Mcl-1, a Bcl-2 family member, plays a central role in the ability of cancer cells to resist apoptosis and growth arrest, yet effective clinical treatments targeting it remain out of reach. A recent study by Guo Wei and colleagues utilized a high-throughput screening method to analyze a library of compounds, to identify those that preferentially repress \textit{MCL1} expression [1]. By inhibiting \textit{MCL1} expression relative to that of other Bcl-2 family members (both anti and pro-apoptotic), cells were induced into apoptotic cell death. Their screen identified a number of commercially available compounds, including several drugs widely used in cancer chemotherapy, and suggests their use could improve treatment outcomes in Mcl-1-dependent tumors.

Bcl-2 family proteins: major arbitrators of cell survival

Members of the Bcl-2 family are highly conserved proteins intimately involved in regulating cell survival. Bcl-2 family members can be divided into three groups based on function and structural homology between the four conserved Bcl-2 homology (BH) domains. Of these, the most highly pro-survival members share multiple BH domains, including Bcl-2, Bcl-xL and Mcl-1. One of the major survival mechanisms, for which there has been significant clinical interest, is through heterodimerization with pro-apoptotic family members (BH3-only type) through a cleft consisting of multiple BH domains [2]. Despite structural and functional similarities, Mcl-1 possesses distinct characteristics that set it apart from other Bcl-2 family members. For instance, the aforementioned BH3-only binding cleft within Mcl-1 differs from its congeners and this is a major reason why some BH3 inhibitors fail to recognize it. For instance, the expression of \textit{MCL1} contributes to resistance to the novel Bcl-2/Bcl-xL inhibitor ABT-263 (Abbott Laboratories, Abbott Park, IL, USA) [3]. Also, from a drug development standpoint, the Mcl-1 protein has a particularly short half-life of a few hours, and undergoes rapid degradation. From a potential toxicity standpoint, \textit{MCL1} expression is essential for the survival and function of hematopoietic stem cells [4].

Complicating its role as an anti-apoptotic agent, Mcl-1 appears to have distinct additional functions, such as resisting chemotherapy-induced senescence [5]. Conversely, ablation of \textit{MCL1} in experimental systems results in enhanced sensitivity to chemotherapy, and can induce dramatic levels of apoptosis and senescence even in untreated tumors [5,6]. Regulating the balance between apoptosis and senescence is a key function of the Bcl-2 family. Given the powerful anti-apoptotic and anti-senescence abilities of Mcl-1 in particular, it is not surprising that cancers have taken advantage of these pathways to promote survival and growth.

Targeting Mcl-1 and the Bcl-2 family for cancer therapy

Mcl-1 plays a unique role in tumorigenesis and cancer progression; the \textit{MCL1} locus (as well as \textit{BCL2}) is one of the most highly amplified in all human cancers, with a
Although Mcl-1 was initially studied in hematopoietic tumors, it is clear that many solid tumors are also dependent on this survival factor. Despite the importance of Bcl-2 molecules like Mcl-1, clinically effective inhibitors remain elusive. For example, in clinical trials the anti-sense oligonucleotide Genasense (Genta, Berkeley Heights, NJ, USA), specifically targeting BCL2 mRNA for degradation, has shown little promise. Similarly, the pan-Bcl-2 inhibitors (which target Mcl-1) AT-101 (Ascenta Therapeutics, Malvern, PA, USA) and Obatoclax (Cephalon, Frazer, PA, USA) showed promise in pre-clinical models, but have yet to demonstrate significant clinical benefit. Interestingly, ABT-263 (Navitoclax), which does not target Mcl-1, has shown some activity in a variety of cancers, but with significant toxicities [8]. Many of the clinically relevant Bcl-2 family targeting drugs are shown in Figure 1.

These inhibitors assume that the main function of Mcl-1 is through its BH3-binding pocket, a supposition that is now being challenged [5]. This concern can be partially addressed through the use of agents that reduce MCL1 expression by decreased binding/sequestration of BH3-only proteins. Drugs such as ABT-737 and ABT-263 inhibit the BH3 domains of Bcl-2 and Bcl-xL, while Mantoclax inhibits Mcl-1. AT-101 and Obatoclax inhibit the BH3 domain of all Bcl-2 family members. While inhibition of the BH3-binding domains enhances apoptosis, the domains of Mcl-1 that resist non-apoptotic processes (for example, senescence) remain unaffected.

A high-throughput approach to MCL1 repression

With the need for more specific Mcl-1 inhibitors in mind, Guo Wei and colleagues [1] screened for small molecule repressors of MCL1 expression. Using a Luminex bead-based method capable of examining global mRNA levels, the screen focused on compounds that repressed MCL1 expression (while not immediately causing cell death) relative to 48 other pro-apoptotic genes. Screening a library of 2,922 compounds obtained from the Broad Institute Chemical Biology Program, which included 530 US Food and Drug Administration-approved drugs, gave 24 candidates, of which 7 were chosen for further study based on commercial availability (likely excluding many newly discovered inhibitors) and clear dose-related effects.

This approach holds promise for the development of more specific Mcl-1 inhibitors, which could ultimately lead to more clinically effective treatments.

Figure 1. Clinically relevant strategies for specific inhibition of Bcl-2 family proteins. (a) Inhibition of the BH3-binding domain leads to apoptosis by decreased binding/sequestration of BH3-only proteins. Drugs such as ABT-737 and ABT-263 inhibit the BH3 domains of Bcl-2 and Bcl-xL, while Mantoclax inhibits Mcl-1. AT-101 and Obatoclax inhibit the BH3 domain of all Bcl-2 family members. While inhibition of the BH3-binding domains enhances apoptosis, the domains of Mcl-1 that resist non-apoptotic processes (for example, senescence) remain unaffected. (b) Repression of Bcl-2 family protein production leads to both apoptotic and non-apoptotic growth arrest by limiting the availability of each protein. Specific inhibitors have been designed, such as Genasense, an antisense oligonucleotide that targets BCL2 mRNA. On the other hand, the compounds identified by Wei et al. preferentially affect MCL1. Future combinations of these therapies could hold the key for more clinically effective treatments.
replication of MCL1 mRNA levels. These compounds included triptolide (a herbal extract possessing pro-apoptotic effects), the transcription inhibitors 5,6-dichloro-benzimidazole riboside and actinomycin D, a kinase inhibitor 5-iodotubercidin, and the anthracycline drugs doxorubicin, daunorubicin and epirubicin. Resistance to these compounds could be induced by ectopic MCL1 expression, and RNA interference (RNAi) knockdown of MCL1 could phenocopy the transcriptional profiles of drug treatment in multiple tumor cell lines.

The compounds found induced similar transcriptional responses despite having differing cytotoxic mechanisms, strongly suggesting these agents caused a generalized repression of transcription. This hypothesis was strengthened by the fact that the anthracycline drugs identified, known for their inhibition of DNA topoisomerase II, induced markedly different transcriptional profiles compared with etoposide, another topoisomerase inhibitor, which did not affect MCL1.

These data indicate that the specificity of the transcription repressing (TR) compounds for MCL1 is primarily due to the short half-life of Mcl-1 protein, in that its high rate of degradation reduced its relative production while transcription was globally repressed. In effect, these compounds act as indirect suppressors of MCL1 expression, highlighting the limitations of the screening strategy. While using general transcription repression rather than specific inhibition has the advantage of identifying a wide array of compounds utilizing potentially novel mechanisms, clearly this approach cannot distinguish between target-specific mechanisms (that is, those that act directly on MCL1) and general mechanisms that happen to affect MCL1 preferentially. While the compounds chosen for further study induced similar indirect effects on MCL1 expression, it is possible that other compounds identified in the screen, but not yet tested, could be more specific repressors. In fact, other studies have found that low levels of doxorubicin, identified in this screen, do not significantly alter MCL1 expression [5].

Cancer is a heterogeneous disease, and tumor cells can have differing dependencies on pro-survival mechanisms regulated by Bcl-2 family proteins. Indeed, while conducting their screens, Wei et al. [1] identified a number of cell lines that continued to resist apoptosis after knockdown of MCL1 by TR compounds or RNAi. The authors returned to their genomic screening model to identify genes whose expression correlated with sensitivity or resistance to MCL1 repression. Genetic profiles (gene copy number and expression data) for over 18,000 genes and known mutation data for 34 genes over 72 cell lines were correlated with relative sensitivity to TR compounds. The screen identified a single candidate: the closely related Bcl-2 family member Bcl-xL. High BCL2L2 (the gene encoding Bcl-xL) expression correlated with resistance to MCL1 repression while low BCL2L2 expression was associated with sensitivity. Both Mcl-1 and Bcl-xL could bind the pro-apoptotic proteins Bim and Bak, and the release of these molecules contributed to apoptosis induced by TR compound treatment, suggesting a level of redundancy not previously reported.

Given that MCL1 expression is a known resistance mechanism for inhibition of other BCL2 genes, this result is not surprising. Further, it suggests that transcription-repressing drugs would be most efficacious in cancers possessing low BCL2L2 expression. Also, using the technology employed by Wei et al., additional drugs could be discovered by focusing on BCL2L2 expression to synergize with those already identified that affect MCL1.

The clinical promise of transcriptional repressors

An intriguing aspect of this study is that many of the identified TR compounds are already in clinical use, particularly the anthracycline drugs. The evidence that they exert at least part of their cytotoxic effect via repression of MCL1 is novel, and suggests that regimens designed to take advantage of this process could enhance its efficacy. Further studies to identify optimal dosing and scheduling are clearly needed. With the apparent importance of MCL1 expression in many cancers, the hope is that beneficial synergies could be found, particularly in combination with other Bcl-2 family inhibitors. Finally, the chemical genomic approach taken by Wei et al. has promise as a method to discover other novel specific transcription repressors of MCL1 and other genes, and as a means of identifying possible combinations of existing chemotherapeutics to optimize their efficacy. This strategy could be especially helpful in combating therapy-resistant cancers, and used in personalized cancer-treatment models with a goal of optimally inhibiting specific genes and pathways.

Abbreviations
BH domain, Bcl-2 homology domain; RNAi, RNA interference; TR, transcription repressing.

Competing interests
The authors declare that they have no competing interests.

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References
1. Wei G, Margolin AA, Haery L, Brown E, Cucolo L, Julian B, Shehata S, Kung AL, Beroukhim R, Golub TR: Chemical genomics identifies small-molecule MCL1 repressors and BCL-xL as a predictor of MCL1 dependency. Cancer Cell 2012, 21:547-562.
2. Danial NN, Korsmeyer SJ: Cell death: critical control points. Cell 2004, 116:205-219.
3. van Delf MF, Wei AH, Mason KD, Vandenberg CJ, Chen L, Czabanot PE, Willis SN, Scott CL, Day CL, Cory S, Adams JM, Roberts AW, Huang DC: 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, 10:389-399.
4. Opferman JT, Iwasaki H, Ong CC, Suh H, Mizuno S, Akashi K, Korsmeyer SJ: Obligate role of anti-apoptotic MCL-1 in the survival of hematopoietic stem cells. Science 2005, 307:1101-1104.

5. Bolesta E, Pfannenstiel LW, Demelash A, Lesniewski ML, Tobin M, Schlanger SE, Nallar SC, Papadimitrou JC, Kalvakolanu DV, Gastman BR: Inhibition of mcl-1 promotes senescence in cancer cells: implications for preventing tumor growth and chemotherapy resistance. Mol Cell Biol 2012, 32:1879-1892.

6. Glaser SP, Lee EF, Trounson E, Bouillet P, Wei A, Fairlie WD, Izon DJ, Zuber J, Rappaport AR, Herold MJ, Alexander WS, Lowe SW, Robb L, Strasser A: Anti-apoptotic Mcl-1 is essential for the development and sustained growth of acute myeloid leukemia. Genes Dev 2012, 26:120-125.

7. Boroukhim R, Merkel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, Barretina J, Boehm JS, Dobson J, Urashima M, Mc Henry KT, Pinchback RM, Ligon AH, Cho YJ, Haery L, Greulich H, Reich M, Winckler W, Lawrence MS, Weir BA, Tanaka KE, Chiang DY, Bass AJ, Loo A, Hoffman C, Prensner J, Liefeld T, Gao Q, Yecies D, Signoretti S, et al.: The landscape of somatic copy-number alteration across human cancers. Nature 2010, 463:899-905.

8. Wilson WH, O’Connor OA, Czuczman MS, LaCasce AS, Gerecitano JF, Leonard JP, Tulipale A, Dunleavy K, Xiong H, Chiu YL, Cui Y, Busman T, Elmore SW, Rosenberg SH, Krivoshik AP, Enschede SH, Humenickhouse RA: Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. Lancet Oncol 2010, 11:1149-1159.

9. Gores GJ, Kaufmann SH: Selectively targeting Mcl-1 for the treatment of acute myelogenous leukemia and solid tumors. Genes Dev 2012, 26:305-311.

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