standard therapy in elderly patients with acute myeloid leukemia (AML). *J Clin Oncol*. 2019;37(15_suppl):7001-7001. https://doi.org/10.1200/jco.2019.37.15_suppl.7001
199. Vey N, Delaunay J, Martinelli G, et al. Phase I clinical study of RG7356, an anti-CD44 humanized antibody, in patients with acute myeloid leukemia. *Oncotarget*. 2016;7(22):32532-32542. https://doi.org/10.18632/oncotarget.8687
200. Heuser M, Smith BD, Fiedler W, et al. Clinical benefit of glasdegib plus low-dose cytarabine in patients with de novo and secondary acute myeloid leukemia: long-term analysis of a phase II randomized trial. *Ann Hematol*. 2021;100(5):1181-1194. https://doi.org/10.1007/s00277-021-04465-4
201. Mehlman C, Takam Kamga P, Costantini A, et al. Baseline hedgehog pathway activation and increase of plasma Wnt1 protein are associated with resistance to immune checkpoint inhibitors in advanced non-small-cell lung cancer. *Cancers (Basel)*. 2021;13(5):1107. https://doi.org/10.3390/cancers13051107
202. Chang WH, Lai AG. Aberrations in Notch-Hedgehog signalling reveal cancer stem cells harbouring conserved oncogenic properties associated with hypoxia and immunoevasion. *Br J Cancer*. 2019;121(8):666-678. https://doi.org/10.1038/s41416-019-0572-9
203. Zhao S, Chang SL, Linderman JJ, Feng FY, Luker GD. A comprehensive analysis of CXCL12 isoforms in breast cancer(1,2). *Transl Oncol*. 2014;7(3):429-438. https://doi.org/10.1016/j.tranon.2014.04.001
204. Luker GD, Yang J, Richmond A, et al. At the bench: pre-clinical evidence for multiple functions of CXCR4 in cancer. *J Leukoc Biol*. 2021;109(5):969-989. https://doi.org/10.1002/JLB.2BT1018-715RR
205. Keating GM. Plerixafor: a review of its use in stem-cell mobilization in patients with lymphoma or multiple myeloma. *Drugs*. 2011;71(12):1623-1647. https://doi.org/10.2165/11206040-000000000-00000
206. Dubrovska A, Hartung A, Bouchez LC, et al. CXCR4 activation maintains a stem cell population in tamoxifen-resistant breast cancer cells through AhR signalling. *Br J Cancer*. 2012;107(1):43-52. https://doi.org/10.1038/bjc.2012.105
207. Singh S, Srivastava SK, Bhardwaj A, Owen LB, Singh AP. CXCL12-CXCR4 signalling axis confers gemcitabine resistance to pancreatic cancer cells: a novel target for therapy. *Br J Cancer*. 2010;103(11):1671-1679. https://doi.org/10.1038/sj.bjc.6605968
208. Ahn S, Saha A, Clark R, Kolonin MG, DiGiovanni J. CXCR4 and CXCR7 signaling promotes tumor progression and obesity-associated epithelial-mesenchymal transition in prostate cancer cells. *Oncogene*. 2022;41(41):4633-4644. https://doi.org/10.1038/s41388-022-02466-9
209. Gupta N, Ochiai H, Hoshino Y, et al. Inhibition of CXCR4 enhances the efficacy of radiotherapy in metastatic prostate cancer models. *Cancers (Basel)*. 2023;15(4).
210. Nengroo MA, Maheshwari S, Singh A, et al. CXCR4 intracellular protein promotes drug resistance and tumorigenic potential by inversely regulating the expression of death receptor 5. *Cell Death Dis*. 2021;12(5):464. https://doi.org/10.1038/s41419-021-03730-8
211. Gatti M, Pattarozzi A, Bajetto A, et al. Inhibition of CXCL12/CXCR4 autocrine/paracrine loop reduces viability of human glioblastoma stem-like cells affecting self-renewal activity. *Toxicology*. 2013;314(2-3):209-220. https://doi.org/10.1016/j.tox.2013.10.003
212. Gassenmaier M, Chen D, Buchner A, et al. CXC chemokine receptor 4 is essential for maintenance of renal cell carcinoma-initiating cells and predicts metastasis. *Stem Cells*. 2013;31(8):1467-1476. https://doi.org/10.1002/stem.1407
213. Cooper TM, Sison EAR, Baker SD, et al. A phase 1 study of the CXCR4 antagonist plerixafor in combination with high-dose cytarabine and etoposide in children with relapsed or refractory acute leukemias or myelodysplastic syndrome: A Pediatric Oncology Experimental Therapeutics Investigators' Consortium study (POE 10-03). *Pediatr Blood Cancer*. 2017;64(8). https://doi.org/10.1002/pbc.26414
214. Andtbacka RHI, Wang Y, Pierce RH, et al. Mavorixafor, an orally bioavailable CXCR4 antagonist, increases immune cell infiltration and inflammatory status of tumor microenvironment in patients with melanoma. *Cancer Res Commun*. 2022;2(8):904-913. https://doi.org/10.1158/2767-9764.CRC-22-0090
215. Biasci D, Smoragiewicz M, Connell CM, et al. CXCR4 inhibition in human pancreatic and colorectal cancers induces an integrated immune response. *Proc Natl Acad Sci USA*. 2020;117(46):28960-28970. https://doi.org/10.1073/pnas.2013644117
216. Fortunato O, Belisario DC, Compagno M, et al. CXCR4 inhibition counteracts immunosuppressive properties of metastatic NSCLC stem cells. *Front Immunol*. 2020;11:02168. https://doi.org/10.3389/fimmu.2020.02168
217. Allard B, Longhi MS, Robson SC, Stagg J. The ectonucleotidases CD39 and CD73: novel checkpoint inhibitor targets. *Immunol Rev*. 2017;276(1):121-144. https://doi.org/10.1111/imr.12528
218. Mittal SK, Cho KJ, Ishido S, Roche PA. Interleukin 10 (IL-10)-mediated immunosuppression: MARCH-I induction regulates antigen presentation by macrophages but not dendritic cells. *J Biol Chem*. 2015;290(45):27158-27167. https://doi.org/10.1074/jbc.M115.682708
219. Ruffini PA. The CXCL8-CXCR1/2 axis as a therapeutic target in breast cancer stem-like cells. *Front Oncol*. 2019;9:40. https://doi.org/10.3389/fonc.2019.00040
220. Han ZJ, Li YB, Yang LX, et al. Roles of the CXCL8-CXCR1/2 Axis in the Tumor Microenvironment and Immunotherapy. *Molecules*. 2021;27(1):137. https://doi.org/10.3390/molecules27010137
221. Piro G, Carbone C, Agostini A, et al. CXCR1/2 dual-inhibitor ladarixin reduces tumour burden and promotes immunotherapy

response in pancreatic cancer. *Br J Cancer*. 2023;128(2):331-341. https://doi.org/10.1038/s41416-022-02028-6
222. Khwaja A, Bjorkholm M, Gale RE, et al. Acute myeloid leukaemia. *Nat Rev Dis Primers*. 2016;2:16010. https://doi.org/10.1038/nrdp.2016.10
223. Stelmach P, Trumpp A. Leukemic stem cells and therapy resistance in acute myeloid leukemia. *Haematologica*. 2023;108(2):353-366. https://doi.org/10.3324/haematol.2022.280800
224. Jen EY, Ko CW, Lee JE, et al. FDA approval: gemtuzumab ozogamicin for the treatment of adults with newly diagnosed CD33-positive acute myeloid leukemia. *Clin Cancer Res*. 2018;24(14):3242-3246. https://doi.org/10.1158/1078-0432.CCR-17-3179
225. Allen C, Zeidan AM, Bewersdorf JP. BiTEs, DARTS, BiKEs and TriKEs-are antibody based therapies changing the future treatment of AML? *Life (Basel)*. 2021;11(6).
226. Valent P, Bauer K, Sadovnik I, et al. Cell-based and antibody-mediated immunotherapies directed against leukemic stem cells in acute myeloid leukemia: perspectives and open issues. *Stem Cells Transl Med*. 2020;9(11):1331-1343. https://doi.org/10.1002/sctm.20-0147
227. DeAngelo DJ, Jonas BA, Liesveld JL, et al. Phase 1/2 study of uproleselan added to chemotherapy in patients with relapsed or refractory acute myeloid leukemia. *Blood*. 2022;139(8):1135-1146. https://doi.org/10.1182/blood.2021010721
228. Jalil AR, Andrechak JC, Discher DE. Macrophage checkpoint blockade: results from initial clinical trials, binding analyses, and CD47-SIRPalpha structure-function. *Antib Ther*. 2020;3(2):80-94. https://doi.org/10.1093/abt/tbaa006
229. Garcia-Manero G, Goldberg AD, Winer ES, et al. Eprenetapopt combined with venetoclax and azacitidine in TP53-mutated acute myeloid leukaemia: a phase 1, dose-finding and expansion study. *Lancet Haematol*. 2023;10(4):e272-e283. https://doi.org/10.1016/S2352-3026(22)00403-3
230. Lane AA, Stein AS, Garcia JS, et al. Safety and efficacy of combining tagraxofusp (SL-401) with azacitidine or azacitidine and venetoclax in a phase 1b study for CD123 positive AML, MDS, or BPDCN. *Blood*. 2021;138(Supplement 1):2346-2346. https://doi.org/10.1182/blood-2021-147486
231. Naval Daver PM, Aribi A, Martinelli G, et al. AML-262 pivekimab sunirine (PVEK, IMGN632) triplet with azacitidine and venetoclax shows broad activity in adverse genetic subsets of relapsed/refractory acute myeloid leukemia and reduced infusion-related reactions. *Clin Lymphoma Myeloma Leuk*. 2022;22(Supplement 2):S231-S232.
232. Zeidan AM, Westermann J, Kovacsovics T, et al. P582: first results of a phase II study (STIMULUS-AML1) investigating sabatolimab + azacitidine + venetoclax in patients with newly diagnosed acute myeloid leukemia. *HemaSphere*. 2022;6:481-482. https://doi.org/10.1097/01.hs9.0000845216.33320.a2
233. Waclawiczek A, Leppa AM, Renders S, et al. Combinatorial BCL-2 family expression in acute myeloid leukemia stem cells predicts clinical response to azacitidine/venetoclax. *Cancer Discov*. 2023;13(6).
234. Norsworthy KJ, By K, Subramaniam S, et al. FDA approval summary: glasdegib for newly diagnosed acute myeloid leukemia. *Clin Cancer Res*. 2019;25(20):6021-6025. https://doi.org/10.1158/1078-0432.CCR-19-0365
235. Pemmaraju N, Konopleva M. Approval of tagraxofusp-erzs for blastic plasmacytoid dendritic cell neoplasm. *Blood Adv*. 2020;4(16):4020-4027. https://doi.org/10.1182/bloodadvances.2019000173
236. Lawrence R, Watters M, Davies CR, Pantel K, Lu Y-J. Circulating tumour cells for early detection of clinically relevant cancer. *Nat Rev Clin Oncol*. 2023;20(7):487-500. https://doi.org/10.1038/s41571-023-00781-y
237. Peitzsch C, Tyutyunnykova A, Pantel K, Dubrovska A. Cancer stem cells: the root of tumor recurrence and metastases. *Semin Cancer Biol*. 2017;44:10-24. https://doi.org/10.1016/j.semcancer.2017.02.011
238. Pantel K, Hille C, Scher HI. Circulating tumor cells in prostate cancer: from discovery to clinical utility. *Clin Chem*. 2019;65(1):87-99. https://doi.org/10.1373/clinchem.2018.287102
239. Schniewind I, Hadiwikarta WW, Grajek J, et al. Cellular plasticity upon proton irradiation determines tumor cell radiosensitivity. *Cell Rep*. 2022;38(8):110422. https://doi.org/10.1016/j.celrep.2022.110422
240. Pushpakom S, Iorio F, Eyers PA, et al. Drug repurposing: progress, challenges and recommendations. *Nat Rev Drug Discov*. 2019;18(1):41-58. https://doi.org/10.1038/nrd.2018.168
241. Hirsch HA, Iliopoulos D, Tsichlis PN, Struhl K. Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. *Cancer Res*. 2009;69(19):7507-7511. https://doi.org/10.1158/0008-5472.CAN-09-2994
242. Wilson BE, Armstrong AJ, de Bono J, et al. Effects of metformin and statins on outcomes in men with castration-resistant metastatic prostate cancer: secondary analysis of COU-AA-301 and COU-AA-302. *Eur J Cancer*. 2022;170:296-304. https://doi.org/10.1016/j.ejca.2022.03.042
243. Micha JP, Rettenmaier MA, Bohart RD, Goldstein BH. A phase II, open-label, non-randomized, prospective study assessing paclitaxel, carboplatin and metformin in the treatment of advanced stage ovarian carcinoma. *J Gynecol Oncol*. 2023;34(2):e15. https://doi.org/10.3802/jgo.2023.34.e15
244. Soliman PT, Westin SN, Iglesias DA, et al. Everolimus, letrozole, and metformin in women with advanced or recurrent endometrioid endometrial cancer: a multi-center, single arm, phase II study. *Clin Cancer Res*. 2020;26(3):581-587. https://doi.org/10.1158/1078-0432.CCR-19-0471
245. Mukha A, Dubrovska A. Metabolic targeting of cancer stem cells. *Front Oncol*. 2020;10:537930. https://doi.org/10.3389/fonc.2020.537930
246. Huang ME, Ye YC, Chen SR, et al. Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood*. 1988;72(2):567-572.
247. Stahl M, Tallman MS. Acute promyelocytic leukemia (APL): remaining challenges towards a cure for all. *Leuk Lymphoma*. 2019;60(13):3107-3115. https://doi.org/10.1080/10428194.2019.1613540
248. Puschel J, Dubrovska A, Gorodetska I. The multifaceted role of aldehyde dehydrogenases in prostate cancer stem cells. *Cancers (Basel)*. 2021;13(18):4703. https://doi.org/10.3390/cancers13184703
249. Ahmed M, Jinks N, Babaei-Jadidi R, et al. Repurposing antibacterial AM404 as a potential anticancer drug for targeting colorectal cancer stem-like cells. *Cancers (Basel)*. 2019;12(1):106. https://doi.org/10.3390/cancers12010106
250. He X, Smith SE, Chen S, et al. Tumor-initiating stem cell shapes its microenvironment into an immunosuppressive barrier and protumorigenic niche. *Cell Rep*. 2021;36(10):109674. https://doi.org/10.1016/j.celrep.2021.109674
251. Marin-Acevedo JA, Kimbrough EO, Lou Y. Next generation of immune checkpoint inhibitors and beyond. *J Hematol Oncol*. 2021;14(1):45. https://doi.org/10.1186/s13045-021-01056-8
252. Chaurasiya S, Chen NG, Warner SG. Oncolytic virotherapy versus cancer stem cells: a review of approaches and mechanisms. *Cancers (Basel)*. 2018;10(4):124. https://doi.org/10.3390/cancers10040124
253. Griffin GK, Wu J, Iracheta-Vellve A, et al. Epigenetic silencing by SETDB1 suppresses tumour intrinsic immunogenicity. *Nature*. 2021;595(7866):309-314. https://doi.org/10.1038/s41586-021-03520-4
254. Zhang SM, Cai WL, Liu X, et al. KDM5B promotes immune evasion by recruiting SETDB1 to silence retroelements. *Nature*. 2021;598(7882):682-687. https://doi.org/10.1038/s41586-021-03994-2
255. Peitzsch C, Gorodetska I, Klusa D, et al. Metabolic regulation of prostate cancer heterogeneity and plasticity. *Semin*

Cancer Biol. 2022;82:94-119. https://doi.org/10.1016/j.semcancer.2020.12.002
256. Xia P, Dubrovska A. CD98 heavy chain as a prognostic biomarker and target for cancer treatment. *Front Oncol*. 2023;13:1251100. https://doi.org/10.3389/fonc.2023.1251100
257. Kahya U, Koseer AS, Dubrovska A. Amino acid transporters on the guard of cell genome and epigenome. *Cancers (Basel)*. 2021;13(1):125. https://doi.org/10.3390/cancers13010125
258. Chaudhary A, Raza SS, Haque R. Transcriptional factors targeting in cancer stem cells for tumor modulation. *Semin Cancer Biol*. 2023;88:123-137. https://doi.org/10.1016/j.semcancer.2022.12.010
259. Llombart V, Mansour MR. Therapeutic targeting of "undruggable" MYC. *EBioMedicine*. 2022;75:103756. https://doi.org/10.1016/j.ebiom.2021.103756
260. Allen TM, Brehm MA, Bridges S, et al. Humanized immune system mouse models: progress, challenges and opportunities. *Nat Immunol*. 2019;20(7):770-774. https://doi.org/10.1038/s41590-019-0416-z
261. Graham AL. Naturalizing mouse models for immunology. *Nat Immunol*. 2021;22(2):111-117. https://doi.org/10.1038/s41590-020-00857-2
262. Hammel JH, Cook SR, Belanger MC, Munson JM, Pompano RR. Modeling immunity in vitro: slices, chips, and engineered tissues. *Annu Rev Biomed Eng*. 2021;23:461-491. https://doi.org/10.1146/annurev-bioeng-082420-124920
263. Hofer M, Lutolf MP. Engineering organoids. *Nat Rev Mater*. 2021;6(5):402-420. https://doi.org/10.1038/s41578-021-00279-y