Evidence of TCR and BCR Clonal Dynamics with Enitociclib Monotherapy in Patients with MYC+ Non-Hodgkin Lymphoma

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INTRODUCTION

- Non-Hodgkin Lymphoma (NHL) is the seventh most common cancer in men and the sixth most common cancer in women. The disease accounts for 4% of all cancers in the United States. In 2023, an estimated 80,550 people (44,880 men and 35,670 women) in the United States will be diagnosed with NHL.
- A subset of these patients are diagnosed with high-grade B-cell lymphoma with MYC and BCL2 rearrangement, a subtype also known as double-hit diffuse large B cell lymphoma (DH-DLBCL). MYC and BCL2 gene rearrangements result in dysregulated activation of translocation fusion proteins that drive oncogenic transcription and anti-apoptotic signaling, respectively. DH-DLBCL patients have poor prognosis compared with patients with other DLBCL subtypes and are generally refractory to standard of care chemotherapy.
- MYC is a pleiotropic transcription factor, which controls cell cycle, DNA damage repair, and cell metabolism by regulating numerous target genes. MYC mRNA has a short half-life; therefore, high rates of MYC transcription are necessary to drive oncogenic signaling.
- Enitociclib (VIP152) is a potent and selective CDK9 inhibitor with robust MYC downregulation (1). We have previously reported safety, efficacy and MYC downregulation from 16 patients with MYC+ NHL treated with enitociclib monotherapy, including 2 patients with double-hit diffuse large B-cell lymphoma (DH-DLBCL) who achieved complete metabolic remissions (CR) for 5.5+ and 4.0+ years (2,3) respectively and a patient with transformed follicular lymphoma (tFL) who has been on study for 1.33 years and achieved stable disease (SD).
- DH-DLBCL is characterized by MYC and BCL2/BCL6 rearrangements, and MYC+ NHL patients are known to be refractory to immune-oncology (IO) therapies.

Herein, we evaluate the IO effect of enitociclib monotherapy in the blood of MYC+ NHL patients by tracking T cell and B cell clonal dynamics (**Figure 1**).

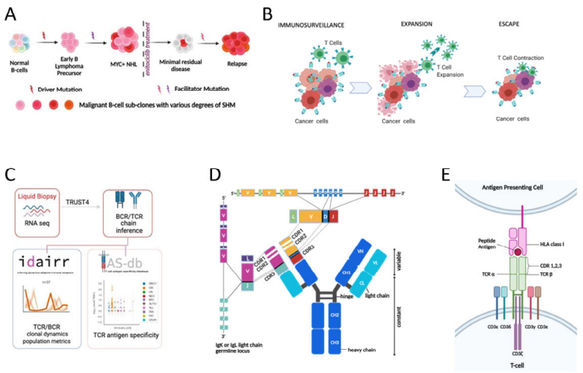

Figure 1- Tracing B-cell and T-cell clonal dynamics in MYC+ NHL patients treated with 30mg weekly enitociclib. (**A**) Schematic representation of malignant B-cell clonal dynamics (**B**) and of T-cell immunosurveillance, expansion on antigen targeting and potential immune escape, representing the expansion and contraction of the anticancer T-cell response. (**C**) Schematic representation of bioinformatics pipeline developed to track B- and T cell dynamics in lymphoma. (**D**) Schematic representation of the B-cell receptor immunoglobulin heavy and light chain (**E**) and the peptide major histocompatibility class I (MHC CI) complex and T-cell receptor binding.

SHM: somatic hypermutation, TCR: T cell receptor, BCR: B cell receptor, db: database, Ig: Immunoglobulin, CDR: complementarity-determining region, HLA: human leukocyte antigen

METHODS

Patient population
Enitociclib is being evaluated in a Phase 1 trial in patients with NHL receiving 30 mg i.v. once weekly (NCT02635672). From the previously reported 16-patient cohort (3), 15 have evidence of MYC+ and had whole blood collections taken before and after enitociclib dosing but only 12 patients had samples sequenced from at least two enitociclib doses (204 samples).

RNA sequencing
RNA was purified from whole blood and bulk RNA seq performed by Illumina stranded total RNA prep with RiboZeroTM plus rRNA depletion and globin reduction RNA library preparation with strand-specific sequencing 50M paired reads (Discovery Life Science, AL, USA).

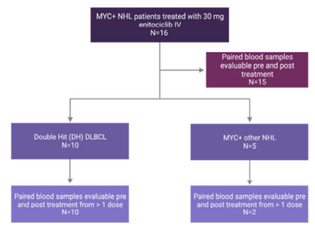

Figure 2- Patient Flow Chart
IV: intravenous

Bioinformatics pipeline and adaptive immune repertoire (AIRR) analysis
T-cell receptor (TCR) and B-cell receptor (BCR) sequences were reconstructed using the TRUST4 algorithm (4). Receptor repertoire richness, Simpson's clonality and dynamics were calculated using the IDAIR pipeline (5). The CDR3 beta sequence of the TCR (CDR3b) sequences were mapped to experimentally validated TCR-antigen pairs in the TAS-db (6): a harmonized, T-cell antigen specificity database that combines CEDAR, VDJdb, McPAS-TCR, IEDB, TCR3d and PATCRdb. Clonality and richness of Ig kappa/ Ig lambda chains (IGKC/IGLC) of the B-cell receptor and of the T cell receptor CDR3 β chain (TRBC) after 2 weekly doses of enitociclib at baseline and the difference in clonality *D*= IGKC-IGLC. As *D* tends to -1, the sample is more clonal in IGLC, and as *D* tends to 1, the sample is more clonal in IGKC. For association with response to enitociclib, patients were separated into two groups: those with high initial IGKC or IGLC clonality, and association with enitociclib response over the course of treatment was tested using the Mann-Whitney test with Benjamini-Hochberg correction for small numbers.

RESULTS

MYC+ NHL patients' baseline characteristics, demographics and evaluable blood samples for TCR and BCR analysis

- Baseline characteristics and demographics of the 15-patient cohort with evaluable samples collected pre- and post-treatment are described in **Tables 1** and **2** include: 13 men; median age 69 (21-84) years; median prior therapy 3 (1-8); and includes DH-DLBCL (10; 2 CR, 8 clinical progression [PD]), and other MYC+ NHL patients (5; triple hit-DLBCL [1 PD], Burkitt lymphoma [1 PD], mantle cell lymphoma [1 PD], Richter DLBCL [1 PD], and transformed follicular lymphoma [1 stable disease, on study for 1.33 years and in Cycle 23]).
- Since the timecourse for clonal dynamics observation is at least 2 weeks, only 12 patients had evaluable blood sample collections over 2 weeks analyzed by RNA seq in this patient cohort **(Figure 2**). Up to a maximum of 20 peripheral blood samples per patient were collected and analyzed.

Table 1. Baseline Characteristics

	DH-DLBCL (N=10)	NHL-Other (N=5)	Total (N=15)
ECOG Performance Status, n(%)			
0	0	3 (60.0)	3 (20.0)
1	8 (80.0)	0	8 (53.3)
2	2 (20.0)	2 (40.0)	4 (26.7)
Number of Prior Systemic Anti-Cancer Therapies			
1	1 (10.0)	1 (20.0)	2 (13.3)
2	4 (40.0)	0	4 (26.7)
>= 3	5 (50.0)	4 (80.0)	9 (60.0)
n	10	5	15
Mean (SD)	3.80 (2.440)	4.00 (2.550)	3.87 (2.386)
Median	2.5	4.0	3.0
Min, Max	1.0, 7.0	1.0, 8.0	1.0, 8.0
Refractory to last treatment, n(%)	3 (30.0)	1 (20.0)	4 (26.7)
Bulky Disease > 5 cm, n(%)	7 (70.0)	5 (100.0)	12 (80.0)
Staging at Study Entry, n(%)			
Ann Arbor STAGE II	1 (10.0)	0	1 (6.7)
Ann Arbor STAGE III	3 (30.0)	0	3 (20.0)
Ann Arbor STAGE IV	6 (60.0)	4 (80.0)	10 (66.7)
Ann Arbor UNKNOWN	0	1 (20.0)	1 (6.7)
Diagnosis, n(%)			
All other NHL	0	5 (100.0)	5 (33.3)
DH-DLBCL	10 (100.0)	0	10 (66.7)

Table 2. Patient Demographics

	DH-DLBCL (N=10)	NHL-Other (N=5)	Total (N=15)
Sex			
Female	1 (10.0)	1 (20.0)	2 (13.3)
Male	9 (90.0)	4 (80.0)	13 (86.7)
Race			
Black or African American	0	1 (20.0)	1 (6.7)
Not Reported	1 (10.0)	0	1 (6.7)
White	9 (90.0)	4 (80.0)	13 (86.7)
Ethnicity			
Hispanic or Latino	1 (10.0)	0	1 (6.7)
Not Hispanic or Latino	9 (90.0)	5 (100.0)	14 (93.3)
Age (years)			
N	10	5	15
Mean (SD)	69.60 (7.633)	60.20 (22.174)	66.47 (14.106)
Median	68.0	69.0	69.0
Min, Max	58.0, 84.0	21.0, 74.0	21.0, 84.0

Adaptive immune repertoire (AIRR) analysis of BCR light chains (IGKC or IGLC) demonstrate clonality changes observed after 2 weekly doses of enitociclib treatment - Lower clonality associates with response.

- Dynamics of malignant B-cell clones were observed by tracking the clonality and frequencies IGKC or IGLC of the B-cell receptor (BCR). We hypothesized that malignant B cell clones can be identified by high frequency of IGKC or IGLC and calculated IGKC and IGLC clonality, and the difference in clonality D= IGKC-IGLC.
- The disappearance of a high frequency IGLC clone in a DH-DLBCL patient with complete response, compared to an increase in a high frequency IGLC clone in a patient with progressive disease.
- Lower median IGKC/IGLC clonality associates with enitociclib response over the course of treatment; however, the small sample size (9 PD, 1 SD, 2 CR) in each response category limits generalizability.

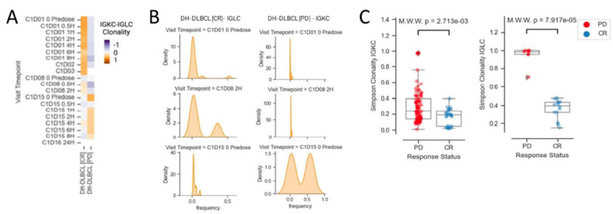

Figure 3. BCR light chains (IGKC or IGLC) clonality changes are observed in patients after 2 weekly doses of enitociclib treatment and lower clonality associates with response. (A) Heatmap of D, the difference in clonality between IGKC and IGLC at each timepoint over the enitociclib treatment course for a patient with complete response [CR] and a patient with progressive disease [PD]. As D tends to -1, the sample is more clonal in IGLC, and as D tends to 1, the sample is more clonal in IGKC. **(B)** Plot of kernel density estimate of the frequency distribution of IGLC over 3 timepoints, for [CR] patient and for [PD] patient. **(C)** Lower Simpson clonality of dominant IGKC or IGLC over enitociclib treatment course associates with response. Box and scatter plot of IGKC (left) or IGLC (right) clonality PD vs. CR, total 11 patients, cycle 1 (C1) enitociclib predose to day 15, Mann-Whitney test with Benjamini-Hochberg correction (M.W.W.).

Clearance of the highest frequency B-cell IGLC clone with 2 weeks enitociclib treatment is observed with simultaneous expansion of a high frequency T-cell TRBC clone in a DH-DLBCL patient with a durable CR.

- Three patients had clinical benefit from enitociclib treatment, and in one DH-DLBCL [CR] patient, clearance of the highest frequency IGLC with enitociclib treatment is observed with simultaneous expansion and contraction of a high frequency TRBC. We hypothesize that these T-cell clonal dynamics could represent T-cell expansion upon antigen targeting and contraction with decrease in tumor burden.

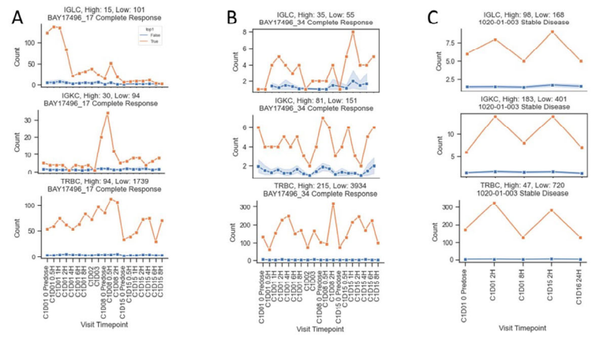

Figure 4- Clearance of the highest frequency B-cell IGLC clone with enitociclib treatment is observed with simultaneous expansion of a high frequency T-cell TRBC clone in a complete responder. Lineplots of count of highest frequency IGLC (red, top), IGKC (red, middle) and TRBC (red, bottom) for patient (**A**) DH-DLBCL [CR] (**B**) the second DH-DLBCL [CR] (**C**) transformed FL [SD, on study in Cycle 23] from Cycle 1 Day 1 predose to Cycle 1 Day 15 after 2 weekly doses of 30mg enitociclib. This contrasts with all low frequency IGLC clones (blue, top) and TRBC (blue, bottom).

Expansion of a highest frequency TRBC clone in a DH-DLBCL patient with durable complete response to enitociclib treatment does not map to any known T-cell antigen specificity database and may be oncogenic.

- In one DH-DLBCL patient who achieved a CR, clearance of the highest frequency IGLC with enitociclib treatment is observed with simultaneous expansion and contraction of a high frequency TRBC. We hypothesize that these T-cell clonal dynamics could represent T-cell expansion upon antigen targeting and contraction with decrease in tumor burden.
- To test whether this T-cell clone had known antigen specificity, we queried TAS-db and found CDR3b mostly mapped to several epitopes of CMV. Although most TRBC map to viral specificities in this patient, this top TRBC is unmapped to publicly known antigens suggesting the possibility of a cancer-specific TRBC.

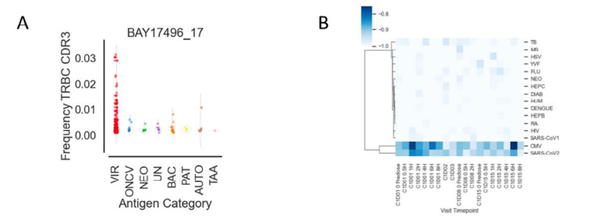

Figure 5- Expansion of a highest frequency T-cell TRBC clone in a DH-DLBCL patient with durable complete response to enitociclib treatment does not map to any known T-cell antigen specificity database and may be oncogenic. (**A**) Violin and strip plot of the sum of TRBC counts over the course of enitociclib treatment for patient DH-DLBCL [CR] who has observed B-cell IGLC clearance (**B**) Heatmap of the sum of CDR3 TRBC frequencies that are mapped to T-cell antigen specificities in the TAS-db over the course of treatment for the same patient from Figure 4A.

CONCLUSIONS

- Adaptive immune B-cell receptor (BCR) and T-cell receptor (TCR) chains were identified from RNA sequencing performed on peripheral blood samples collected before and during enitociclib treatment. Clonality and richness sample metrics were calculated for CDR3s from B-cell receptor light chains (IGKC and IGLC) and T-cell receptor beta chain (TRBC).
- For each patient, the potential dominant disease clone was identified as the highest frequency either IGKC and IGLC before treatment and showed that lower median IGKC/IGLC clonality over the first 2 weeks of treatment is associated with achieving a complete response.
- Three patients derived clinical benefit from enitociclib treatment; 2 patients with DH-DLBCL achieving complete remission [CR] and 1 patient with tFL who achieved stable disease [SD] and after 23 cycles (1.33 years) remains on study.
- Of the three patients who derived clinical benefit, one of these patients, a DH-DLBCL [CR], with a complete response, we identified a high frequency TRBC that covaried with the frequency of a dominant IGLC disease clone that is unmapped in TAS-db.
- These data suggest that an IO mechanism of action may contribute to the durable CR (5.5+ years) observed in a DH-DLBCL patient treated with enitociclib monotherapy. Analysis in expanded datasets is required to support this preliminary observation including following active patients.

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