A pilot study of the use of dynamic analysis of cell-free DNA from aqueous humor and vitreous fluid for the diagnosis and treatment monitoring of vitreoretinal lymphomas

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Supplementary Data

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Supplementary material and methods

Cytology, IHC, and IGH clonality analyses

Cytological evaluations of undiluted VF samples were performed using May-Grünwald-Giemsa staining. IHC staining for CD20 (L26, Ascend BIO, Guangzhou, China, 1:100) and CD3 (SP1, Dako, Glostrup, Denmark, 1:250) was performed using automated staining (Ventana Medical Systems, Tucson, AZ), according to the manufacturer's protocol.

Genomic DNA was extracted and purified from 200 µL of VF using the TIANGEN DNA Mini kit DP316, according to the manufacturer's instructions. DNA concentration and quality were analyzed using a Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA) following manufacturer's instructions and agarose gel electrophoresis, respectively. IGH gene rearrangements were detected using PCR, as previously described, with the consensus primers, BIOMED-2 FR3 and FR2.[1]

Interleukin measurements

The IL-6 and IL-10 levels in AH/VF samples were measured using Becton Dickinson bead-based Cytometric Bead Arrays (Human IL-6 Flex Set, No.558276, BD Bioscience, San Jose, CA, Human IL-10 Flex Set, No.558274, BD Bioscience, San Jose, CA), following the manufacturer's instructions. IL-6 and IL-10 concentrations in the AH samples from patients and controls were determined using standard curves.

Statistics

Mutation allele frequency comparisons between paired AH/VF/CSF samples were calculated using the t-test (two-sided) and labeled as follows: *, $p < 0.1$; **, $p < 0.05$; ***, $p <$

0.01. Mutational frequency comparisons between VRL and PCNSL patients were analyzed using the Fisher's exact test. A p-value of less than 0.05 was considered statistically significant.

References

1. van Dongen JJ, Langerak AW, Bruggemann M, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia. 2003;17(12):2257-2317.

Supplementary Figures

List of supplementary figures

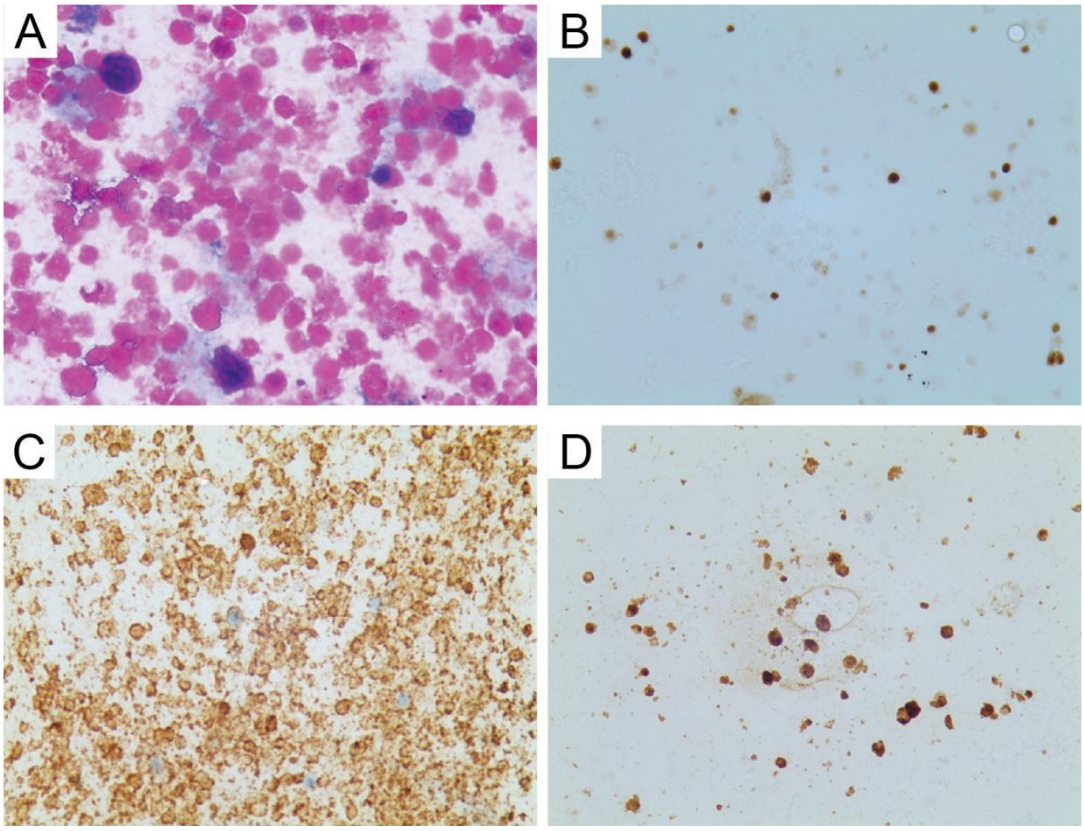

Figure S1. Cytologic and immunohistochemistry (IHC) images of a representative VRL patient (V4)

(A) The May-Grünwald-Giemsa staining (400x magnification) shows a hypercellular aspirate with large atypical cells and small lymphocytes. Atypical cells had a moderate to abundant basophilic cytoplasm, round to ovary nuclei, inconspicuous nucleoli, and condensed chromatin. IHC showed that the large atypical cells were positive for (B) Ki67, (C) CD20, and (D) CD79a (400x magnification).

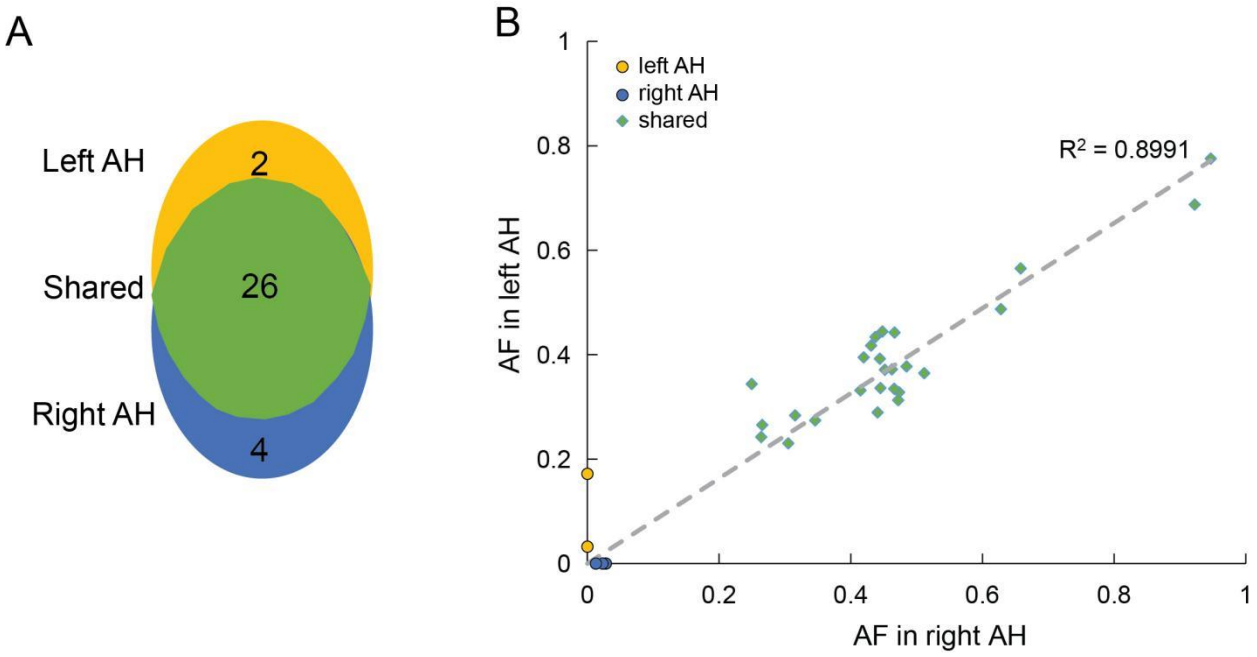

Figure S2. Mutational comparisons between paired left and right AH samples in patient V5

(A) The number of alterations detected in both or only in the left or right AH samples from patient V5. (B) The AFs of mutations in matched left and right AH samples are shown by the dot plot. Shared mutations are represented by green squares and mutations detected only in the left or right AH samples are represented by yellow and blue circles, respectively. AH: aqueous humor; AF: allele frequency

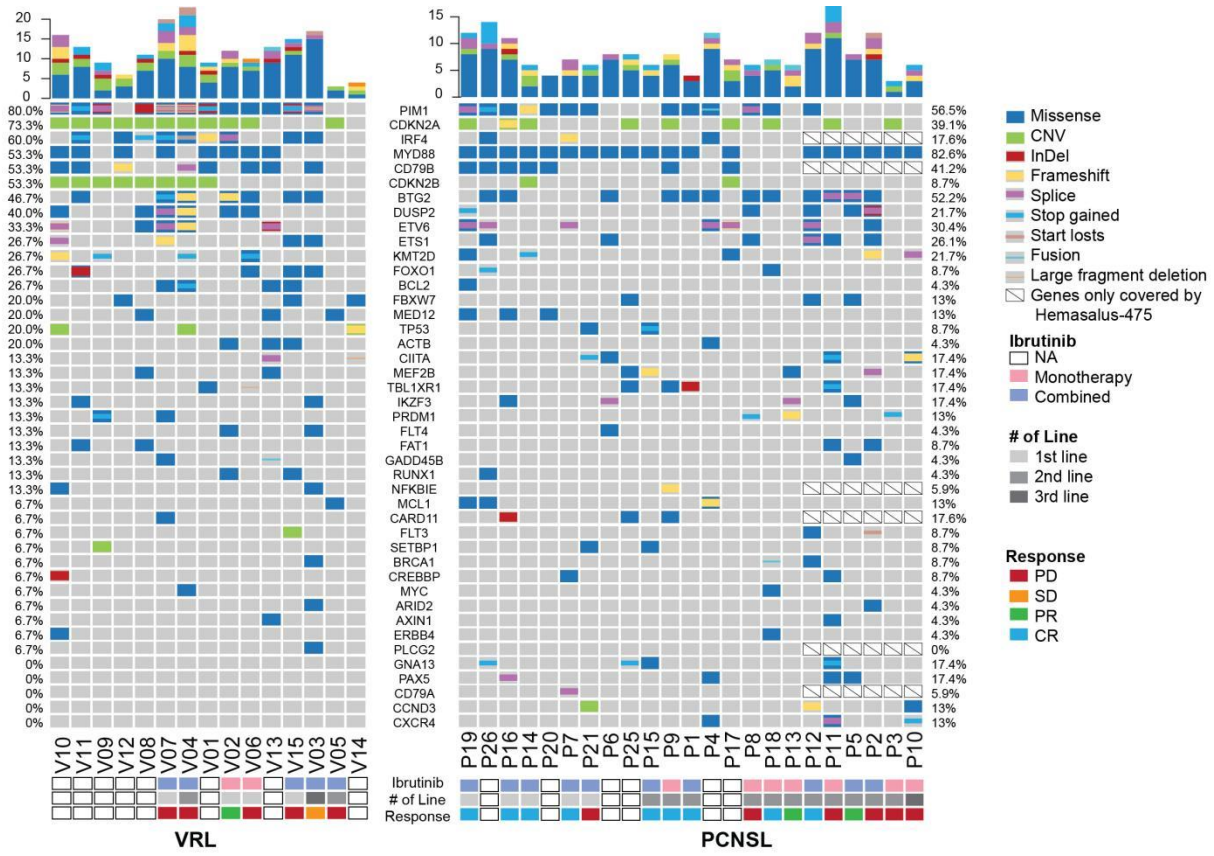

Figure S3. Concurrent mutations in baseline samples from VRL and PCNSL patients

The concurrent mutations in 15 VRL and 23 PCNSL patients are shown in the left and right panels. All mutation types are indicated in the legend. Due to differences in the sequencing panels used, the tissue samples from six PCNSL patients (P2-3, P5, P10-12) were analyzed using a prior version of the sequencing panel, which did not include the genes represented by slashes. VRL: vitreoretinal lymphoma; PCNSL: primary central nervous system lymphoma; PD: progressed disease; SD: stable disease; PR: partial response; CR: complete response; CNV: copy number variant; InDel: Insertion–deletion mutation

Supplementary Tables

List of supplementary tables

*Table S1-S3 are provided in separated excel files

Table S4. Clinicopathologic data of 15 VRL patients

Patient	Age	Sex	Ibrutinib Treatment	B-cell clonality	Vitreous opacity	Location	Subtype	AH Sampling	VF Sampling	CSF ctDNA
V1	58	M	NA	+	+++	Vitreous	Synchronous VRL and PCNSL	Baseline	Baseline	+
V2	45	F	1st-line	NA	++	Vitreous	Primary VRL	Sequential	Baseline	Not tested
V3	51	M	3rd-line	+	0~++	Vitreous	Primary VRL	Sequential	Baseline	-
V4	52	F	2nd-line	+	++	Vitreous	Primary VRL	Baseline	Baseline	Not tested
V5	64	F	2nd-line	NA	+	Subretinal	Primary VRL (subsequent CNS involvement)	Sequential	NA	+
V6	56	M	1st-line	NA	+	Subretinal	Primary VRL (subsequent CNS involvement)	NA	Sequential	+
V7	38	M	1st-line	+	++	Vitreous	Primary VRL	Baseline	NA	-
V8	63	F	NA	NA	+	Subretinal	Primary VRL	Baseline	NA	Not tested
V9	65	F	NA	NA	++	Vitreous	Primary VRL	NA	Baseline	Not tested
V10	46	M	NA	NA	+~+++	Vitreous, Subretinal	Synchronous VRL and PCNSL	NA	Baseline	+
V11	63	M	NA	NA	+++	Vitreous, Subretinal	Primary VRL	Baseline	NA	Not tested
V12	68	M	NA	NA	+	Vitreous, Subretinal	Primary VRL	Baseline	NA	Not tested
V13	62	F	NA	NA	++	Vitreous, Subretinal	Primary VRL	NA	Baseline	Not tested
V14	55	F	NA	NA	+~++	Subretinal	Primary VRL	NA	Baseline	Not tested
V15	56	M	1st-line	NA	+++	Vitreous	Primary VRL	Sequential	NA	+

Abbreviation: F (Female); M (Male); NA (not available); + (positive)

Table S5. The therapeutic responses of ibrutinib in 17 PCNSL and 7 PVRL cases

Patient	Subtype	# of Line	*MYD88*	*CD79B*	Details	Best Response
P12	PCNSL	2nd	mutant	NA	Pembrolizumab+Ibrutinib+TMZ	CR
P18	PCNSL	2nd	wt	wt	Ibrutinib	CR
P1	PCNSL	2nd	mutant	wt	Sintilimab+LEN+Ibrutinib	CR
P15	PCNSL	2nd	mutant	wt	Sintilimab+LEN+Ibrutinib	CR
P16	PCNSL	1st	mutant	mutant	MIT	CR
P9	PCNSL	2nd	mutant	mutant	Ibrutinib	CR
P14	PCNSL	1st	mutant	mutant	Rituximab+Ibrutinib+TMZ	CR
P7	PCNSL	1st	mutant	wt	MIT	CR
P19	PCNSL	1st	mutant	mutant	MIT	CR
P5	PCNSL	2nd	mutant	NA	Ibrutinib+Pemetrexed	PR
P13	PCNSL	2nd	wt	wt	Ibrutinib	PR
P2	PCNSL	2nd	mutant	NA	Ibrutinib+Pemetrexed	PD
P11	PCNSL	2nd	mutant	NA	Ibrutinib	PD
P3	PCNSL	2nd	mutant	NA	Ibrutinib	PD
P8	PCNSL	2nd	wt	wt	Ibrutinib	PD
P21	PCNSL	1st	mutant	wt	MIT	PD
P10	PCNSL	3rd	mutant	NA	Ibrutinib	PD
V2	VRL	1st	mutant	wt	Ibrutinib	PR
V3	VRL	3rd	wt	mutant	Toripalimab+Ibrutinib	SD
V4	VRL	2nd	wt	mutant	Sintilimab+Ibrutinib	PD
V5	VRL	2nd	wt	wt	Ibrutinib+LEN	PD
V6	VRL	1st	mutant	mutant	Ibrutinib	PD
V7	VRL	1st	mutant	wt	Ibrutinib+LEN	PD
V15	VRL	1st	wt	wt	Ibrutinib+LEN	PD

Abbreviation: wt: wild-type; NA: not available; LEN: lenalidomide; TMZ: temozolomide; MIT:methotrexate+ibrutinib+temzolomide; CR: complete response; PR: partial response; SD: stable disease; PD: progressed disease