

# Drug-Tolerant Persister Cells in Cancer Therapy Resistance

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## ABSTRACT

One of the current stumbling blocks in our fight against cancer is the development of acquired resistance to therapy, which is attributable to approximately 90% of cancer-related deaths. Undercutting this process during treatment could significantly improve cancer management. In many cases, drug resistance is mediated by a drug-tolerant persister (DTP) cell subpopulation present in tumors, often referred to as persister cells. This review provides a summary of currently known persister cell subpopulations and approaches to target them. A specific DTP cell subpopulation with elevated levels of aldehyde dehydrogenase (ALDH) activity has stem cell-like characteristics and a high level of plasticity, enabling them to switch

rapidly between high and low ALDH activity. Further studies are required to fully elucidate the functions of ALDH-high DTP cells, how they withstand drug concentrations that kill other cells, and how they rapidly adapt under levels of high cellular stress and eventually lead to more aggressive, recurrent, and drug-resistant cancer. Furthermore, this review addresses the processes used by the ALDH-high persister cell subpopulation to enable cancer progression, the ALDH isoforms important in these processes, interactions of ALDH-high DTPs with the tumor microenvironment, and approaches to therapeutically modulate this subpopulation in order to more effectively manage cancer.

## Introduction

Cancer remains a serious public health issue affecting people across the globe. In the United States, it is the second leading cause of death, with the most common forms being lung, breast, prostate, and colorectal cancers (1). Despite investing billions of dollars in cancer research and the development of a vast arsenal of anticancer therapies, drug resistance remains the most serious factor hindering effective treatment. Unfortunately, small molecule-based therapies (2), and immunotherapies (3) that can be initially effective, eventually, fail due to the development of drug resistance (4). Although these therapeutic approaches frequently eliminate the majority of actively proliferating bulk tumor cells, “drug-tolerant persister” (DTP) cells can remain and play a key role in disease relapse mediating drug resistance (5), which then leads to approximately 90% of cancer-related deaths (6). DTPs have been increasingly identified as playing important roles in drug resistance through unique cellular mechanisms, which can involve inhibiting cell death signals (7, 8), altering cell metabolism (9), changing DNA damage repair (10), undergoing an epithelial-to-mesenchymal transition (9), increasing drug efflux from

cells (11), inactivating the drugs (12), and epigenetic cellular reprogramming (involving DNA methylation, acetylation, and methylation of histones; ref. 13).

### Prevalence of DTPs in cancers

DTPs have been reported in many cancers, including pancreatic ductal adenocarcinoma (14), acute myeloid leukemia (15), chronic myeloid leukemia (16), B-cell acute lymphoblastic leukemia (17), breast cancer (18), prostate cancer (19), colon cancer (20), basal cell carcinoma (20), and melanoma (20). The prevalence of DTPs in many cancers underscores their importance in the disease progression and for management during treatment. Generally, DTPs in cancers are thought to be rare, slowly replicating cells distinguished by unique transcriptional and epigenetic profiles (21). However, the proliferation rates of DTPs remain controversial, with some reports suggesting slow proliferation (7), whereas other studies suggest that they are rapidly proliferating or grow at rates similar to non-DTP cells (7, 9). As a group, DTPs generally seem to exhibit resistance to a wide variety of chemotherapeutics (22), radiotherapy (23), and targeted therapies (7).

### Clonal evolution, selection, and the quiescent state of DTPs

Because of the clonal-based selection that occurs during solid tumor development, DTPs evolve as clones in this environment of high cellular heterogeneity and undergo a unique selective process (7, 12, 24, 25). Generally, they evolve to have highly flexible energy consumption capabilities, which facilitate rapid adaptation to a changing microenvironment (12, 22). DTPs are thought to develop from cancer cells having stem cell-like characteristics, where they have retained some of these characteristics enabling enhanced survival (25). For example, lineage barcoding and single-cell transcriptomics have been used to demonstrate the development of drug resistance in stem-like glioblastoma cells treated with dasatinib (26). As a potential cancer stem cell population, they can also facilitate the development of cellular heterogeneity within tumors (24, 27). Within these heterogeneous cellular masses, DTPs can be found with distinct molecular fingerprints, which enable unique resistance capabilities against diverse therapeutics (24). For example, a hypoxic tumor microenvironment causes the upregulation of certain genes like hypoxia inducible factor 1

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subunit alpha (*HIF1 $\alpha$* ) and glucose transporter 1 (*GLUT1*), which are beneficial for DTP evolution (28). Remaining in a quiescent state is also a characteristic of DTPs, where the cells can remain dormant when exposed to drugs whose action requires proliferation or a high level of metabolic activity for efficacy (28). Many mechanisms that underlie the survival of DTP cells still remain to be identified as well as specific therapies to target them; however, stemness and cellular plasticity seem to be particularly important to their survival (12).

### Stemness of DTPs

Stemness is one of the key factors thought to be responsible for DTP drug resistance because stem cells tend to be slowly proliferating and resistant to therapy due to this characteristic (29). The presence of stemness-related gene expression also tends to be associated with a poor patient prognosis, and cells with combined properties of stemness (30), drug resistance (30), and dormancy (29) have been identified in multiple tumor types (31). A challenge to study the involvement of cells having stem cell-like properties in drug resistance is that the anticancer drug treatment is tested on the entire tumor population and not just those that have stem cell-like characteristics (32). Therefore, it has been difficult to quantify the exact involvement of stem cells in the development of drug resistance. This quandary makes the elucidation of DTP drug resistance mechanisms and DTP cells biomarker identification important for the clinical management of patients with cancer.

### Plasticity of DTPs

The stem cell-like characteristics of DTPs also provide them with a high level of plasticity, tumorigenicity, multipotency, and self-renewal potential (9, 33, 34). However, there is strong agreement that DTP cells can rapidly interconvert between phenotypes, which may be epigenetically mediated (35). Soluble factors and location can affect DTP cell self-renewal capacity; therefore, stemness is likely a transient state of enhanced plasticity, modulated by microenvironmental signals, including interactions with niche elements, tumor, nontumoral cells, soluble factors, and anticancer therapies (9).

### Phenotypic switching of DTPs

The rapid changes in phenotypes or phenotypic switching seem to be a property governed through cellular plasticity and can alter the expression of molecules, which are currently being used to define DTP cell subpopulations, which can be problematic when studying them. Tumor cell phenotypic state transitions following drug treatment appear to be very frequent and chaotic, thus generating high levels of heterogeneity, which constitutes the underlying foundation of drug resistance (9). Phenotypic switching also has led to challenges when tracking the fate of these cells (34). Therefore, many of the currently used markers for DTP cells can be transient, dynamic, and variable, rapidly changing in response to the extracellular environment (34). To circumvent this concern, the stem cell-like characteristics of DTPs have been studied using genetic barcoding in order to track the cellular fate and the downstream progeny of DTP cells (36). To accomplish successful tracking, lentiviral infection systems tag stem cells in order to track cell hierarchies and the evolution of DTPs (37). This approach has been useful during serial xenografting to follow subclones to highlight the functional robustness of cancer cell hierarchies and DTPs (38–40).

### Stromal-cell interactions with DTPs

Targeting the complex cell–cell interactions of DTPs with other stromal cells might also be required to fully understand their biology

and to successfully eliminate these cells. For example, the tumor microenvironment (TME) can be a crucial determinant of DTP cell survival and evolution (9, 41). Soluble molecules released by various cells present in the tumor stroma can initiate persister cell survival programs (25). Factors produced by endothelial cells like nitric oxide activates the Notch pathway in glioblastoma, cancer-associated fibroblasts produce hepatocyte growth factor, osteopontin, and stromal-derived factor 1 $\alpha$ , which activates the WNT pathway in colorectal cancer (42, 43); and tumor-associated macrophages in breast (44), as well as brain cancer (45), can aid persister cell survival (45, 46).

Recently, exosomes and macrovesicles produced by niche cells in the TME have been shown to aid drug resistance mediated by DTPs (47). For example, microvesicles produced by breast cancer-associated fibroblasts can transfer miR-221 to cancer cells, thus increasing the drug-resistant CD133<sup>hi</sup> stem cell population (48). Therefore, targeting the components of the TME might be a useful therapeutic strategy to control DTPs. Targeting an acidic TME by altering the pH (49), inflammatory immune signaling (50), the abnormal extracellular matrix (51), the cancer-associated fibroblasts (52) or endothelial cells (53) are strategies being evaluated, which have the potential to elucidate the complex process that enables the DTPs to develop resistance to drugs (51).

### DTPs and immune system evasion

One of the intriguing features of DTPs is immune cell evasion preventing their elimination. Their involvement in adaptive immunotherapy resistance also remains elusive. A recent research report in a mouse organotypic spheroid *ex vivo* model demonstrated the formation of a distinct subpopulation of cells resistant to immune-checkpoint inhibitor anti-PD-1 antibody therapy (54). These cells displayed stem cell antigen Sca1/Snai1 and displayed resistance to CD8<sup>+</sup> T-cell-mediated death. Surprisingly, these DTPs relied on anti-apoptotic proteins called BIRC2/3, and inhibiting them in combination with anti-PD-1 treatment improved tumor cell death *in vivo* (54). Although PD-1/PD-L1 immunotherapy in patients with cancer can be successful, many patients relapse owing to adaptive resistance. There is a pressing need to learn more about how DTPs elude immune responses, which is starting to be dissected (55). For example, Zhang and colleagues have reported that ALDH2 is involved in mediating alcohol-induced colorectal cancer immune escape by preventing PD-L1 from ubiquitin-dependent degradation (55). Moreover, combination therapy of PD-1 blockade along with inhibition of ALDH2 led to an increase in tumor-infiltrating lymphocytes infiltration, thereby preventing immune cell evasion (55). This approach could be further developed as a novel strategy to target ALDH<sup>+</sup> DTPs (56).

### Therapeutically targeting DTPs

The stem cell-like plasticity of DTPs have been explored therapeutically in order to develop better approaches to overcome drug resistance. However, this approach has been complex because drug resistance mediated by DTPs plasticity does not appear to be caused by a single event, but rather a combination of changes; thus, a multimodal approach will likely be needed (22). For example, inhibitors targeting epigenetic changes like histone deacetylases (57), lysine demethylases (KDM; ref. 58), antiestrogen therapy to suppress proliferation signals (59), reactivation of dormant cells through IFN $\alpha$  (60), and granulocyte colony-stimulating factor (60) could be used alone or in combination. Moreover, the elimination of quiescent cells by using mithramycin to activate ferroptosis can be useful in targeting stemness in DTPs (61). It is generally thought that a successful approach will have to combine agents that modulate the epigenetic, transcriptional,

and translational processes occurring in these cells (22). In summary, clinical targeting of persister cells will remain a major challenge in the future, which will require unraveling the mechanisms leading to their survival using preclinical cell and tumor-based modeling.

## Current Types of DTP Cells

Several types of DTP cells have been characterized but many types remain to be identified. Current persister cell subpopulations can be identified through expression of ABCB5 (62), CD133 (63), CD271 (34), JARID1B (34), or aldehyde dehydrogenase (ALDH; Fig. 1; ref. 64). However, a complication is that some DTP subpopulations have overlapping markers such as those simultaneously having ALDH activity and being CD44, and CD133 positivity (65). DTPs expressing multiple markers remain an important area requiring further research.

### Persister cells expressing ABCB5

The *ABCB5* gene belongs to the ABC drug transporter family of proteins that actively expels drugs via efflux mechanisms to facilitate chemotherapeutic resistance in cancers of the colon, lung, and skin (62, 65). ABCB5 has been shown to promote therapeutic resistance to 5-fluorouracil in human colon cancer cells and ABCB5-mediated chemoresistance is positively regulated by *c-MYC* (66). Molecular silencing of *ABCB5* in COLO-320 cells led to a decrease in the survival rate following treatment with 5-fluorouracil. Moreover, in contrast to the parental Caco-2 cells, 5-fluorouracil-resistant Caco-2 cells expressed high levels of ABCB5 and had a higher survival rate after treatment. These results suggest that targeting ABCB5 would be a potential way to overcome 5-fluorouracil resistance in colon cancer (66). Melanoma subpopulations with persister cell characteristics have also been found to have strong ABCB5 expression and tumor-initiating traits (62). This subpopulation was highly chemoresistant and targeting it with an anti-ABCB5 mAb inhibited tumor development in mouse models. ABCB5 expression is elevated in metastatic melanoma in comparison to melanocytic nevi or primary tissues. Nodal metastatic lesions showed higher ABCB5 expression levels than cells from visceral metastasis. Furthermore, using *in vivo* lineage tracing experiments, ABCB5<sup>+</sup> cells gave rise to both ABCB5<sup>+</sup> and ABCB5<sup>-</sup> cells (62). These ABCB5<sup>+</sup> cells had a distinct ability for self-renewal as well as differentiation in comparison to ABCB5<sup>-</sup> cells, which exclusively gave rise to ABCB5<sup>-</sup> cells at lower rates. Finally, the authors also demonstrated that ABCB5<sup>+</sup> cells were more tumorigenic than ABCB5<sup>-</sup> melanoma cells in SCID mice (62). Targeting these ABCB5<sup>+</sup> persister cells to overcome drug resistance was tested using etoposide and carboplatin in merkel cell carcinoma (MCC) cell lines MKL-1 and WaGa (67). MCC cell lines were subjected to increasing doses of etoposide or carboplatin in the presence and absence of anti-ABCB5 blocking antibodies, showing that blocking ABCB5 antibody led to increased sensitivity to these agents (67). Thus, targeting ABCB5 represents one strategy to reduce the impact of persister cells in cancer recurrence.

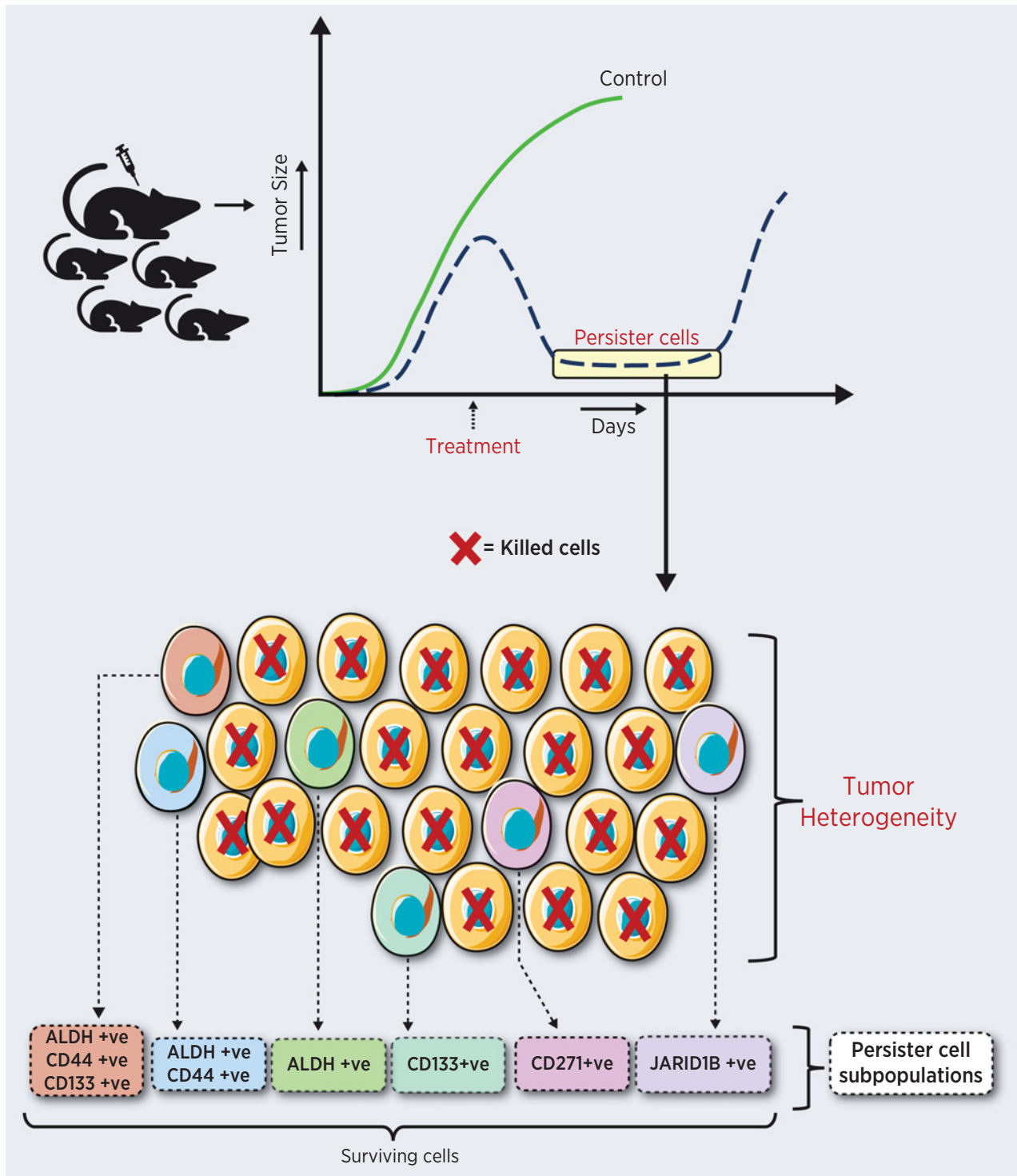
### Persister cells expressing CD133

The membrane glycoprotein CD133 is a well-established stem cell marker, which is present in many tissues and linked to chemoresistance (68). Expression analysis using qPCR and immunoblotting showed high CD133 expression in cisplatin resistance of KATO-III cells (Cis-KATO-III) compared with parental KATO-III cells, indicating that CD133 regulates cisplatin resistance in KATO-III cells. Furthermore, proliferation and apoptosis assays on flow-sorted CD133<sup>+</sup> Cis-KATO-III cells that were transfected with Sh-CD133

and pc-CD133 showed that CD133 inhibition decreased cell viability and increased cell death upon cisplatin treatment (68). Thus, CD133 is a potential target to overcome cisplatin resistance in gastric cancer. Similarly, patient-derived CD133<sup>+</sup> and CD133<sup>-</sup> melanoma cells were tested for resistance to MAPK inhibitors (63). Increasing doses of dabrafenib and/or trametinib were given to human melanoma cells, either before or after they were separated into CD133<sup>+</sup> and CD133<sup>-</sup> subpopulations. After high-dose treatment, the proportion of CD133<sup>+</sup> cells increased in parental CD133-mixed lines (both CD133<sup>+</sup> and CD133<sup>-</sup>). In addition, substantially higher IC<sub>50</sub>s for FACS sorted CD133<sup>+</sup> cells were observed with combination and single MAPK signaling pathway inhibitors. Sensitivity to dabrafenib and trametinib was enhanced after siRNA-based knockdown of CD133. Furthermore, microarray results of CD133<sup>+</sup> cells demonstrated that several ABC transporter genes were upregulated and knockdown of CD133 via siRNA decreased expression of *ABCG2*. Also, ABCG2 inhibition led to the sensitization of CD133<sup>+</sup> cells to dabrafenib and trametinib (63). The ABCB5 drug efflux transporter was found to be specifically expressed on CD133<sup>+</sup> cell phenotype-expressing subpopulations within clinical human malignant melanomas (69). Targeting CD133 positive persister cells using T-cell therapy (70), natural killer cells (71), aptamers (72, 73), immunotoxins (74, 75), and antibody-conjugated nanoparticles (76) all led to some level of tumor inhibitory efficacy, suggesting potential for preventing cancer recurrence.

### Persister cells expressing CD271

The cell surface molecule CD271 (low-affinity nerve growth factor receptor; p75<sup>NTR</sup>) is expressed in a DTP subpopulation in melanoma, esophageal, and hypopharyngeal carcinomas (77, 78), which have stem cell-like properties (22). CD271 is a neurotrophin receptor and a member of the tumor necrosis factor receptor superfamily, having roles in cell survival, differentiation, and migration (79). Increased expression of CD271 is linked to a poor clinical outcome in oral squamous cell carcinoma (80), hypopharyngeal (78), and esophageal cancer (81, 82). CD271 was found to be differentially expressed in osteosarcomas. CD271<sup>+</sup> osteosarcoma cells displayed stem cell-like properties including drug resistance, differentiation, tumorigenicity, and self-renewal capacity (83). When CD271<sup>+</sup> and CD271<sup>-</sup> sorted cells from osteosarcoma cells (SAOS2, U2OS, and MNNG/HOS) were treated with *cis*-diamminedichloroplatinum (DDP), CD271<sup>+</sup> cells exhibited enhanced resistance to DDP. Also, ABCG2 (involved in drug resistance), Bcl-2 (anti-apoptotic factor), and DNA-PKcs (involved in DNA repair and promoting survival) were highly expressed in CD271<sup>+</sup> cells in comparison to CD271<sup>-</sup> cells. Furthermore, subcutaneous injection of CD271<sup>+</sup> cells from MNNG/HOS cells formed tumors more frequently than negative counterparts, suggesting that CD271<sup>+</sup> cells are more oncogenic. These findings suggest that CD271 provides resistance against DDP in osteosarcoma (83). In melanoma (33), squamous cell carcinoma (84), lung cancer (85), and breast cancer (86), CD271 expression was transient and associated with a slow-cycling population (34). Multiple stress factors including glucose deprivation, hypoxia, and drug exposure increased CD271 expression in melanoma cells (33, 85). The expression of CD271 was also found in DTP cells with high ALDH activity in breast cancer, non-small cell lung cancer, and esophageal squamous cell carcinoma (57, 77, 87, 88). CD271-mediated drug resistance by suppression of p53 activity (89) and protection from reactive oxygen species (ROS; ref. 90). In melanoma, CD271 expression is associated with resistance to MAPK pathway inhibitors (33, 34). CD271 can be targeted for therapeutic intervention, but direct targeting is complicated because of



**Figure 1.**

Emergence of persister cells. Mice injected with tumors and treated with and without drugs show different tumor kinetics. The green line shows the untreated control group with a high tumor burden. A rise in tumor size and shrinkage of tumor upon initialization of treatment leading to the emergence of drug-resistant persister cells is depicted by the blue line. In a tumor with a heterogeneous cell population, treatment with the drug would lead to the killing of most cells, but the drug-resistant persister cells expressing ABCB5, CD133, CD271, JARID1B, ALDH, ALDH<sup>+</sup>/CD44<sup>+</sup>, or ALDH<sup>+</sup>/CD133<sup>+</sup>/CD44<sup>+</sup> could survive, leading to relapse and recurrent resistant disease development.

the multiple critical roles of CD271 in the central nervous system (91). However, an anti-CD271 antibody-based approach inhibited melanoma metastasis (92, 93), and this observation can be examined in other cancers.

#### Persister cells expressing JARID1B/KDM5B

JARID1B/KDM5B is a histone 3K4 demethylase that regulates gene transcription (94). It is involved in the pathobiology of melanoma, mesothelioma, cholangiocarcinoma, endometrial carcinoma, lung, breast, pancreatic, ovarian, and cervical cancer (95). A slow-cycling subpopulation of 1% to 5% of melanoma cells having high JARID1B expression and stem cell-like properties gave rise to rapidly proliferating progeny (96). Stable knockdown of JARID-1B in long-term cultures and serial xenotransplantation experiments led to a cessation of melanoma growth (96). Furthermore, JARID1B was found to be not necessary for melanoma initiation, although it was needed for growth in culture and tumor development leading to metastatic disease progression (96, 97). The demethylase activity of KDM5 was essential for the survival of DTPs treated with CPI-455, a pan-KDM5 inhibitor, which led to an increase in global H3K4 trimethylation (H3K4me<sub>3</sub>; ref. 98). Pretreatment of cancer cells with the KDM5-specific inhibitor eliminated a subpopulation of cancer cells that played a role in cancer progression (98). A complication of targeting JARID1B is that there is functional redundancy across KDM5 family members, which makes it critical to know the unique functional role(s) of each family member in order to have agents targeting the specific ones active in particular DTP subpopulations (99). Compounds 54j, 54k, GSK467, and GSK-J1 have all been developed to target JARID-1B with low IC<sub>50</sub> values, which could suggest toxicity (95). This will be an important area of research in the future to target the JARID-1B DTP subpopulation in cancers.

#### Persister cells expressing ALDHs

The ALDHs are a group of 19 oxidoreductive enzymes that are important for cellular survival by detoxification of aldehydes, converting them into carboxylic acids (100, 101). ALDHs are required for the proper development, differentiation, and maintenance of stem cells (101, 102). They protect against ROS (87) and are involved in the biosynthesis of retinoic acid (RA; ref. 101). ALDH activity is high in cancers of the liver (103), prostate (104), colon (105), brain (106), lung (107), breast (108), pancreas (109), stomach (110), ovary (111), esophagus (112), neck (113), and in melanoma (9, 64). High levels of ALDH activity is generally associated with poor clinical prognosis for patients (102).

#### ALDH as a Central Marker for DTPs

ALDH could be a central marker of DTPs for research and clinical applications. The ALDH<sup>+</sup> cells have a fundamental role in various metabolic and cellular processes, leading to drug resistance (114). ALDH-dependent drug resistance was initially discovered in hematopoietic progenitors and cell lines where overexpression of ALDH1A1/3A1 increased resistance to cyclophosphamide active metabolites (115). Furthermore, high expression of ALDH contributes to resistance against various cytotoxic drugs, including cisplatin, dacarbazine (116), and cytarabine (Ara-C; ref. 117). In mantle cell lymphoma (MCL) patients, rare slow cycling, drug-resistant, highly clonogenic ALDH-positive cells were found to be the cause of relapse (118). ALDH<sup>hi</sup> CD44<sup>+</sup> breast cancer cells have been found to play a crucial role in mediating breast cancer metastasis (119). ALDH<sup>hi</sup> CD44<sup>+</sup> breast cancer cells are also resistant to taxanes and anthracyclines as well as radiation. ALDH<sup>hi</sup> CD44<sup>+</sup> cells and not

ALDH<sup>low</sup> CD44<sup>-</sup> cells expressed significantly higher amounts of proteins that are involved in therapy resistance, such as CHK1, GST $\pi$ , and p-glycoprotein (120). Treatment of these cells with ALDH inhibitors diethylaminobenzaldehyde (DEAB) or all-trans RA (ATRA) made them more vulnerable to killing using standard cancer therapies, such as paclitaxel and doxorubicin (120).

There is strong clinical support suggesting that ALDH expression is a potentially useful marker of drug-resistant disease recurrence. A tissue microarray using specimens from 68 malignant melanoma patients with comprehensive pathologic and clinical follow-up suggested that higher levels of ALDH1 activity were linked to improved outcomes (121). However, the authors did not investigate the potential involvement of other ALDH isoforms, which could complicate their interpretation. These observations were similar to those reported in lung cancer (122) but are contradictory for leukemia (123) and breast cancer (124, 125), where higher levels of ALDH1 were associated with a poor prognosis. These contradictory observations suggest that ALDH1 is a ubiquitous enzyme playing different functions in various organ systems, which implies that the various isoforms could play distinct roles in different cancer types (121). The size of the ALDH gene family and the heterogeneity of isoform activity in different tumor types will make it difficult to target ALDH DTPs using the same approach for all cancers unless a broad-spectrum inhibitor can be identified for clinical use.

#### Phenotypic Switching between ALDH<sup>+</sup> and ALDH<sup>-</sup> Cells

There is rapid interconversion of cells with high ALDH activity into those with low activity and vice versa. The role this process plays in cancer progression remains to be elucidated. Melanoma cell lines, xenografts, and patient biopsies have been identified, having cell subpopulations with high levels of ALDH activity (116). The ALDH<sup>+</sup> cells have been found to give rise to ALDH<sup>-ve</sup> cells, but the reverse conversion was less common, showing that ALDH<sup>+</sup> cells had a greater ability to restore ALDH heterogeneity in tumors (116). Interestingly, the authors did not find any specific differences between ALDH<sup>+</sup> and ALDH<sup>-ve</sup> cells with respect to *in vitro* colony formation and *in vivo* tumor initiation (116). Also, the anti-melanoma drugs lexatimumab and dacarbazine had equal potency for killing both subpopulations (116). A small subpopulation of ALDH expressing DTPs have been found to cause melanoma initiation when utilizing limiting dilution studies in NOD/SCID mice, which led Boonyaratanakornkit and colleagues to conclude that 0.005% of ALDH<sup>+</sup> cells were melanoma-initiating cells (64). However, 25% of ALDH<sup>+</sup> cells derived from human metastatic melanoma were shown to be melanoma-initiating cells in the NOD/SCID12rg(NSG) mice (64). These cells had no distinct phenotype and were capable of clonogenic proliferation and self-renewal (64).

#### Regulation of DTP Stemness by ALDHs

Pathways have been identified that link ALDH activity to stem cell-like characteristics in cancer cells. For example, the Hedgehog (Hh) signaling pathway, important for vertebrate development, tissue regeneration, and stem cell development plays an important role in ALDH DTPs (126, 127). Inhibition of the Hh/GLI signaling cascade reduced self-renewing capacity and tumorigenicity of ALDH<sup>high</sup> cells. Furthermore, the Hh/GLI cascade was able to modulate the SRY-box transcription factor 2 (SOX2) directly, thereby assisting ALDH<sup>+</sup> cells in forming melanomas (128). Cells forming melanomas have been

found to have high expression levels of ALDH1A1 and ALDH1A3. In NOD/SCID as well as NOD SCID gamma (NSG) mice, ALDH<sup>+</sup> melanoma cells were more tumorigenic than ALDH<sup>-</sup> cells (129). In addition, they demonstrated that ALDH enzymes are involved in the regulation of melanoma-initiating cells. Molecular silencing of ALDH1A using RNAi reduced cell viability, apoptosis, cell-cycle arrest, and decreased tumorigenesis by these cells (129).

Many of the stem cell markers found in ALDH<sup>+</sup> cells from patient-derived tumor tissues were RA-directed target genes and RA response elements (129). Thus, targeting RA signaling might inhibit the stem cell properties present in ALDH<sup>+</sup> cells to inhibit the melanoma-initiating properties (129). Tumor cells also altered ALDH activity levels in endothelial cells. Ohmura-Kakutani and colleagues in 2014 characterized stem-like tumor endothelial cells (TEC; ref. 130). TECs from melanoma xenografted nude mice showed higher levels of ALDH but not those from normal endothelial cells derived from normal skin (NEC). Moreover, ALDH<sup>high</sup> TECs were able to form more tubes and sustained them for longer periods in a matrigel-based experiment than ALDH<sup>low</sup> TECs. Also, ALDH<sup>high</sup> TECs exhibited higher levels of VEGFR2 than ALDH<sup>low</sup> TECs. In addition, ALDH was found in the tumor blood vessels of melanoma and oral carcinoma mice models but not in normal blood vessels. According to these studies, ALDH<sup>high</sup> TECs have an angiogenic phenotype and may play key functions in tumor angiogenesis (130).

## Multiple ALDH Isoforms Are Active in DTPs

Total ALDH activity in a tumor is the result of the activity of multiple isoforms, with ALDH 1, 2, and 3 appearing to be the most active in melanoma (5). Melanoma cancer stem cells have also been reported to have high ALDH activity, predominantly expressing ALDH1A and ALDH1A3. Knockdown of ALDH1A using RNAi technology led to cell-cycle arrest, apoptosis, decreased cell culture viability, reduced tumorigenesis, and sensitized melanoma cells to drug-induced cell death (129). ALDH1A1 expression was linked to chemoresistance of cultured and xenografted ovarian, stomach, and pancreatic cancers (102). Studies on ALDH1A1 expression have been linked with poorer progression-free survival and overall survival in patients with clear cell renal carcinoma and breast cancer treated with neoadjuvant therapy (131–133). Given the fundamental importance of ALDHs in a variety of normal cellular processes (134), as well as their role in malignancies (114), and persist cell biology (9), targeting ALDHs therapeutically would be complex. It might require a better understanding of the tissue-specific expression and unique role of the different isoforms in specific tumor types.

## Targeting ALDH Expressing DTPs with Broad-Spectrum ALDH Inhibitors

In a tumor, only a small subpopulation is ALDH expressing DTPs. Therefore, targeting these cells specifically with drugs would leave the main population unaffected, necessitating the identification of approaches targeting both populations. These unique strategies might involve combination approaches (Fig. 2) or treatment with an ALDH inhibitor only after killing the non-ALDH expressing cells. The ALDH expressing cells could be targeted using isoform or broad-spectrum ALDH inhibitors. Isoform-specific ALDH inhibitors have been developed but seem to be ineffective for treating cancers since inhibiting one, appears to boost the activity of another (100). This can be

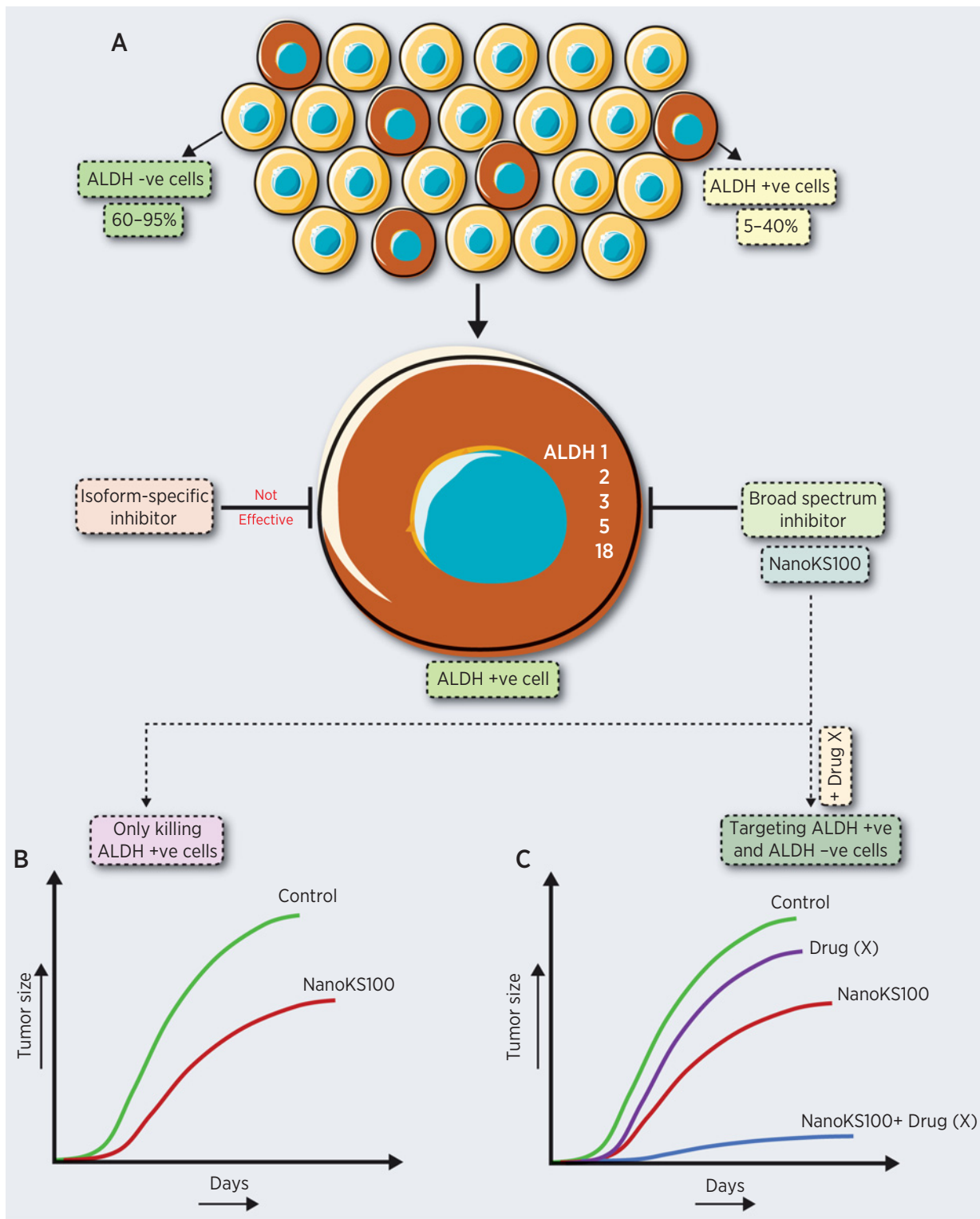
mitigated by using broad-spectrum ALDH inhibitors, but these seem to be toxic (5), which until recently has been a major problem (5). Many broad-spectrum drugs have been developed to target ALDH family members, for example, a pan-ALDH1A family inhibitor 673A has been developed and found to trigger necroptosis in ovarian stem cells, eradicating tumors in several ovarian cancer models (135). In another study, individuals with advanced lung cancer who received a triple combination therapy of cisplatin, vinorelbine, and the ALDH inhibitor disulfiram (DSF) had approximately 10% disease-free survival rate after 3 years of treatment, compared with patients who received just cisplatin and vinorelbine (136). Unfortunately, DSF has been shown to have toxicity, short *in vivo* half-life, and multiple off-target effects (135). So, although broad-spectrum ALDH inhibitors can be effective, toxicity is a major factor limiting use in cancer (135). Another approach that can reduce toxicity-related concerns involves using a prodrug that is converted to an active cancer-killing agent in the presence of ALDH activity (137). For example, the antibiotic prodrug, 5-nitrofurans can be bioactivated by ALDH-1A1/1A3 enzymes that are expressed in melanomas (137). High ALDH1 expressing cells exhibited increased sensitivity to nifuroxazide, regardless of melanoma genotype (137).

Another approach to limit the toxicity-related concerns associated with broad-spectrum ALDH inhibitors involves drug formulation. Recent studies suggest that loading broad-spectrum ALDH inhibitors into nanoliposomal formulations can reduce toxicity to levels useful for cancer treatment (138). The most promising current agent is nanoKS100 (Fig. 2), developed on a structure-activity-based chemical synthesis approach. It is a multi-ALDH isoform inhibitor of ALDH1A1, ALDH2A1, and ALDH3A1 (5). The compound was very toxic in animals until it was loaded into a nanoliposomal formulation, which eliminated the toxicity but remarkably retained the effective antitumor efficacy (5). The potential use of nanoKS100 alone or in combination with other agents is shown in Fig. 2, where it is predicted to synergize with current approaches to prevent drug resistance mediated by the DTP ALDH expressing subpopulation of cells. Dimethyl ampal thiolester (DIMATE; ref. 139) is a similar agent to nanoKS100 but inhibits the ALDH1 and 2 isoforms only. DIMATE is being evaluated in clinical trials in Europe but it appears that toxicity is limiting its development and now nanoliposomal formulations are being tested to circumvent this concern (140). It is unclear as to why nanoliposomal formulations are effective at reducing the toxicity of broad-spectrum ALDH inhibitors but they do generally have a high biocompatibility index, greater stability, better solubility, extended-release, and reduced toxicity compared with native drugs, which could be leading to reduced toxicity of nanoKS100 (138).

## Identifying Tumor Types and Therapies Enriching ALDH<sup>+</sup> Cell Subpopulations for Effective ALDH Inhibitor Treatment

For treating ALDH DTPs in cancers, a comprehensive understanding of tumor types and therapies that mostly enrich ALDH<sup>+</sup> DTP state would be critical for success. A recent study by Kawakami and colleagues demonstrated this concept using single-cell analysis on the basis of stem cell lineage-related and gastric cell lineage-related gene expression in patient-derived stomach cancer cell models. The analyses showed that 5-fluorouracil causes a dynamic shift in cell heterogeneity. Post 5-fluorouracil treatment stem cell-related genes were enriched in residual cancer cells. Upon subsequent analysis of these cells, ALDH1A3 emerged as a prominent stem cell marker in these





**Figure 2.**

Tumor heterogeneity and targeting ALDH cells. **A**, Tumors are highly heterogeneous and both ALDH<sup>+</sup> cells and ALDH<sup>-</sup> cells can be found. The percentage of ALDH<sup>+</sup> cells can be 60% to 95%, whereas ALDH<sup>-</sup> cells can be 5% to 40%. Inhibition of ALDH<sup>+</sup> cells with isoform-specific inhibitors is not effective as it can lead to an increase in the activity of other forms. In contrast, treatment with a broad-spectrum inhibitor like NanoKS100 would kill the ALDH<sup>+</sup> cells, leading to tumor regression as seen in **B**. Combination therapy [NanoKS100 along with the standard-of-care drug (X)] might lead to more effective tumor regression than either approach alone (NanoKS100 or drug alone) as seen in **C**.

DTPs. Among the members of the ALDH family, ALDH1A3 was preferentially overexpressed in 5-FU or SN38 (DNA topoisomerase I inhibitor) treated cells. RNA interference-based knockdown of ALDH1A3 decreased the number of persister cells and tumor burden in a mouse xenograft model (141). ALDH1A3 expression has also been reported in cells treated with paclitaxel and cisplatin (141). Thus, particular therapies seem to enrich the ALDH<sup>+</sup> DTP state but the particular isoform varies, which could complicate effective ALDH inhibitor treatment without a corresponding diagnostic analysis of isoform activity. For example, ALDH5A1 was expressed in breast cancer (142); ALDH3A1 in lung cancer (143) and prostate cancer (144); and ALDH1A3 in melanoma, glioma, and breast cancer (141). It remains unclear whether a different therapy in a particular cancer type would lead to increased activity of the same ALDH isoform.

The therapeutic window for eliminating ALDH DTPs might also vary and not require prolonged treatment but need enrichment of the ALDH<sup>+</sup> subpopulation prior to targeting them. Targeting the ALDH subpopulations of 10% to 20% in melanoma cell lines led to a 60% reduction in tumor volume (5). Therefore, it is possible that enriching the ALDH<sup>+</sup> population using (V600E) BRAF therapy in melanoma could be used to increase the ALDH DTP subpopulation. Targeting the expanded ALDH<sup>+</sup> subpopulation with a broad-spectrum ALDH inhibitor, such as nanoKS100, might then achieve an even more substantial and durable response. This possibility remains to be examined.

## Complications Due to Overlap of ALDH Positivity with Other DTP Markers

Multiple studies suggest an overlap of ALDH positivity with other DTP markers. For example, pancreatic cancer stem cells (PCSC) express markers like CD44<sup>+</sup>, CD133<sup>+</sup> along with high ALDH activity (145, 146). A study by Ma and colleagues, in 2008 reported that in hepatocellular carcinoma (HCC), ALDH1A1 is preferentially expressed in the CD133<sup>+</sup> subfraction. Furthermore, in liver cancer cell lines, expression analysis of various ALDH isoforms and their enzymatic activity revealed ALDH activity to be positively correlated with the expression of CD133 (147). However, Tanaka and colleagues, in 2015 reported that ALDH1A1 was not co-expressed with DTP markers such as CD133 (148) in HCC. In another study, where the expression of CD133 and ALDH enzymatic activity were analyzed in 108 human ovarian cancer samples, 13% of the individuals tested were fully negative for ALDH activity and 26% were completely negative for CD133 staining. Both markers were shown to be variable in expression within the samples, and there was no statistically significant link between ALDH enzymatic activity and CD133 expression when both were evaluated in the same tumor sample (149). In 2015 Kuo and colleagues,

performed a flow-cytometric-based correlative analysis of JARID1B expression with known stem cell markers like CD133, and ALDH activity in neuroblastoma cells. They reported that JARID1B knockdown negatively modulated stemness markers and significantly decreased the proportions of ALDH<sup>+</sup> and CD133<sup>+</sup> in comparison to wild-type neuroblastoma cells (150). Thus, in cases where there is an overlap of ALDH<sup>+</sup> DTPs with other DTP markers, a combination approach targeting ALDH and the other DTP markers might be more effective. However, in tumors where ALDH<sup>+</sup> DTPs comprise the majority of cells, ALDH inhibitors could be quite effective.

## Conclusion

Many important questions remain regarding ALDH-positive DTP cells and their role in drug resistance. Questions that remain are related to the optimal experimental conditions in cell culture and in animals that could be used to mimic their behavior in patients developing drug resistance. Another question involves the heterogeneity of ALDH DTP cells and whether slow-cycling, increased metabolism, or plasticity could be used as a marker for those cells leading to drug resistance. It also remains unclear whether ALDH activity is the best marker or whether a pathway or process regulated by ALDH might be more easily studied in the clinic. Drugs specifically targeting the ALDH DTP cells also remain a challenge. Isoform-specific inhibitors do not seem useful for cancer treatment, and those targeting multiple isoforms can be toxic. A recent advance might have solved this concern by using nanoparticle formulations. However, it would be important to determine the mechanism through which the nanoliposomal formulation kills cancer tumor cells without having significant toxicity. Another issue would be the involvement of DTP cells with high ALDH activity in double resistant (BRAF/MEKi) populations. Finally, the effects of ALDH inhibitors on the immune system remain to be studied, as well as, whether ALDH inhibitors can be combined with immune therapy to more effectively eliminate the DTP cells.

## Authors' Disclosures

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## References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin* 2021;71:7-33.
2. Bozic I, Allen B, Nowak MA. Dynamics of targeted cancer therapy. *Trends Mol Med* 2012;18:311-6.
3. Nakhoda SK, Olszanski AJ. Addressing recent failures in immuno-oncology trials to guide novel immunotherapeutic treatment strategies. *Pharmaceut Med* 2020;34:83-91.
4. Bai R, Chen N, Li L, Du N, Bai L, Lv Z, et al. Mechanisms of cancer resistance to immunotherapy. *Front Oncol* 2020;10:1-12.
5. Dinavahi SS, Gowda R, Gowda K, Bazewicz CG, Chirasani VR, Battu MB, et al. Development of a novel multi-isoform ALDH inhibitor effective as an anti-melanoma agent. *Mol Cancer Ther* 2020;19:447-59.
6. Bukowski K, Kciuk M, Kontek R. Mechanisms of multidrug resistance in cancer chemotherapy. *Int J Mol Sci* 2020;21.
7. Cabanos HF, Hata AN. Emerging insights into targeted therapy-tolerant persister cells in cancer. *Cancers (Basel)* 2021;13:1-26.



8. Radhakrishna VK, Siravegna G, Hu H, Lockerman E, Kalsy A, Lee D, et al. Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. *Nat Med* 2016;22:262–9.
9. De Conti G, Dias MH, Bernards R. Fighting drug resistance through the targeting of drug-tolerant persister cells. *Cancers (Basel)* 2021;13:1–15.
10. Fahrer J. Switching off DNA repair—how colorectal cancer evades targeted therapies through adaptive mutability. *Signal Transduct Target Ther* 2020;5:1–2.
11. Wang X, Zhang H, Chen X. Drug resistance and combating drug resistance in cancer. *Cancer Drug Resist* 2019;2:141–60.
12. Karki P, Angardi V, Mier JC, Orman MA. A transient metabolic state in melanoma persister cells mediated by chemotherapeutic treatments. *bioRxiv* 2021;432154.
13. Ellis L, Atadja PW, Johnstone RW. Epigenetics in cancer: Targeting chromatin modifications. *Mol Cancer Ther* 2009;8:1409–20.
14. Viale A, Pettazzoni P, Lyssiotis CA, Ying H, Sánchez N, Marchesini M, et al. Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. *Nature* 2014;514:628–32.
15. Farge T, Saland E, de Toni F, Aroua N, Hosseini M, Perry R, et al. Chemotherapy-resistant human acute myeloid leukemia cells are not enriched for leukemic stem cells but require oxidative metabolism. *Cancer Discov* 2017;7:716–35.
16. Kuntz EM, Baquero P, Michie AM, Dunn K, Tardito S, Holyoake TL, et al. Targeting mitochondrial oxidative phosphorylation eradicates therapy-resistant chronic myeloid leukemia stem cells. *Nat Med* 2017;23:1234–40.
17. Shojaei S, Caesar R, Buchner M, Park E, Swaminathan S, Hurtz C, et al. Erk negative feedback control enables Pre-B cell transformation and represents a therapeutic target in acute lymphoblastic leukemia. *Cancer Cell* 2015;28:114–28.
18. Risom T, Langer EM, Chapman MP, Rantala J, Fields AJ, Boniface C, et al. Differentiation-state plasticity is a targetable resistance mechanism in basal-like breast cancer. *Nat Commun* 2018;9:3815.
19. Kim JK, Jung Y, Wang J, Joseph J, Mishra A, Hill EE, et al. TBK1 regulates prostate cancer dormancy through mTOR inhibition. *Neoplasia (United States)* 2013;15:1064–74.
20. Mullard A. Stemming the tide of drug resistance in cancer. *Nat Rev Drug Discov* 2020;19:221–3.
21. Qin S, Jiang J, Lu Y, Nice EC, Huang C, Zhang J, et al. Emerging role of tumor cell plasticity in modifying therapeutic response. Vol. 5, *Signal transduction and targeted therapy*; 2020.
22. De Angelis ML, Francescangeli F, La Torre Z, Zeuner A. Stem cell plasticity and dormancy in the development of cancer therapy resistance. *Front Oncol* 2019;9:1–14.
23. Zhao Y, Song Y, Zhao R, Zhao M, Huang Q. Gene panel of persister cells as a prognostic indicator for tumor repopulation after radiation. *Front Oncol* 2020;10:1–12.
24. Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol* 2018;15:81–94.
25. Vallette FM, Olivier C, Lézot F, Oliver L, Cochonneau D, Lalier L, et al. Dormant, quiescent, tolerant and persister cells: four synonyms for the same target in cancer. *Biochem Pharmacol* 2019;162:169–76.
26. Eyler CE, Matsunaga H, Hovestadt V, Vantine SJ, Van Galen P, Bernstein BE. Single-cell lineage analysis reveals genetic and epigenetic interplay in glioblastoma drug resistance. *Genome Biol* 2020;21:1–21.
27. Ramirez M, Rajaram S, Steininger RJ, Osipchuk D, Roth MA, Morinishi LS, et al. Diverse drug-resistance mechanisms can emerge from drug-tolerant cancer persister cells. *Nat Commun* 2016;7:1–8.
28. Fluegen G, Avivar-Valderas A, Wang Y, Padgen MR, Williams JK, Nobre AR, et al. Phenotypic heterogeneity of disseminated tumour cells is preset by primary tumour hypoxic microenvironments. *Nat Cell Biol* 2017;19:120–32.
29. Phi LTH, Sari IN, Yang YG, Lee SH, Jun N, Kim KS, et al. Cancer stem cells (CSCs) in drug resistance and their therapeutic implications in cancer treatment. *Stem Cells Int* 2018;2018:5416923.
30. Zeng H, Ji J, Song X, Huang Y, Li H, Huang J, et al. Stemness related genes revealed by network analysis associated with tumor immune microenvironment and the clinical outcome in lung adenocarcinoma. *Front Genet* 2020;11:1–15.
31. Recasens A, Munoz L. Targeting cancer cell dormancy. *Trends Pharmacol Sci* 2019;40:128–41.
32. Wang T, Narayanaswamy R, Ren H, Torchilin VP. Combination therapy targeting both cancer stem-like cells and bulk tumor cells for improved efficacy of breast cancer treatment. *Cancer Biol Ther* 2016;17:698–707.
33. Menon DR, Das S, Krepler C, Vultur A, Rinner B, Schauer S, et al. A stress-induced early innate response causes multidrug tolerance in melanoma. *Oncogene* 2015;34:4448–59.
34. Menon DR, Hammerlindl H, Torrano J, Schaidler H, Fujita M. Epigenetics and metabolism at the crossroads of stress-induced plasticity, stemness and therapeutic resistance in cancer. *Theranostics* 2020;10:6261–77.
35. Pfister SX, Ashworth A. Marked for death: targeting epigenetic changes in cancer. *Nat Rev Drug Discov* 2017;16:241–63.
36. Bhang HEC, Ruddy DA, Radhakrishna VK, Causi JX, Zhao R, Hims MM, et al. Studying clonal dynamics in response to cancer therapy using high-complexity barcoding. *Nat Med* 2015;21:440–8.
37. Oren Y, Tsabar M, Cuoco MS, Amir-Zilberstein L, Cabanos HF, Hütter JC, et al. Cycling cancer persister cells arise from lineages with distinct programs. *Nature* 2021;596:576–82.
38. Porter SN, Baker LC, Mittelman D, Porteus MH. Lentiviral and targeted cellular barcoding reveals ongoing clonal dynamics of cell lines in vitro and in vivo. *Genome Biol* 2014;15:R75.
39. Nolan-Stevaux O, Tedesco D, Ragan S, Makhanov M, Chenchik A, Ruefli-Brasse A, et al. Measurement of cancer cell growth heterogeneity through lentiviral barcoding identifies clonal dominance as a characteristic of in vivo tumor engraftment. *PLoS One* 2013;8:e67316.
40. Umkehrer C, Holstein F, Formenti L, Jude J, Froussios K, Neumann T, et al. Isolating live cell clones from barcoded populations using CRISPRa-inducible reporters. *Nat Biotechnol* 2021;39:174–8.
41. Milanovic M, Fan DNY, Belenki D, Däbritz JHM, Zhao Z, Yu Y, et al. Cancer stemness. *Nat Publ Gr* 2018.
42. Wu F, Yang J, Liu J, Wang Y, Mu J, Zeng Q, et al. Signaling pathways in cancer-associated fibroblasts and targeted therapy for cancer. *Signal Transduct Target Ther* 2021;6:1–35.
43. Charles N, Ozawa T, Squatrito M, Bleau AM, Brennan CW, Hambarzumyan D, et al. Perivascular nitric oxide activates notch signaling and promotes stem-like character in PDGF-induced glioma cells. *Cell Stem Cell* 2010;6:141–52.
44. Gómez V, Eykyn TR, Mustapha R, Flores-Borja F, Male V, Barber PR, et al. Breast cancer-associated macrophages promote tumorigenesis by suppressing succinate dehydrogenase in tumor cells. *Sci Signal* 2020;13:1–13.
45. Grégoire H, Roncali L, Rousseau A, Chérel M, Delneste Y, Jeannin P, et al. Targeting tumor associated macrophages to overcome conventional treatment resistance in glioblastoma. *Front Pharmacol* 2020;11:1–17.
46. Guadagno E, Presta I, Maisano D, Donato A, Pirrone CK, Cardillo G, et al. Role of macrophages in brain tumor growth and progression. *Int J Mol Sci* 2018;19:1–19.
47. Shen S, Faouzi S, Souquere S, Roy S, Routier E, Libenciu C, et al. Melanoma persister cells are tolerant to BRAF/MEK inhibitors via ACOX1-mediated fatty acid oxidation. *Cell Rep* 2020;33:108421.
48. Wei Y, Lai X, Yu S, Chen S, Ma Y, Zhang Y, et al. Exosomal miR-221/222 enhances tamoxifen resistance in recipient ER-positive breast cancer cells. *Breast Cancer Res Treat* 2014;147:423–31.
49. Webb BA, Chimenti M, Jacobson MP, Barber DL. Dysregulated pH: a perfect storm for cancer progression. *Nat Rev Cancer* 2011;11:671–7.
50. Zollo M, Di Dato V, Spano D, De Martino D, Liguori L, Marino N, et al. Targeting monocyte chemotactic protein-1 synthesis with bindarit induces tumor regression in prostate and breast cancer animal models. *Clin Exp Metastasis* 2012;29:585–601.
51. Zhong S, Jeong JH, Chen Z, Chen Z, Luo JL. Targeting tumor microenvironment by small-molecule inhibitors. *Transl Oncol* 2020;13:57–69.
52. Li M, Li M, Yin T, Shi H, Wen Y, Zhang B, et al. Targeting of cancer-associated fibroblasts enhances the efficacy of cancer chemotherapy by regulating the tumor microenvironment. *Mol Med Rep* 2016;13:2476–84.
53. Soares J, Espadinha M, Raimundo L, Ramos H, Gomes AS, Gomes S, et al. DIMP53-1: a novel small-molecule dual inhibitor of p53-MDM2/X interactions with multifunctional p53-dependent anticancer properties. *Mol Oncol* 2017;11:612–27.

54. Sehgal K, Portell A, Ivanova EV, Lizotte PH, Mahadevan NR, Greene JR, et al. Dynamic single-cell RNA sequencing identifies immunotherapy persister cells following PD-1 blockade. *J Clin Invest* 2021;131:1–17.
55. Zhang H, Xia Y, Wang F, Luo M, Yang K, Liang S, et al. Aldehyde dehydrogenase 2 mediates alcohol-induced colorectal cancer immune escape through stabilizing PD-L1 expression. *Adv Sci (Weinheim, Baden-Wurttemberg, Ger.)* 2021;8:2003404.
56. Liu C, Qiang J, Deng Q, Xia J, Deng L, Zhou L, et al. ALDH1A1 activity in tumor-initiating cells remodels myeloid-derived suppressor cells to promote breast cancer progression. *Cancer Res* 2021;81:5919–34.
57. Guler GD, Tindell CA, Pitti R, Wilson C, Nichols K, KaiWai Cheung T, et al. Repression of stress-induced LINE-1 expression protects cancer cell subpopulations from lethal drug exposure. *Cancer Cell* 2017;32:221–37.
58. Liao BB, Sievers C, Donohue LK, Gillespie SM, William A, Miller TE, et al. Plasticity and drug tolerance. *2018*;20:233–46.
59. Abderrahman B, Jordan VC. Rethinking extended adjuvant antiestrogen therapy to increase survivorship in breast cancer. *JAMA Oncol* 2018;4:15–6.
60. Essers MAG, Trumpp A. Targeting leukemic stem cells by breaking their dormancy. *Mol Oncol* 2010;4:443–50.
61. Vanner RJ, Renke M, Gallo M, Selvadurai HJ, Lee L, Kushida M, et al. HHS Public Access 2015;26:33–47.
62. Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, et al. Identification of cells initiating human melanomas. *Nature* 2008;451:345–9.
63. Simbulan-Rosenthal CM, Gaur A, Zhou H, Abdussamad M, Qin Q, Dougherty R, et al. CD133 is associated with increased melanoma cell survival after multikinase inhibition. *J Oncol* 2019;2019:6486173.
64. Boonyaratankornkit JB, Yue L, Strachan LR, Scalapino KJ, Leboit PE, Lu Y, et al. Selection of tumorigenic melanoma cells using ALDH. *J Invest Dermatol* 2010;130:2799–808.
65. Ma J, Lin JY, Alloo A, Wilson BJ, Schatton T, Zhan Q, et al. Isolation of tumorigenic circulating melanoma cells. *Biochem Biophys Res Commun* 2010;402:711–7.
66. Kugimiya N, Nishimoto A, Hosoyama T, Ueno K, Enoki T, Li TS, et al. The c-MYC-ABC5 axis plays a pivotal role in 5-fluorouracil resistance in human colon cancer cells. *J Cell Mol Med* 2015;19:1569–81.
67. Kleffel S, Lee N, Lezcano C, Wilson BJ, Sobolewski K, Saab KR, et al. carcinoma growth. *2017*;136:838–46.
68. Lu R, Zhao G, Yang Y, Jiang Z. Inhibition of CD133 overcomes cisplatin resistance through inhibiting PI3K /AKT / mTOR signaling pathway and autophagy in CD133-positive gastric cancer cells. *Technol Cancer Res Treat* 2019;18:1–11.
69. El-Khattouti A, Sheehan NT, Monico J, Drummond HA, Haikel Y, Brodell RT, et al. CD133+ melanoma subpopulation acquired resistance to caffeic acid phenethyl ester-induced apoptosis is attributed to the elevated expression of ABCB5: significance for melanoma treatment. *Cancer Lett* 2015;357:83–104.
70. Zhao L, Yang Y, Zhou P, Ma H, Zhao X, He X, et al. Targeting CD133 high colorectal cancer cells *in vitro* and *in vivo* with an asymmetric bispecific antibody. *J Immunother* 2015;38:217–28.
71. Schmohl JU, Felices M, Oh F, Lenvik AJ, Lebeau AM, Panyam J, et al. Engineering of anti-CD133 trispecific molecule capable of inducing NK expansion and driving antibody-dependent cell-mediated cytotoxicity. *Cancer Res Treat* 2017;49:1140–52.
72. Alibolandi M, Abnous K, Anvari S, Mohammadi M, Ramezani M, Taghdisi SM. CD133-targeted delivery of self-assembled PEGylated carboxymethylcellulose-SN38 nanoparticles to colorectal cancer. *Artif Cells, Nanomedicine Biotechnol* 2018;46(sup1):1159–69.
73. Shigdar S, Qiao L, Zhou SF, Xiang D, Wang T, Li Y, et al. RNA aptamers targeting cancer stem cell marker CD133. *Cancer Lett* 2013;330:84–95.
74. Swaminathan SK, Olin MR, Forster CL, Cruz KSS, Panyam J, Ohlfest JR. Identification of a novel monoclonal antibody recognizing CD133. *J Immunol Methods* 2010;361:110–5.
75. Damek-Poprawa M, Volgina A, Korostoff J, Sollecito TP, Brose MS, O'Malley BW, et al. Targeted inhibition of CD133+ cells in oral cancer cell lines. *J Dent Res* 2011;90:638–45.
76. Glumac PM, Lebeau AM. The role of CD133 in cancer: a concise review. *Clin Transl Med* 2018;1–14.
77. Li S, Yue D, Chen X, Wang L, Li J, Ping Y, et al. Epigenetic regulation of CD271, a potential cancer stem cell marker associated with chemoresistance and metastatic capacity. *Oncol Rep* 2015;33:425–32.
78. Imai T, Tamai K, Oizumi S, Oyama K, Yamaguchi K, Sato I, et al. CD271 defines a stem cell-like population in hypopharyngeal cancer. *PLoS One* 2013;8:e62002.
79. Mochizuki M, Tamai K, Imai T, Sugawara S, Ogama N, Nakamura M, et al. CD271 regulates the proliferation and motility of hypopharyngeal cancer cells. *Sci Rep* 2016;6:1–12.
80. Kiyosue T, Kawano S, Matsubara R, Goto Y, Hirano M, Jinno T, et al. Immunohistochemical location of the p75 neurotrophin receptor (p75NTR) in oral leukoplakia and oral squamous cell carcinoma. *Int J Clin Oncol* 2013;18:154–63.
81. Okumura T, Tsunoda S, Mori Y, Ito T, Kikuchi K, Wang TC, et al. The biological role of the low-affinity p75 neurotrophin receptor in esophageal squamous cell carcinoma. *Clin Cancer Res* 2006;12:5096–103.
82. Tsunoda S, Okumura T, Ito T, Mori Y, Soma T, Watanabe G, et al. Significance of nerve growth factor overexpression and its autocrine loop in oesophageal squamous cell carcinoma. *Br J Cancer* 2006;95:322–30.
83. Tian J, Li X, Si M, Liu T, Li J. CD271+ osteosarcoma cells display stem-like properties. *PLoS One* 2014;9:e98549.
84. Murillo-Sauca O, Chung MK, Shin JH, Karamboulas C, Kwok S, Jung YH, et al. CD271 is a functional and targetable marker of tumor-initiating cells in head and neck squamous cell carcinoma. *Oncotarget* 2014;5:6854–66.
85. EA Al M DM, Menon DR, Stark MS, Torrano J, Hammerlindl H, et al. Distinct histone modifications denote early stress-induced drug tolerance in cancer. *Oncotarget* 2018;9:8206–22.
86. Verbeke S, Meignan S, Lagadec C, Germain E, Hondermarck H, Adriaenssens E, et al. Overexpression of p75NTR increases survival of breast cancer cells through p21waf1. *Cell Signal* 2010;22:1864–73.
87. Raha D, Wilson TR, Peng J, Peterson D, Yue P, Evangelista M, et al. The cancer stem cell marker aldehyde dehydrogenase is required to maintain a drug-tolerant tumor cell subpopulation. *Cancer Res* 2014;74:3579–90.
88. Tomellini E, Touil Y, Lagadec C, Julien S, Ostyn P, Ziental-Gelus N, et al. Nerve growth factor and prongf simultaneously promote symmetric self-renewal, quiescence, and epithelial to mesenchymal transition to enlarge the breast cancer stem cell compartment. *Stem Cells* 2015;33:342–53.
89. Zhou X, Hao Q, Liao P, Luo S, Zhang M, Hu G, et al. Nerve growth factor receptor negates the tumor suppressor p53 as a feedback regulator. *Elife* 2016;5:1–22.
90. Mi Z, Rogers DA, Mircics ZK, Schor NF. P75NTR-dependent modulation of cellular handling of reactive oxygen species. *J Neurochem* 2009;110:295–306.
91. Vidal A, Redmer T. Decoding the role of CD271 in Melanoma. *Cancers (Basel)* 2020;12:1–19.
92. Morita S, Mochizuki M, Wada K, Shibuya R, Nakamura M, Yamaguchi K, et al. Humanized anti-CD271 monoclonal antibody exerts an anti-tumor effect by depleting cancer stem cells. *Cancer Lett* 2019;461:144–52.
93. Ngo M, Han A, Lakatos A, Sahoo D, Hachey SJ, Weiskopf K, et al. Antibody therapy targeting CD47 and CD271 effectively suppresses melanoma metastasis in patient-derived xenografts. *Cell Rep* 2016;16:1701–16.
94. He R, Kidder BL. H3K4 demethylase KDM5B regulates global dynamics of transcription elongation and alternative splicing in embryonic stem cells. *Nucleic Acids Res* 2017;45:6427–41.
95. Jose A, Shenoy GG, Rodrigues GS, Kumar NAN, Munisamy M, Thomas L, et al. Histone demethylase KDM5B as a therapeutic target for cancer therapy. *Cancers (Basel)* 2020;12:1–16.
96. Roesch A, Fukunaga-Kalabis M, Schmidt EC, Zabierowski SE, Brafford PA, Vultur A, et al. A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. *Cell* 2010;141:583–94.
97. Yamamoto S, Wu Z, Russnes HG, Takagi S, Peluffo G, Vaske C, et al. JARID1B is a luminal lineage-driving oncogene in breast cancer. *Cancer Cell* 2014;25:762–77.
98. Vinogradova M, Gehling VS, Gustafson A, Arora S, Tindell CA, Wilson C, et al. An inhibitor of KDM5 demethylases reduces survival of drug-tolerant cancer cells. *Nat Chem Biol* 2016;12:531–8.
99. Brier ASB, Loft A, Madsen JGS, Rosengren T, Nielsen R, Schmidt SF, et al. The KDM5 family is required for activation of pro-proliferative cell cycle genes during adipocyte differentiation. *Nucleic Acids Res* 2017;45:1743–59.
100. Koppaka V, Thompson DC, Chen Y, Ellermann M, Nicolaou KC, Juvonen RO, et al. Aldehyde dehydrogenase inhibitors: a comprehensive review of the pharmacology, mechanism of action, substrate specificity, and clinical application. *Pharmacol Rev* 2012;64:520–39.
101. Vassalli G. Aldehyde dehydrogenases: Not just markers, but functional regulators of stem cells. *Stem Cells Int* 2019;2019:3904645.

102. Tomita H, Tanaka K, Tanaka T, Hara A. Aldehyde dehydrogenase 1A1 in stem cells and cancer. *Oncotarget* 2016;7:11018–32.
103. Lingala S, Cui YY, Chen X, Ruebner BH, Qian XF, Zern MA, et al. Immunohistochemical staining of cancer stem cell markers in hepatocellular carcinoma. *Exp Mol Pathol* 2010;89:27–35.
104. Van Den Hoogen C, Van Der Horst G, Cheung H, Buijs JT, Lippitt JM, Guzmán-Ramírez N, et al. High aldehyde dehydrogenase activity identifies tumor-initiating and metastasis-initiating cells in human prostate cancer. *Cancer Res* 2010;70:5163–73.
105. Lugli A, Iezzi G, Hostettler I, Muraro MG, Mele V, Tornillo L, et al. Prognostic impact of the expression of putative cancer stem cell markers CD133, CD166, CD44s, EpCAM, and ALDH1 in colorectal cancer. *Br J Cancer* 2010;103:382–90.
106. Jelski W, Laniewska-Dunaj M, Orywal K, Kochanowicz J, Rutkowski R, Szmítowski M. The diagnostic value of alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) measurement in the sera of patients with brain tumor. *Arch Med Sci* 2017;13:346–52.
107. Ucar D, Cogle CR, Zucali JR, Ostmark B, Scott EW, Gray BA, et al. *Cancer*. 2010; 178:48–55.
108. Tanei T, Morimoto K, Shimazu K, Seung JK, Tanji Y, Taguchi T, et al. Association of breast cancer stem cells identified by aldehyde dehydrogenase 1 expression with resistance to sequential paclitaxel and epirubicin-based chemotherapy for breast cancers. *Clin Cancer Res* 2009;15:4234–41.
109. Rasheed ZA, Yang J, Wang Q, Kowalski J, Freed I, Murter C, et al. Prognostic significance of tumorigenic cells with mesenchymal features in pancreatic adenocarcinoma. *J Natl Cancer Inst* 2010;102:340–51.
110. Wakamatsu Y, Sakamoto N, Oo HZ, Naito Y, Uraoka N, Anami K, et al. Expression of cancer stem cell markers ALDH1, CD44 and CD133 in primary tumor and lymph node metastasis of gastric cancer. *Pathol Int* 2012;62:112–9.
111. Deng S, Yang X, Lassus H, Liang S, Kaur S, Ye Q, et al. Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers. *PLoS One* 2010;5:e10277.
112. Zhang G, Ma L, Xie YK, Miao XB, Jin C. Esophageal cancer tumorspheres involve cancer stem-like populations with elevated aldehyde dehydrogenase enzymatic activity. *Mol Med Rep* 2012;6:519–24.
113. Clay MR, Tabor M, Owen JH, Carey TE, Bradford CR, Wolf GT, et al. Single-marker identification of head and neck squamous cell carcinoma cancer stem cells with aldehyde dehydrogenase. *Head Neck* 2010;32:1195–201.
114. Januchowski R, Wojtowicz K, Zabel M. The role of aldehyde dehydrogenase (ALDH) in cancer drug resistance. *Biomed Pharmacother* 2013;67:669–80.
115. Moreb JS, Muhoczy D, Ostmark B, Zucali JR. RNAi-mediated knockdown of aldehyde dehydrogenase class-1A1 and class-3A1 is specific and reveals that each contributes equally to the resistance against 4-hydroperoxycyclophosphamide. *Cancer Chemother Pharmacol* 2007;59:127–36.
116. Prasmickaite L, Engesaeter B, Skrbno N, Hellenes T, Kristian A, Oliver NK, et al. Aldehyde dehydrogenase (ALDH) activity does not select for cells with enhanced aggressive properties in malignant melanoma. *PLoS One* 2010;5:e10731.
117. Magni M, Shammah S, Schiró R, Mellado W, Dalla-Favera R, Gianni AM. Induction of cyclophosphamide-resistance by aldehyde-dehydrogenase gene transfer. *Blood* 1996;87:1097–103.
118. Brennan SK, Meade B, Wang Q, Merchant AA, Kowalski J, Matsui W. Mantle cell lymphoma activation enhances bortezomib sensitivity. *Blood* 2016;116:4185–92.
119. Croker AK, Allan AL. Cancer stem cells: implications for the progression and treatment of metastatic disease: stem cells review series. *J Cell Mol Med*. 2008; 12:374–90.
120. Croker AK, Allan AL. Inhibition of aldehyde dehydrogenase (ALDH) activity reduces chemotherapy and radiation resistance of stem-like ALDH hiCD44 + human breast cancer cells. *Breast Cancer Res Treat* 2012;133:75–87.
121. Taylor LA, Abraham RM, Tahirovic E, Van Belle P, Li B, Huang L, et al. High ALDH1 expression correlates with better prognosis in tumorigenic malignant melanoma. *Mod Pathol* 2017;30:634–9.
122. Dimou A, Neumeister V, Agarwal S, Anagnostou V. Measurement of aldehyde dehydrogenase 1 expression defines a group with better prognosis in patients with non-small cell lung cancer. *AJPA* 2012;181:1436–42.
123. Ran D, Schubert M, Pietsch L, Taubert I, Wuchter P, Eckstein V, et al. Aldehyde dehydrogenase activity among primary leukemia cells is associated with stem cell features and correlates with adverse clinical outcomes. *Exp Hematol* 2009; 37:1423–34.
124. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* 2007;1:555–67.
125. Morimoto K, Kim SJ, Tanei T, Shimazu K, Tanji Y, Taguchi T, et al. Stem cell marker aldehyde dehydrogenase 1-positive breast cancers are characterized by negative estrogen receptor, positive human epidermal growth factor receptor type 2, and high Ki67 expression. *Cancer Sci* 2009;100:1062–8.
126. Carballo GB, Honorato JR, De Lopes GPF, Spohr T, CLDSE. A highlight on Sonic hedgehog pathway. *Cell Commun Signal* 2018;16:1–15.
127. Dhanyamraju PK, Patel TN, Dovat S. Medulloblastoma: “Onset of the molecular era.” *Mol Biol Rep* 2020;47:9931–37.
128. Santini R, Pietrobono S, Pandolfi S, Montagnani V, D’Amico M, Penachioni JY, et al. SOX2 regulates self-renewal and tumorigenicity of human melanoma-initiating cells. *Oncogene* 2014;33:4697–708.
129. Luo Y, Dallaglio K, Chen Y, Robinson WA, Robinson SE, McCarter MD, et al. ALDH1A isozymes are markers of human melanoma stem cells and potential therapeutic targets. *Stem Cells* 2012;30:2100–13.
130. Ohmura-Kakutani H, Akiyama K, Maishi N, Ohga N, Hida Y, Kawamoto T, et al. Identification of tumor endothelial cells with high aldehyde dehydrogenase activity and a highly angiogenic phenotype. *PLoS One* 2014;9:1–17.
131. Alamgeer M, Ganju V, Kumar B, Fox J, Hart S, White M, et al. Changes in aldehyde dehydrogenase-1 expression during neoadjuvant chemotherapy predict outcome in locally advanced breast cancer. *Breast Cancer Res* 2014; 16:1–13.
132. Li J, Zhang B, Yang YF, Jin J, Liu YH. Aldehyde dehydrogenase 1 as a predictor of the neoadjuvant chemotherapy response in breast cancer a meta-analysis. *Med (United States)* 2018;97:1–6.
133. Wang K, Chen X, Zhan Y, Jiang W, Liu X, Wang X, et al. Increased expression of ALDH1A1 protein is associated with poor prognosis in clear cell renal cell carcinoma. *Med Oncol* 2013;30:574.
134. Vasilou V, Nebert DW. Analysis and update of the human aldehyde dehydrogenase (ALDH) gene family. *Hum Genomics* 2005;2:138–43.
135. Chefetz I, Grimley E, Yang K, Hong L, Ekaterina V, Suci R, et al. HHS public access. 2020;26:3061–75.
136. Nechushtan. Clinical trial results a phase IIb trial assessing the addition of disulfiram to chemotherapy for the treatment of metastatic non-small cell lung cancer. *Oncologist* 2015;366–7.
137. Sarvi S, Crispin R, Lu Y, Zeng L, Hurley TD, Houston DR, et al. ALDH1 bio-activates nifuroxazide to eradicate ALDH high melanoma-initiating cells. *Cell Chem Biol* 2018;25:1456–69.
138. Aguilar-Pérez KM, Avilés-Castrillo JI, Medina DI, Parra-Saldivar R, Iqbal HMN. Insight into nanoliposomes as smart nanocarriers for greening the twenty-first century biomedical settings. *Front Bioeng Biotechnol* 2020;8:1–23.
139. Aramini B, Masciale V, Bianchi D, Manfredini B, Banchelli F, D’Amico R, et al. ALDH expression in angiosarcoma of the lung: a potential marker of aggressiveness? *Front Med* 2020;7:1–9.
140. Rebolledo-Rios R, Venton G, Sánchez-Redondo S, Iglesias i Felip C, Fournet G, González E, et al. Dual disruption of aldehyde dehydrogenases 1 and 3 promotes functional changes in the glutathione redox system and enhances chemosensitivity in nonsmall cell lung cancer. *Oncogene* 2020;39:2756–71.
141. Kawakami R, Mashima T, Kawata N, Kumagai K, Migita T, Sano T, et al. ALDH1A3-mTOR axis as a therapeutic target for anticancer drug-tolerant persister cells in gastric cancer. *Cancer Sci* 2020;111:962–73.
142. Kaur H, Mao S, Li Q, Sameni M, Krawetz SA, Sloane BF, et al. RNA-Seq of human breast ductal carcinoma in situ models reveals aldehyde dehydrogenase isoform 5A1 as a novel potential target. *PLoS One* 2012;7:e50249.
143. Patel M, Lu L, Zander DS, Sreerama L, Coco D, Moreb JS. ALDH1A1 and ALDH3A1 expression in lung cancers: correlation with histologic type and potential precursors. *Lung Cancer*. 2008;59:340–9.
144. Yan J, De Melo J, Cutz J-C, Aziz T, Tang D. Aldehyde dehydrogenase 3A1 associates with prostate tumorigenesis. *Br J Cancer* 2014;110:2593–603.
145. Tang DG, Patrawala L, Calhoun T, Bhatia B, Choy G, Schneider-Broussard R, et al. Prostate cancer stem/progenitor cells: identification, characterization, and implications. *Mol Carcinog*. 2007;46:1–14.

146. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005;65:10946–51.
147. Ma S, Chan KW, Lee TK-W, Tang KH, Wo JY-H, Zheng B-J, et al. Aldehyde dehydrogenase discriminates the CD133 liver cancer stem cell populations. *Mol Cancer Res* 2008;6:1146–53.
148. Tanaka K, Tomita H, Hisamatsu K, Nakashima T, Hatano Y, Sasaki Y, et al. ALDH1A1-overexpressing cells are differentiated cells but not cancer stem or progenitor cells in human hepatocellular carcinoma. *Oncotarget* 2015;6:24722–32.
149. Ricci F, Bernasconi S, Porcu L, Erba E, Panini N, Fruscio R, et al. ALDH enzymatic activity and CD133 positivity and response to chemotherapy in ovarian cancer patients. *Am J Cancer Res* 2013;3:221–9.
150. Kuo Y, Liu Y, Adebayo BO, Shih P. JARID1B expression plays a critical role in chemoresistance and stem cell-like phenotype of neuroblastoma cells. 2015; 1–14.