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Mitochondrial Priming of Chronic Lymphocytic Leukemia Patients Associates Bcl-x_L Dependence with Alvocidib Response

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Letter to the Editor

Chronic lymphocytic leukemia (CLL) patient outcomes may benefit from targeting therapies to individuals with favorable molecular profiles. We assessed mitochondrial functionality in apoptosis signaling for CLL patient response to alvocidib (flavopiridol). Pretreatment patient specimens from Trials OSU0055 and EFC6663 (relapsed/refractory patients) were assessed for mitochondrial outer membrane permeabilization following BH3-only peptides incubation. Training and test cohorts analyses established association between Bim and Hrk biomarkers and patient response that benefitted from inclusion of trisomy 12 status. Separately, tumor lysis syndrome (TLS) occurrence associated with Bad priming that benefitted from ECOG status and patient age inclusions. Taken together, Bim and Hrk association with CLL patient response are consistent with Bcl-x_L-dependence as a driver of alvocidib efficacy. Concurrently, Bad association with TLS, indicative of Bcl-2 dependency, may identify patients at risk for treatment-related toxicity.

Alvocidib has demonstrated single-agent clinical activity in relapsed/refractory CLL patients in Phase I and Phase II clinical studies although a limitation in the clinical activity was

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Supplementary information is available at Leukemia's website.

Conflict-of-interest disclosure

WEP, CD, RJL, NB, ME, and MHC are employees of Eutropics, Inc. SLW and DB are employees of Tolero Pharmaceuticals, Inc. The remaining authors declare no conflicts-of-interest. JCB and MRG are co-inventors of a patent pending for the use of alvocidib.

originally limited presumably due to high protein binding in plasma, attenuating levels of available drug patients.[1–2] However, Byrd and colleagues recently identified a hybrid IV dosing schedule that utilizes a 30-minute loading dose (30 mg/m^2) followed by a 4-hour infusion (50 mg/m^2). This novel dosing schedule has allowed for improved single-agent activity in relapsed and refractory CLL patients in multiple clinical studies with overall response rates between 30–53%, including favorable responses in high-risk patients with 17p13.1 deletions.[3–6] Response rate limitations have been compounded by treatment-related toxicities including TLS. Patient enrichment strategies that include molecular and cytogenetic approaches may enhance development of alvocidib in CLL by targeting drug to patients most likely to exhibit response and least likely to experience adverse events.

Assessment of intrinsic mitochondrial apoptosis pathways by functional profiling has recently shown encouraging results for patient stratification strategies in treatment of hematologic malignancies.[7–8] This profiling of Bcl-2 family members serves to elucidate the extent that pro-apoptotic sensitizers (i.e. Puma, Noxa, Bad, Hrk) and activators (i.e. Bim) may regulate activity of anti-apoptotic proteins (i.e. Mcl-1, Bcl-2, Bcl-x_L). Indeed, previous investigations have established that alvocidib potentiates pan-BH3-mimetic activity through up-regulation of BH3-only proteins with coordinate down-regulation of their anti-apoptotic counterparts.[9] Previous studies assessing alvocidib efficacy in CLL patients ex vivo and expression levels of Bcl-2 family proteins, including Mcl-1, were not able to establish correlation with clinical efficacy.[10]. Based on these observations, we have investigated the functional context of intrinsic apoptosis BH3-only proteins by mitochondrial priming assessment as a surrogate for cellular response to pro-apoptotic cues to provide a predictive strategy for CLL patient management.[7,11]

Patients were randomly segregated into 2 cohorts that would serve as proof-of-principle (n=30) and validation sets (n=32); cohorts were established merely on the basis of specimen tissue bank (Patient information provided in Supplemental Table 1.) Percent priming (i.e. quantifiable propensity of a given BH3 peptide to induce mitochondrial depolarization relative to an uncoupling control agent) for each peptide is summarized in Supplemental Table 2 for patients who exhibited partial response (PR,) stable disease (SD,) or progressive disease (PD). In the training set, only Bim(0.1) and Hrk elicited significance (p=.014 and p=.0098, respectively) between biomarker and PR, SD, and PD patients (regression analyses). These two markers validated (Bim p=.0051; Hrk p=.015) in the second set. When the two sets were combined (n=62), regression indicated Bim(0.1) and Hrk were both significant (Bim(0.1) p=.0027 and Hrk p=.00046, respectively; P-value significance at <0.01 for Bonferroni correction as described in Methods)(Figure 1A). Although not considered in the core group of patients initially identified and examined in this study, a subset of patients from OSU trial 0491 for which there were sufficient available vials was considered as a secondary validation set. Here, Hrk priming in PD/SD patients displayed mean = 6.4%, and PR patients mean = 22.2% (n=13; 4PD/SD, 9 PR). Although not statistically powered, the trend towards Hrk priming correlation with clinical response was consistent (Supplemental Figure 2).

Figure 1B indicates dot plot depictions of Bim(0.1) and Hrk relative to response when the profiling data is further analyzed as PD/SD versus PR. To test the ability of either Bim(0.1)

or Hrk to serve as predictive biomarker of response, we employed AUC analysis to determine the sensitivity and specificity of this biomarker;Bim AUC = 0.73 (CI[.60–.85]; p=0.00037) and Hrk AUC = 0.73 (CI[.61–.86]; p=.00022) (significant P-value<0.01 employing Bonferroni correction). Bim(0.1) displays a p = .04 while Hrk indicates a p=. 0039 (significant P-value<0.01). Adjusted analyses of Bim(0.1) and Hrk priming in which we accounted for trisomy12 status as a second covariate improved the AUC to 0.83(CI[.71–. 95]; p<.0001) (Figure 1C). In contrast to Hrk, trisomy12 status did not improve the model when added with Bim(0.1).

Trisomy 12 is a frequent event in CLL, occurring in approximately 20% of patients[12] generally carrying an intermediate prognosis.[12–13] The frequency of trisomy 12 positive patients in the EFC6663 study is in line this (8 of the 62 (13%)). Although small in number, all eight of the patients on study experienced clinical benefit (7 PR, 1 SD) and whether this extends to a larger patient population needs to be explored. Approximately 35–50% of trisomy 12 positive patients also have a mutation in *NOTCH1*,[14] conferring adverse outcomes in some series.[15] This patient stratification strategy could benefit from layering of BH3 profiling to stratify patients most likely to benefit from alvocidib in future studies.

BH3 profiling biomarkers were analyzed relative to progression free survival (PFS) and overall survival (OS); no significant association between biomarker and outcome was observed (Supplemental Figure 3) although there was a trend between Bim(0.1) and OS (p=. 03) and between Hrk and PFS (p=.08) suggesting that an appropriately powered study may demonstrate more significant correlation between these parameters..

As TLS remains a concern in patients prescribed certain therapies, including alvocidib, we sought to determine if BH3 profiling could be used to identify patients at risk for experiencing toxicity. Supplemental Table 3 summarizes association of BH3 profiling biomarkers with TLS status for 48 patients for which toxicity data was available. Here, both higher Bad and Puma(10) priming scores were significantly associated with TLS (Figure 2A) by Wilcoxon (p=.0083 and p=.0080, respectively) and logistic regression (p=.012 and p=.012, respectively). None of the other BH3 profiling biomarkers was significant (p>.05). The AUC statistic indicated that each marker held a significant ability to predict TLS status; Puma(10) AUC = 0.75 (p=.00079) and Bad AUC = .75 (p = .00072). Puma(10) and Bad when added together did not improve predictive capacity of either marker independently.

Patient age (p=.034 Wilcoxon; p= .067 logistic regression) and baseline ECOG status (p=. 021 Wilcoxon, p=.031 logistic regression) showed significant association or borderline association with toxicity (Table 3). Multivariate analyses, with inclusion of both patient age and ECOG status to Bad BH3 profiling resulted in AUC increase from 0.75 to 0.85, and improved p = .0007 to p < .0001 (Figure 2B).

Mitochondrial priming readout from Hrk and Bim(0.1), both with capacity to bind to and modulate activity of anti-apoptotic Bcl-x_L, are the markers statistically associated with response indicating this clinical endpoint is driven by Bcl-x_L dependency. Patients likely to exhibit TLS are predicted by Bad priming, indicative toxicity may be driven by its anti-apoptotic binding partner Bcl-2. One plausible mechanism to explain the observed

phenomenon is that clinical response and TLS are occurring via action of alvocidib on two clonally related but biologically distinct cell subpopulations, one driven by Bcl-x_L and the other by Bcl-2. Interestingly, both subpopulations would by definition comprise B-cell populations as the BH3 profiling assay here is performed on a patient specimen after purification and elimination of non-B-cells. Additional work will be required to further characterize the presence and composition of these distinct subpopulations. Priming by Noxa, a specific pro-apoptotic modulator of anti-apoptotic Mcl-1, is not associated with either alvocidib response or TLS, consistent with Mcl-1 not being a driver mechanism.

Conceivably, patients could be stratified by cytogenetics and BH3 profiling to increase the overall response rate in patients from 35–50% as observed in previous clinical studies. In the current patient set, 23 of the 62 analyzed patients achieved a PR to alvocidib (37.1%.) In our analyses, the combination of Hrk + trisomy12 yielded an AUC of 0.83. By closer examination of the ROC curves, 95.6% of responder patients were identified (sensitivity) concurrently to discrimination of 66.7% of likely PD/SD patients (specificity). If the predictive value of such an applied biomarker may be to triage the likely non-responder patients (here the triaging of 26 PD/SD patients, while also mis-identifying 1 likely PR patient), then the response rate increases from 37.1% to 62.9% (an overall 69.5% improvement of response rate). Similarly, TLS incidence could be decreased from 13% observed in the EFC6663 study to a minimal number moving forward. While larger cohorts will be required to fully define the extent of medical utility for such diagnostics, thes eearly observations may hold promise for alvocidib impact in CLL patient options and improved outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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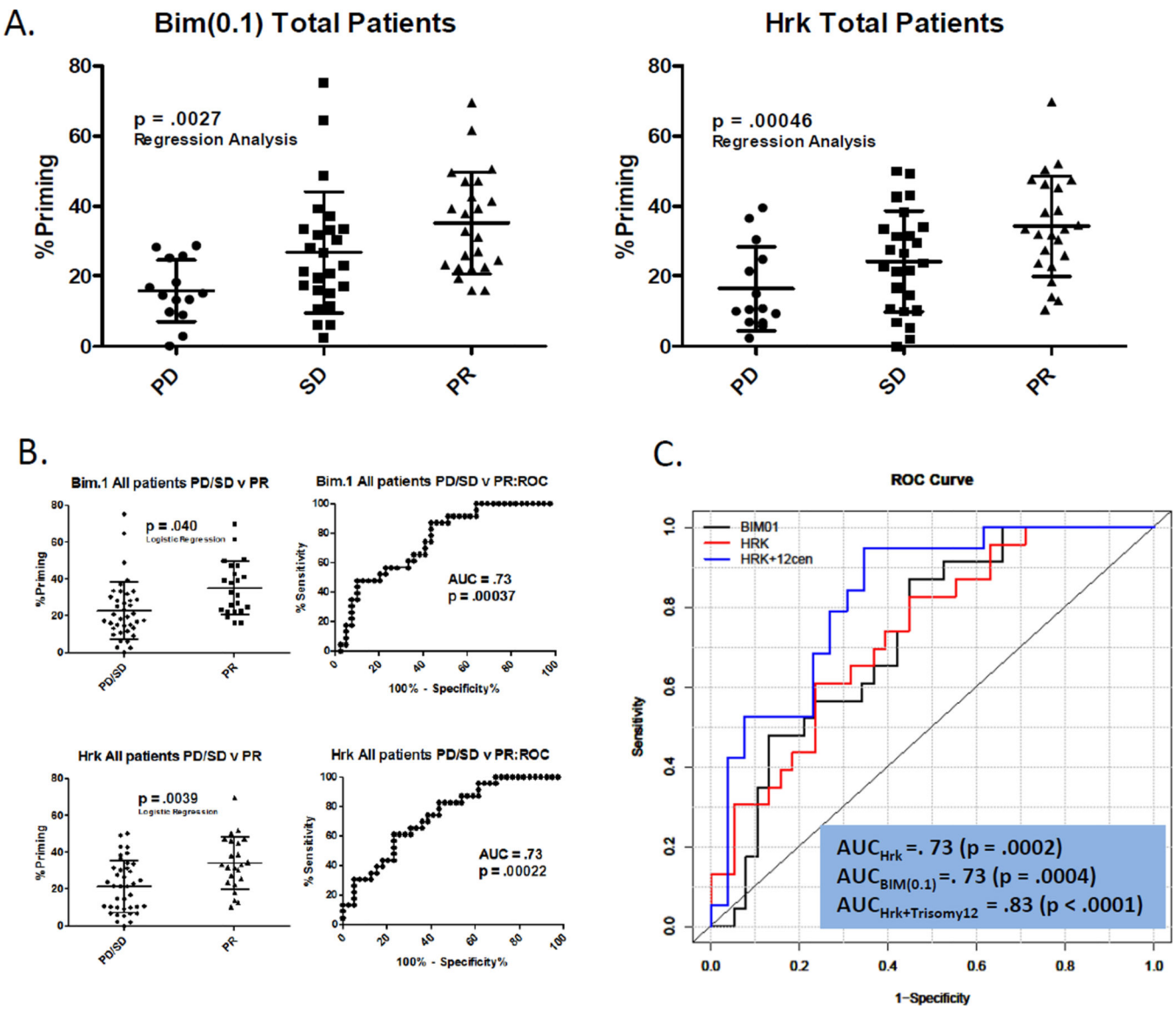

Figure 1. Bim and Hrk BH3 profiling of CLL patients are correlated with alvocidib response

A. Dot-plot depictions of the combined data set by stratification of response into 3 categories (PD, SD, PR). Note that increased priming trends are observed for both Bim(0.1) and Hrk from PD to SD and then from SD to PR. B. Dot-plot and ROC-plot depictions of Bim(0.1) and Hrk display response discrimination (2 groups: PD/SD, PR). C. Chromosome 12 trisomy multivariate analysis adds to Hrk prediction of CLL patient clinical response to alvocidib. While both Bim(0.1) and Hrk display AUC from ROC-plot depictions of 0.73, Hrk models benefit from inclusion of significant clinical adjustment variable trisomy12 to yield the increased AUC of 0.83 ($p < .0001$).

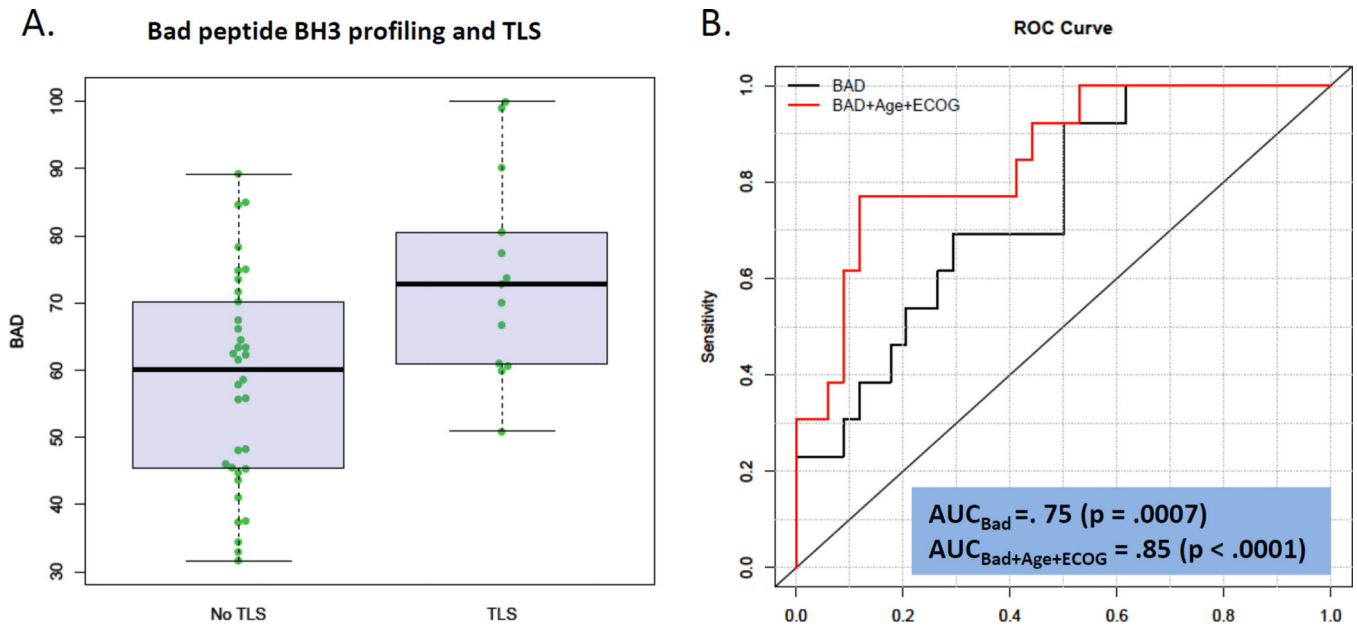

Figure 2. Bad peptide BH3 profiling correlates with TLS in CLL patients following treatment with alvocidib

A. Dot plot depictions indicate that higher Bad BH3 profiling readout values are significantly associated with the presence of TLS versus those patients who did not experience TLS. B. The Bad AUC from ROC-plot analysis was 0.75; this improved to 0.85 when combined with clinical adjustment variables age and ECOG status.