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BH3 profiling identifies BCL-2 dependence in adult patients with early T-cell progenitor acute lymphoblastic leukemia

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Abstract:

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BH3 profiling identifies BCL-2 dependence in adult patients with early T-cell progenitor acute lymphoblastic leukemia

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Running Title: Adult ETP-ALL and T-ALL are BCL-2 Dependent

In a previous study from our group, we reported that pediatric early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) is BCL-2 dependent, while pediatric T-cell acute lymphoblastic leukemia (T-ALL) is BCL-XL dependent.[1] However, therapeutic success of adult ALL is different from pediatric ALL and less understood. Thus, the aim of this study was to identify anti-apoptotic BCL-2 family protein dependencies and sensitivities to BH3 mimetics of adult ETP-ALL and T-ALL lymphoblasts. By measuring direct mitochondrial permeabilization and cell death assays, we validated BH3 profiling as a predictor for BCL-2 dependence in adult ETP-ALL and BCL-2/BCL-XL co-dependence in adult T-ALL. Further, we revealed substantial on-target cytotoxicity of venetoclax and navitoclax, suggesting that this combination of BH3 mimetics is potentially efficacious for adults with T-ALL.

T-ALL results from malignant transformation of immature T-cells, accounting for 10-15% of childhood and 20-25% of adult ALL cases.[2] Long-term survival rates for standard risk childhood T-ALL have shown striking improvements to 80-90%, yet response outcomes in adults remain much lower (40%), possibly due to high induction therapy-related toxicities.[3] In general, the causes for poor response in adult T-ALL are incompletely understood.

Among ALL, the high-risk ETP-ALL subtype originating from clonal expansion of recently immigrated thymocytes, has a significantly worse outcome in adults.[4] ETP-ALL retains multipotent differentiation capacities and resemblance to hematopoietic stem cells or myeloid progenitor cells. Targeted next-generation sequencing[5] showed that adult ETP-ALL presents similar mutation profiles as its pediatric equivalent.[6] Further, similar to pediatric ETP-ALL, adult ETP-ALL has inferior outcomes to chemotherapy than non-ETP-ALL.[6] However, implementation of allogenic transplant after achieving first complete response with standard chemotherapy regimens improved the overall prognosis of patients with ETP-ALL, similar to the rest of the T-ALL cohort in high-risk patients.[6] Nevertheless, the 5-year overall survival rate of adults with ETP-ALL is only 49%.[6] These outcomes highlight the unmet need for new therapeutic approaches for adult ETP-ALL.

BH3 mimetics, small molecule antagonists of BCL-2 family proteins, have recently shown clinical success in various hematological malignancies, including chronic lymphocytic leukemia (CLL)[7,8], acute myeloid leukemia (AML)[9], and ALL.[10,11] Studies from our group[1,12–15] and several others[16–18] showed that BH3 profiling can predict tumor cells' functional dependency on BCL-2. In turn, BH3 profiling to identify BCL-2 dependence directed clinical success of the FDA-approved venetoclax (BCL-2 inhibitor) in CLL[19] and AML.[13,14] In practice, BH3 profiling involves exposing the mitochondria to synthetic BH3 peptides and subsequently measuring the ensuing mitochondrial outer membrane permeabilization (MOMP).[20] When selective peptides are used, e.g. BAD, heightened mitochondrial sensitivity indicates greater dependence on an individual anti-apoptotic protein, e.g. BCL-2 and BCL-XL.[21]

To determine the anti-apoptotic dependencies of adult ETP-ALL, we performed BH3 profiling on 15 primary adult ETP-ALL samples obtained at diagnosis (Figure 1A and Supplemental Table). We exploited the different binding affinities of BAD and HRK BH3 peptides to distinguish between BCL-2 dependence (high BAD, low/no HRK priming) and BCL-XL dependence (equal BAD and HRK priming) (Figure 1B). All samples showed robust

mitochondrial depolarization in response to the BAD peptide as compared to the HRK peptide and DMSO control, suggesting primary dependence on BCL-2 (Figure 1C). Despite heterogeneity among samples, the mean of BAD-induced cytochrome c release was significantly higher compared to the HRK peptide, further confirming BCL-2 dependence in ETP-ALL ($p<0.0001$, Figure 1D). It is important to note that blasts from only Patient A showed higher priming above threshold by HRK (>20%), whereas the remaining 14 patients crossed priming threshold only in response to BAD peptide (Spearman $r=0.71$, $p<0.01$; Figure 1E). Furthermore, BAD and BAD-HRK had a statistically significant association, suggesting that mitochondrial depolarization caused by the BAD peptide in ETP-ALL samples were driven by their BCL-2 dependence (Spearman $r=0.54$, $p<0.05$; Figure 1E). All samples were obtained after informed patient consent under IRB approved Dana-Farber Cancer Institute, MD Anderson Cancer Center, National University Hospital Singapore, and Singapore General Hospital collection protocols.

Having seen primary dependence of adult ETP-ALL on BCL-2, we next hypothesized that ETP-ALL tumors are sensitive to BH3 mimetics, particularly BCL-2 inhibitor. We measured direct mitochondrial sensitivity to BH3 mimetics venetoclax (selectively antagonizes BCL-2) and navitoclax (antagonizes BCL-2 and BCL-XL). Both drugs showed increased cytochrome c release as compared to DMSO, and venetoclax was a better inducer of mitochondrial priming compared to navitoclax, which can be explained by the higher binding affinity of venetoclax ($K_i<0.010$ nM)[22] over navitoclax ($K_i=0.044$ nM)[22] for BCL-2 ($p<0.05$; Figure 1F-G). In four of the samples, we also measured mitochondrial sensitivity to a selective BCL-XL antagonist (A-1331852). None of these samples induced mitochondrial priming response to A-1331852 (Figure 1F-G). We further found that BH3 profiling using BAD peptide predicted on-target cellular sensitivity to BH3 mimetics as shown by a significant correlation between cytochrome c release induced by the BH3 peptide vs. cytochrome c release caused by venetoclax and navitoclax (Supplemental Figure 1A). To validate whether mitochondrial sensitivity to venetoclax predicted by BH3 profiling results in apoptosis of ETP-ALL cells, we performed cell death assays using annexin V. We exposed ETP-ALL primary tumors to venetoclax, navitoclax, and A-1331852, focusing on these BH3 mimetics because of their clinical relevance and advancement in hematological cancer treatment. While responses were heterogeneous, all samples tested were sensitive to both venetoclax and navitoclax, emphasizing that BCL-2 dependence sensitizes adult ETP-ALL blasts to apoptosis (Figure 1H).

Having shown selective apoptotic dependence of adult ETP-ALL on BCL-2, we next evaluated if adult T-ALL shows selective survival dependency on specific members of BCL-2 family proteins. We performed BH3 profiling on 22 primary adult T-ALL samples collected at diagnosis (Figure 1A and Supplemental Table). Contrary to adult ETP-ALL, 18 out of 22 samples showed mitochondrial depolarization in response to both BAD and HRK peptides compared to DMSO controls ($p<0.0001$; Figure 2A-B). Of note, the mean of BAD and HRK-induced cytochrome c release was significantly higher compared to DMSO, indicating co-dependence on both BCL-2 and BCL-XL ($p<0.0001$; Figure 2A-B). BAD-induced mitochondrial sensitivity of T-ALL tumors was relatively higher than HRK ($p<0.0001$), indicating greater sensitivity to BCL-2 inhibition. We found a modest association between BAD and HRK (Spearman $r=0.47$, $p<0.05$) and moderate association between BAD and BAD-HRK (Spearman $r=0.62$, $p<0.01$), further indicating primary dependence on BCL-2 but also co-dependence on BCL-XL (Figure 2C). We then verified our findings by measuring direct mitochondrial priming

sensitivity to venetoclax, navitoclax, and A-1331852. Venetoclax ($p<0.0001$) and navitoclax ($p<0.0001$) caused superior cytochrome c release compared to DMSO (Figures 2D-E). Similar to ETP-ALL, venetoclax elicited slightly higher priming across samples than navitoclax ($p<0.01$; Figure 2D-E). Importantly, A-1331852 induced significant MOMP in T-ALL patients samples ($p<0.01$) compared to DMSO control. However, it did not out-perform navitoclax ($p=0.13$; Figure 2D-E), as we predicted from BH3 profiling using direct peptide sensitivity responses. To further test this, we compared mitochondrial priming induced by the BAD peptide to the cytochrome c release induced by the corresponding BH3 mimetic, venetoclax, as well as the HRK peptide compared to A-1331852. We observed a significant correlation for BAD vs. venetoclax (Spearman $r=0.66$, $p<0.001$) and BAD vs navitoclax ($r=0.72$, $p<0.001$), indicating cytochrome c release caused by BH3 peptides is comparable to direct mitochondrial permeabilization caused by BH3 mimetics (Supplemental Figure 1B).

Because we observed robust mitochondrial depolarization in responses to both BAD and HRK peptides, we hypothesized that T-ALL tumors would show enhanced cytotoxic response to BH3 mimetics drugs targeting BCL-2 and BCL-XL. Next, we performed cell death assays using annexin V on T-ALL primary tumors treated with venetoclax, navitoclax, and A-1331852. Despite heterogeneity, we observed sensitivity to venetoclax, and more than half of samples showed higher cell death in response to navitoclax and/or A-1331852, reiterating that therapeutic antagonism in T-ALL should target both BCL-2 and BCL-XL proteins (Figure 2F and Supplemental Figure 1C).

To further evaluate mechanistic function, we performed western blot and co-immunoprecipitation studies to assess protein expression and binding between pro- and anti-apoptotic BCL-2 family proteins on 7 samples. Using PBMCs as a control , both ETP-ALL and T-ALL samples demonstrated BIM:BCL-2 binding; however, BIM:BCL-XL binding was only observed in T-ALL tumors, suggesting that BIM is primarily sequestered by BCL-2 in ETP-ALL and co-sequestered by BCL-2 and BCL-XL in T-ALL (Supplemental Figure 2A). Comparison of baseline BCL-2 family protein expression of BCL-2 revealed that ETP-ALL patients had higher BCL-2/BCL-XL ratio compared to T-ALL (Supplemental Figure 2B). ETP-ALL has lower levels MCL-1 and p53 proteins compared to T-ALL (Supplemental Figure 2B).

We next asked if there is age-dependent differences in mitochondrial priming between adult vs. pediatric ALL.[1] We found that mean of BAD-induced mitochondrial priming in pediatric T-ALL was higher than adult T-ALL counterparts (60.5%[1] vs. 55.5%; Supplemental Figure 3A). The same was true for pediatric ETP-ALL vs. adult ETP-ALL (71.4%[1] vs. 63.5%; Supplemental Figure 3A). Collectively, ETP-ALL was more sensitive to BAD-induced mitochondrial depolarization compared to T-ALL (68.3% vs. 57.5%, $p<0.01$; Figure 2G and Supplemental Figure 3B).[1] Of note, we carried out a multi-center study providing us the unique opportunity to evaluate sensitivity of BH3 mimetics in adult patients with different demographics, with representation from both Western and Asian populations (Supplemental Table). Irrespective of demographics, adult ETP-ALL and T-ALL patients from both regions show increased mitochondrial sensitivity to BAD peptide compared to DMSO (Supplemental Figure 3C). Further investigation extends beyond the scope of this study, but this suggests that vulnerabilities to BCL-2 family proteins may be conserved across different ethnicities.

Our previous findings on pediatric T-ALL led to the clinical testing of venetoclax in combination with hyperCVAD in relapsed/refractory (R/R) T-ALL patients.[1,19,23] However, whether adult patients with T-ALL also show the selective pattern of anti-apoptotic dependence related to differentiation stage of T-cell had not been previously reported. While most samples accrued in our current study are from pre-venetoclax era, we obtained clinical response data on venetoclax based combinations from three ETP-ALL patients and three T-ALL patients. Four of six patients achieved complete response to venetoclax based therapy regimens as predicted from BH3 profiling, with one patient's status pending and one patient passing from their disease (Figure 2H). Together, this suggests that ETP-ALL and T-ALL both display dependency on BCL-2 that can be targeted therapeutically. This further emphasize that combining BH3 mimetics with standard of care drugs for ALL may meaningfully improve patient responses. Additionally, the results from a recent Phase I study (NCT03181126) in R/R B-ALL and T-ALL using a combination of venetoclax and navitoclax showed that dual dependence on BCL-2 and BCL-XL is maintained in the relapse settings.[10] The reported overall response rate in R/R ALL within all subgroups was 59.9% (n=28/47), with pediatric subgroups showing a higher overall response rate of 75% (9/12) on combination therapy with venetoclax and navitoclax.[10] An independent group also confirmed that 72.7% of T-ALL patients (n=8/11) showed predominant baseline BCL-2 dependency and switched to BCL-XL or BCL-2/BCL-XL dependence on treatment with BH3 mimetics.[10]

Since clinical data in adults with ETP-/T-ALL remains pending on whether combination of venetoclax and navitoclax is superior to venetoclax alone, our study elaborates this gap by elucidating discrete cellular dependencies on BCL-2 and BCL-XL, particularly on treatment-naïve samples. These details help to choose BH3 mimetics therapy for adult T-ALL patients (Figure 2I). Importantly, BCL-2 dependence is heterogeneous by hematological cancer type; CLL is homogeneously BCL-2 dependent,[24] AML is co-dependent on BCL-2 and MCL-1,[13] BCP-ALL is heterogeneously dependent on BCL-2,[17] pediatric ETP-ALL is selectively BCL-2 dependent,[1] and pediatric T-ALL is selectively BCL-XL dependent.[1] Thus, these discrepancies emphasize the necessity of studying each cancer and age group independently to truly understand which BH3 mimetics may be clinically effective. Further, despite study limitations of small sample size due to the rarity of adult ALL occurrence and poor cell viability in some samples (<50% baseline viability), BH3 profiling distinctly identified BCL-2 and BCL-XL dependencies that correlated with BH3 mimetics sensitivity and clinical response, a major advantage compared to frank cell death or cell viability measurements requiring longer incubation times. Our study validated that BH3 profiling will continue to be a valuable functional tool to personalize medicine through identifying protein dependencies and drug vulnerabilities in adult ALL patients.

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Contribution: S.B., M.K., and A.L. designed the laboratory studies. J.S.G., N.J., J.S.M., W.Y.J., G.C.W., and M.O. coordinated the study, provided clinical samples, regulatory oversight, and collected the study data. S.B., E.A.O., K.S.B., and S.R. performed BH3 profiling, cell death assays, and analyzed the data. A.N.M. performed immunoprecipitation studies and analyzed data. S.B. and E.A.O. wrote the manuscript. All authors read, critically reviewed, and approved the final manuscript.

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References

1. Chonghaile TN, Roderick JE, Glenfield C, et al. Maturation stage of T-cell acute lymphoblastic leukemia determines BCL-2 versus BCL-XL dependence and sensitivity to ABT-199. *Cancer Discov*. 2014;4(9):1074-1087. doi:10.1158/2159-8290.CD-14-0353

2. Van Vlierberghe P, Ferrando A. The molecular basis of T cell acute lymphoblastic leukemia. *J Clin Invest*. 2012;122(10):3398-3406. doi:10.1172/JCI61269

3. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. *Lancet*. 2013;381(9881). doi:10.1016/S0140-6736(12)62187-4

4. Jain N, Lamb AV, O'Brien S, et al. Early T-cell precursor acute lymphoblastic leukemia/lymphoma (ETP-ALL/LBL) in adolescents and adults: a high-risk subtype. *Blood*. 2016;127(15):1863-1869. doi:10.1182/blood-2015-08-661702

5. Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinforma Oxf Engl*. 2013;29(1):15-21. doi:10.1093/bioinformatics/bts635

6. Bond J, Graux C, Lhermitte L, et al. Early Response-Based Therapy Stratification Improves Survival in Adult Early Thymic Precursor Acute Lymphoblastic Leukemia: A Group for

Research on Adult Acute Lymphoblastic Leukemia Study. *J Clin Oncol Off J Am Soc Clin Oncol*. 2017;35(23):2683-2691. doi:10.1200/JCO.2016.71.8585

7. Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med*. 2016;374(4):311-322. doi:10.1056/NEJMoa1513257

8. Seymour JF, Kipps TJ, Eichhorst B, et al. Venetoclax–Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. *N Engl J Med*. 2018;378(12):1107-1120. doi:10.1056/NEJMoa1713976

9. DiNardo CD, Jonas BA, Pullarkat V, et al. Azacitidine and Venetoclax in Previously Untreated Acute Myeloid Leukemia. *N Engl J Med*. 2020;383(7):617-629. doi:10.1056/NEJMoa2012971

10. Pullarkat VA, Lacayo NJ, Jabbour E, et al. Venetoclax and Navitoclax in Combination with Chemotherapy in Patients with Relapsed or Refractory Acute Lymphoblastic Leukemia and Lymphoblastic Lymphoma. *Cancer Discov*. Published online January 1, 2021. doi:10.1158/2159-8290.CD-20-1465

11. Richard-Carpentier G, Jabbour E, Short NJ, et al. Clinical Experience With Venetoclax Combined With Chemotherapy for Relapsed or Refractory T-Cell Acute Lymphoblastic Leukemia. *Clin Lymphoma Myeloma Leuk*. 2020;20(4):212-218. doi:10.1016/j.clml.2019.09.608

12. Bhatt S, Pioso MS, Olesinski EA, et al. Reduced Mitochondrial Apoptotic Priming Drives Resistance to BH3 Mimetics in Acute Myeloid Leukemia. *Cancer Cell*. 2020;38(6):872-890.e6. doi:10.1016/j.ccell.2020.10.010

13. Pan R, Hogdal LJ, Benito JM, et al. Selective BCL-2 inhibition by ABT-199 causes on-target cell death in acute myeloid leukemia. *Cancer Discov*. 2014;4(3):362-375. doi:10.1158/2159-8290.CD-13-0609

14. Konopleva M, Pollyea DA, Potluri J, et al. Efficacy and Biological Correlates of Response in a Phase II Study of Venetoclax Monotherapy in Patients with Acute Myelogenous Leukemia. *Cancer Discov*. 2016;6(10):1106-1117. doi:10.1158/2159-8290.CD-16-0313

15. Anderson MA, Deng J, Seymour JF, et al. The BCL2 selective inhibitor venetoclax induces rapid onset apoptosis of CLL cells in patients via a TP53-independent mechanism. *Blood*. 2016;127(25):3215-3224. doi:10.1182/blood-2016-01-688796

16. Frismantas V, Dobay MP, Rinaldi A, et al. Ex vivo drug response profiling detects recurrent sensitivity patterns in drug-resistant acute lymphoblastic leukemia. *Blood*. 2017;129(11):e26-e37. doi:10.1182/blood-2016-09-738070

17. Seyfried F, Demir S, Hörl RL, et al. Prediction of venetoclax activity in precursor B-ALL by functional assessment of apoptosis signaling. *Cell Death Dis*. 2019;10(8):1-12. doi:10.1038/s41419-019-1801-0

18. Kuusanmäki H, Kytölä S, Vänttinen I, et al. Ex vivo venetoclax sensitivity testing predicts treatment response in acute myeloid leukemia. *Haematologica*. Published online December 15, 2022. doi:10.3324/haematol.2022.281692

19. Del Gaizo Moore V, Schlis KD, Sallan SE, Armstrong SA, Letai A. BCL-2 dependence and ABT-737 sensitivity in acute lymphoblastic leukemia. *Blood*. 2008;111(4):2300-2309. doi:10.1182/blood-2007-06-098012

20. Ryan J, Montero J, Rocco J, Letai A. iBH3: simple, fixable BH3 profiling to determine apoptotic priming in primary tissue by flow cytometry. *Biol Chem*. 2016;397(7):671-678. doi:10.1515/hsz-2016-0107

21. Certo M, Del Gaizo Moore V, Nishino M, et al. Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. *Cancer Cell*. 2006;9(5):351-365. doi:10.1016/j.ccr.2006.03.027

22. Souers AJ, Leverson JD, Boghaert ER, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med*. 2013;19(2):202-208. doi:10.1038/nm.3048

23. Alford SE, Kothari A, Loeff FC, et al. BH3 Inhibitor Sensitivity and Bcl-2 Dependence in Primary Acute Lymphoblastic Leukemia Cells. *Cancer Res*. 2015;75(7):1366-1375. doi:10.1158/0008-5472.CAN-14-1849

24. Del Gaizo Moore V, Brown JR, Certo M, Love TM, Novina CD, Letai A. Chronic lymphocytic leukemia requires BCL2 to sequester prodeath BIM, explaining sensitivity to BCL2 antagonist ABT-737. *J Clin Invest*. 2007;117(1):112-121. doi:10.1172/JCI28281

Figure 1. Adult ETP-ALL has increased dependency on BCL-2 rather than BCL-XL for survival.

(A) Experimental schematic for baseline BH3 profiling. Cytochrome c release was measured by gating on blats. (B) Binding affinities of BH3 peptides (BAD and HRK) and BH3 mimetics (venetoclax, navitoclax, and A-1331852) for anti-apoptotic proteins BCL-2 and BCL-XL. (C/D) FACS-based BH3 profiles for BAD (BCL-2 and BCL-XL dependence) and HRK (BCL-XL dependence). One-way ANOVA analysis for % cytochrome c release between BAD vs. DMSO, BAD vs. HRK. (E) Spearman correlation between % cytochrome c release for BAD vs. HRK, BAD vs. BAD-HRK. Data is normalized to DMSO. (F/G) FACS-based BH3 profiles for venetoclax, navitoclax, and A-1331852. One-way ANOVA analysis for % cytochrome c release between venetoclax vs. DMSO, navitoclax vs. DMSO, and venetoclax vs. navitoclax. (H) Cell death assays using annexin V for adult ETP-ALL samples treated with venetoclax, navitoclax, or A-1331852 for 8 hours. Data are plotted as percentage of live cells compared to DMSO controls. UN = untreated. Note: Gating on adult ETP-ALL primary blast samples. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$; ns = no significance. (C)/(F) Dotted line represents threshold for significant priming determined by DMSO±3xSD.

Figure 2. Adult T-ALL specimens are primarily dependent on BCL-2 and partially dependent on BCL-XL for survival. (A/B) FACS-based BH3 profiles using BAD (BCL-2 and BCL-XL dependence) and HRK (BCL-XL dependence) peptides. One-way ANOVA analysis of % cytochrome c release between BAD vs. DMSO and HRK vs. DMSO; BAD vs. HRK. (C) Spearman correlation between % cytochrome c release for BAD vs. HRK, BAD vs. BAD-HRK. (D/E) FACS-based BH3 profiles for venetoclax, navitoclax, and A-1331852. One-way ANOVA analysis for % cytochrome c release between venetoclax vs. DMSO, navitoclax vs. DMSO, A-1331852 vs. DMSO, venetoclax vs. navitoclax, venetoclax vs. A-1331852 and navitoclax vs. A-1331852. (F) Cell death assays using annexin V for adult T-ALL primary samples treated with venetoclax, navitoclax, or A-1331852 for 8 hours. Refer to Supplemental Figure 1 for additional samples. Data are plotted as the percentage of live cells compared to DMSO controls. UN = untreated. Mean ± SD of three replicates. (G) Dot plot of BAD peptide response vs. HRK peptide response in adult ETP-ALL (green) and T-ALL (black). Red shows probable BCL-2 dependence; blue shows probable BCL-XL dependence. (H) Clinical response timelines of Patient M, Patient O, Patient N, Patient 15, Patient 19, and Patient 20. CR, complete response; PR, partial response; MRD, minimal residual disease. (I) Proposed schematic on prediction of venetoclax and navitoclax efficacy by BCL-2 and BCL-XL dependence. Note: Gating on adult T-ALL primary blast samples. Priming was normalized to DMSO. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$; ns = no significance. (A)/(D) Dotted line represents threshold for significant priming determined by DMSO±3xSD.

Figure 1

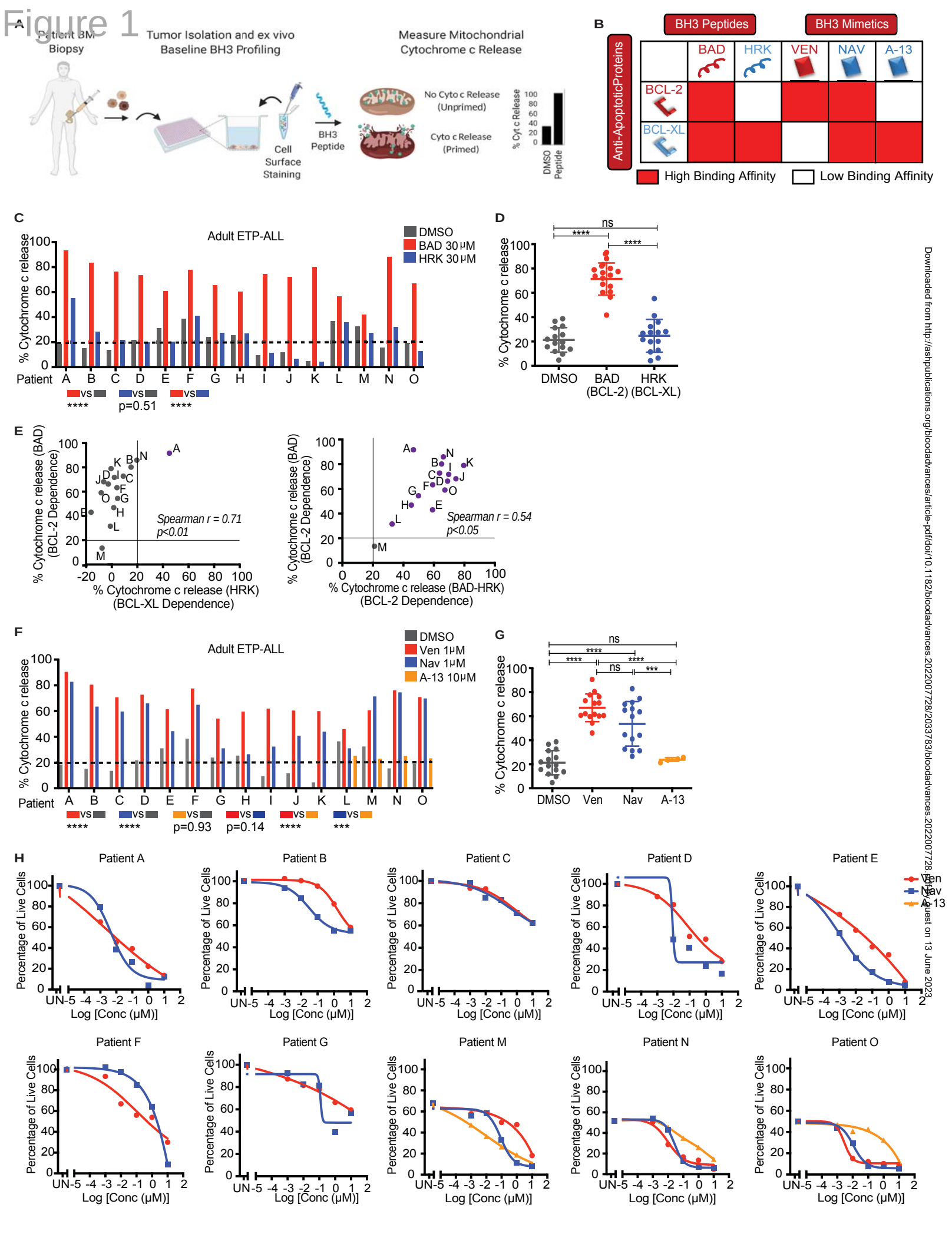

Figure 2