WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 22, 802-821. Research Article ISSN 2277–7105

MCL1 INHIBITOR BRD-810 KILLS CANCER CELLS WHILE MINIMIZING RISK OF CARDIOTOXICITY

Akanksha N. Mote*[1] and Karan D. Atpadkar[2]

India.

Article Received on
25 Sept. 2024,

Revised on 16 Oct. 2024,
Accepted on 06 Nov. 2024

DOI: 10.20959/wjpr202422-34586

***Corresponding Author**
Akanksha N. Mote
India.

ABSTRACT

The MCL1 gene is regularly increased in cancer and codes for the antiapoptotic protein myeloid cell leukemia 1 (MCL1), which confers resistance to the current standard of care. In this manner, MCL1 is an attractive anticancer target. Here we depict BRD-810 as a strong and particular MCL1 inhibitor and its key design guideline of fast systemic clearance to possibly minimize region beneath the curve-driven toxicities associated with MCL1 inhibition. Cell death escape is one of the most prominent features of cancer cells and closely connected to the dysregulation of members of the Bcl-2 family of proteins. Among those, the anti-apoptotic family member myeloid cell leukemia-1 (MCL-1) acts as a master regulator of apoptosis in different human malignancies. Irrespective of its unfavorable structure profile, independent research efforts recently led to the era of highly strong MCL-1 inhibitors that are currently assessed in clinical trials.

INTRODUCTION

MCL-1 is among the top genes amplified in several cancers and is implicated in cancer progression, drug resistance and poor forecast. It ensures cancer cells from apoptosis and decreases their sensitivity to targeted agents or chemotherapeutics.[1-5] The intrinsic apoptosis pathway represents the most prominent cell death signaling cascade and is primar ily controlled by the BCL-2 family of proteins. This can be split into pro-survival/anti-apoptotic (BCL-2, BCL xL, MCL-1, BCL-W, BFL1), effector (BAK, BAX, BOK), BH3-only activator (BIM, Offered, Puma) and sensitizer (NOXA, Bad, BMF, BIK, Hrk) proteins.[6] Resistance to modified cell death, termed apoptosis, is a trademark of cancer.[7] Each cell is prepared to sense extrinsic and intrinsic stretch signals and maintain a proper adjust of proapoptotic and

antiapoptotic factors, which ultimately decides the life or death of the cell. In malignant cells, the B cell lymphoma 2 (Bcl-2) protein family has a central role and myeloid cell leukemia 1 (MCL1), as one of the key antiapoptotic variables of that protein family, has been recognized to promote abnormal cell survival.[8] Focusing on myeloid cell leukemia-1 (MCL 1) protein is a effective procedure to induce apoptosis and overcome tumor resistance to chemotherapy and focused on treatment. Different procedures to restrain the antiapoptotic action of MCL-1 protein, including transcription, translation, and the degradation of MCL-1 protein, have been tried. Neutralizing MCL-1's function by focusing on its interactions with other proteins via BCL-2 interacting mediator (BIM)S2A has been shown to be an similarly effective approach. Encouraged by the design of venetoclax and its efficacy in chronic lymphocytic leukemia, researchers have developed other BCL-2 homology (BH3) mimetics—particularly MCL-1 inhibitors (MCL-1i)—that are currently in clinical trials for various cancers. While extensive reviews of MCL-1i are accessible, basic analyses focusing on the challenges of MCL-1i and their optimization are lacking. In this review, we examine the current information regarding clinically relevant MCL-1i and focus on predictive biomarkers of response, mechanisms of resistance, major issues associated with use of MCL-1i, and the future utilize of and maximization of the benefits from these agents.

Mcl-1 gene amplification was most elevated among numerous tumors. Essentially, MCL-1 protein has been implicated in both tumorigenesis and chemotherapeutic resistance.[9,10] Consistent with this statement, it has been shown that, in xenograft models, knockdown of MCL-1 decreased the proliferation rate of cancer cells to a greater degree than that seen in controls.[11] Conversely, increased incidence of B-cell lymphoma was noticed in transgenic mice overexpressing MCL-1.[12] MCL-1 overexpression has moreover been implicated in resistance to both targeted treatment[13-15] and conventional chemotherapy, including taxol, cisplatin, erlotinib, and cytarabine.[16-18] There was selection for RAS-mutant clones in patients with acute myelogenous leukemia (AML) treated with venetoclax. These clones mediated resistance to venetoclax through MCL-1 upregulation, and cells were resensitized to the drug through the inhibition of MCL-1. Other than, concomitant utilize of MCL-1 inhibitors was better than other combination regimens in venetoclax resistant models.[19] The overexpression of MCL-1 is related to cisplatin resistance.[20] Depleting MCL-1 has reversed resistance to cisplatin and doxorubicin in osteosarcoma cell lines in vitro and xenograft tumors in vivo.[21] MCL-1 amplification has been found in subsets of wild-type fibroblast growth factor receptor urothelial cancer, and its degradation by erdafitinib synergized BCL-

xL/BCL-2 inhibitors.[22]

Multi functional roles of MCL 1

Myeloid-cell leukemia 1 (MCL-1) was found, isolated from the human myeloid leukemia cell line ML-1.[23] MCL-1 was found to have near sequence similarities with BCL-2 and both genes shared "surprising" oncogenic properties: they sustained cell survival but did not promote cell multiplication.[23] Nearly thirty a long time afterward BCL-2 and MCL-1 are the most prominent members of the BCL-2 family and well known for their anti-apoptotic role in health and disease.

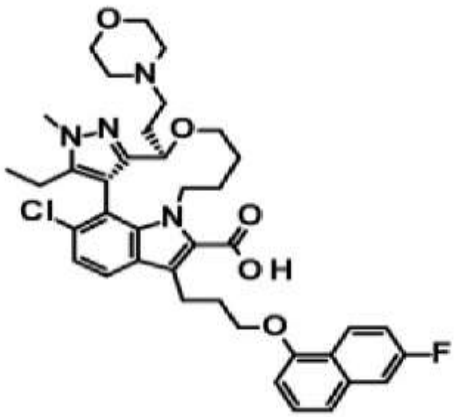
MCL-1 shares the presence of BCL-2 homology regions (BH1) and a carboxy-terminal transmembrane localization domain with other pro-survival family members.[24] The alpha helix of the BH3 domain is fundamental for coordinate interactions between BCL-2 family individuals.[25] Anti-apoptotic family individuals counting MCL-1 frame a hydrophobic groove (composed of BH1-BH3 domains), where four hydro phobic binding pockets (P1–P4) guide the interaction with hydrophobic residues (h1-h4) of BH3 domains.

As well as playing a role in resistance to treatment, raised MCL-1 can really drive haematopoietic tumour development.[26] This oncogenic role for MCL-1 may be broad as the MCL1 locus is one of the most as often as possible amplified regions of the human genome across a wide variety of cancers including breast cancer.[27] Later evidence from in vitro tests recommends an vital role for MCL-1 in breast cancer cell survival, especially in triple-negative (TN) breast cancer particularly interacts with MCL-1 inhibits metastases of TN breast cancer cell lines in xenograft models.[28] There is a require for unused therapeutic options to reduce the mortality burden of breast cancer. Given the emergence of BH3-mimetic drugs capable of targeting MCL-1 we investigated the expression and useful requirement for MCL-1 in breast cancer, systematically testing this through a combination of human breast tumour tissue analysis with relationship to clinicopathological data; breast cancer cell line testing in vitro and in vivo; and for the to begin with time appear a role for MCL-1 in mammary tumorigenesis using a genetically designed mouse model.

Multi functional roles of BRD-10

We present a modern profoundly potent and specific MCL1 inhibitor, BRD-810, that quickly induces apoptosis in vitro and manages strong antitumor effects in vivo. Given the brief half-life of the MCL1 protein and the irreversible nature of apoptosis, we reasoned that a rapidly cleared MCL1 inhibitor would be an effective antitumor operator with potential for less toxicity than longer-acting agents. We report here our characterization of BRD-810 in tumor models and in hi PS cell-derived cardiomyocytes. The results suggest that BRD-810 is suitable for clinical development.

BRD-810 was recognized during a medicinal chemistry campaign tasked with balancing cellular potency and in vivo clearance within a macro cyclic MCL1 inhibitor class. We next investigated the downstream cellular effects of disruption of MCL1 and proapoptotic protein complexes. Treating the breast cancer cell line HMC1-8 with increasing concentrations of BRD-s810 led to the activation of caspase 3 and induction of cell death. These effects were dose dependent and occurred at comparable concentrations of BRD-810 (half-maximal effective concentration.



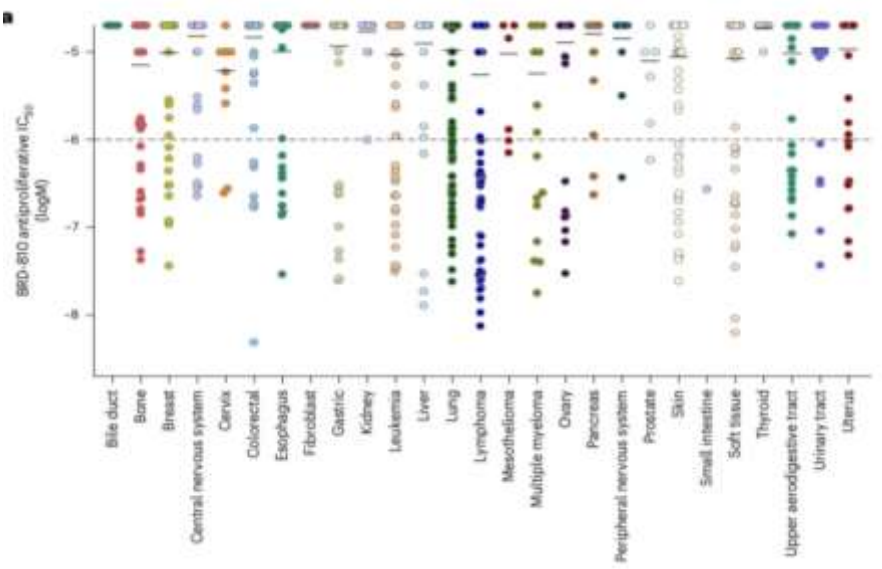
RESULT

MCL1 inhibitor BRD-810 triggers apoptosis in cancer cells

To examine the potential of BRD-810 to displace the complexes of MCL1 and proapoptotic proteins in intact cells, we created a quantitative ELISA assay. This assay measures the interaction of local MCL1 protein and BAK or BIM protein at increasing doses of BRD-810 and at distinctive exposure time points. The IC50 of BRD-810 required to disrupt the MCL1–BAK complex in cells was calculated to be 1.2 nM on normal. In contrast, disruption of the MCL1–BIM complex by BRD-810 was clearly dose and time dependent and longer exposure times were required to completely disrupt the complex, reflecting the longer target residence time of BIM on MCL1 protein.[28] Co immunoprecipitation assays appeared comparable complex disruption dynamics when cells were exposed to increasing concentrations of BRD-810.

We next investigated the downstream cellular impacts of disruption of MCL1 and proapoptotic protein complexes. These impacts were dose dependent and occurred at comparable concentrations. To determine whether BRD-810 induced cell killing through the intrinsic apoptotic pathway, we generated BAX-deficient and BAK-deficient HMC1-8 cells utilizing clustered frequently interspaced short palindromic repeats (CRISPR)–Cas9 gene altering to compare those double-knockout cells to their isogenic HMC1-8 Cas9 control cells with respect to their sensitivity to BRD-810. Whereas the HMC1-8 Cas9 control cells behaved like the parental HMC1-8 cells, knockout of BAX and BAK secured cells from BRD-810-mediated induction of apoptosis and cell killing. This proposes that BRD-810-induced cell death indeed happens through an on-target mechanism activating the intrinsic apoptotic pathway.

To illustrate the MCL1 specificity of BRD-810 in intact cells, we measured caspase activation and development inhibition in diffuse expansive B cell lymphoma (DLBCL) lines known to be either MCL1 or Bcl-XL dependent. To establish BRD-810 as a particular MCL1 inhibitor.



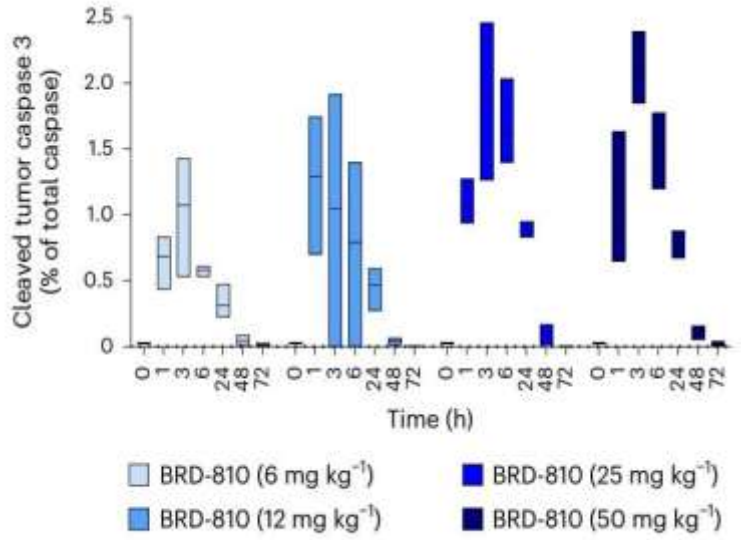
Candidate biomarker for BRD-810 susceptibility

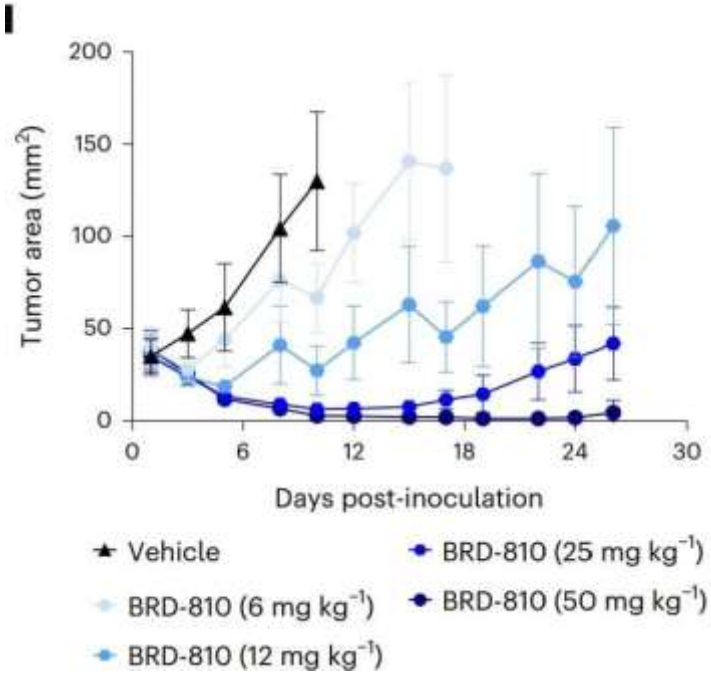
To comprehensively assess the antiproliferative action of BRD-810.50. Taking after a 5-day incubation of cell line pools with BRD-810, antiproliferative activity was observed at submicromolar concentra tions acrosbroad range of strong and hematological cancer models, including breast cancer, lung cancer, melanoma, sarcoma, lymphoma and leukemia.

Table 2 | Antiproliferative activity of BRD-810 and other MCL1 inhibitors

	BRD-810	AMG176	AZD5991	S64315
Cell culture medium unbound fraction (%)	3.1	1.0	2.1	52.2
Antiproliferative IC_{50} (nM)				
Hematological cancer cell lines				
SU-DHL-10	0.1	0.9	ND	1.6
SU-DHL-5	0.2	1.2	0.3	0.5
KMS-12-BM	0.3	1.9	0.7	ND
AMO-1	0.3	1.3	1.2	1.7
RPMI 8226	0.3	3.0	1.3	ND
OPM-2	0.4	1.2	0.7	2.7
MOLP-8	0.5	2.3	0.8	3.9
JJN-3	0.9	5.7	1.5	3.5
KMS-12-PE	1.5	12.0	4.4	ND
Solid cancer cell lines				
DMS114	0.6	2.9	1.5	2.9
A-427	0.6	5.8	1.1	7.3
HCC-1187	0.9	3.4	1.1	2.6
SNU398	1.1	5.1	1.1	3.7
HCC-2157	1.4	2.7	2.0	4.2
PA-1	1.8	6.5	2.2	9.2
NCI-H82	1.8	4.9	3.4	19.1
SNU-16	2.0	6.0	5.6	38.0
A-431	2.2	6.6	4.6	37.0
HMC1-8	2.3	6.0	6.9	23.0

To better understand the biological drivers of BRD-810 specificity, we utilized Bayesian variable selection methods to identify a subset of standard genomic features of cell lines that best clarified the measured MCL1 inhibitor affectability. Interests, sensitivity was less related with MCL1 protein or mRNA expression (Extended Information Fig. 3c,d).





Suggesting that MCL1 dependency is more predicted by how much Bcl-XL is show to compensate for blocked MCL1 protein, as well as how much proapoptotic BAK, the favored binding accomplice to MCL1 and Bcl-XL, can be liberated by MCL1–BAK disruption.

Targeting of MCL 1 with small molecule inhibitors

The poor selectivity and low affinity of the to begin with inhibitors and the reduced bioavailability of others delayed the clinical improvement of powerful MCL-1 inhibitors. Particular targeting of MCL-1 is especially challenging due to its expansive, surface-exposed hydrophobic BH3 binding groove.[29,30] A few of the putative MCL-1 inhibitors, which demonstrated high adequacy in preclinical studies, showed various off-target impacts not related with the direct restraint of MCL-1 (e.g., upregulation of NOXA).[31] In any case, during the evolution of MCL-1 inhibitors, their affinities improved from small scale molar to subnanomolar which finally empowered the design and synthesis of highly powerful and particular clinical-grade MCL-1 inhibitors . Up to date, three distributed compounds moved into clinical trials.

Different drug development strategies driven to the later advancement and clinical interpretation of potent MCL-1 inhibitors. These compounds are characterized by their impressive potency, on-target movement (i.e., BAK dependent induction of apoptosis), a stabilizing impact on MCL-1 protein at least in non-sensitive cells, an inverse correlation between movement and BCL-xL expression levels as well as their special action in hematological malignancies. These characteristics are shared among the mentioned MCL-1 inhibitors, suggesting that they can be utilized to characterize this novel drug class.[32]

Targeting MCL1 has appeared great potential in cancer treatment. Be that as it may, it is vital that MCL1 too has an important role in early embryonic development[33] and in the survival of numerous ordinary cell lineages.[34] MCL1 is highly expressed in the myocardium and is vital for mitochondrial homeostasis and the induction of autophagy in cardiomyocytes.[35] Deficiency of MCL1 in murine hearts leads to rapid and fatal cardiomyopathy, and inhibition of MCL1 in human cardiomyocytes results in a severe contractile defect.[36] Furthermore, deletion of MCL1 triggers the loss of hematopoietic stem cells, lymphocytes, and neutrophils.[37,38–40] MCL1 too con tributes to the maintenance of hepatic integrity in murine livers[41], and its absence in murine hepatocytes causes chronic liver damage and hepatocarcinogenesis.[42] Based on these studies, MCL1 depletion may present potential undesirable cardiotoxicity, hepatotoxicity, and hematological toxicity, especially in combination with other cytotoxic drugs. Hence, there is a require to recognize a therapeutic window in which cancer cells are more sensitive than normal cells to the loss of MCL1.

Interests, a few groups have found that the loss of a single allele of MCL1 can kill c-MYC-driven lymphoma cells, an alteration that is well tolerated in normal cells.[43,44] In addition, improving therapeutic methods to particularly convey the inhibitors to cancer tissues will be another choice to reduce side impacts on ordinary tissues.

Cell killing dynamics of BRD-810

Having established that the rapidly cleared BRD-810 is sufficient to maximally induce apoptosis in vivo, we next evaluated the antitumor efficacy of BRD-810 in the same MOLP-8 cancer cell model. Once-weekly dosing of BRD-810 led to a dose-dependent inhibition of cancer cell growth, again with a maximum effect reached at 25 mg kg−1. Like most MCL1 inhibitors, BRD-810 does not inhibit murine Mcl1; as such, the on-target toxicity of BRD-810 cannot be assessed in mice. However, as one measure of off-target toxicity, we observed no impact on body weight following once-weekly dosing.

METHODS

MCL-1inhibitors and up-regulation of MCL-1 protein

The MCL-1upregulate MCL-1protein in cell lines and in essential patient samples. This finding has not been seen with other BH3 mimetics that target BCL-2 or BCL-xL. Fluorescence reverberation energy transfer studies have confirmed that this up regulation connects with engagement of theMCL-1i into theMCL-1 protein. Hence, MCL-1 upregulation can be considered as a biomarker for MCL-1i target engagement.[45] This up regulation was related to the expanded stability of MCL-1 or maybe than to expanded translation. As of late,[46] appeared that the MCL-1i-induced stabilityofMCL-1 protein is basically due to defective ubiquitination ofMCL-1.

Proteins, BH3-derived peptides and compounds

MCL1 protein utilized for in vitro natural assays (surface plasmon resonance (SPR), HTRF and X-ray crystal lography), was expressed and purified as already depicted utilizing a bacterial expression system.[47] Bcl-2 and Bcl-XL protein for HTRF assays was obtained from BPS Bioscience. BH3-derived peptides were acquired from Bio syntan with the following sequences: Noxa, biotin-PEG2-PEG2-PAELEVE-Nva-ATQLRRFGDKLNFRQKLL-amide; Bad, biotin-PEG2-PEG2-NLWAAQ RYGRELRR-Nle-SDEFVDSFKK-amide. Compounds 1 and 2 and BRD 810 were synthesized as described in WO 2017198341 (compound 1), WO 2019096905 (compound 2) and WO 2020236556 (BRD-810).

Co crystallization of MBP–MCL1 bound to BRD-810

Crystallization was fulfilled utilizing our already reported method[47] with a few modifications to give a rapid, reliable crystallization system with improved co crystallization success rates for low-solubility compounds. Briefly, 2.5 µl of solubilized BRD-810 solution (1 mM in 20% PEG400, 40% methyl prednisolone and 40% DMSO) was placed on the side of a thin-walled PCR tube. To improve compound solubilization, this drop was rapidly flushed with 7.5 µl of maltodextrin-binding protein (MBP)–MCL 1, mixed completely and allowed to incubate for 15 min on ice. This completed mixture was sealed and allowed to equilibrate against 400 µl of 1.5 M NaCl. Protein crystals appeared inside 1 day and continued to grow for approximately 7 days. A single crystal with measurements of approximately 40 µM × 80 µM × 200 µM was then collected straightforwardly from the crystallization drop in a nylon cryo loop and flash-frozen by rapidly diving into liquid nitrogen.

X-ray diffraction datasets were collected from solidified single crystals at the ALBA synchrotron and prepared and refined with the programs xia2 and DIALS from the CCP4 program suites.[48-50] Automated refinement and ligand identification were performed utilizing DIMPLE and a known MBP–MCL1 protein as a model (Protein Data Bank (PDB) 4WGI). Iterative model modifying and refinement were performed utilizing the programs Coot and Phenix.[51,52]

Cardiac toxicity evaluation

ECGs were measured using a Cardiofax ECG 9620 electrocardiograph. Heart tissue for histopathology was collected at the end of the study and embedded in paraffin, sectioned, mounted on glass slides, stained with hematoxylin and eosin and evaluated his to pathologically by a board-certified veterinary pathologist blinded to treatment groups. For the troponin I assay, supernatants (50 µl per well) were collected from each well of the 96-well plates in culture and were immediately frozen at −80 °C. On the day of the assay, the supernatant samples were thawed at room temperature and were used to perform the troponin I AlphaLISA assay according to the manufacturer's instructions. The standard curve of the assay was generated using a nonlinear regression 4-PL sigmoidal curve fit and the LLOD (lower level of detection) was calculated at 1.8 pg ml−1 cTnI. Positive control and test samples with a notable cardiotoxic effect (exceeding the 1,000 pg ml−1 threshold value) were found within the dynamic range of the assay.

DISCUSSION

There are 2 major issues to be overcome with respect to the clinical application of current MCL-1. To begin with, reports of MCL-1 cardiotoxicity are disturbing and raise safety concerns. Second, analysts must determine how to fit MCL-1into treatment calculations to identify the patients likely to benefit from the drugs. The mechanism of MCL-1mediated incident cardiotoxicity is not very clear. MCL-1 has high expression in the myocardium and is essential for maintaining cardiac homeostasis and inducing autophagy in the heart. It has been appeared that "cardiac-specific deletion of MCL-1 in mice" driven to mitochondrial brokenness, impaired autophagy, hypertrophy, and cardiomyopathy with distorted ultrastructure of disorganized sarcomeres and swollen mitochondria.[53-55] Interests, concomitant BAX/BAK knockout in these mice models generally protected the lethality and impaired cardiac function but not the ultrastructure changes of the mitochondria caused by the MCL-1 deletion.[54] Moreover, double knockout of MCL-1 and cyclophilin D, which controls the mitochondrial permeability transition pore, amplified survival and delayed the progression to heart failure.[55] These data may indicate that the cell death associated with MCL-1 deletion contributes to the observed cardiac dysfunction, independently of mitochondrial brokenness. In spite of the fact that the creators did not particularly think about the impact of this mitochondrial dysfunction on the MCL-1 deletion induced cardiomyopathy, they predicted that the observed mitochondrial dysfunction may too contribute to the observed cardiotoxicity, owing to the importance of mitochondria to the cardiac function.[56] They showed that MCL-1 deletion in murine embryonic fibroblast and hepatocyte distorted mitochondrial morphology with abnormal cristae and imperfect electron transport system. In any case, the direct impacts of MCL-1i are different since MCL-1i upregulate, or maybe than deplete, MCL-1.[58,59,60] cells", leading to generally poor cardiomyocyte performance.[57] These impacts have been seen with high doses of the drug and/or prolonged periods of treatment. Treating the hiPSC-CMs with 100 nM S63845 for two weeks impaired cardiomyocyte beating with mitochondrial dysfunction and impeded calcium influx, in spite of live cells, indicating that the impeded cardiomyocyte beating might be independent of cell death.

The contrasting discoveries of these studies with no separation by disease subtype could be clarified if a prognostic role for MCL-1 occurred as it were in specific subsets of patients. More recently, MCL-1 was shown to be widely expressed in breast cancer cellls, notwithstanding of subtype or ER status in spite of the fact that these considers did not report

understanding outcome. Intriguingly low levels of MCL-1 protein were correlated with poor prognosis in a cohort of Luminal A breast cancer patients.[61] The same consider detailed differential associations between MCL1 mRNA levels and prognosis depending on whether patients had received treatment. Intriguingly, as no relationship was found between MCL-1 protein and transcript levels in breast cancer samples[62] it suggests to us that pathways changing MCL-1 interpretation or protein stability have an impact on patient survival.

It is vital to determine how to fit MCL-1 into treatment algorithms for ideal benefits to patients. It shows up that, compared to solid tumors, hematologic malignancies appear special sensitivity to MCL-1.[63,64] Preclinical data demonstrate that MCL-1i are effective in cell lines and tumors that are dependent on MCL-1 for survival. The classic example of this is MM, in which MCL-1 shows up to act as a guardian against apoptosis. Accordingly, there is a good chance for MCL-1i to be successful in patients with MM.[63] AML, on the other hand, appears double or heterogenous reliance on BCL-2/MCL-1. Be that as it may, an approach to testing combination treatments with MCL-1 is required. Future studies are required to decide the ideal combination treatment approaches including MCL-1. It is attainable that the transitory utilize of MCL-1 in mechanism-based combinations may advantage patients without untoward cardiotoxicity.

Amplification of the MCL1 locus is one of the most frequent substantial genetic events in human cancer and overexpression of MCL1 in reaction to chemotherapy or targeted agents is a frequent cause of cancer resistance to current treatments.[64-66] In any case, given recent reports of cardiac safety signals during the early clinical development of MCL1 inhibitors and the emerging biology of MCL1 in normal cardiomyocyte function[67] the key address is whether inhibition of MCL1 is safe to pursue for the treatment of cancer. Here, we present BRD-810, a quickly cleared inhibitor of MCL1 that has strong in cancer cells .We appear that BRD-810 binds to the BH3 groove of MCL1 and blocks sequestration of proapoptotic proteins to rapidly induce caspase activation in MCL1-dependent cell lines with superior potency when compared to other clinical-stage MCL1 inhibitors. BRD-810 induced cancer cell death in a large board of hematologic and cancer cell lines, reflecting its broad potential to advance apoptosis indeed in treatment-refractory settings. Whereas hematologic cancer cell lines were on normal more sensitive to BRD-810 treatment than cancer cells, sensitivity to BRD-810 was observed across cancer cells indications and we propose the ratio of BCL2L1 to BAK mRNA as a potential biomarker for patient stratification in ongoing and

future MCL1 inhibitor trials. This biomarker profile is in line with earlier studies and resonates with the understanding that Bcl-XL can compensate for MCL1 loss whereas BAK is a proapoptotic protein required to induce the intrinsic apoptotic pathway.[68-70]

CONCLUSION

Clinical observations clearly emphasize MCL-1 as a therapeutic target for many cancers. In spite of the fact that MCL-1 that specifically neutralize MCL-1's function are being tested in stage 1 clinical trials, our knowledge about the utilize of MCL-1i in the clinic is right now restricted and depends upon how successful the clinical trials are. Optimizing and designing new potent and particular MCL-1 is critically required in light of the rising role of MCL-1 in tumorigenesis and therapeutic resistance. Identifying biomarkers of response and resistance will direct us to way better uses of MCL-1i in the clinic. In addition, gaining a more profound understanding of the impacts of MCL-1on the non-antiapoptotic work of MCL-1 may offer assistance improve the safety profiles of MCL-1i. Efforts from chemists and pharmaceutical interests, the enthusiasm of scientists to carve out ideal MCL-1i utilization and combination methodologies, and clinical endeavors and observations are at their peak levels and will help make MCL-1 a clinical target.

At the same time, MCL1 is among the most exceedingly over expressed pathologic proteins over all cancers, including strong tumor malignancies in liver, breast, non-small cell lung (NSCLC), urothelial or pancreatic cancer, as well as hematological cancers including acute myeloid or chronic lymphocytic leukemia and non-Hodgkin lymphoma. The overexpression of MCL1 is commonly linked to poor prognosis and resistance to radiation treatment and chemotherapy. Interests, MCL1 was illustrated to too mediate resistance against focused on treatments counting BRAF inhibitors, receptor tyrosine kinase inhibitors and multikinase inhibitors that are commonly used in modern cancer therapy.

Our objective is to outline the function of bromodomain proteins in different biological contexts and to provide insights on the useful role of the bromodomain in these processes from the lessons learned by analyzing the cellular impacts of small-molecule bromodomain inhibitors as potential anti-cancer agents. It can be anticipated that more combinatorial helpful choices will be created to circumvent the development of drug resistance in cancer, with BRD inhibitors emerging as key players in combinatorial treatments, targeting the underlying epigenetic regulatory systems across various cancer types.

REFERENCE

1. Krajewski S, et al. Am J Pathol, 1995; 146: 1309-19.
2. Beroukhim R, et al. Nature, 2010; 463: 899–905. https://doi. org/10.1038/nature08822.
3. Ramsey HE, et al. Cancer Discov, 2018; 8: 1566–81. https:// doi.org/10.1158/2159-8290.CD-18-0140.
4. Balko JM, et al. Cancer Discov, 2014; 4: 232–45. https://doi. org/10.1158/2159-8290.CD-13-0286.
5. Wei SH, et al. Cancer Chemother Pharmacol, 2008; 62: 1055–64. https://doi.org/10.1007/s00280-008-0697-7.
6. Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and dis ease: the balancing act of BCL-2 family proteins. Nat Rev Mol Cell Biol., 2019; 20: 175–9.
7. anahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. Cell, 2011; 144: 646–674.
8. Beroukhim, R. et al. The landscape of somatic copy-number alteration across human cancers. Nature, 2010; 463: 899–905.
9. Campbell KJ, Dhayade S, Ferrari N, Sims AH, Johnson E, Mason SM, et al. MCL 1 is a prognostic indicator and drug target in breast cancer. Cell Death Dis., 2018; 9(2): 1–14. doi: 10.1038/s41419-017-0035-2.
10. Perciavalle RM, Opferman JT. Delving deeper: MCL-1's contributions to normal and cancer biology. Trends Cell Biol., 2013; 23(1): 22–9. doi: 10.1016/j.tcb.2012.08.011.
11. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. Thelandscape of somatic copy-number alteration across human cancers. Nature, 2010; 463(7283): 899–905. doi: 10.1038/nature08822.
12. Zhou P, Levy NB, Xie H, Qian L, Lee C-YG, Gascoyne RD, et al. MCL1 transgenic mice exhibit a high incidence of b-cell lymphoma manifested as a spectrum of histologic subtypes. Blood J Am Soc Hematol, 2001; 97(12): 3902–9. doi: 10.1182/ blood.V97.12.3902.
13. Ramsey HE, Fischer MA, Lee T, Gorska AE, Arrate MP, Fuller L, et al. A novel MCL1 inhibitor combined with venetoclax rescues venetoclax-resistant acute myelogenous leukemia. Cancer Discov, 2018; 8(12): 1566–81. doi: 10.1158/2159 8290.CD-18-0140 .
14. Van Delft MF, Wei AH, Mason KD, Vandenberg CJ, Chen L, Czabotar PE, et al. The BH3 mimetic ABT-737 targets selective bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if mcl-1 is neutralized. Cancer Cell, 2006; 10(5): 389–99. doi: 10.1016/j.ccr.2006.08.027

15. Merino D, Kelly GL, Lessene G, Wei AH, Roberts AW, Strasser A. BH3-mimetic drugs: blazing the trail for new cancer medicines. Cancer Cell., 2018; 34(6): 879–91. doi: 10.1016/j.ccell.2018.11.004.
16. Balko JM, Giltnane JM, Wang K, Schwarz LJ, Young CD, Cook RS, et al. Molecular profiling of the residual disease of triple-negative breast cancers after neoadjuvant chemotherapy identifies actionable therapeutic targets. Cancer Discov, 2014; 4(2): 232–45. doi: 10.1158/2159-8290.CD-13-0286.
17. Wei S-H, Dong K, Lin F, Wang X, Li B, Shen J-j, et al. Inducing apoptosis and enhancing chemosensitivity to gemcitabine via RNA interference targeting mcl-1 gene in pancreatic carcinoma cell. Cancer Chemother Pharmacol, 2008; 62(6): 1055–64. doi: 10.1007/s00280-008-0697-7.
18. Wertz IE, Kusam S, Lam C, Okamoto T, Sandoval W, Anderson DJ, et al. Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. Nature, 2011; 471(7336): 110–4. doi: 10.1038/nature09779.
19. Bhatt S, Pioso MS, Olesinski EA, Yilma B, Ryan JA, Mashaka T, et al. Reduced mitochondrial apoptotic priming drives resistance to BH3 mimetics in acute myeloid leukemia. Cancer Cell, 2020; 38(6): 872–90.e6. doi: 10.1016/j.ccell.2020.10.010.
20. Michels J, Obrist F, Vitale I, Lissa D, Garcia P, Behnam-Motlagh P, et al. MCL-1 dependency of cisplatin-resistant cancer cells. Biochem Pharmacol, 2014; 92(1): 55–61. doi: 10.1016/j.bcp.2014.07.029 .
21. Osaki S, Tazawa H, Hasei J, Yamakawa Y, Omori T, Sugiu K, et al. Ablation of MCL1expression by virally induced microRNA-29 reverses chemoresistance in human osteosarcomas. Sci Rep., 2016; 6: 28953. doi: 10.1038/srep28953.
22. Ohtsu A, Arai S, Sawada T, Kato M, Maeno Y, Miyazawa Y, et al. Fibroblast growth factor receptor inhibitor erdafitinib promotes mcl-1 degradation and synergistically induces apoptosis with bcl-xL/Bcl-2 inhibitor in urothelial cancer cells. Biochem Biophys Res Commun, 2022; 628: 76–83. doi: 10.1016/j.bbrc.2022.08.083.
23. Kozopas KM, Yang T, Buchan HL, Zhou P, Craig RW. MCL1, a gene expressed in programmed myeloid cell differentiation, has sequence similarity to BCL2. Proc Natl Acad Sci U S A., 1993; 90: 3516–20.
24. Petros AM, Olejniczak ET, Fesik SW. Structural biology of the Bcl-2 family of proteins. Biochim Biophys Acta Mol Cell Res., 2004; 1644: 83–94.
25. Sattler M, et al. Structure of Bcl-xL-Bak peptide complex: recognition between regulators of apoptosis. Science., 1997; 275: 983–6.

26. Campbell, K. J. et al. Elevated Mcl-1 perturbs lymphopoiesis, promotes trans formation of hematopoietic stem/progenitor cells, and enhances drug resis tance. Blood, 2010; 116: 3197–3207.
27. Beroukhim, R. et al. The landscape of somatic copy-number alteration across human cancers. Nature, 2010; 463: 899–905.
28. Dahal, L., Kwan, T. O. C., Hollins, J. J. & Clarke, J. Promiscuous and selective: how intrinsically disordered BH3 proteins interact with their pro-survival partner MCL-1. J. Mol. Biol., 2018; 430: 2468–2477.
29. Czabotar PE, et al. Structural insights into the degradation of Mcl-1 induced by BH3 domains. Proc Natl Acad Sci U S A., 2007; 104: 6217–22.
30. Day CL, et al. Solution structure of prosurvival Mcl-1 and characteriza tion of its binding by proapoptotic BH3-only ligands. J Biol Chem., 2005; 280: 4738–44.
31. Soderquist R, Eastman A. BCL2 inhibitors as anticancer drugs: a plethora of misleading BH3 mimetics. Mol Cancer Ther., 2016; 15: 2011–7.
32. Ramsey HE, et al. A Novel MCL1 inhibitor combined with venetoclax rescues venetoclax-resistant acute myelogenous leukemia. Cancer Discov, 2018; 8: 1566–81.
33. Opferman, J.T. etal. Obligateroleofanti-apoptotic MCL-1 in the survival of hematopoietic stem cells. Science, 2005; 307: 1101–1104. 139. Rinkenberger, J. L., Horning, S., Klocke, B., Roth, K. & Korsmeyer, S. J.Mcl-1 deficiency results in peri-implantation embryonic lethality. Genes Dev., 2000; 14: 23–27.
34. Perciavalle, R.M. & Opferman, J.T.Delvingdeeper:MCL-1’s contributions to normal and cancer biology. Trends Cell Biol., 2013; 23: 22–29.
35. Thomas, R. L. & Gustafsson, A.B. MCL1iscritical formitochondrial function and autophagy in the heart. Autophagy, 2013; 9: 1902–1903.
36. Rasmussen, M. L. et al. MCL-1 inhibition by selective BH3 mimetics disrupts mitochondrial dynamics causing loss of viability and functionality of human cardiomyocytes. iScience, 2020; 23: 101015.
37. Opferman, J. T. et al. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. Nature, 2003; 426: 671–676.
38. Dzhagalov, I., Dunkle, A. & He, Y. W. The anti-apoptotic Bcl-2 family member Mcl-1 promotes T lymphocyte survival at multiple stages. J. Immunol, 2008; 181: 521–528.
39. Dzhagalov, I., St John, A. & He, Y. W. The antiapoptotic protein Mcl-1 is essential for the survival of neutrophils but not macrophages. Blood, 2007; 109: 1620–1626.

40. Hikita, H. et al. Mcl-1 and Bcl-xL cooperatively maintain integrity of hepatocytes in developing and adult murine liver. Hepatology, 2009; 50: 1217–1226.
41. Weber, A. et al. Hepatocyte-specific deletion of the antiapoptotic protein myeloid cell leukemia-1 triggers proliferation and hepatocarcinogenesis in mice. Hepatology, 2010; 51: 1226–1236.
42. Kelly, G. L. et al. TargetingofMCL-1killsMYC-drivenmouseandhuman lymphomas even when they bear mutations in p53. Genes Dev., 2014; 28: 58–70.
43. Grabow, S. et al. Critical B-lymphoid cell intrinsic role of endogenous MCL-1 in c-MYC-induced lymphomagenesis. Cell Death Dis., 2016; 7: e2132.
44. Grabow, S., Delbridge, A.R., Aubrey, B. J., Vandenberg, C.J. & Strasser, A. Loss of a single Mcl-1 allele inhibits MYC-driven lymphomagenesis by sensitizing Pro-B cells to apoptosis. Cell Rep., 2016; 14: 2337–2347.
45. Tron AE, Belmonte MA, Adam A, Aquila BM, Boise LH, Chiarparin E, et al. Discovery of mcl-1-specific inhibitor AZD5991 and preclinical activity in multiple myeloma and acute myeloid leukemia. Nat Commun, 2018; 9(1): 1–14. doi: 10.1038/ s41467-018-07551-w
46. Tantawy SI, Sarkar A, Hubner S, Tan Z, Wierda WG, Eldeib A, et al. Mechanisms of MCL-1 protein stability induced by MCL-1 antagonists in b-cell malignancies. Clin Cancer Res., 2023; 29(2): 446–57. doi: 10.1158/1078-0432.CCR-22 2088.
47. Clifton, M. C. et al. A maltose-binding protein fusion construct yields a robust crystallography platform for MCL1. PLoS ONE, 2015; 10: e0125010.
48. Winter, G. xia2: an expert system for macromolecular crystallography data reduction. J. Appl. Cryst., 2010; 43: 186–190.
49. Winter, G. et al. DIALS as a toolkit. Protein Sci., 2022; 31: 232–250.
50. Winn, M. D. et al. Overview of the CCP4 suite and current developments. Acta Crystallogr. D Biol. Crystallogr, 2011; 67: 235–242.
51. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. Acta Crystallogr. D Biol. Crystallogr, 2010; 66: 486–501.
52. Liebschner, D. et al. Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix. Acta. Crystallogr. D Struct. Biol., 2019; 75: 861–877.
53. Moyzis AG, Leon LJ, Najor RH, Gustafsson AB. MCL-1 regulates mitochondrial fission and mitophagy. Circulation, 2017; 136(1): A14178–A. doi: 10.1161/res.119.suppl_1.375

54. Wang X, Bathina M, Lynch J, Koss B, Calabrese C, Frase S, et al. Deletion of MCL-1 causes lethal cardiac failure and mitochondrial dysfunction. Genes Dev., 2013; 27(12): 1351–64. doi: 10.1101/gad.215855.113
55. ThomasRL, RobertsDJ, KubliDA, LeeY, QuinsayMN, OwensJB, etal. Lossof MCL-1leads to impaired autophagy and rapid development of heart failure. Genes Dev., 2013; 27(12): 1365–77. doi: 10.1101/gad.215871.113
56. Kuznetsov AV, Margreiter R. Heterogeneity of mitochondria and mitochondrial function within cells as another level of mitochondrial complexity. Int J Mol Sci., 2009; 10(4): 1911–29. doi: 10.3390/ijms10041911
57. Rasmussen ML, Taneja N, Neininger AC, Wang L, Robertson GL, Riffle SN, et al. MCL-1 inhibition by selective BH3 mimetics disrupts mitochondrial dynamics causing loss of viability and functionality of human cardiomyocytes. Iscience, 2020; 23(4): 101015. doi: 10.1016/j.isci.2020.101015
58. Kotschy A, Szlavik Z, Murray J, Davidson J, Maragno AL, Le Toumelin-Braizat G, et al. The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models. Nature, 2016; 538(7626): 477–82. doi: 10.1038/nature19830
59. Caenepeel S, Brown SP, Belmontes B, Moody G, Keegan KS, Chui D, et al. AMG176, a selective MCL1 inhibitor, is effective in hematologic cancer models alone and in combination with established therapies. Cancer Discov, 2018; 8(12): 1582–97. doi: 10.1158/2159-8290.CD-18-0387
60. Tron AE, Belmonte MA, Adam A, Aquila BM, Boise LH, Chiarparin E, et al. Discovery of mcl-1-specific inhibitor AZD5991 and preclinical activity in multiple myeloma and acute myeloid leukemia. Nat Commun, 2018; 9(1): 1–14. doi: 10.1038/ s41467-018-07551-w.
61. Young, A. I. et al. MCL-1 inhibition provides a new way to suppress breast cancer metastasis and increase sensitivity to dasatinib. Breast Cancer Res., 2016; 18: 125.
62. O'Driscoll, L. et al. Expression and prognostic relevance of Mcl-1 in breast cancer. Anticancer Res., 2004; 24: 473–482.
63. Caenepeel S, Brown SP, Belmontes B, Moody G, Keegan KS, Chui D, et al. AMG176, a selective MCL1 inhibitor, is effective in hematologic cancer models alone and in combination with established therapies. Cancer Discov, 2018; 8(12): 1582–97. doi: 10.1158/2159-8290.CD-18-0387
64. Tron AE, Belmonte MA, Adam A, Aquila BM, Boise LH, Chiarparin E, et al. Discovery of mcl-1-specific inhibitor AZD5991 and preclinical activity in multiple myeloma and

acute myeloid leukemia. Nat Commun, 2018; 9(1): 1–14. doi: 10.1038/ s41467-018-07551-w.

65. Kaufmann, S. H. et al. Elevated expression of the apoptotic regulator MCL-1 at the time of leukemic relapse. Blood, 1998; 91: 991–1000.
66. Nakano, T., Go, T., Nakashima, N., Liu, D. & Yokomise, H. Overexpression of antiapoptotic MCL-1 predicts worse overall survival of patients with non-small cell lung cancer. Anticancer Res., 2020; 40: 1007–1014.
67. Shi, X., Liu, S., Kleeff, J., Friess, H. & Büchler, M. W. Acquired resistance of pancreatic cancer cells towards 5-fluorouracil and gemcitabine is associated with altered expression of apoptosis-regulating genes. Oncology, 2002; 62: 354–362.
68. Wang, X. et al. Deletion of MCL-1 causes lethal cardiac failure and mitochondrial dysfunction. Genes Dev., 2013; 27: 1351–1364.
69. Caenepeel, S. et al. AMG 176, a selective MCL1 inhibitor, is effective in hematologic cancer models alone and in combination with established therapies. Cancer Discov, 2018; 8: 1582–1597.
70. Wolf, P., Schoeniger, A. & Edlich, F. Pro-apoptotic complexes of Bax and Bak on the outer mitochondrial membrane. Biochim. Biophys. Acta Mol. Cell Res., 2022; 1869: 119317.
71. Dzhagalov, I., Dunkle, A. & He, Y.-W. The anti-apoptotic Bcl-2 family member MCL-1 promotes T lymphocyte survival at multiple stages. J. Immunol, 2008; 181: 521–528.
72. Ulrike Rauh 1, BRD-810 is a highly selective MCL1 inhibitor with optimized in vivo clearance and robust efficacy in solid and hematological tumor model, https://doi.org/10.1038/s43018-024-00814-
73. Arnold Bolomsky1, MCL-1 inhibitors, fast-lane development of a new class of anti-cancer agent, Bolomsky et al. J Hematol Oncol, 2020; 13: 173.
74. Samuel P Boyson, Functional Roles of Bromodomain Proteins in Cancer, roles of Bromodomain Proteins in Cancer. Cancers, 2021; 13: 3606. https://doi.org/10.3390/ cancers1314360.
75. Xiaowei Wu Abstract 1, Ubiquitination and deubiquitination of MCL1 in cancer: deciphering chemoresistance mechanisms and providing potential therapeutic options, Official journal of the Cell Death Differentiation Association, Wu et al. Cell Death and Disease, 2020; 11: 55.
76. Shady I. Tantawy, Decoding the mechanism behind MCL-1 inhibitors: A pathway to understanding MCL-1 protein stability, : Mechanisms of MCL-1 protein stability induced

by MCL-1 antagonists in B-cell malignancies. Clinical Cancer Res., 2023; 29: 446–57. https://doi.org/10.1158/1078-0432.ccr-22-2088.

77. Andrew W. Roberts 1, BCL2 and MCL1 inhibitors for hematologic malignancies, 30 September 2021; 138: 13.
78. Shady I. Tantawy1, Targeting MCL-1 protein to treat cancer: opportunities and challenges, 2023; 13. | https://doi.org/10.3389/fonc.2023.1226289.
79. Matilde Murga1, Emerging concepts in drug discovery for cancer therapy, Molecular Oncology, 2022; 16: 3757–3760.
80. Clint Mitchell1, Inhibition of MCL-1 in breast cancer cells promotes cell death in vitro and in vivo, DOI: 10.4161/cbt.10.9.13273.
81. Kirsteen J. Campbell, MCL-1 is a prognostic indicator and drug target in breast cancer, Campbell et al. Cell Death and Disease, 2018; 9: 19.