Longitudinal Plasma cfDNA and Genomic Profiling during Chemotherapy in Canine Multicentric Lymphoma: A Pilot Study

Supplementary Materials

Table S1. Detection performance of two dilution-based analytical models across nominal variant fractions.

Nominal VAF	Synthetic-reference model	Canine gDNA model
≥ 1.00%	99.60%	99.81%
0.50%	90.60%	90.20%
0.25%	72.00%	67.00%

Detection rates represent the proportion of predefined positive variant sites successfully detected at each nominal VAF, pooled across two independent library preparation and sequencing replicates per dilution level. Both dilution-based models were evaluated using 10 ng of DNA input per library. These observed site-level detection rates were used to characterize analytical performance and do not represent a formally established LOD95.

Table S2. Sequencing quality metrics and locus-specific coverage of matched buffy coat genomic DNA at plasma-positive variant positions.

Dog	Time point	DNA type	DNA input amount (ng)	Library conc. (ng/μl.)	Library pooling amount (ng)	fold 80	Mean DP at plasma-positive loci	Median DP at plasma-positive loci	Min DP at plasma-positive loci	>300x (%) at plasma-positive loci	>500x (%) at plasma-positive loci
Dog 1	T0	gDNA	30	121	100	1.2	1241	1284	340	100.0%	95.8%
	T1	gDNA	30	118	100	1.3	1268	1285	350	100.0%	97.2%
	T2	gDNA	30	118	100	1.2	1101	1093	344	100.0%	91.0%
Dog 2	T0	gDNA	30	260	100	1.3	1590	1445	225	99.2%	96.2%
	T1	gDNA	30	126	100	1.3	1160	1044	176	98.1%	93.2%
	T2	gDNA	30	85.4	100	1.2	1641	1587	365	100.0%	98.5%
	Tx	gDNA	30	140	100	1.2	1673	1574	293	99.6%	98.5%
Dog 3	T0	gDNA	30	145	100	1.2	1792	1825	391	100.0%	99.4%
	T1	gDNA	30	145	100	1.2	1689	1690	301	100.0%	99.4%
	T2	gDNA	30	103	100	1.2	889	868	192	97.7%	89.0%
Dog 4	T0	gDNA	30	107	100	1.3	753	752	302	100.0%	87.1%
	T1	gDNA	30	143	100	1.2	956	944	366	100.0%	91.4%
	T2	gDNA	30	140	100	1.4	861	855	275	96.8%	88.2%
Dog 5	T0	gDNA	30	145	100	1.2	957	935	239	100.0%	94.5%
	T1	gDNA	30	145	100	1.2	959	930	238	100.0%	95.5%
	T2	gDNA	30	145	100	1.2	943	940	242	100.0%	92.7%
Dog 6	T0	gDNA	30	127	100	1.2	1146	1105	287	99.0%	94.8%
	T1	gDNA	30	116	100	1.3	908	876	216	96.9%	94.8%
Dog 7	T0	gDNA	30	171	100	1.2	878	842	395	100.0%	96.5%
	T1	gDNA	30	97.7	100	1.3	774	698	362	100.0%	94.7%

Sequencing depth was evaluated at genomic positions corresponding to variants detected in the matched plasma samples. Mean, median, and minimum depth values represent read-level coverage at these positions in the corresponding buffy coat samples. Coverage percentages indicate the proportion of evaluated plasma-positive positions with matched buffy coat sequencing depth >300× or >500×. Variant detection in buffy coat DNA was determined using Mutect2 PASS calls. The absence of a PASS call was interpreted as no variant detected under the applied calling and filtering criteria, rather than definitive evidence of variant absence.

Table S3. DNA input and read-level sequencing coverage at plasma-positive variant positions across longitudinal plasma cfDNA samples.

Dog	Time point	DNA type	DNA input amount (ng)	Library conc. (ng/μL)	Library pooling amount (ng)	fold 80	Mean DP at plasma-positive loci	Median DP at plasma-positive loci
Dog 1	T0	cfDNA	10	159	350	1.3	2681	2224
	T1	cfDNA	10	147	350	1.3	2001	1905
	T2	cfDNA	10	40	350	1.2	1768	1709
Dog 2	T0	cfDNA	10	78	350	1.2	2133	1931
	T1	cfDNA	10	57	350	1.2	1758	1674
	T2	cfDNA	9.7	42	350	1.2	1935	1844
	Tx	cfDNA	10	100	350	1.3	2982	2702
Dog 3	T0	cfDNA	10	116	350	1.4	2747	2713
	T1	cfDNA	10	86	350	1.3	2210	2136
	T2	cfDNA	10	91	350	1.2	1832	1650
Dog 4	T0	cfDNA	10	194	350	1.3	1971	1826
	T1	cfDNA	10	174	350	1.3	1905	1625
	T2	cfDNA	10	61	350	1.1	1590	1520
Dog 5	T0	cfDNA	10	89	350	1.2	1892	1812
	T1	cfDNA	10	149	350	1.2	1959	1970
	T2	cfDNA	10	75	350	1.2	1934	1859
Dog 6	T0	cfDNA	10	145	350	1.3	3049	2641
	T1	cfDNA	10	160	350	1.3	2193	2165
Dog 7	T0	cfDNA	10	154	350	1.2	2136	2299
	T1	cfDNA	10	114	350	1.2	2009	1989

Nineteen plasma cfDNA libraries were prepared using 10 ng of input DNA, whereas the Dog 2 T2 library was prepared using the entire available amount of 9.7 ng. Sequencing depth represents read-level coverage at genomic positions corresponding to plasma-detected variants. Although unique molecular identifiers were incorporated during library preparation, UMI-based consensus generation was not performed; therefore, independent molecular depth was not determined. Coverage metrics should not be interpreted as direct estimates of sensitivity at 0.5% VAF.

Table S4. Longitudinal measurements of tumor burden (sumLD) and plasma cfDNA concentration for all dogs at each sampling time point (T0, T1, T2, and follow-up where available).

Dog	Time point	sumLD (cm)	cfDNA (ng/mL)	**Δ**sumLD (%)	Δ cfDNA (%)	Clinical Response
Dog 1	T0	10.5	2640.0			
	T1	0.0	33.58	-100%	-99%	CR
	T2	0.0	3.89	—	-88%	CR
Dog 2	T0	12.4	4.74			
	T1	11.5	4.66	-7%	-2%	SD
	T2	3.1	2.43	-73%	-48%	CR
	Tx	9.0	20.79	190%	756%	PD
Dog 3	T0	14.5	25.26			
	T1	5.5	19.90	-62%	-21%	PR
	T2	5.0	4.43	-9%	-78%	PR
Dog 4	T0	10.5	7.04			
	T1	0.0	50.34	-100%	615%	PR
	T2	0.0	2.53	—	-95%	CR
Dog 5	T0	18.7	5.96			
	T1	2.7	7.98	-86%	34%	PR
	T2	0.0	5.34	-100%	-33%	CR
Dog 6	T0	13.5	120.75			
	T1	3.9	23.63	-71%	-80%	PR
Dog 7	T0	28.5	8.05			
	T1	24.5	4.47	-14%	-44%	SD

Percentage changes in sumLD and plasma cfDNA concentration were calculated relative to the immediately preceding available sampling time point for each dog. Accordingly, T1 values were compared with T0, T2 values with T1, and the relapse-associated Tx values with T2. These interval-specific percentage changes differ from the baseline-referenced T0–T2 comparisons presented in Table 2 and Figure 5. Percentage changes were not calculated when the reference value was zero and are indicated by a dash.

Table S5. Plasma DNA quantification and recovery characteristics across longitudinal sampling time points in seven dogs with multicentric B-cell lymphoma.

Dog	Time point	Blood volume (mL)	plasma volume (mL)	plasma/blood (%)*	Qubit DNA concentration (ng/μL)	DNA elution volume (μL)	total cfDNA amount (ng)**	cfDNA amount/ml of plasma (ng/mL)***	T0 – T2 reduction (%)
Dog 1	T0 (pre-treatment baseline)	6.5	4	61.5%	352§	30	10560.0	2640.00	
	T1 (~7 days; on-treatment)	6.5	3.6	55.4%	4.03	30	120.9	33.58	
	T2 (~30 days; on-treatment)	7.5	3.4	45.3%	0.441	30	13.2	3.89	99.9%
Dog 2	T0 (pre-treatment baseline)	6.5	2.7	41.5%	0.427	30	12.8	4.74	
	T1 (~7 days; on-treatment)	6.8	2.8	41.2%	0.435	30	13.1	4.66	
	T2 (~30 days; on-treatment)	7.86	4	50.9%	0.324	30	9.7	2.43	48.7%
	Tx (Recurrence)	7	2.8	40.0%	1.94	30	58.2	20.79	
Dog 3	T0 (pre-treatment baseline)	7.5	3.8	50.7%	2.4	40	96.0	25.26	
	T1 (~7 days; on-treatment)	7.5	4	53.3%	1.99	40	79.6	19.90	
	T2 (~30 days; on-treatment)	8	4	50.0%	0.591	30	17.7	4.43	82.5%
Dog 4	T0 (pre-treatment baseline)	6.5	2.8	43.1%	0.493	40	19.7	7.04	
	T1 (~7 days; on-treatment)	9	3.2	35.6%	5.37	30	161.1	50.34	
	T2 (~30 days; on-treatment)	8.1	4	49.4%	0.337	30	10.1	2.53	64.1%
Dog 5	T0 (pre-treatment baseline)	11	2.6	23.6%	0.469	33	15.5	5.96	
	T1 (~7 days; on-treatment)	7.9	2.5	31.6%	0.499	40	20.0	7.98	
	T2 (~30 days; on-treatment)	7.5	2.9	38.7%	0.5	31	15.5	5.34	10.4%
Dog 6	T0 (pre-treatment baseline)	7.5	4	53.3%	16.1	30	483.0	120.75	
	T1 (~7 days; on-treatment)	6.5	4	61.5%	3.15	30	94.5	23.63	
Dog 7	T0 (pre-treatment baseline)	8	3.2	40.0%	0.859	30	25.8	8.05	
	T1 (~7 days; on-treatment)	7	2.5	35.7%	0.486	23	11.2	4.47	

* Plasma recovery (%) was calculated as the plasma volume divided by the collected blood volume, multiplied by 100.

** Total recovered DNA (ng) was calculated as the measured DNA concentration (ng/μL) multiplied by the DNA elution volume (μL).

*** Plasma DNA concentration (ng/mL) was calculated as the total recovered DNA amount (ng) divided by the input plasma volume (mL).

§ The baseline sample from Dog 1 exceeded the quantification range of the Qubit dsDNA HS Assay and was therefore quantified using the Qubit dsDNA BR Assay. All other measurements were obtained using the HS assay.

DNA concentrations represent total fluorometrically quantified double-stranded DNA recovered from plasma and do not distinguish cell-free DNA from potential genomic DNA contamination. Direct fragment-size analysis of extracted plasma DNA was not performed.

T0–T2 reduction (%) was calculated for dogs with paired samples as [(T0 plasma DNA concentration − T2 plasma DNA concentration) / T0 plasma DNA concentration] × 100. Excluding Dog 1, reductions ranged from 10.4% to 82.5%, with a median of 56.4% among the four remaining dogs.