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Functional profiling of venetoclax sensitivity can predict clinical response in multiple myeloma

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In a phase I clinical trial of Venetoclax in patients with t(11;14) relapsed/refractory MM, 40% of the patients treated achieved an objective response(1). While this response rate with a single agent in MM is impressive, it demonstrates that t(11;14) is not an optimal biomarker for response to venetoclax. Functional profiling of BCL2 family members has been shown to predict responses to therapy in myeloma and other diseases(2). Therefore, we set out to determine how *ex vivo* sensitivity to venetoclax corresponds to clinical response and resistance.

We performed *ex vivo* apoptosis assays on a cohort of 76 samples collected from 65 patients (Supplemental Table 1). Consistent with previous preclinical results we found that samples from patients with t(11;14) myeloma were significantly more sensitive than samples from t(11;14)-negative patients (Median IC50 70 vs. 910 nM, $P=0.0176$, Figure 1A)(3). However, a range of sensitivity was observed in both populations.

The samples appeared to cluster into three groups based on sensitivity. Thirty of the 76 total samples (39.5%) had an IC50 of 100 nM or less (Figure 1B, green bars), consisting primarily from t(11;14)-positive samples (73.3%). A second cohort of 20 samples (26.3%) had an IC50 from 170 to 450 nM (Figure 1B, yellow bars). In the remaining 26 samples (34.2%) the IC50 was 900 nM or greater (range 900–18400 nM, Figure 1B, red bars). The presence of t(11;14) was the only significant difference between the sensitive group and the

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Conflict of Interest

NJB funding and honorarium from Celgene and Janssen

PM and JDL employment, Abbvie

LTH, research funding, Abbvie

SL consultancy Takeda, Celgene, Novartis, Abbvie, Amgen, Janssen, Bristol-Myers Squibb

AKN advisory board or consultancy, Amgen, Adaptive Technologies, Janssen, Celgene, Spectrum Pharmaceuticals, Bristol-Myers Squibb, GlaxoSmithKline, Takeda

JLK Consultancy, Abbvie, Janssen, Bristol-Myers Squibb, Takeda, Data Monitoring Committee, Karyopharm, Pharmacyclics

LHB, consultancy, Abbvie, honorarium, AstraZeneca.

others (Supplemental Table 2). Twenty patients received venetoclax as a single agent (N=10) or in combination with dexamethasone (N=9) or daratumumab/dexamethasone (N=1) and 18 had *ex vivo* sensitivity tested immediately prior to venetoclax treatment (Supplemental Tables 1 and 3). A patient with non-secretory disease and another that withdrew consent prior to completing one cycle were not included in this analysis. Ten of the 16 remaining patients had a clinical response (PR or better), 9 of which were in the most sensitive cohort. Only 1 of the 6 non-responding patients was in the most sensitive group (Figure 1C). Thus, the sensitivity of the assay is 0.9 while the specificity is 0.83 (Fisher's exact test, P=0.0076). Examples of *ex vivo* concentration-response curves and clinical responses are provided in Supplemental Figure 1. Together these data suggest that *ex vivo* pre-treatment sensitivity can predict patient responses to venetoclax.

Of the patients who responded to venetoclax, 3 had both a pre-treatment and post-relapse sample that was analyzed (Figure 1D). MM93 had an initial IC50 of 8 nM and achieved a PR for 8 months (Supplemental Figure 2). In the post-treatment sample the IC50 increased to 70 nM (Figure 1D, left panel). While this would still be considered a sensitive sample in the *ex vivo* analysis, it does represent a nearly 9-fold increase in IC50 post-treatment. MM100 had non-secretory disease however while on venetoclax the patient demonstrated a hematologic recovery. This "response" was transient and the patient became cytopenic after 3 months (Supplemental Figure 2). Consistent with development of venetoclax resistance, *ex vivo* testing indicated that the IC50 had increased from 4 nM to 1870 nM (Figure 1D). MM91 had an initial IC50 of 20 nM when the combination of daratumumab, venetoclax and dexamethasone was initiated. The patient had a VGPR that lasted for over 11 months before progression. The post-treatment IC50 increased over 400-fold to 8100 nM (Figure 1D). Finally, for two patients who were treated, no pre-treatment sample was available however a post-relapse sample was tested for *ex vivo* sensitivity. In both cases the sample tested as moderately or highly resistant to venetoclax (Figure 1D, right panel). MM77 achieved a sCR that was maintained for 15 months. The post-relapse sample was highly resistant to *ex vivo* venetoclax treatment (IC50, 12000 nM). MM99 had stable disease for nearly 6 months before treatment was stopped. The post-treatment sample displayed sensitivity consistent with our pre-treatment non-responder population (IC50, 200 nM). Thus, post-treatment *ex vivo* testing reflects development of venetoclax resistance.

In addition to testing serial samples from patients who were treated with venetoclax we were able to test samples from 6 patients who were not treated with venetoclax to determine if clonal changes associated with other therapy could influence *ex vivo* sensitivity. We observed a decrease in *ex vivo* IC50 with 2 patients, an increase in *ex vivo* IC50 in 3 patients and 1 patient who had no change (Supplementary Figures 3 and 4).

We previously demonstrated that dexamethasone could synergize with venetoclax by increasing the expression of the pro-apoptotic BH3-only protein BIM and subsequent priming of BCL2(4). This combination resulted in an increase in the response rate of t(11;14)-positive patients from 40% in the initial study to 65%(5). We analyzed the ven/dex combination in 46 samples, including our original cohort of 9. Only 11 samples demonstrated *ex vivo* sensitivity to dexamethasone alone as defined by induction of at least 25% cell death. Therefore, the majority of responses observed are due to sensitization while

in the 11 that showed dexamethasone sensitivity we cannot rule out additive or synergistic effects if the cells were also sensitive to venetoclax. The decrease in IC50 with dexamethasone was significant in all three sensitivity cohorts. In the most sensitive cohort, the median IC50 decreased from 40 nM to 9.5 nM with dexamethasone (P 0.0001). However, 5/20 samples were sensitive to dexamethasone alone suggesting that this change could be in part due to an additive response to two active drugs (Figure 2A). Dexamethasone had a more profound effect on the intermediate population where 7/10 samples had an IC50 shift to the most sensitive cohort (Figure 2B, median IC50 325 nM vs. 60 nM with dexamethasone, P=0.0020). While 5/10 samples were sensitive to dexamethasone alone, they are not considered sensitive to venetoclax alone so this is unlikely to be an additive response, more likely reflecting synergy or sensitization. Similar results were observed in the most resistant population where 8/15 samples shifted to an IC50 consistent with a more sensitive cohort (median IC50 3250 nM vs. 810 nM for the combination, P 0.0001). Only 1 of these samples was sensitive to dexamethasone alone. Amazingly 3 of these samples were now in the most sensitive cohort demonstrating a greater than one log shift in the venetoclax IC50 (Figure 2C). Of the 10 samples that were sensitized by dexamethasone to an IC50 consistent with the sensitive cohort only half were t(11;14) suggesting this may be an effective way to increase the response rate of venetoclax in both t(11;14)-positive and -negative myeloma.

Together our data represent an alternative approach to precision medicine that utilizes functional testing rather than targeting of acquired mutations or structural variations(6,7). The latter have had limited success due to the heterogeneity of these events within a tumor or availability of targeted therapy (8) (9). Targeting tumor dependence on BCL2 for survival, which is typically not based on mutations or translocations, is a promising new approach and has been used effectively in CLL (10) (11). Our results build on the pre-clinical BH3 profiling and *ex vivo* testing with venetoclax first used to demonstrate BCL2 dependence in t(11;14) myeloma (3, 12, 13).

We applied functional profiling of BCL2 dependence to a large series of myeloma patient samples and consistent with previous findings demonstrated that t(11;14) was the only commonly tested marker that was associated with *ex vivo* venetoclax sensitivity(3). However consistent with previous studies of cell lines, patients samples, and the phase I venetoclax monotherapy trial, t(11;14) samples can be resistant to venetoclax and non-t(11;14) samples can be sensitive(1). Therefore, we focused on the functional responses of the samples as opposed to genomic alterations to define BCL2 dependence. Since these cohorts included patients treated with venetoclax (both pre-and post-treatment) we were able to determine what level of *ex vivo* drug sensitivity associated with clinical response. There was a strong correlation with *ex vivo* sensitivity as 90% of the responding patients fell in the most sensitive cohort and 83% of the non-responding patients were in the other 2 cohorts. This compares favorably to the use of expression ratios, which worked best when the ratio was high (1). Moreover, all post-treatment samples showed a significant increase in their IC50. While the total number of treated patients evaluated in this study is small it is the first example of a patient cohort where clinical and *ex vivo* responses were both measured.

Our findings also demonstrate the dynamic nature of BCL2 dependence and the importance of when sensitivity is tested relative to initiation of treatment. We observed significant changes in sensitivity of samples taken from patients at different points of treatment. This could reflect clonal heterogeneity of myeloma and the effects of previous treatments on the makeup of the disease at any given time(14, 15). Alternatively, it could be due to changes in mitochondrial priming associated with therapy(16).

Finally, *ex vivo* testing demonstrates the ability of dexamethasone to convert some venetoclax-insensitive or even -resistant cells to clinically sensitive samples. This combination has already been shown to have significant activity in t(11;14) patients compared to single agent venetoclax(5). Our data point to the possibility of using this combination in a broader spectrum of myeloma patients beyond the 15–20% that are t(11;14)-positive.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Kumar S, Kaufman JL, Gasparetto C, Mikhael J, Vij R, Pegourie B, et al. Efficacy of venetoclax as targeted therapy for relapsed/refractory t(11;14) multiple myeloma. *Blood*. 2017;130(22):2401–9. [PubMed: 29018077]
2. Ni Chonghaile T, Sarosiek KA, Vo TT, Ryan JA, Tammareddi A, Moore Vdel G, et al. Pretreatment mitochondrial priming correlates with clinical response to cytotoxic chemotherapy. *Science*. 2011;334(6059):1129–33. [PubMed: 22033517]
3. Touzeau C, Dousset C, Le Gouill S, Sampath D, Levenson JD, Souers AJ, et al. The Bcl-2 specific BH3 mimetic ABT-199: a promising targeted therapy for t(11;14) multiple myeloma. *Leukemia*. 2014;28(1):210–2. [PubMed: 23860449]
4. Matulis SM, Gupta VA, Nooka AK, Hollen HV, Kaufman JL, Lonial S, et al. Dexamethasone treatment promotes Bcl-2 dependence in multiple myeloma resulting in sensitivity to venetoclax. *Leukemia*. 2016;30(5):1086–93. [PubMed: 26707935]
5. Kaufman JL, Gasparetto CJ, Mikhael J, Moreau P, Touzeau C, Vij R, et al. Phase 1 Study of Venetoclax in Combination with Dexamethasone As Targeted Therapy for t(11;14) Relapsed/Refractory Multiple Myeloma. *Blood*. 2017;130(Suppl 1):3131-.
6. Friedman AA, Letai A, Fisher DE, Flaherty KT. Precision medicine for cancer with next-generation functional diagnostics. *Nature reviews Cancer*. 2015;15(12):747–56. [PubMed: 26536825]
7. Letai A. Functional precision cancer medicine-moving beyond pure genomics. *Nature medicine*. 2017;23(9):1028–35.
8. Cheng ML, Solit DB. Opportunities and Challenges in Genomic Sequencing for Precision Cancer Care. *Ann Intern Med*. 2018;168(3):221–2. [PubMed: 29310131]
9. Moscow JA, Fojo T, Schilsky RL. The evidence framework for precision cancer medicine. *Nat Rev Clin Oncol* 2018;15(3):183–92. [PubMed: 29255239]
10. Levenson JD. Chemical parsing: Dissecting cell dependencies with a toolkit of selective BCL-2 family inhibitors. *Mol Cell Oncol* 2016;3(1):e1050155.

11. 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. *The Journal of clinical investigation*. 2007;117(1):112–21. [PubMed: 17200714]
12. 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;30(3):761–4. [PubMed: 26174630]
13. 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*. 2011;118(14):3901–10. [PubMed: 21835956]
14. Lohr JG, Stojanov P, Carter SL, Cruz-Gordillo P, Lawrence MS, Auclair D, et al. Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy. *Cancer cell*. 2014;25(1):91–101. [PubMed: 24434212]
15. Walker BA, Wardell CP, Melchor L, Brioli A, Johnson DC, Kaiser MF, et al. Intracloal heterogeneity is a critical early event in the development of myeloma and precedes the development of clinical symptoms. *Leukemia*. 2014;28(2):384–90. [PubMed: 23817176]
16. Montero J, Sarosiek KA, DeAngelo JD, Maertens O, Ryan J, Ercan D, et al. Drug-induced death signaling strategy rapidly predicts cancer response to chemotherapy. *Cell*. 2015;160(5):977–89. [PubMed: 25723171]

Figure 1. Ex Vivo Response of Patient-Derived Plasma Cells to Venetoclax.

Buffy coat cells were treated with Venetoclax for 24 h and apoptosis of the plasma cells was determined as described in the Supplemental Methods section. Dose curves using concentrations of 0, 10, 100 and 1000 nM venetoclax were used to calculate the IC₅₀ for each patient sample. **A.** The samples were clustered based on the presence (N=31) or absence (N=36) of the t(11;14) translocation and graphed according to IC₅₀. Whiskers extend from 10–90%, $P=0.0176$. Post-venetoclax treatment samples were removed from this analysis due to the effects of acquired resistance. **B.** Samples separated into 3 categories

according to venetoclax sensitivity; Green – highly sensitive, IC_{50} up to 100 nM, Yellow – intermediate sensitivity, IC_{50} 170 nM – 450 nM, Red – resistant, IC_{50} 900 nM. Asterisks denote samples from t(11;14)-positive patients **C.** Clinical response of 16 patients graphed versus IC_{50} . Progressive disease (PD) N = 4, Single agent venetoclax; stable disease (SD) N = 2, single agent venetoclax. Partial response (PR) N = 4, venetoclax monotherapy; very good partial response (VGPR) N = 2, 1 single agent venetoclax, 1 venetoclax plus daratumumab and dexamethasone; stringent complete response sCR N = 4, 2 venetoclax monotherapy, 2 venetoclax plus dexamethasone. Dotted line represents the cutoff for the most sensitive cohort (100 nM), solid lines represent the median IC_{50} for each response. Symbol colors represent sensitivity as shown in Figure 1B. **D. (Left)** Ven IC_{50} for MM91, MM100, and MM93 before treatment and upon progression. **(Right)** Post-Ven IC_{50} for MM77 and MM99. Pre-treatment samples were not obtained.

Figure 2. Combining Dexamethasone with Venetoclax Decreases IC₅₀ and Shifts Sample Classification.

Buffy coat cells were isolated and treated with Ven as before, +/- 0.5 μ M Dex. **A.** IC₅₀ changes in samples sensitive to Ven alone. **B.** Shifts in IC₅₀ of samples displaying intermediate sensitivity to Ven. **C.** IC₅₀ shifts in samples resistant to Ven alone. Symbol colors represent sensitivity as shown in Figure 1B.