Functional Precision Profiling: The Way Forward for Personalized Medicine

Lyndsey Flanagan¹, Siobhan Glavey²,³*, Triona Ní Chonghaile¹

¹Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland
²Department of Pathology, Royal College of Surgeons in Ireland, Dublin, Ireland
³Department of Haematology, Beaumont Hospital, Dublin 9, Ireland

*Correspondence should be addressed to Siobhan Glavey; siobhanglavey@rcsi.ie

Received date: April 30, 2021, Accepted date: May 25, 2021

Copyright: ©2021 Flanagan L, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

List of Abbreviations

AML: Acute Myeloid Leukemia; BAD: Bcl-2 associated agonist of cell death; BAK: Bcl-2 homologous antagonist killer; BAX: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; Bcl-W: B-cell lymphoma-w; Bcl-xL: B-cell lymphoma-extra-large; Bfl-1: Bcl-2-related gene expressed in fetal liver; BH: Bcl-2 Homology; BIK: Bcl-2 Interacting Killer; BIM: Bcl-2-like protein 1; BMF: Bcl-2 Modifying Factor; CCND1: Cyclin D1; CD: Cluster of Differentiation; CLL: Chronic Lymphocytic Leukemia; FDA: Food and Drug Administration; HRK: Harakiri; JC-1: 1st J-aggregate-forming cationic dye; Mcl-1: Myeloid cell leukemia 1; MM: Multiple Myeloma; MOMP: Mitochondrial Outer Membrane Permeabilization; NOXA: Latin for damage; PCL: Plasma Cell Leukemia; PUMA: p53 Upregulated Modulator; sPCL: Secondary Plasma Cell Leukemia; tBID: Truncated BH3 interacting-domain death agonist

Introduction

Multiple Myeloma (MM) is a malignancy of the antibody-producing plasma cells found in the bone marrow [1]. In recent years, we have witnessed significant improvements made in both the diagnostic criteria and novel therapies for MM, resulting in the prolonged survival of MM patients. Approved novel therapies include proteasome inhibitors (bortezomib and carfilzomib), immunomodulatory drugs (thalidomide, lenalidomide & pomalidomide), monoclonal antibodies (daratumumab, elotuzumab & isatuximab) and B-cell maturation antigens (Belantamab) [2,3]. Recently, CAR T cell therapy has been FDA approved for MM treatment [4]. Despite these advancements, MM remains an incurable cancer with suboptimal overall survival, with many patients developing relapsed/refractory MM. Plasma cell leukemia (PCL) is a rare and aggressive variant of MM. PCL is classified as either primary PCL, which develops de novo, or secondary PCL, that can arise in the late and advanced stages of MM [5]. The present diagnostic criteria for PCL include the number of circulating plasma cells exceeding 2 x 10⁹/L and/or >20% plasma cells in the total leucocyte count [6]. In both forms, PCL clinically resembles late-stage MM, with patients experiencing anemia, bone marrow failure, recurring bacterial infections renal insufficiency and hyperviscosity. Secondary PCL is a rare occurrence that is seen in approximately 1% of all MM, however it should be noted that MM therapy has increased survival in approximately 1% of all MM, however incidence is increasing as a result of the prolonged survival of MM patients [7]. Secondary PCL has a poor prognosis, is unresponsive to treatment and has a median survival of 1.3 months [7]. The underlying mechanisms that are involved in the transformation of MM to secondary PCL remain elusive. Owing to the rarity of this disease, data is limited with regards to therapeutic options. This underlines the importance of individual case reports and small case series for secondary PCL, as they aid the advancement of therapeutic treatment options for this difficult and challenging disease.

Bcl-2 Family Proteins

Human MM cells are reliant on Bcl-2 family proteins for survival [8,9]. Bcl-2 proteins are regulators of the mitochondrial apoptotic pathway. The anti-apoptotic Bcl-2 proteins (Bcl-2, Bcl-xL, Mcl-1, Bfl-1) prevent mitochondrial outer membrane permeabilization (MOMP) by binding and inhibiting pro-apoptotic proteins [10-15]. Upon activation, the pro-apoptotic BAX and BAK proteins homo-oligomerise and insert in to the outer mitochondrial...
membrane and form pores, which allows the release of cytochrome into the cytosol [16-18]. Lastly, are the pro-apoptotic BH3-only proteins which are further divided into activators and sensitizers. The activators (BIM, tBID, full length PUMA and NOXA) promote apoptosis through directly activating BAX and BAK [19-24]. The sensitizers (NOXA, HRK, BMF, BAD, BIK) bind to the pro-survival proteins (Bcl-2, Bcl-xL, Mcl-1), thereby inhibiting their anti-apoptotic function [25-30].

Development of BH3 Mimetics

The development of small molecule inhibitors to selectively target Bcl-2 proteins took over two decades of research, likely due to the difficult nature of disrupting protein-protein interactions. Ultimately, BH3 mimetics were developed using technical innovations such as structure-activity-relationship by nuclear magnetic resonance [31,32]. To be classified as a BH3 mimic, the compound needs to not only display a high affinity to the targeted Bcl-2 protein, but also be able to induce cell death in a BAX/BAK dependent manner [33]. The first BH3 mimic to be developed, ABT-737, bound to Bcl-2, Bcl-W and Bcl-xL in a manner analogous to the BAD BH3 domain [32,34]. The limiting factor with ABT-737 was the lack of bioavailability, which resulted in the development of ABT-263, or Navitoclax. ABT-263 inhibited both Bcl-2 and Bcl-xL and showed promising results in early clinical studies. Unfortunately, ABT-263 caused a dose-limiting thrombocytopenia due to on-target inhibition of Bcl-xL in platelets [35,36]. To overcome this adverse side effect, a selective Bcl-2 inhibitor was developed. ABT-199 is the first BH3 mimetic to be approved by the FDA first for the treatment of chronic lymphocytic leukemia (CLL) and then for the treatment of acute myeloid leukemia (AML) in combination with demethylating agents [37-39] (Figure 1). In MM, Bcl-2 dependence was initially identified in a subset of patients, characterized by the presence of a translocation in cyclin D1 (CCND1) t(11;14) [40]. Sensitivity to ABT-199 is associated with t(11;14), which was confirmed across a panel of MM cell lines and patient samples [41,42]. In a Phase I study in relapsed/refractory MM, with ABT-199 as a single agent, the overall response rate in t(11;14) patients was 40%, which was exceptionally impressive for a single agent study in the relapsed/refractory setting [43]. There is very limited data on therapeutic options for patients with sPCL, due to the lack of prospective clinical trials specific for patients with PCL. However, our recent report is a single case study demonstrating a rapid and deep response in one patient with venetoclax [44]. However, further data is needed to support the use of this agent in larger numbers of PCL patients. It demonstrates the potential use of venetoclax as a single agent oral treatment, where therapeutic options are extremely limited.

Figure 1: Schematic of Bcl-2 protein and BH3 mimetic interactions. A) Anti-apoptotic proteins prevent MOMP by inhibiting both pro-apoptotic proteins and BH3-only proteins. B) WEHI-539 is a selective Bcl-xL inhibitor, ABT-263 inhibits Bcl-xL, Bcl-W and Bcl-2, ABT-199 is a selective Bcl-2 inhibitor and Mcl-1 is inhibited by both AMG-176 and S68345.
BH3 Profiling as a Potential Biomarker

Since the development of ABT-199, we now have selective BH3 mimetics that are being developed that target Bcl-xL (WEHI-539) and Mcl-1 (AMG-176) [45,46]. Additionally, there are CDK9 inhibitors that can target Mcl-1 [47]. The challenge now is to try and match the right targeted BH3-mimetic to the right patient, in a personalized approach. In the Phase III BELLINI trial, evaluating the combination of venetoclax (ABT-199), dexamethasone and bortezomib in relapsed/refractory MM, the patients with the t(11;14) translocation were particularly sensitive [48]. However, a proportion of patients without the t(11;14) translocation were also sensitive, suggesting that Bcl-2 dependence may exist outside of this cytogenic subgroup. This highlights the importance of developing a biomarker that can identify patients who will benefit from ABT-199 treatment, which is extremely relevant to avoid toxicity and maximize therapeutic benefit. To try to tackle this issue we conducted a study at RCSI and Beaumont Hospital, where we have combined the use of ex-vivo BH3 mimetic treatment of primary MM bone marrow samples, along BH3 profiling to identify anti-apoptotic dependence in primary MM samples. BH3 profiling is a state-of-the-art functional assay that was co-developed in the Dana Farber Cancer Institute [49,50]. The basis of BH3 profiling is to expose the mitochondria to known concentrations of BH3 peptides derived from the BH3 domains of pro-apoptotic BH3-only proteins of the Bcl-2 family. The mitochondria are exposed to these synthetic BH3 peptides for a defined length of time and the resulting MOMP is measured using flow cytometry or by plate reader [51]. Through the use of careful controls, comparisons can be made between the different cell lines and tissues towards sensitivity to intrinsic apoptosis and anti-apoptotic dependence [52,53]. The BAD peptide binds to Bcl-2/Bcl-xL/Bcl-W, while the HRK peptide binds selectively to Bcl-xL and NOXA peptides binds only to Mcl-1. Therefore, the selective binding of the synthetic BH3 peptides to the different anti-apoptotic proteins enables survival dependencies to be uncovered [54].

Methods

In our recent publication entitled “Secondary plasma cell leukaemia treated with single agent venetoclax” [44], we assessed if precision functional profiling correctly identified Bcl-2 dependence in MM cells (Figure 2). Primary myeloma patient bone marrow samples were collected following written informed consent and with local ethics committee approval. The primary MM bone marrow samples were processed using CD138 microbeads (Miltenyi Biotech) to isolate the myeloma cells as per standard protocol. Following this, the CD138 MM cells

Figure 2: Schematic of processing technique for primary MM bone marrow samples. Red cell lysis is used to remove red blood cells from primary MM bone marrow sample before incubating with CD138 beads. Primary sample suspension is run through MACS magnetic cell sorter and CD138+ MM cells are used for BH3 profiling or ex-vivo BH3 mimetic treatment.
are seeded and treated *ex-vivo* with BH3 mimetics or used in the BH3 profiling assay. The primary MM cells are treated for 16 hours with a panel of BH3 mimetics (ABT-199; selective Bcl-2 inhibitor, ABT-263; selective Bcl-2 and Bcl-xL inhibitor, WEHI-539; selective Bcl-xL inhibitor, AMG-176; selective Mcl-1 inhibitor). The cells are then stained with Annexin V/PI and cell viability post BH3 mimetic treatment is measured. The cells that are put aside for the BH3 profiling get stained and permeabilized with JC-1 and digitonin, before they are exposed to BH3 peptides and loss of MOMP is measured by a plate reader. CD138+ MM cells are processed immediately following bone marrow collection and analyzed within 24 hours to maintain cell viability. The volume of experiments that can be performed using primary patient MM samples are limited by the number of CD138+ cells we can isolate from a patient’s sample. To perform BH3 profiling we need at least $1.5 \times 10^6$ cells, while *ex-vivo* BH3 mimetic treatments require $1 \times 10^6$ cells, therefore the downstream processing is highly dependent on the number of isolated CD138+ cells.

**Results**

By comparing the pre-clinical data generated from both the BH3 profile and *ex-vivo* BH3 mimetics, we can learn a considerable amount from the patient sample. Our case report was the first to demonstrate that ABT-199 induced a rapid hematologic and clinical response in a patient with hyperviscosity syndrome that otherwise would have undoubtedly been rapidly fatal. In this case, BH3 profiling of the sPCL sample demonstrated a response to the BAD peptide and ABT-199, consistent with BCL-2 dependence. This Bcl-2 dependence was also confirmed by assessing sensitivity to *ex-vivo* BH3 mimetic treatment by flow cytometry. This assay outperformed cytogenetics at our institute in terms of turn-around time and aided in clinical decision making. Even before the cytogenetic analysis in the clinic had revealed that this sPCL had a t(11;14) translocation and potentially was dependent on Bcl-2, the pre-clinical data had already demonstrated using two different techniques that this would be the case. For rare cancers such as sPCL, assessing the sample *ex-vivo* using techniques such as BH3 profiling and *ex-vivo* BH3 mimetic treatment could offer incredible insight into what treatment would best suit the patient. This is especially important when there is no standard therapeutic regimen, and the patient may have already received and be refractory to the standard anti-myeloma therapies.

**Conclusion and Recommendation**

This case report demonstrates the importance of clinicians and scientists working in collaboration. The pre-clinical data serves as proof of concept that BH3 profiling and *ex-vivo* BH3 mimetic sensitivity may be used as a biomarker in predicting patient response to therapy in real time. In this particular case, the t(11;14) translocation was present and indicated that there would be a potential Bcl-2 dependence, which was confirmed by both BH3 profiling and *ex-vivo* BH3 mimetic sensitivity. However, had that translocation not been present, there would not have been an indication from genetics as to which, if any, anti-apoptotic protein the patient sample was dependent on. Therefore, the functional assay BH3 profiling is an incredibly useful technique, as it can distinguish the anti-apoptotic dependence and demonstrate which BH3 mimetic the sample may be sensitive too. BH3 profiling will also be highly relevant in the case of resistance to ABT-199, as it can also identify switching of anti-apoptotic dependence in a patients sample [55]. There is evidence in acute lymphocytic leukemia from patients treated with venetoclax and navitoclax of switching from Bcl-2 dependence to other anti-apoptotic proteins such as Bcl-xL and Mcl-1 following treatment [56,57]. As more BH3 mimetics receive FDA approval to be used in the clinic, it becomes apparent that a fast effective biomarker is required to offer new therapeutic options to sPCL, patients. BH3 profiling has the potential to predict the patient’s response to treatment and help tailor the treatment to the specific patient to allow us to treat the right patient, at the right time with the right drug. Individual case reports aid in the advancement of therapeutic options for difficult and challenging diseases such as sPCL, where there are no clinical trials.

**References**

1. Landgren O, Weiss BM. Patterns of monoclonal gammopathy of undetermined significance and multiple myeloma in various ethnic/racial groups: support for genetic factors in pathogenesis. Leukemia. 2009 Oct;23(10):1691-7.

2. Moreau P, Attal M, Facon T. Frontline therapy of multiple myeloma. Blood. 2015 May 14;125(20):3076-84.

3. Lei M, Kim EB, Branagan A, Lou U, Zemel M, Raje N. Current management and emerging treatment strategies for multiple myeloma. Rinsho Ketsueki. 2019;60(9):1243-56.

4. Timmers M, Roex G, Wang Y, Campillo-Davo D, Van Tendeloo VF, Chu Y, et al. Chimeric antigen receptor-modified T cell therapy in multiple myeloma: beyond B cell maturation antigen. Frontiers in Immunology. 2019 Jul 16;10:1613.

5. Kosmo MA, Gale RP. Plasma cell leukemia. Seminars in Hematology. 1987 Jul 1;24(3):202-208.

6. Kyle RA, Maldonado JE, Bayrd ED. Plasma cell leukemia. Report on 17 cases. Archives of Internal...
Tiedemann RE, Gonzalez-Paz N, Kyle RA, Santana-Davila R, Price-Troska T, Van Wier SA, et al. Genetic aberrations and survival in plasma cell leukemia. Leukemia. 2008 May;22(5):1044-52.

8. Wuilleme-Toumi S, Robillard N, Gomez P, Moreau P, Le Gouill S, Avet-Loiseau H, et al. Mcl-1 is overexpressed in multiple myeloma and associated with relapse and shorter survival. Leukemia. 2005 Jul;19(7):1248-52.

9. Touzeau C, Ryan J, Guerriero J, Moreau P, Chonghaile TN, Le Gouill S, et al. BH3 profiling identifies heterogeneous dependency on Bcl-2 family members in multiple myeloma and predicts sensitivity to BH3 mimetics. Leukemia. 2016 Mar;30(3):761-4.

10. Boise LH, González-García M, Postema CE, Ding L, Lindsten T, Turka LA, et al. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell. 1993 Aug 27;74(4):597-608.

11. Choi SS, Park IC, Yun JW, Sung YC, Hong SI, Shin HS. A novel Bcl-2 related gene, Bfl-1, is overexpressed in stomach cancer and preferentially expressed in bone marrow. Oncogene. 1995 Nov 1;11(9):1693-8.

12. Cleary ML, Sklar J. Nucleotide sequence of at (14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint-cluster region near a transcriptionally active locus on chromosome 18. Proceedings of the National Academy of Sciences. 1985 Nov 1;82(21):7439-43.

13. Gibson L, Holmgreen SP, Huang DC, Bernard O, Copeland NG, Jenkins NA, et al. bcl-w, a novel member of the bcl-2 family, promotes cell survival. Oncogene. 1996 Aug 1;13(4):665-75.

14. Kozopas KM, Yang T, Buchan HL, Zhou P, Craig RW. MCL1, a gene expressed in programmed myeloid cell differentiation, has sequence similarity to BCL2. Proceedings of the National Academy of Sciences. 1993 Apr 15;90(8):3516-20.

15. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t (14;18) chromosome translocation. Science. 1984 Nov 30;226(4678):1097-9.

16. Kuwana T, Mackey MR, Perkins G, Ellisman MH, Latterich M, Schneider R, et al. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell. 2002 Nov 1;111(3):331-42.

17. Antonsson B, Montessuit S, Lauper S, Eskes R, Martinou JC. Bax oligomerization is required for channel-forming activity in liposomes and to trigger cytochrome c release from mitochondria. Biochemical Journal. 2000 Jan 15;345(2):271-8.

18. Korsmeyer SJ, Wei MC, Saito MT, Weiler S, Oh KJ, Schlesinger PH. Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome c. Cell Death & Differentiation. 2000 Dec;7(12):1166-73.

19. Nakano K, Voutsen KH. PUMA, a novel proapoptotic gene, is induced by p53. Molecular Cell. 2001 Mar 1;7(3):683-94.

20. Yu J, Zhang L, Hwang PM, Kinzler KW, Vogelstein B. PUMA induces the rapid apoptosis of colorectal cancer cells. Molecular Cell. 2001 Mar 1;7(3):673-82.

21. Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, Yamashita T, et al. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science. 2000 May 12;288(5468):1053-8.

22. Chen HC, Kanai M, Inoue-Yamauchi A, Tu HC, Huang Y, Ren D, et al. An interconnected hierarchical model of cell death regulation by the BCL-2 family. Nature Cell Biology. 2015 Oct;17(10):1270-81.

23. Dai H, Smith A, Meng XW, Schneider PA, Pang YP, Kaufmann SH. Transient binding of an activator BH3 domain to the Bak BH3-binding groove initiates Bak oligomerization. Journal of Cell Biology. 2011 Jul;194(1):39-48.

24. Kim H, Rafiuddin-Shah M, Tu HC, Jeffers JR, Zambetti GP, Hsieh JJ, et al. Hierarchical regulation of mitochondrion-dependent apoptosis by BCL-2 subfamilies. Nature Cell Biology. 2006 Dec;8(12):1348-58.

25. Letai A, Bassik MC, Walensky LD, Sorcinelli MD, Weiler S, Korsmeyer SJ. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. Cancer Cell. 2002 Sep 1;2(3):183-92.

26. Leshchiner ES, Braun CR, Bird GH, Walensky LD. Direct activation of full-length proapoptotic BAK. Proceedings of the National Academy of Sciences. 2013 Mar 12;110(11):E986-95.

27. Gavathiotis E, Reyna DE, Davis ML, Bird GH, Walensky LD. BH3-triggered structural reorganization drives the activation of proapoptotic BAX. Molecular Cell. 2010 Nov 12;40(3):481-92.

28. Gavathiotis E, Suzuki M, Davis ML, Pitter K, Bird GH, Katz SG, et al. BAX activation is initiated at a novel interaction site. Nature. 2008 Oct;455(7216):1076-81.
29. Mérino D, Giam M, Hughes PD, Siggs OM, Heger K, O’Reilly LA, et al. The role of BH3-only protein Bim extends beyond inhibiting Bcl-2-like prosurvival proteins. Journal of Cell Biology. 2009 Aug 10;186(3):355-62.

30. Wei MC, Lindsten T, Mootha VK, Weiler S, Gross A, Ashiya M, et al. tBID, a membrane-targeted death ligand, oligomerizes BAK to release cytochrome c. Genes & Development. 2000 Aug 15;14(16):2060-71.

31. Shuker SB, Hajduk PJ, Meadows RP, Fesik SW. Discovering high-affinity ligands for proteins: SAR by NMR. Science. 1996 Nov 29;274(5292):1531-4.

32. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Auger DJ, Belli BA, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature. 2005 Jun;435(7042):677-81.

33. Lessene G, Czabotar PE, Colman PM. BCL-2 family antagonists for cancer therapy. Nature Reviews Drug Discovery. 2008 Dec;7(12):989-1000.

34. Kline MP, Rajkumar SV, Timm MM, Kimlinger TK, Haug JL, Lust JA, et al. ABT-737, an inhibitor of Bcl-2 family proteins, is a potent inducer of apoptosis in multiple myeloma cells. Leukemia. 2007 Jul;21(7):1549-60.

35. Zhang H, Nimmer PM, Tahir SK, Chen J, Fryer RM, Hahn KR, et al. Bcl-2 family proteins are essential for platelet survival. Cell Death & Differentiation. 2007 May;14(5):943-51.

36. Roberts AW, Seymour JF, Brown JR, Wierda WG, Kipps TJ, Khaw SL, et al. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. Journal of Clinical Oncology. 2012 Feb 10;30(5):488-96.

37. Roberts AW, Davids MS, Page JM, Kahl BS, Puvvada SD, Gerecitano JF, et al. Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. New England Journal of Medicine. 2016 Jan 28;374(4):311-22.

38. DiNardo CD, Stein EM, de Botton S, Roboz GJ, Altman JK, Mims AS, et al. Durable remissions with ivosidenib in IDH1-mutated relapsed or refractory AML. New England Journal of Medicine. 2018 Jun 21;378(25):2386-98.

39. DiNardo CD, Pratz K, Pullarkat V, Jonas BA, Arellano M, Becker PS, et al. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. Blood. 2019 Jan 3;133(1):7-17.

40. Bodet L, Gomez-Bougie P, Touzeau C, Dousset C, Descamps G, Maiga S, et al. ABT-737 is highly effective against molecular subgroups of multiple myeloma. Blood, The Journal of the American Society of Hematology. 2011 Oct 6;118(14):3901-10.

41. Touzeau C, Dousset C, Le Guouil S, Sampath D, Leverson JD, Souers AJ, et al. The Bcl-2 specific BH3 mimic ABT-199: a promising targeted therapy for t (11; 14) multiple myeloma. Leukemia. 2014 Jan;28(1):210-2.

42. Touzeau C, Ryan J, Guerriero J, Moreau P, Chonghaile TN, Le Guouil S, et al. BH3 profiling identifies heterogeneous dependency on Bcl-2 family members in multiple myeloma and predicts sensitivity to BH3 mimetics. Leukemia. 2016 Mar;30(3):761-4.

43. Kumar S, Kaufman JL, Gasparetto C, Mikhail J, Vij R, Pegourie B, et al. Efficacy of venetoclax as targeted therapy for relapsed/refractory t (11; 14) multiple myeloma. Blood. 2017 Nov 30;130(22):2401-9.

44. Glavey SV, Flanagan L, Bleach R, Kelly C, Quinn J, Ní Chonghaile T, et al. Secondary plasma cell leukaemia treated with single agent venetoclax. British Journal of Haematology. 2020 Aug;190(4):e242-5.

45. Lessene G, Czabotar PE, Sleebs BE, Zobel K, Lowes KN, Adams JM, et al. Structure-guided design of a selective BCL-X L inhibitor. Nature Chemical Biology. 2013 Jun;9(6):390-7.

46. Caenepeel S, Brown SP, Belmontes B, Moody G, Keegan KS, Chui D, et al. AMG 176, a selective MCL1 inhibitor, is effective in hematologic cancer models alone and in combination with established therapies. Cancer Discovery. 2018 Dec 1;8(12):1582-97.

47. O’Reilly E, Dhami SP, Baev DV, Ortuçay C, Halpin-McCormick A, Morrell R, et al. Repression of Mcl-1 expression by the CDC7/CDK9 inhibitor PHA-767491 overcomes bone marrow stroma-mediated drug resistance in AML. Scientific Reports. 2018 Oct 25;8(1):15752.

48. Moreau P, Chanan-Khan A, Roberts AW, Agarwal AB, Faron T, Kumar S, et al. Promising efficacy and acceptable safety of venetoclax plus bortezomib and dexamethasone in relapsed/refractory MM. Blood, The Journal of the American Society of Hematology. 2017 Nov 30;130(22):2392-400.

49. Letai A. BH3 domains as BCL-2 inhibitors: prototype cancer therapeutics. Expert Opinion on Biological Therapy. 2003 Apr 1;3(2):293-304.

50. Certo M, Moore VD, Nishino M, Wei G, Korsmeyer S, Armstrong SA, et al. Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. Cancer Cell. 2006 May 1;9(5):351-65.

51. Ryan J, Letai A. BH3 profiling in whole cells by
Flanagan L, Glavey S, Chonghaile TN. Functional Precision Profiling: The Way Forward for Personalized Medicine. J Clin Haematol. 2021; 2(3): 73-79.

fluorimeter or FACS. Methods. 2013 Jun 1;61(2):156-64.

52. Chonghaile TN, Sarosiek KA, Vo TT, Ryan JA, Tammareddi A, Moore VD, et al. Pretreatment mitochondrial priming correlates with clinical response to cytotoxic chemotherapy. Science. 2011 Nov 25;334(6059):1129-33.

53. Deng J, Carlson N, Takeyama K, Dal Cin P, Shipp M, Letal A. BH3 profiling identifies three distinct classes of apoptotic blocks to predict response to ABT-737 and conventional chemotherapeutic agents. Cancer Cell. 2007 Aug 14;12(2):171-85.

54. Chonghaile TN, Roderick JE, Glenfield C, Ryan J, Sallan SE, Silverman LB, et al. Maturation stage of T-cell acute lymphoblastic leukemia determines BCL-2 versus BCL-XL dependence and sensitivity to ABT-199. Cancer Discovery. 2014 Sep 1;4(9):1074-87.

55. Di Grande A, Peirs S, Donovan PD, Van Trimpont M, Morscio J, Lintermans B, et al. The spleen as a sanctuary site for residual leukemic cells following ABT-199 monotherapy in ETP-ALL. Blood Advances. 2021 Apr 13;5(7):1963-76.

56. Punnoose EA, Leerson JD, Peale F, Boghaert ER, Belmont LD, Tan N, et al. Expression profile of BCL-2, BCL-XL, and MCL-1 predicts pharmacological response to the BCL-2 selective antagonist venetoclax in multiple myeloma models. Molecular Cancer Therapeutics. 2016 May 1;15(5):1132-44.

57. Pullarkat VA, Lacayo NJ, Jabbour E, Rubnitz JE, Bajel A, Laetsch TW, et al. Venetoclax and navitoclax in combination with chemotherapy in patients with relapsed or refractory acute lymphoblastic leukemia and lymphoblastic lymphoma. Cancer Discovery. 2021 Feb 16. doi: 10.1158/2159-8290.CD-20-1465.