MINIMAL RESIDUAL DISEASE ASSOCIATED WITH MITOCHONDRIAL DNA IN CHILDHOOD B-CELL LYMPHOMAS

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MINIMAL RESIDUAL DISEASE ASSOCIATED WITH MITOCHONDRIAL DNA IN CHILDHOOD B-CELL LYMPHOMA Purpose of Study: Children and adolescents with B-cell Lymphoma (BCL) have a high Event Free Survival (EFS), however, high risk children with recurrent or refractory disease have an overall low EFS with poor prognosis. Evaluating peripheral blood (PB) and bone marrow (BM) specimens for clonal cells using IgVH primer pools can detect minimal residual disease (MRD). Specimens that have MRD may also have high copies of mitochondrial DNA (mtDNA) per cell as a result of a compensatory survival mechanism which may contribute to recurrent disease. The purpose of this study is to evaluate the potential value of MRD and mtDNA in patients with BCL. **Methods Used:** PB and BM specimens from four consented children with advance BCL were submitted at three timepoints: entry, 3 weeks after chemotherapy initiation and 9 weeks. DNA from these specimens were assessed for MRD through semi-nested real-time PCR via evaluation of IgH VH family usage and mtDNA copy number/cell was also assessed. **Summary of Results:** As expected, all four patients exhibited positive MRD and high mtDNA levels at entry. Following treatment until CR evaluation, a general decrease in mtDNA copy number/cell was noted. After the 9 week time point, MRD was undetectable in three of the four patients. **Conclusions:** These initial results demonstrate the potential use of mtDNA and MRD assessment in PB and BM from children treated for BCL. This may be particularly useful in identifying risk for relapse in advance BCL patients if future analyses are able to show clinical significance of MRD and high mtDNA copy numbers.

	Entry				3 Weeks				9 Weeks			
Patient Number	**MRD Result (BM)**	**Mitochondria Per Cell (BM)**	**MRD Result (PB)**	**Mitochondria Per Cell (PB)**	**MRD Result (BM)**	**Mitochondria Per Cell (BM)**	**MRD Result (PB)**	**Mitochondria Per Cell (PB)**	**MRD Result (BM)**	**Mitochondria Per Cell (BM)**	**MRD Result (PB)**	**Mitochondria Per Cell (PB)**
001	Positive (VH4)	386	Positive (VH4)	636	N/R	N/R	N/R	N/R	N/R	N/R	Negative	169
002	N/A	N/A	Positive (VH4)	27	Positive (VH4 both BMR/L)	54 (BMR), 51 (BML)	Positive (VH4)	46	Positive (VH4 both BMR/L)	85 (BMR), 53 (BML)	Negative	228
003	Positive (VH4 both BMR/L)	21 (BMR), 27 (BML)	Positive (VH4)	76	Positive (VH4)	121	Positive (VH4)	96	Positive (VH4 both BMR/L)	28 (BMR), 16 (BML)	Negative	16
004	Positive (VH4 BMR), Negative (BML)	96 (BMR), 90 (BML)	Positive (VH4)	440	N/R	N/R	N/R	N/R	N/R	N/R	Positive (VH4)	123
005	Positive (VH4 both BMR/L)	216	Negative	516	N/R	N/R	N/R	N/R	N/R	N/R	Positive (VH4)	296
006	Positive (VH4)	76	N/A	N/A	N/R	N/R	N/R	N/R	N/R	N/R	Positive (VH4)	505
007	Positive (VH4)	76	Positive (VH4)	206	N/R	N/R	N/R	N/R	N/R	N/R	Positive (VH4)	284
008	Positive (VH4)	78	Positive (VH4)	348	N/R	N/R	N/R	N/R	N/R	N/R	Positive (VH4)	169
009	Positive (VH4)	220	Positive (VH4)	198	Negative	77	Positive (VH4)	67	Positive (VH4)	124 (BMR), 121 (BML)	Positive (VH4)	44
010	Positive (VH4)	132	Positive (VH4)	33	N/R	N/R	N/R	N/R	N/R	N/R	Positive (VH4)	196
011	Positive (VH3)	43	Positive (VH3)	79	Positive (VH3)	193	Positive (VH3)	205	Positive (VH3)	215	Positive (VH3)	284
012	N/A	N/A	N/A	N/A	N/A	N/A	Positive (VH4)	78	Positive (VH4 both BMR/L)	64 (BMR), 25 (BML)	Positive (VH4)	84
013	Positive (VH4)	141	Positive (VH4)	354	Positive (VH4)	96	Positive (VH4)	1234	Positive (VH4)	63 (BMR), 124 (BML)	Positive (VH4)	383
014	Positive (VH4 both BMR/L)	131 (BMR), 93 (BML)	Negative	246	Positive (VH4 BMR), Negative (BML)	75 (BMR), 106 (BML)	Positive (VH4)	80	Positive (VH4)	141 (BMR), 260 (BML)	Positive (VH4)	356
015	Positive (VH4 both BMR/L)	95 (BMR), 636 (BML)	Positive (VH4)	306	Positive (VH4 both BMR/L)	60 (BMR), 65 (BML)	Positive (VH4)	108	Positive (VH4 both BMR/L)	177 (BMR), 282 (BML)	Positive (VH4)	58
016	Positive (VH4 BML)	31 (BMR), 40 (BML)	Positive (VH4)	245	N/R	N/R	N/R	N/R	N/R	N/R	Positive (VH4)	58

Table 1. Summary of MRD and mtDNA Analyses of PB and BM
VH3: VH3 Family; VH4: VH4 Family; Numerical values: mtDNA copy number/cell; N/R: No specimen required; N/A: No specimen available. For control, 58 mtDNA copies/cell were found from normal peripheral blood mononuclear cells.

Background

- Children and adolescents with B-cell lymphomas have a high Event Free Survival (EFS)
- However, with recurrent or refractory disease, overall EFS is low with poor prognosis
- Evaluating peripheral blood and bone marrow specimens for clonal cells using IgVH primer pools can detect molecular minimal residual disease (MRD)
- Specimens with MRD may also have high copies of mitochondrial DNA (mtDNA) per cell as a result of a compensatory survival mechanism, which may also contribute to recurrent disease

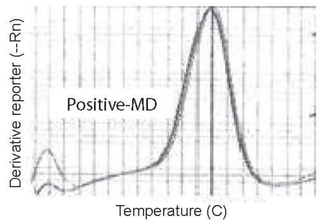
Objectives

- The purpose of this study is to evaluate the potential value of MRD and mtDNA in patients with B-cell lymphomas (BCL)

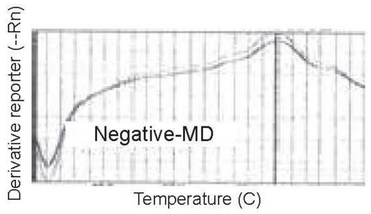
Methods

- Peripheral blood and bone marrow specimens from fifteen children with advanced BCL were submitted at three time points: entry, 3 weeks after chemotherapy initiation and 9 weeks.
- Mononuclear cells were isolated from BM and PB specimens using a Ficoll separation method.
- DNA from these cells were assessed for MRD through semi-nested real-time PCR via evaluation of IgVH family usage
- mtDNA copy number/cell was assessed using nuclear genomic primers encoding the Fas Ligand, and mitochondrial primers encoding for the mitochondrial protein NADH dehydrogenase, subunit 2

A.



B.



C.

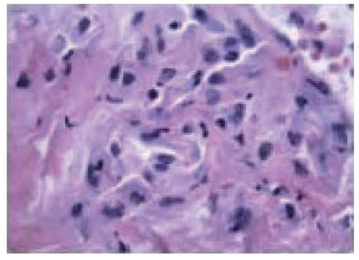


Figure 1. A. Positive-melt curve and amplified product. **B**. Negative-melt curve. **C.** DLBCL H&E stained tissue specimen.
From: Shiramizu B, et. al. *Br J Haematol*. 153:758-763, PMID 21496005, PMC3103617

Discussion

- mtDNA copy numbers may reflect a survival mechanism or remaining disease and may also predict a change in disease status
- The sample numbers are too few to determine if MRD-positive specimens have higher mtDNA copy numbers compared to MRD-negative specimens
- A larger patient cohort, clinical status of each patient during treatment and additional time points for evaluation are needed to determine the use of MRD and mtDNA copy numbers clinically

Conclusions

- Correlations between MRD status and mtDNA show promise as a tool in evaluating patient disease status, as well as response to treatment
- As patients with recurrent or refractory disease generally have a poor prognosis, using MRD and mtDNA copy numbers could be a useful clinical tool in identifying these patients earlier
- Future studies will include additional time points and larger sample sizes with known clinical outcomes

Reference

1. Shiramizu B, et. al. (2011) *Br J Haematol*. 153:758-763, PMID 21496005, PMC3103617
2. Shiramizu B, et. al. (2003) *J Acquir Immune Defic Syndr*. 32:370-4, PMID 12640193.

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