

The Challenge of Drugging Undruggable Targets in Cancer: Lessons Learned from Targeting BCL-2 Family Members

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Abstract The genomic and proteomic revolutions have provided us with an ever-increasing number of mechanistic insights into cancer pathogenesis. Mutated genes and pathologic protein products have emerged as the basis for modern anticancer drug development. With the increasing realization of the importance of disrupting oncogenic protein-protein interaction, new challenges have emerged for classical small molecule and protein-based drug modalities, i.e., the critical need to target flat and extended protein surfaces. Here, we highlight two distinct technologies that are being used to bridge the pharmacologic gap between small molecules and protein therapeutics. With the BCL-2 family of survival proteins as their substrate for intracellular targeting, we conclude that peptide stapling and fragment-based drug discovery show promise to traverse the critical surface features of proteins that drive human cancer.

An unfortunate irony of the revolution in understanding cancer as a genetic disease is that not a single approved therapy directly targets the products of the most frequently activated genes in human cancers. Why, for example, are there as yet no direct-acting inhibitors of c-Myc or K-Ras, oncoproteins that are each mutationally activated in nearly half of all human cancers? Validated by an overwhelming body of high-quality biological data, big-league cancer drivers would seem to represent irresistible targets for drug discovery, so why the dearth of drugs that target them? The answer is that many of the targets in cancer that are most attractive from a biological perspective are considered intractable from a chemical standpoint, beyond the targeting capabilities of established drug discovery technologies. Such chemically intractable targets have come to be branded, in drug discovery parlance, as undruggable. What is it that makes some targets druggable and others undruggable, and what are the prospects for drugging the undruggable ones? Here, we discuss this issue and highlight recent successes with one prominent class of targets previously thought to be as intractable as Myc and Ras, i.e., members of the BCL-2 family. Although this exposition focuses on issues of chemical

tractability for certain targets in the cell death pathway, companion articles in this issue of *Clinical Cancer Research* FOCUS provide the rationale for drugging these critical control points of apoptosis (1–4).

Broadly speaking, there exist only two major structural classes of approved drugs, small molecules and protein therapeutics (also known as biologics). Small molecules typically possess <100 atoms (molecular weight <1,000 Da), which endows them with only modest overall surface area available to contact a protein target. Most proteins literally engulf their small molecule partners in order to maximize hydrophobic contact surface area and produce a stable complex (Fig. 1, *left*). This being the case, the hallmark characteristic of targets considered druggable is the presence on its solvent-accessible surface of a deep invagination lined by hydrophobic amino acid side chains, a so-called hydrophobic pocket (5–7). Protein therapeutics, on the other hand, typically possess many thousands of atoms and therefore have a surfeit of contact surface area available to engage a target. For this reason, protein therapeutics tend to bind relatively flat but extensive surfaces on their targets, and these need not even be particularly hydrophobic (Fig. 1, *right*). Small molecules can usually be tailored to enable rapid passive diffusion across mammalian cell membranes, but protein therapeutics are unable to traverse this cellular barrier. Thus, the targeting range of small molecules is limited to proteins having hydrophobic pockets, whereas the range of protein therapeutics is restricted to extracellular targets. How seriously do these limitations curtail the utility of these two classes of drugs? Surface character analyses of the extensive collection of protein structures now available in the Protein Data Bank suggest that only approximately 3,000 of the ~25,000 proteins encoded in the human genome⁴ possess a hydrophobic pocket suitable for tight

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⁴ <http://www.ornl.gov/sci/techresources/Human.Genome/faq/genenumber.shtml#first>

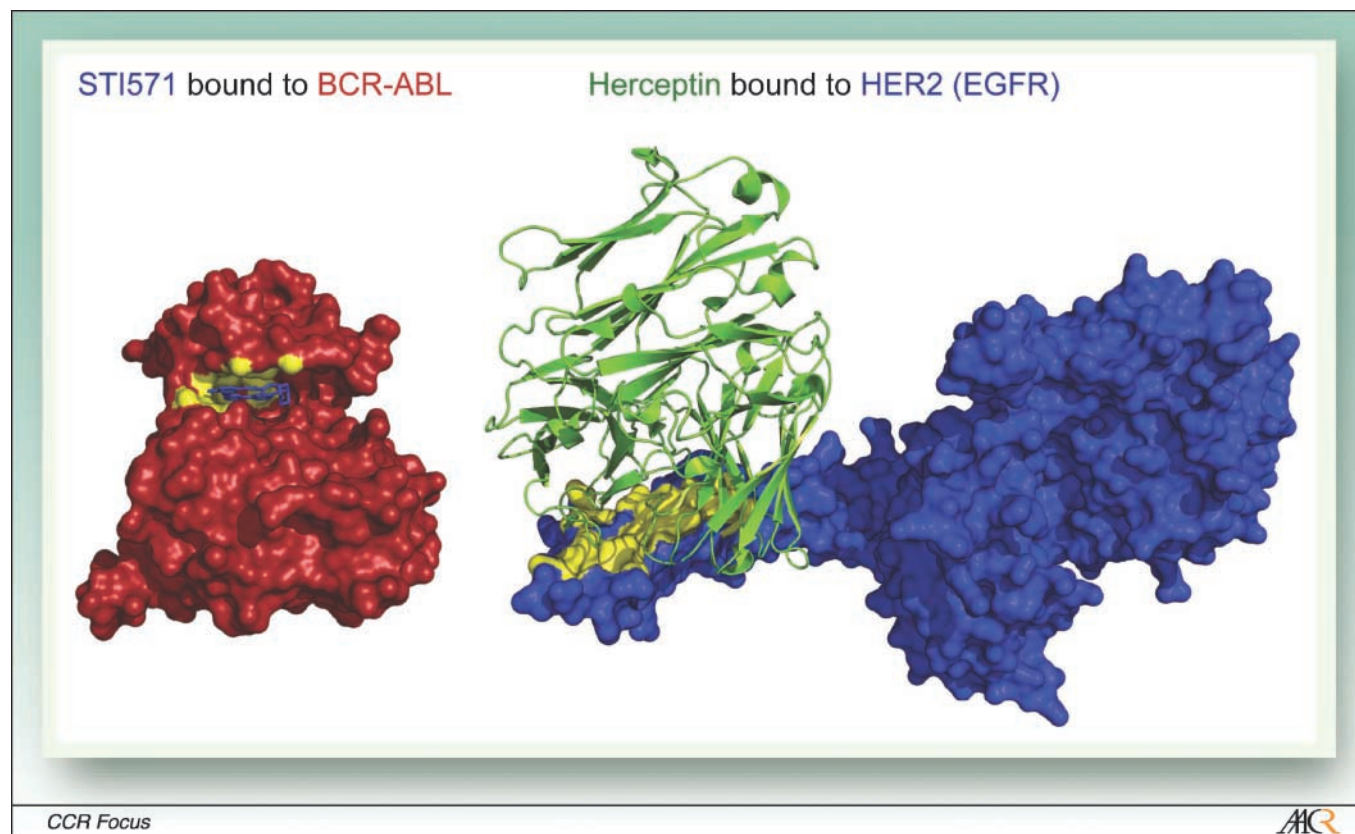


Fig. 1. Surface characteristics of small molecule – protein interactions versus protein-protein interactions. Representative examples of oncogenic protein targets bound to a small molecule or protein therapeutic. Left, X-ray structure (ref. 33) of the Abl kinase domain (*red space-filling model*) bound to STI-571 (imatinib; *blue framework model*). Right, X-ray structure (ref. 34) of the extracellular domain of the epidermal growth factor receptor (*blue space-filling model*) bound to the antigen-combining domain of Herceptin (*green ribbon trace*). In each case, the region of the target protein that is in direct contact with the drug is highlighted in yellow. Note the deep, narrow involution of the Abl surface in contact with STI-571 contact surface, in contrast to the flat, extended patch of the epidermal growth factor receptor surface that contacts Herceptin.

binding of small drug-like molecules (5–7). On the other hand, bioinformatics studies predict that <10% of all human proteins are resident on the cell surface or are secreted.⁵ These considerations lead to the disheartening revelation that the majority of all existing targets, as high as 75% to 80%, are beyond the reach of the two established classes of drugs.

Among the most intractable of targets are those that exert their biological function through engagement in intracellular protein-protein interactions. The combination of flat, extended contact surfaces and intracellular localization makes such targets risky prospects at best for intervention using either biologics or small molecules (8). Representatives of such targets are BCL-2 family members, the biological features of which are discussed in detail elsewhere in this issue of *CCR Focus* (1). The BCL-2 family's network of protein interactions operates at the crossroads of the cellular decision to live or die; aberrations in this decision circuit can enable transformed cells to evade death. Hence, the BCL-2 pathway has, from a biological standpoint, been a compelling target for drug development for more than two decades. The critical event in

BCL-2 family signal propagation is the direct association of a protein containing a BH3 death domain with a multidomain BCL-2 family member. Notwithstanding the diversity of interacting protein pairs, their interaction mode seems to consist of a shallow groove on the multidomain protein serving as a receptor site for docking of the signature α -helical BH3 domain contained within all BCL-2 family members (Figs. 2 and 3; ref. 9). Although intact pairs of these proteins have thus far proven refractory to high-resolution structure determination, numerous nuclear magnetic resonance and X-ray structures of multidomain BCL-2 family members bound to BH3 domain peptides are now available (9–13). On the one hand, these structures put a much-needed molecular face on the target of interest—the interaction surface of the multidomain receptor—but at the same time, they show the lack of deep hydrophobic pockets on that surface (Fig. 3), thus intensifying concerns regarding suitability as a target for small molecule intervention. The unique challenges implicit in the structures of BCL-2 family members bound to BH3 domain peptides suggests that novel drug discovery approaches are required to “drug” these targets. Here, we focus on the two completely distinct strategies that have emerged thus far.

⁵ J. Quackenbush, personal communication.

Peptide Stapling

The realization that most targets are poorly suited to engagement by established drug classes has provided powerful impetus for attempts to discover entirely new structural classes of drugs that could fill the existing gaps in drug discovery space. Of particular interest would be some sort of molecule that combined the versatile surface-recognition properties of biologics with the synthetic manipulability and cell-permeability of small molecules, that is, a synthetic biologic. In attempting to create a synthetic biologic that could target BCL-2 family members, we directed our attention at the outset to BH3 domain peptides because they represented a natural solution to the problem of targeting multidomain BCL-2 family proteins. This positive impetus was, however, weighed against one enormous negative, namely the nearly universal consensus among drug discovery professionals that peptides cannot be drugs.

Peptides in general suffer from several major drawbacks. Most are unable to maintain a well-defined three-dimensional

structure, existing instead as a vast ensemble of conformational isomers that interconvert on the nanosecond time scale. Embedded in a BH3-only protein, the BH3 domain is locked into an α -helical structure through scaffolding interactions with the neighboring parts of the protein; deprived of this scaffolding, BH3 peptides exhibit little if any propensity to form an α -helix (14, 15). This floppiness of BH3 peptides, indeed, of all noncyclic peptides, severely weakens their receptor binding because the substantial entropic penalty associated with adopting an ordered, bioactive structure factors into the overall binding energetics. Secondly, the hydrolytic active sites of proteases can gain ready access to the exposed amide linkages of disordered peptides but not the nested peptide bonds of folded structures, and therefore, unfolding of the peptide causes a dramatic increase in its rate of proteolytic degradation. Lastly, exposed backbone amides have significant ionic character and are therefore strongly solvated in water. These attributes impede passive diffusion across the outer cell membrane and promote rapid clearance *in vivo* through renal

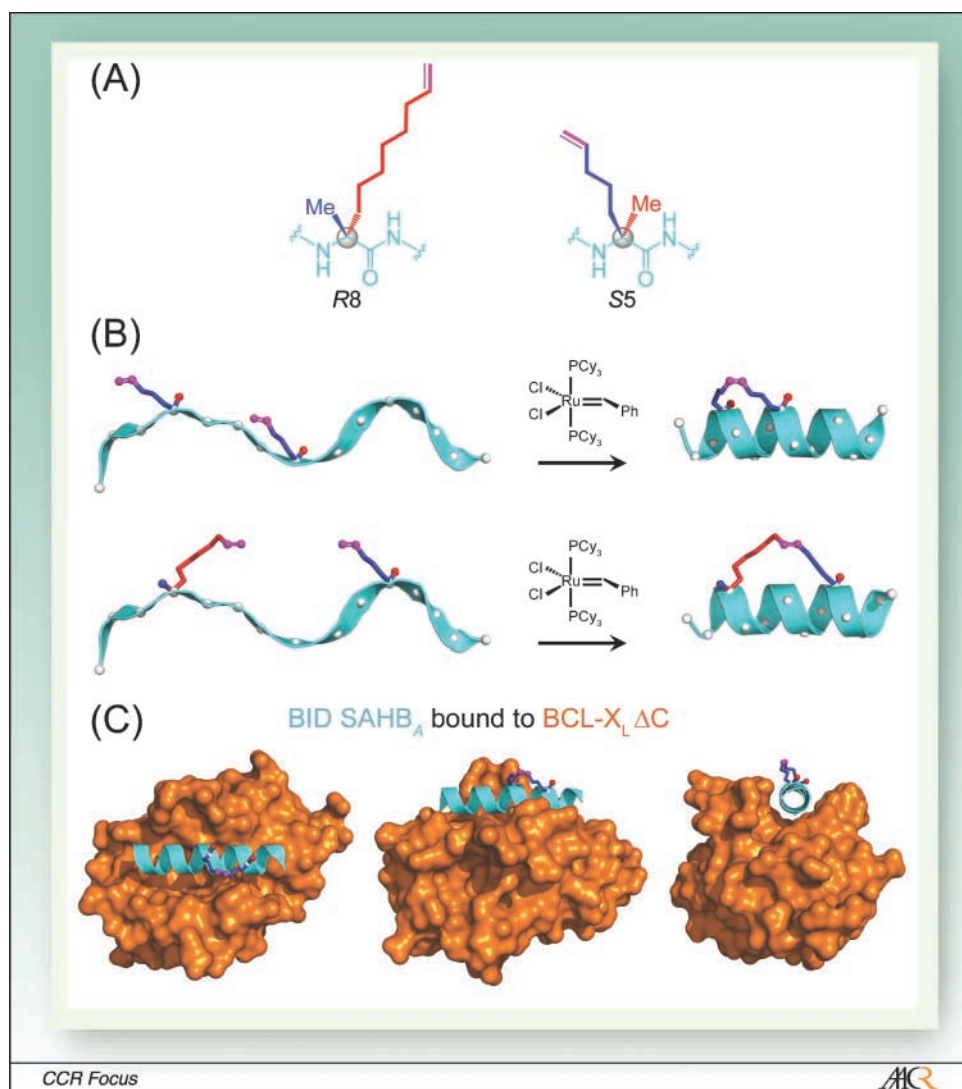


Fig. 2. Overview of peptide stapling. *A*, structures of the two designer amino acids used to introduce all-hydrocarbon staples into peptides. Both possess an α -methyl group and an α -alkenyl group, but they are of opposite stereochemical configuration and differ in length at the alkenyl chain. *B*, closure of the macrocyclic ring by Ru-mediated olefin metathesis enforces the α -helical structure in an otherwise poorly ordered peptide. For $i + 4$ stapling, two units of the same designer amino acid, S5, are incorporated at sites separated by three intervening residues from the original peptide; for $i + 7$ stapling, one unit of R8 is incorporated on the NH₂-terminal side and one unit of S5 on the COOH-terminal side, separated by six original residues (red ball, α -methyl group). The $i + 4$ staple spans one turn of the helix, whereas the $i + 7$ staple spans two turns. *C*, model of the stapled peptide BID-SAHB_A (cyan) bound to BCL-X_L Δ C (orange), viewed from three different perspectives. This model was constructed grafting the staple, in the appropriate position, onto the structure of the BAD BH3 bound to BCL-X_L Δ C (10), then truncating the ends to correspond with those in BID-SAHB_A.

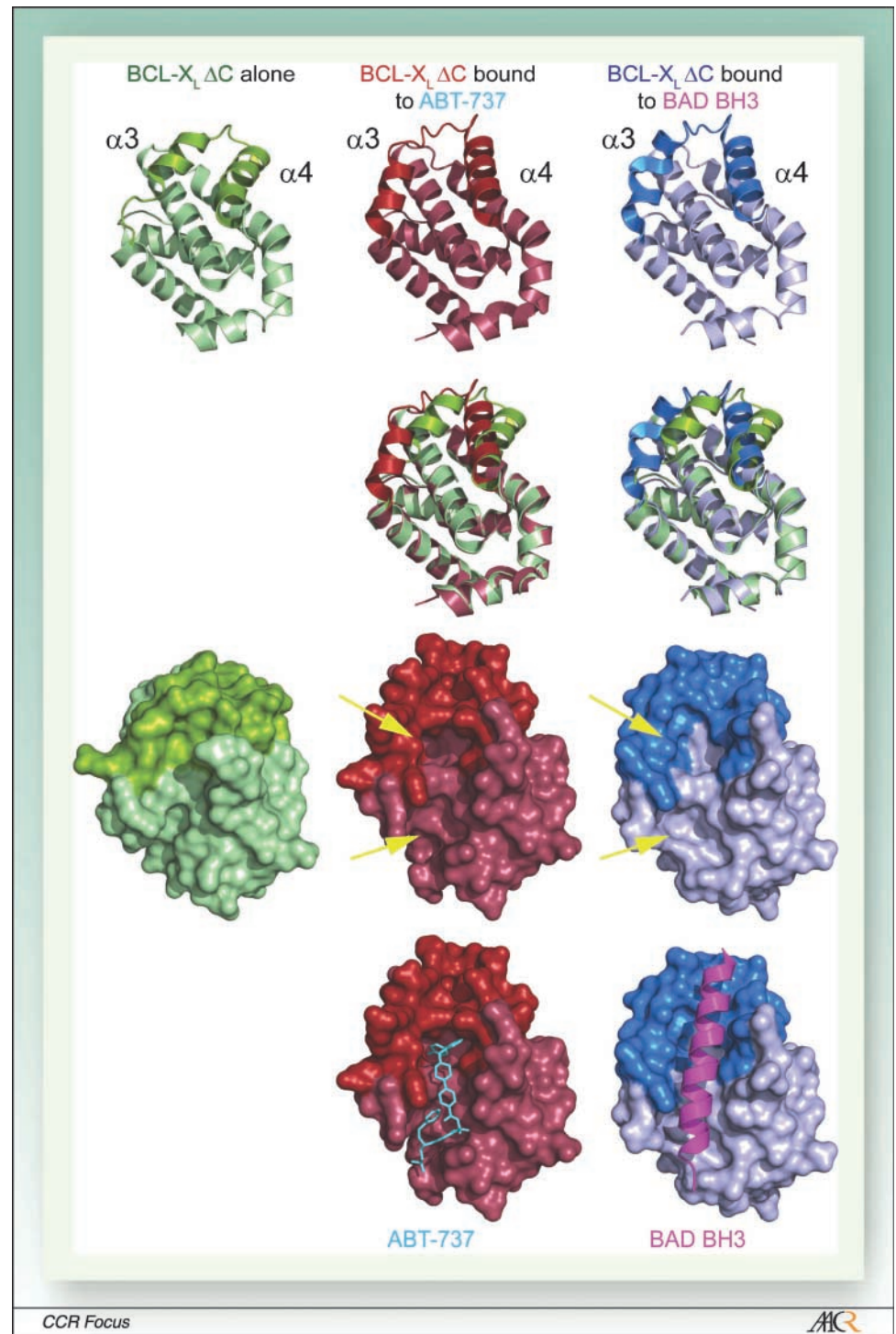


Fig. 3. Structure of Bcl-X_L ΔC and Bcl-X_L ΔC – ligand complexes. Top row, ribbon trace of Bcl-X_L ΔC unliganded (*green*; ref. 35; for an earlier structure at lower resolution, see ref. 31), bound to ABT-737 (*red*; ref. 36), and bound to a BAD BH3 peptide (6); the ligands are not shown and the α3-α4 region is highlighted. Second row, RMSD overlay of unliganded BCL-X_L ΔC (*green*) onto the structures of the protein when bound to ABT-737 (*red*) or a BAD BH3 peptide (*blue*). Note the change in the overall structure of the α3-α4 region that occurs upon ligand binding. Third row, surface representation of the Bcl-X_L ΔC forms corresponding with the top row. Yellow arrows, hydrophobic pockets that become evident in BCL-X_L upon ligand binding but are largely absent in the unliganded protein. Note also the greater depth of the pockets in the ABT-737-liganded form, as compared with the BAD BH3-bound form. Bottom row, surface representations with bound ligands shown.

filtration. Any one of these detriments would severely diminish the prospects of a peptide becoming a drug, and their combination has effectively debilitated the discovery of peptide drugs.

We reasoned that it might be possible to overcome all of these problems in one operation by introducing a synthetic modification that would lock the BH3 peptide into its

bioactive, α-helical conformation. Based on the results of exploratory design studies (16), we used an all-hydrocarbon staple that combines, in one synthetic unit, two very potent α-helix-stabilizing modifications, α-methylation and macrocyclization (10). Introduction of the staple entails a straightforward modification of standard peptide synthesis, with α-methyl, α-alkenyl amino acids being introduced at two

positions along the peptide chain, separated by either three or six intervening residues ($i + 4$ or $i + 7$ spacing, respectively); these spacings place the stapling amino acids on the same face of the α -helix, straddling either one ($i + 4$) or two ($i + 7$) helical turns. The fully elongated, resin-bound peptide is exposed to a ruthenium catalyst that promotes cross-linking of the alkenyl chains through olefin metathesis (17), thereby forming an all-hydrocarbon macrocyclic cross-link.

Our initial studies used a 21-amino acid peptide comprising the BID BH3 domain (14). We chose this particular BH3 domain because the available evidence suggested that BID could interact with both antiapoptotic and proapoptotic multidomain BCL-2 family proteins (18). Based on the results of a limited screen of $i + 4$ and $i + 7$ stapled BID BH3 peptides, termed stabilized α -helices of BCL-2 domains (SAHB), we selected one of the most helical and protease-resistant compounds, BID SAHB_A, for further analysis. Mere introduction of the staple into BID SAHB_A increased its α -helical content from $\sim 20\%$ to $\sim 90\%$ in water at room temperature, increased the *ex vivo* serum half-life 10-fold to ~ 30 h, and increased the affinity for BCL-2 from 269 nM to 39 nM/L (14). Most remarkable, however, was the observation that cells took up BID SAHB_A through an energy-dependent, pinocytotic pathway, such that the peptide was found localized to the outer mitochondrial membrane, in which its direct targets reside, within 24 h of exposure to the intact cells. The unmodified BID BH3 peptide was completely impermeable to cells. In tissue culture, BID SAHB_A alone showed potent cell-killing activity at low micromolar concentrations. Importantly, neither the unmodified BID BH3 nor a control stapled peptide having a single point mutation [BID-SAHB_{A(G→E)}] that weakens BCL-2 binding by 10-fold killed cells at these concentrations. In a murine xenograft model of refractory human leukemia, intravenous dosing of BID SAHB_A at 10 mg/kg/d resulted in substantial reduction of overall tumor burden and an increase of 6 days in the median survival time after only a limited 7-day treatment course. Mice dosed with the control BID-SAHB_{A(G→E)} peptide had a tumor burden comparable with the vehicle-treated mice. The serum half-life of i.v. administered BID SAHB_A in mice, ~ 1.5 h, is exceptionally long for a peptide, and by allometric scaling is expected to give a half-life in humans of ~ 6 to 8 h.

In addition to their development as prototype therapeutics, stapled peptides are also proving invaluable as scientific discovery tools, capable of probing signal transduction pathways both *in vitro* and *in vivo*. Like the pharmacologic drawbacks that arise from peptide unfolding, *in vitro* studies of peptide-protein interactions can similarly be limited by structural instability. For example, the interaction of proapoptotic BAX with BH3 targets BID and BIM, not detectable with unmodified and unstructured peptides (19, 20), is readily shown and quantified when α -helical reinforcement is used in the case of BID and BIM SAHBs (15). Thus, by chemically stabilizing the α -helical structure of peptide domains, and thereby restoring the natural structure that exists within the protein, high-fidelity synthetic ligands can be generated for the investigation of physiologic interactions and their functional implications. Because hydrocarbon staples can

be implanted with surgical precision to avoid critical interaction surfaces, the specificity and selectivity of natural amino acid sequences are preserved. Whereas stapled BIM peptides bind both proapoptotic BAX and all antiapoptotic targets with high affinity, a stapled BAD peptide retains its more restricted binding profile, engaging only antiapoptotic BCL-2, BCL-X_L, and BCL-w (15).⁶ The capacity of SAHBs to simulate the native specificities of BH3 peptides has yielded a pharmacologic toolbox for targeting, either selectively or broadly, the entire range of apoptotic targets. Depending on the clinical context, focused anti-BCL-2 (e.g., BAD SAHB) and/or anti-MCL-1 (e.g., NOXA SAHB) blockade may be desirable, or in especially refractory disease, bifunctional proapoptotic and antiapoptotic engagement (e.g., BID, BIM SAHBs) may best circumvent chemoresistance through pan-antiapoptotic blockade complemented by direct activation of BAX/BAK.

The stapling technology clearly endows a diverse array of BH3 domain peptides with more drug-like properties, but an ever-increasing body of evidence suggests that the stapling technology is much broader in scope. In a recent study (21), we reported that 15-mer peptides derived from the Hdm2 interaction domain of p53, having no sequence relationship to BH3s, bound Hdm2 with subnanomolar K_d and also penetrated cells by an endocytic transport mechanism. These stapled α helices of p53 (SAH-p53) pharmacologically restored the p53 tumor suppressor pathway in Hdm2-overexpressing cancer cells by targeting intracellular Hdm2, resulting in elevated p53 levels and up-regulation of p53 transcriptional targets. Consequently, SAH-p53 compounds, but not inactive point mutants, reactivated apoptosis in cultured Hdm2-overexpressing cells (21). Additional preliminary studies on a wide variety of targets have yielded similarly promising results in biophysical, biochemical, and cellular assay systems, and in animal models of cancer.⁷ In the cases of BH3 domain peptides and Hdm2-interacting peptides, the ability of stapled peptides to phenocopy the binding specificity of their parent protein promises to be particularly valuable in the clinical setting.

Whereas stapling bioactive helices has already yielded important new tool compounds for biological study, do stapled peptides as a class have genuine potential to be next-generation therapeutics? Although vigorous IND-enabling studies of SAHBs are currently under way to address directly their pharmacologic potential, it is encouraging that even at this early stage, BID SAHB_A, which has not been subjected to pharmacologic optimization, exhibits a serum half-life in mice that greatly exceeds that of typical peptides (1.6 h in mice; ref. 14)⁸ and is within range of a clinical candidate. In addition, no evidence of toxicity resulting from the proapoptotic activity of this molecule has thus far been observed in murine models after short-term treatment. Parallel efforts are currently under way to develop therapeutic stapled peptides that target Hdm2, Notch, and, among the more

⁶ Unpublished observations.

⁷ G.L. Verdine and L.D. Walensky, unpublished results.

⁸ Unpublished results.

intransigent targets, c-Myc. Should these efforts prove successful, the likely effect would be to both inform and energize efforts to develop stapled peptides against the abundance of biological targets that employ α -helical motifs to engage in protein-protein interactions.

Fragment-Based Drug Discovery

Consistent with the idea that multidomain BCL-2 family proteins are challenging targets, high-throughput screening of a historical compound collection at Abbott Laboratories failed to furnish high-affinity hit compounds that bind Bcl-X_L. Rosenberg and Fesik therefore decided to apply what has since become known as fragment-based drug discovery (22, 23) to the problem. In fragment-based drug discovery, the goal is to identify two or more structurally simple molecules (fragments) that bind to neighboring but nonoverlapping sites on a receptor surface. By using structural information on these proximal binding interactions as a guide, the fragments can then be connected together via synthetic chemistry to produce a composite molecule that binds more strongly because of energetic additivity. The Abbott group used nuclear magnetic resonance spectroscopy for both the screening and connectivity-guiding aspects of the discovery effort, an approach they had earlier described as structure-activity relationship by nuclear magnetic resonance (24, 25). Iterative rounds of optimization led to a molecule having nanomolar activity against BCL-2 and BCL-X_L (26); this became the development molecule ABT-737 (27). ABT-737 showed potent cytotoxic activity *in vitro* and in mouse xenograft models of human cancers driven by BCL-2 overexpression, including follicular lymphoma, non-small cell lung cancer, chronic lymphocytic leukemia, and acute myelogenous leukemia (27, 28). ABT-737 also potentiated the cell-killing activity of standard chemotherapeutic agents such as etoposide, paclitaxel, and cisplatin (27). A recent study suggested that BCL-2 overexpression enabled primary acute myelogenous leukemia cells *in vitro* to circumvent cell-killing by a FLT3 inhibitor, yet ABT-737 restored and potentiated the activity of this inhibitor (29). An orally bioavailable derivative of ABT-737, ABT-263, entered phase 1/2a testing earlier this year in patients with relapsed or refractory lymphoid malignancies and small cell lung cancer.

ABT-737 and ABT-263 have been described as BAD BH3 mimetics because, like BAD, they interact at high affinity with a specified subgroup of antiapoptotic targets, i.e., BCL-2, BCL-X_L, and BCL-w, but not with Mcl-1 or BFL1/A1 (27). Recent work has shown that cell lines overexpressing MCL-1 and/or BFL1/A1, antiapoptotics outside the high-affinity binding spectrum of ABT-737, are indeed insensitive to ABT-737 (28, 30). These data emphasize the importance of identifying additional design strategies and compounds to expand the arsenal of agents that target the array of apoptotic proteins implicated in the pathogenesis and maintenance of cancer.

Why is it that multidomain BCL-2 family members can be inhibited by small molecules, negating early concerns of intractability raised by the unliganded structure of BCL-X_L Δ C

(31)? The recently reported structure of BCL-X_L Δ C bound to ABT-737 provides insight into this question. Comparison of the unliganded protein structure (Fig. 3, *green*) with that bound to ABT-737 (*red*) shows a substantial conformational reorganization of the protein structure in the vicinity of the ligand-binding site. The most significant backbone changes are, for the most part, localized to the helix-loop-helix element α 3-loop- α 4, as can be seen readily in the RMSD overlay shown in Fig. 3 (*row 2*). On ligand binding, helix α 3 unravels at its NH₂-terminal end and extends in the COOH-terminal direction, whereas α 4 undergoes a rigid-body rotation that is most pronounced at its NH₂ terminus. In addition to these substantial structural reorganizations, a number of less pronounced motions such as rotation of amino acid side chains takes place at the floor of the binding site. The net effect of these structural reorganizations is to form two larger hydrophobic pockets in the BCL-X_L Δ C surface (Fig. 3, *third row, arrows*), into which hydrophobic functional groups on ABT-737 insert themselves (Fig. 3, *fourth row*). As discussed in detail elsewhere (32), a number of small molecules other than ABT-737, including the phase I/II molecule GX15-070, are also believed to target multidomain BCL-2 family members. It will be of great interest to see whether the specific features of protein surface adaptation seen with ABT-737 are a common feature of small molecule recognition.

Such comparative structural analyses further show that BCL-X_L Δ C binding of a BH3 peptide induces a similar reorganization of α 3-loop- α 4, but the pockets at the corresponding sites on the protein surface are considerably less voluminous. The larger overall contact surface in the peptide-protein complex, as compared with the small molecule complex, presumably requires less remodeling of the protein surface to create a landscape suitable for tight binding. That said, the structural plasticity of the protein surface in BCL-2 proteins is apparently critical to productive targeting by both small molecules and synthetic biologics.

Even the most powerful computational technology cannot predict such conformational adaptations, and therefore, it remains the province of trial-and-error to discover those relatively rare examples in which it takes place. In the case of ABT-737, fragment-based drug discovery provided a framework to guide the search for a drug, whereas the discovery of SAHBs emerged by borrowing a page from the playbook of Nature.

Acknowledgments

Two distinct experimental strategies discussed here both bear the unmistakable intellectual and experimental imprint of Stan Korsmeyer, a cancer research pioneer whose commitment to scientific mentorship and meticulous experimentation continues to inspire and guide our work. It is appropriate to consider one component of the Korsmeyer legacy—the notion that undruggable targets present scientifically challenging opportunities that await a fresh outlook. Guided by Stan's boundless enthusiasm and laser-focused commitment to surmounting cancer's most formidable obstacles through innovation and persistence, perhaps researchers soon will find the path to such elusive targets as c-Myc and K-Ras.

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References

- Daniel NN. BCL-2 family proteins: critical checkpoints of apoptotic cell death. *Clin Cancer Res* 2007;13:7254–63.
- Rixe O, Fojo T. Is cell death a critical endpoint for anticancer therapies or is cytostasis sufficient? *Clin Cancer Res* 2007;13:7280–8.
- Amaravadi RK, Thompson CB. The roles of therapy-induced autophagy and necrosis in cancer treatment. *Clin Cancer Res* 2007;13:7271–9.
- Benz EJ, Jr., Nathan DG. Targeting the cell death-survival equation. *Clin Cancer Res* 2007;13:7250–3.
- Hopkins AL, Groom CR. The druggable genome. *Nat Rev Drug Discov* 2002;1:727–30.
- Hopkins AL, Groom CR. Target analysis: a priori assessment of druggability. *Ernst Schering Res Found Workshop* 2003;42:11–7.
- Russ AP, Lampel S. The druggable genome: an update. *Drug Discov Today* 2005;10:1607–10.
- Arkin MR, Wells JA. Small-molecule inhibitors of protein-protein interactions: progressing towards the dream. *Nat Rev Drug Discov* 2004;3:301–17.
- Sattler M, Liang H, Nettlesheim D, et al. Structure of Bcl-xL-Bak peptide complex: recognition between regulators of apoptosis. *Science* 1997;275:983–6.
- Petros AM, Nettlesheim DG, Wang Y, et al. Rationale for Bcl-xL/Bad peptide complex formation from structure, mutagenesis, and biophysical studies. *Protein Sci* 2000;9:2528–34.
- Liu X, Dai S, Zhu Y, Marrack P, Kappler JW. The structure of a Bcl-xL/Bim fragment complex: implications for Bim function. *Immunity* 2003;19:341–52.
- Kvansakul M, van Delft MF, Lee EF, et al. A structural viral mimic of prosurvival Bcl-2: a pivotal role for sequestering proapoptotic Bax and Bak. *Mol Cell* 2007;25:933–42.
- Czabotar PE, Lee EF, van Delft MF, 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.
- Walensky LD, Kung AL, Escher I, et al. Activation of apoptosis *in vivo* by a hydrocarbon-stapled BH3 helix. *Science* 2004;305:1466–70.
- Walensky LD, Pitter K, Morash J, et al. A stapled BID BH3 helix directly binds and activates BAX. *Mol Cell* 2006;24:199–210.
- Schafmeister CJ, Po J, Verdine GL. An all-hydrocarbon cross-linking system for enhancing the helicity and metabolic stability of peptides. *J Am Chem Soc* 2000;122:5891–2.
- Grubbs RH. Olefin metathesis. *Tetrahedron* 2004;60:7114–40.
- Wang K, Yin XM, Chao DT, Milliman CL, Korsmeyer SJ. BID: a novel BH3 domain-only death agonist. *Genes Dev* 1996;10:2859–69.
- Chen L, Willis SN, Wei A, et al. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. *Mol Cell* 2005;17:393–403.
- Willis SN, Fletcher JI, Kaufmann T, et al. Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. *Science* 2007;315:856–9.
- Bernal F, Tyler AF, Korsmeyer SJ, Walensky LD, Verdine GL. Reactivation of the p53 tumor suppressor pathway by a stapled p53 peptide. *J Am Chem Soc* 2006;129:2456–7.
- Hajduk PJ, Greer J. A decade of fragment-based drug design: strategic advances and lessons learned. *Nat Rev Drug Discov* 2007;6:211–9.
- Bartoli S, Fincham CI, Fattori D. Fragment-based drug design: combining philosophy with technology. *Curr Opin Drug Discov Devel* 2007;10:422–9.
- Shuker SB, Hajduk PJ, Meadows RP, Fesik SW. Discovering high-affinity ligands for proteins: SAR by NMR. *Science* 1996;274:1531–4.
- Petros AM, Dinges J, Augeri DJ, et al. Discovery of a potent inhibitor of the antiapoptotic protein Bcl-xL from NMR and parallel synthesis. *J Med Chem* 2006;49:656–63.
- Bruncko M, Oost TK, Belli BA, et al. Studies leading to potent, dual inhibitors of Bcl-2 and Bcl-xL. *J Med Chem* 2007;50:641–62.
- Oltersdorf T, Elmore SW, Shoemaker AR, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* 2005;435:677–81.
- Konopleva M, Contractor R, Tsao T, et al. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. *Cancer Cell* 2006;10:375–88.
- Kohl TM, Hellinger C, Ahmed F, et al. BH3 mimetic ABT-737 neutralizes resistance to FLT3 inhibitor treatment mediated by FLT3-independent expression of BCL2 in primary AML blasts. *Leukemia* 2007;21:1763–72.
- van Delft MF, Wei AH, Mason KD, 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;10:389–99.
- Muchmore SW, Sattler M, Liang H, et al. X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. *Nature* 1996;381:335–41.
- Walensky LD. BCL-2 in the crosshairs: tipping the balance of life and death. *Cell Death Differ* 2006;13:1339–50.
- Nagar B, Bornmann WG, Pellicena P, et al. Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res* 2002;62:4236–43.
- Cho HS, Mason K, Ramyar KX, et al. Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. *Nature* 2003;421:756–60.
- Manion MK, O'Neill JW, Giedt CD, Kim KM, Zhang KY, Hockenbery DM. Bcl-XL mutations suppress cellular sensitivity to antimycin A. *J Biol Chem* 2004;279:2159–65.
- Lee EF, Czabotar PE, Smith BJ, et al. Crystal structure of ABT-737 complexed with Bcl-x(L): implications for selectivity of antagonists of the Bcl-2 family. *Cell Death Differ* 2007;14:1711–3.

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