Clonal B-Cell Expansion and the Potential Challenges to Blood-Based Early Cancer Detection

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INTRODUCTION

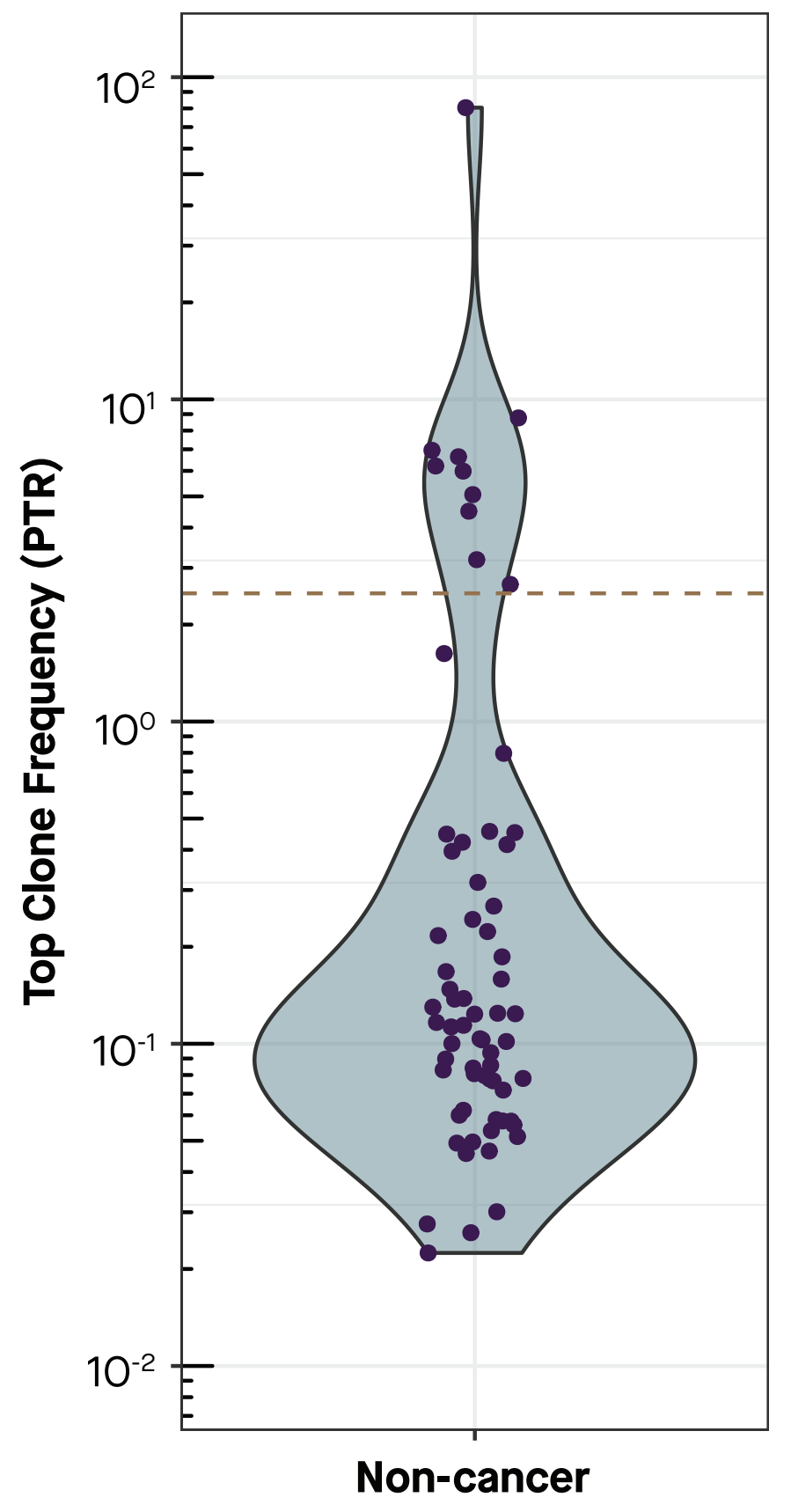
- Confounding signals originating from white blood cells, such as clonal hematopoiesis of indeterminate potential, are a potential source of false positives for blood-based early cancer detection and disease monitoring, with different impacts based on the genomic feature[1-3]
- Precursor conditions, such as monoclonal B-cell lymphocytosis (MBL) and monoclonal gammopathy of undetermined significance (MGUS), are highly prevalent in a potential screening population (MBL: 7% age ≥45; MGUS: 3% age ≥50)[4,5] and are also sources of clonal expansion
- Characterization of B-cell clonal expansion in non-cancer participants to date is limited
  - Previous studies include clonality profiling for DNA of 9 spleen samples[6] and RNA for 10 human peripheral blood mononuclear cell samples[7]
  - The largest study (155 B-cell samples) focused on immunoglobulin heavy chain (*IGHV*) gene usage[8]

OBJECTIVE

- To characterize clonal expansion in a potential cancer screening population of adults aged ≥50 years, we sought to:
  - Characterize the distribution of clonal frequencies for the top clone in a large number of samples from non-cancer participants (n=67) that are representative of a potential screening population
  - Compare clonality in non-cancer participants with clonality in participants with hematological precursor (MBL and MGUS) and neoplastic conditions (chronic lymphocytic leukemia [CLL] and multiple myeloma [MM]) (HPNCs)

KEY RESULTS: CLONAL EXPANSION WAS HIGHLY PREVALENT IN A NON-CANCER POPULATION OF SCREENING AGE, AND THE LEVEL OF EXPANSION WAS POSITIVELY CORRELATED WITH AGE

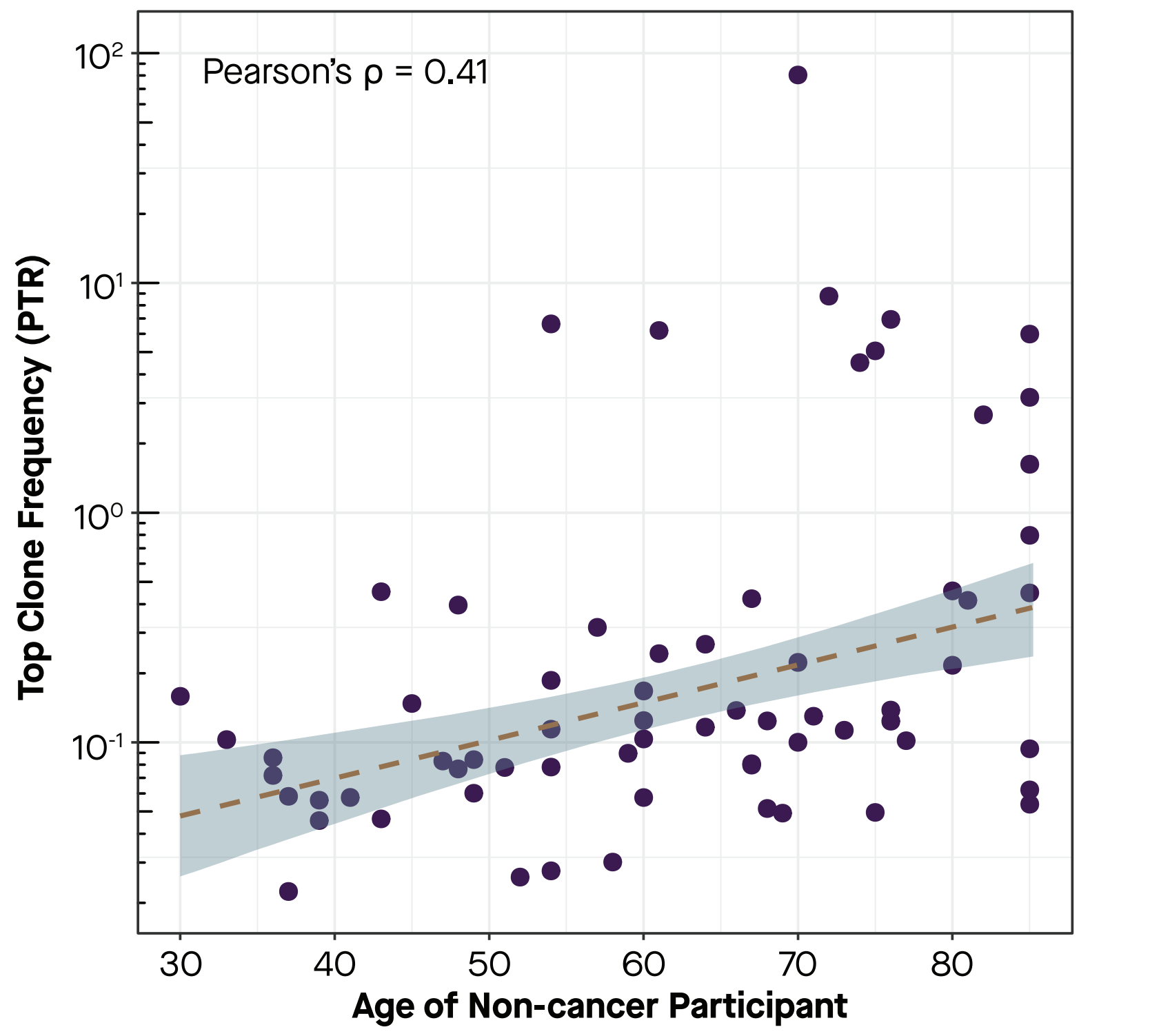
Figure 1. The distribution of top clone frequencies for non-cancer participants.



- For non-cancer samples, 15% (10/67) showed evidence of clonal expansion (top clone frequency >2.5% of total reads; **Table 1; Figure 1**)
  - 9/10 (90%) were monoclonal and 7/9 had a mutation rate >2%, indicating somatic hypermutation.
  - One sample exhibited oligoclonality with 2 clones (percent total reads [PTR] of 6.6% and 3.2%), and both clones showed evidence of somatic hypermutation
- The remaining non-cancer samples (57/67 [85%]) lacked evidence of clonal expansion (**Figure 1**)
  - Median top clone frequency was 0.10% (mean: 0.18%; standard deviation [SD]: 0.24%)
  - Median Simpson clonality was 0.0021 (mean: 0.0030; SD: 0.0030) for the top 10 clones
- An upward trend between the top clone frequency and age (ρ=0.41) was observed, indicating that individuals are more likely to exhibit higher levels of clonal expansion as they age (**Figure 2**)
  - All samples that crossed the threshold of detection were from participants ≥50 years old

The dashed horizontal line indicates the 2.5% threshold for considering clonality, measured as PTR, detected in a sample.

Figure 2. The relationship between B-cell clonal expansion and age for non-cancer participants.



Top clone frequency (PTR) is plotted against the age of each non-cancer participant. The Pearson correlation of the log frequencies vs age is 0.41 (0.43 without the 70-year-old participant with PTR of 80%), indicating a positive correlation between clonality and age. The dashed trend line was generated using a linear model and fitted with robust regression. The frequencies of the top clone are log transformed.

The level of clonal expansion in samples from participants with HPNCs are concordant with the expectations of their clinical status.

- Most of the CLL and MBL samples were detected to have expanded clones that make up >70% of the total reads (**Table 1; Figure 3**)
- Consistent with the observation that small quantities of plasma cells are in circulation, fewer MM and MGUS samples were detected to have evidence of clonal expansion; the top clone frequency was lower in those samples that did have expanded clones (**Table 1; Figure 3**)
  - Notably, 1 MM sample and 3 MGUS samples had a top clone frequency >40%
    - Although the assay used can also detect plasma cell lineages,[9] and since the samples were not cell sorted, we suspect that concurrent lymphoid clonal expansion is responsible for the larger frequencies

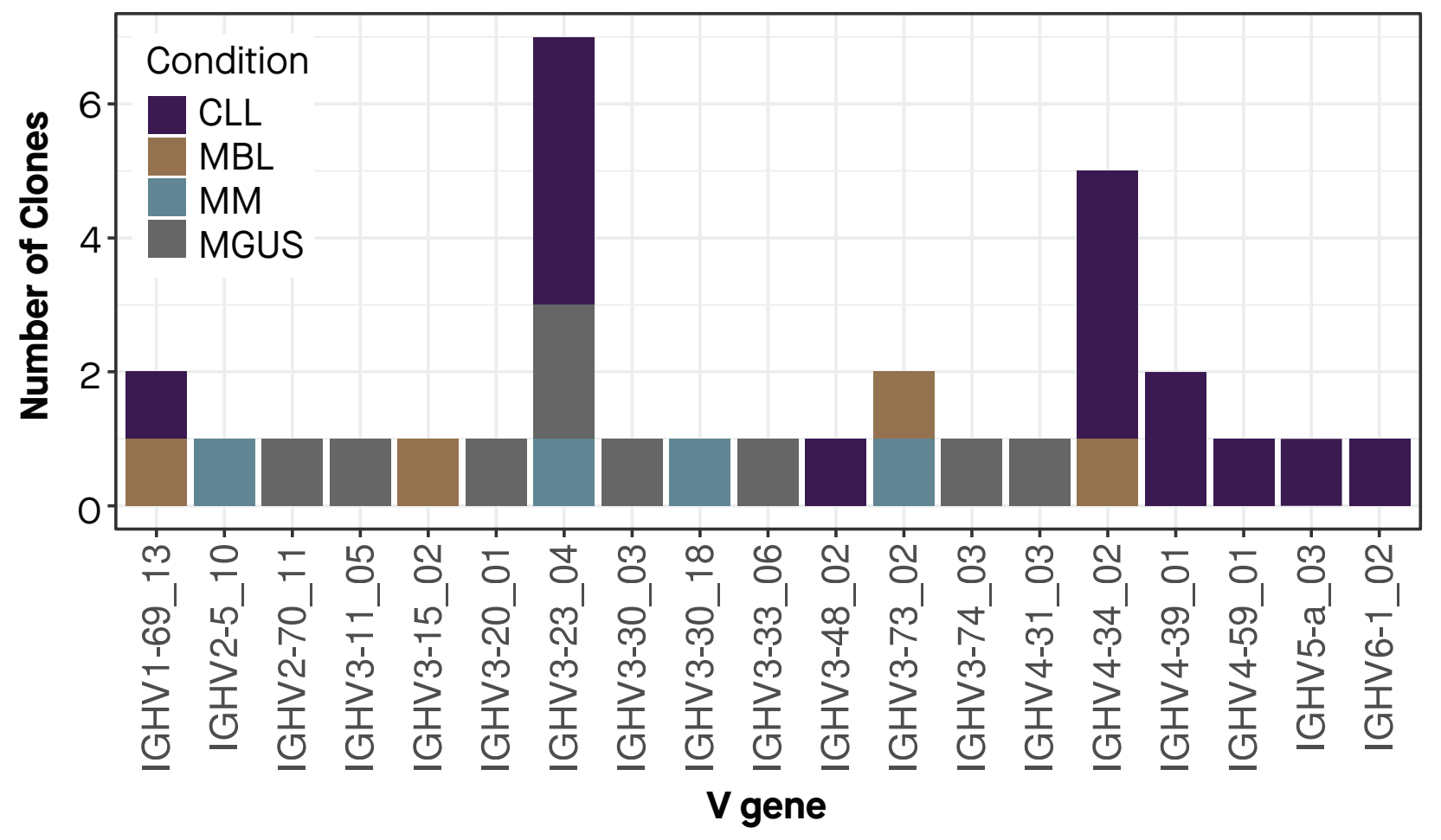
Table 1. Clonal expansion in non-cancer vs HPNC samples.

Participant Type	Participants With Evidence of Clonal Expansion, n/N (%)	Median Top Clone Frequency (PTR)
Non-cancer	10/67 (15)	6.0%
CLL	11/11 (100)	81.0%
MBL	3/4 (75)	48.8%
MM	3/10 (30)	19.1%
MGUS	7/20 (35)	9.5%

Gene usage in CLL/MBL and MM/MGUS samples is consistent with common genes reported in the literature (Figure 4).

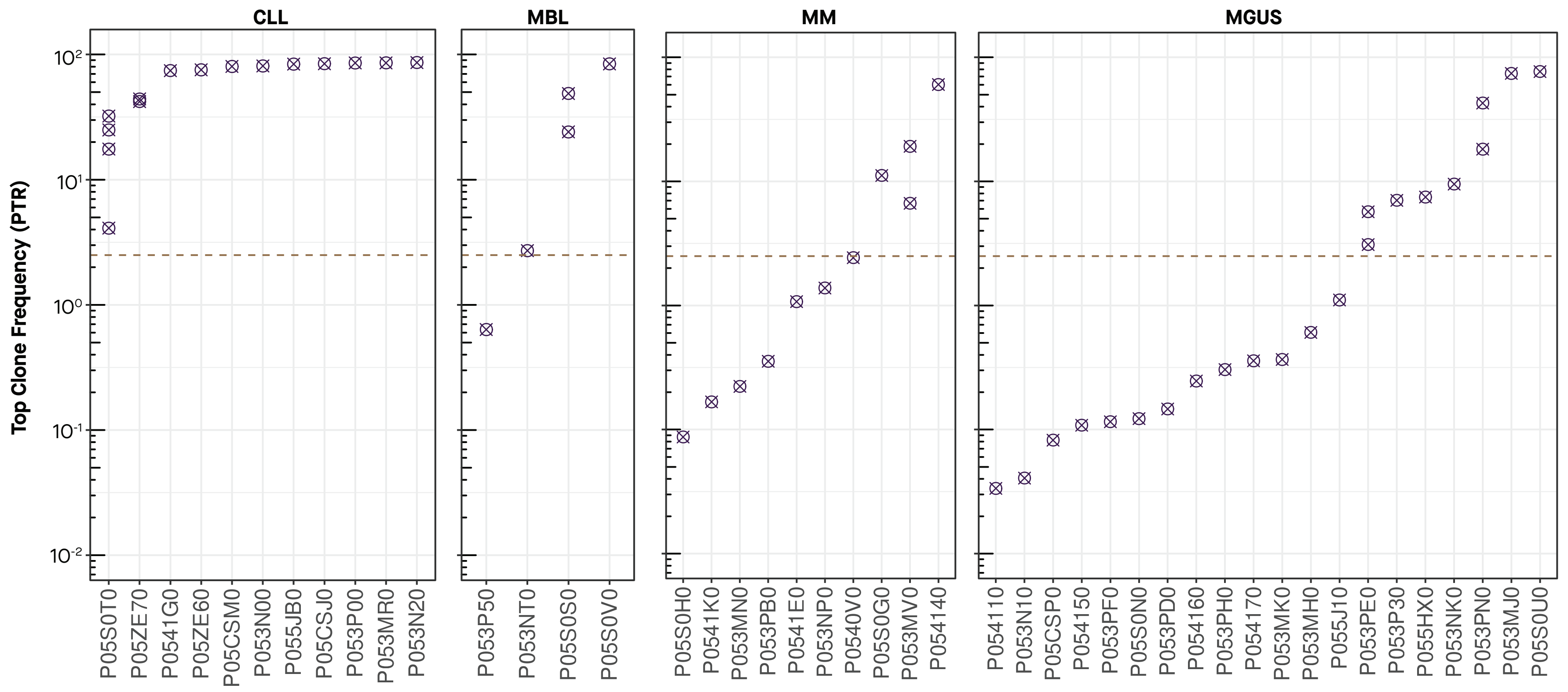
- CLL/MBL cases frequently used genes *IGHV1-69*, *IGHV4-34*, and *IGHV3-23*, consistent with previous studies[10,11]
- MM/MGUS gene usage did not seem to concentrate on particular genes. However, *IGHV3-23* appeared in 3 clones and *IGHV3-30* appeared in 2 clones
  - These have been reported in the literature as the most common genes across a study of >300 individuals[12]

Figure 4. *IGHV* gene usage in samples from study participants with CLL, MBL, MM, and MGUS.



The x-axis lists the IGHV genes identified by the IGH FR1 assay in the study samples. The y-axis shows the number of clones with that gene. If a sample has multiple clones, then multiple clones are represented here.

Figure 3. Expanded clones in samples from participants with HPNCs.



The dashed horizontal line indicates the 2.5% threshold. Each sample is represented along the x-axis and each point represents a clone belonging to a sample. Some samples are polyclonal and both clones are represented if above the threshold.

CONCLUSIONS

- 15% of non-cancer participants in this study had evidence of expanded lymphoid clones with somatic hypermutation
- Top clone frequency was positively correlated with age
  - This observation highlights the higher frequency of HPNCs seen in an older asymptomatic population
- A higher fraction of CLL and MBL cases showed evidence of clonal expansion, and higher levels of clonal expansion were detected as compared to MM and MGUS cases, which was expected due to the limited presence of plasma cells in the circulation
  - It may be possible to detect more cases of MM/MGUS by running additional targets, such as the *IGH* Leader and *IGK* for the kappa light chain[15]
- Larger studies are necessary to estimate the level at which genetic or epigenetic biomarkers are impacted by a shared cancer signal
  - This will help elucidate how to reduce false positives originating from aberrant signals generated by B-cell clonal expansion that are not yet considered cancer

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Disclosures

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METHODS

PARTICIPANT SELECTION

- Buffy coat samples were processed from 112 participants from the Circulating Cancer Genome Atlas (CCGA; NCT02889978) study, including a subset of 67 non-cancer participants (**Table 2**). This subset was selected to be balanced for age and gender (**Table 3**)
- Samples from participants with CLL and MM served as positive controls to characterize the performance of the assay
- Finally, samples from participants with MBL and MGUS were selected in order to compare the level of clonal expansion in non-cancer participants

IMMUNE REPERTOIRE SEQUENCING

- Targeting immunoglobulin gene rearrangements is widely used for assessing clonality in diseases with B-cell proliferation[13]
- The enormous diversity generated by rearrangement of the VDJ regions results in unique sequences that encode the antigen binding regions[14]
- Thus, the genetic material from a population of cells can be interrogated for identical rearrangements that indicate clonal expansion
- In this study, B-cell clonality was assessed using the LymphoTrack *IGH* FR1 Assay Kit from Invivoscribe, Inc (San Diego, CA, USA), which has previously been evaluated clinically,[13] and was optimized from the multiplex PCR assays established by the EuroClonality/BIOMED-2 consortium[15]

SAMPLE PROCESSING

- For samples from CCGA participants, whole blood was collected and isolated into plasma and buffy coat as previously described[3,16]
- A sample with eight tubes of frozen buffy coat for a young healthy donor (18–35; no pre-existing health conditions; non-smoker; viral negative) was sourced commercially (Discovery Life Sciences, Huntsville, AL, USA) and used as the background for the titration sequence
- Genomic DNA (gDNA) was extracted from the buffy coat with the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germany), concentrated to 100 ng/μL, and quantified using the AccuClear assay (Biotium, Inc, Fremont, CA) to ensure sufficient yield (at least 500 ng) available for sequencing
- All samples were sequenced at Invivoscribe, Inc (San Diego, CA, USA) with the LymphoTrack *IGH* FR1 Assay kit
  - The FR1 target was selected based on the results of a previous study in which the LymphoTrack assay was used to detect clonal expansions of a set of hematological diseases[13]
- After sequencing, we required that samples had at least 100,000 reads before we proceeded with analysis (**Figure 5**)
  - Samples that did not meet the criteria were sequenced again
  - Clonal frequencies and sequences were generated with the LymphoTrack software

LIMIT of QUANTIFICATION (LoQ)

- Using 12 titration samples, we determined the LoQ of detecting an unknown clone against a polyclonal background to be 0.1% of the total reads (**Figure 6**)
- To generate the titration sequence, DNA from a CCGA participant with CLL was spiked into background material from the young healthy donor at the following fractions: 0%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5% 1%, 5%, 10%, 50%, and 100%
- The threshold used to determine evidence of clonal expansion was 2.5% of the total reads, which is well above the LoQ

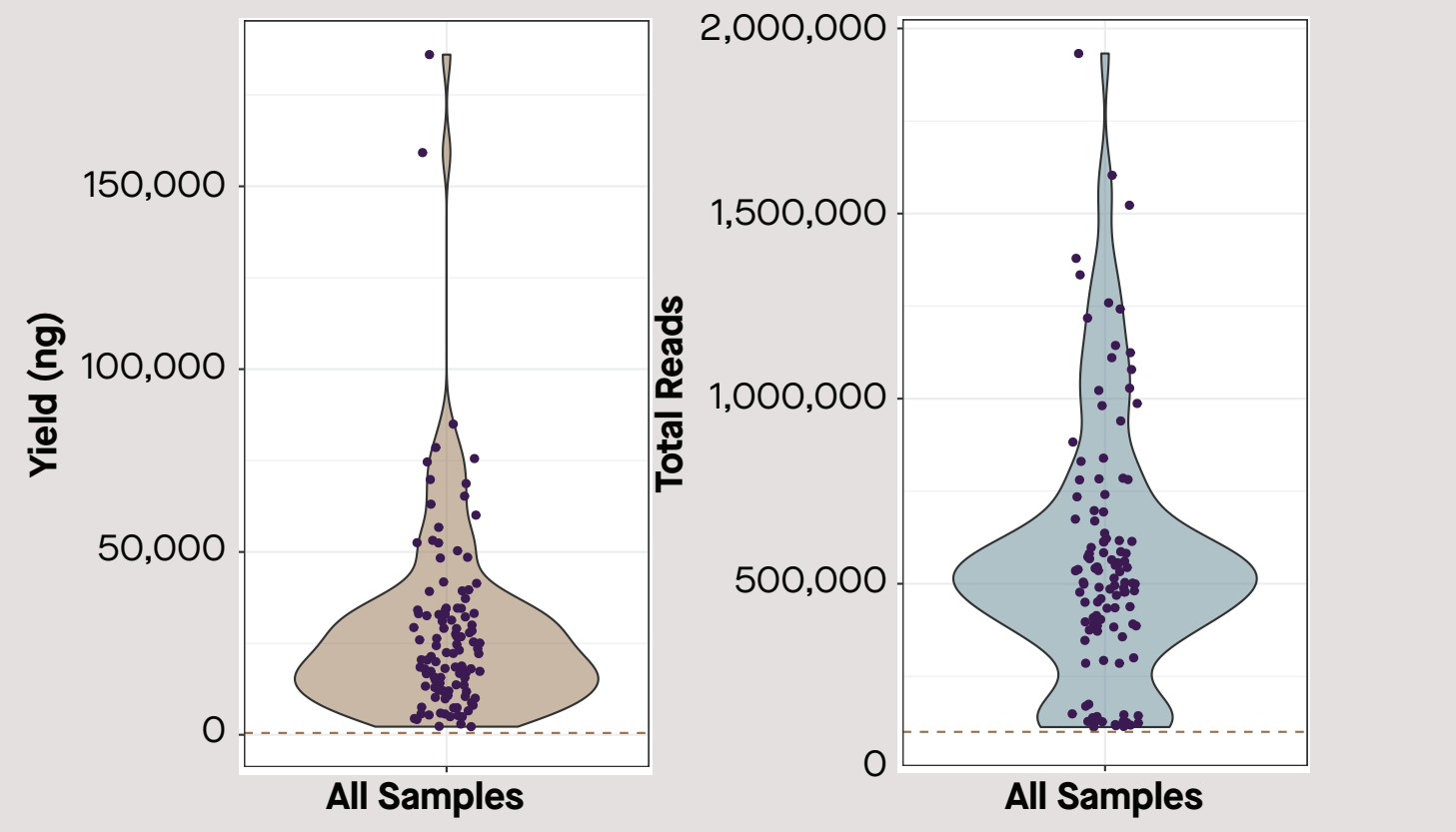
Table 2. Participant cohorts.

Inclusion Criteria	Number of Participants
Non-cancer	67
MBL	4
MGUS	20
Diagnosed CLL	11
Diagnosed MM	10

Table 3. Age of non-cancer participant cohort.

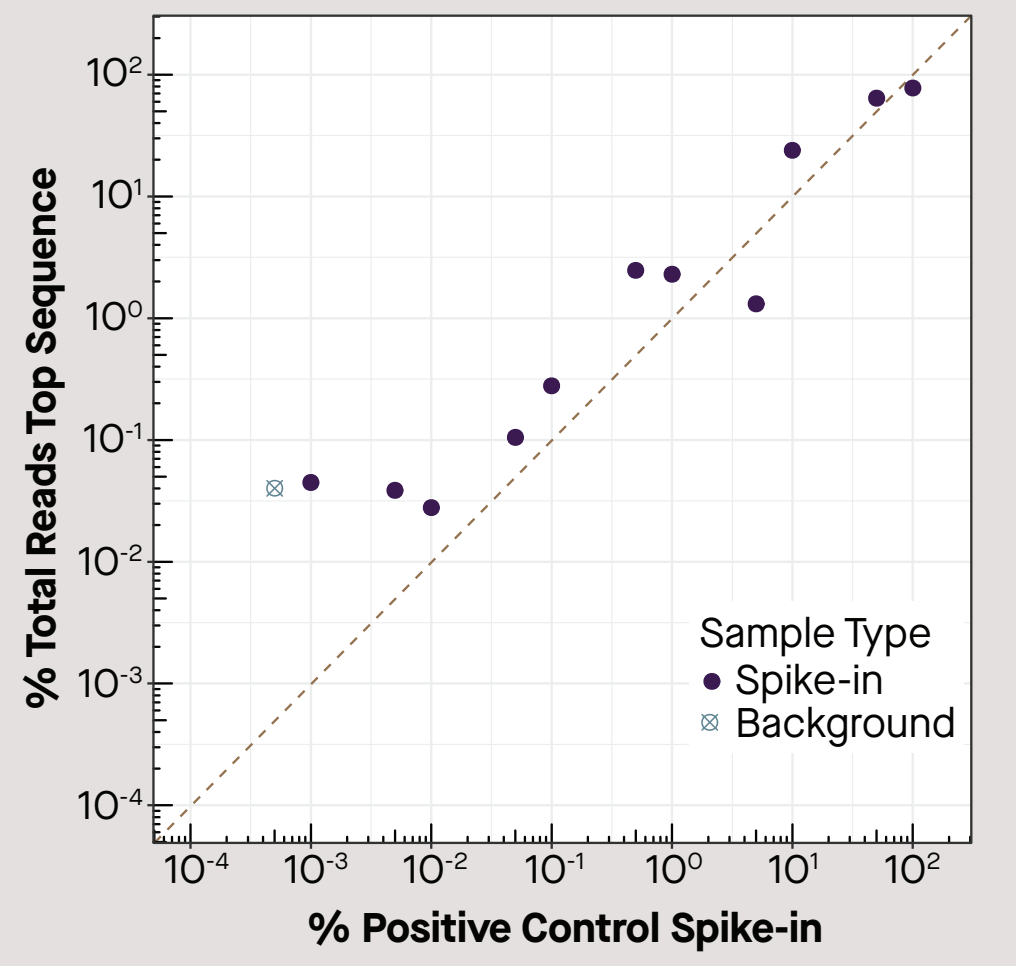
Age Group, years	Male	Female
<40	4	4
40–49	4	5
50–59	6	4
60–69	6	9
70–79	8	5
≥80	7	5
Total	**35**	**32**
Median Age (Range)	**67 (33–85)**	**60 (30–85)**

Figure 5. Sample yield and total reads metrics.



Left: The distribution of the gDNA yield that was extracted from buffy coat samples. The dashed horizontal line shows the 500 ng threshold required for samples to move forward to sequencing.
Right: The distribution of total reads after the IGH FR1 assay was run. The dashed horizontal line shows our requirement of 100,000 reads post sequencing.

Figure 6. LoQ of the *IGH* FR1 assay.



Each point represents a titration sample. The x-axis shows the % of a known CLL sample that was spiked into a sample with DNA from a young and healthy background. The titration samples ranged from 0% CLL spike-in DNA to 100% CLL spike-in DNA. The y-axis shows the clonality, measured as PTR, of the top sequence after targeted sequencing with the IGH FR1 assay.