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Rapid diagnostics of actionable mutations in acute myeloid leukemia using amplicon nanopore sequencing

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Short title: Rapid AML diagnostics using amplicon ONT sequencing

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Conflict of interest

The authors declare no potential conflicts of interest.

Declaration of AI use

No artificial AI tools were used in the preparation or writing of this manuscript.

Availability of data and materials

Genetic data for this study were generated and deposited at Clinical Genomics Uppsala, Science for Life Laboratory, Department of Immunology, Genetics, and Pathology, Uppsala University, Sweden (project number CGU_2024_05, RRID:SCR_014078). Both genetic and clinical data are not publicly available because of the Swedish legislation but are available from the corresponding author (panagiotis.baliakas@igp.uu.se) upon request. Requests should reference the project number “CGU_2024_05.”

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Authors' contributions

RÖ: Conceptualization, data, methodology, visualization, project management, formal analysis, writing–original draft, writing–review and editing. CC: Data selection and analysis, software, methodology, visualization, writing–original draft, writing–review and editing. ELC: Data selection and analysis, methodology, writing–original draft, writing–review and editing. TP: Data selection and analysis, methodology, writing–review and editing. OW: Data selection and analysis, methodology, writing–original draft, writing–review and editing. ARM: Data selection and analysis, writing–review and editing.18 GL: Data selection and analysis, writing–review and editing. FBM: Data selection and analysis, writing–review and editing. JNZ: Data selection and analysis, writing–review and editing. HL: Data selection and analysis, writing–review and editing. PH: Data selection and analysis, writing–review and editing. FD: Data selection and analysis, writing–review and editing. MM: Data selection and analysis, writing–review and editing. IH: Data selection and analysis, visualisation, methodology, writing–review and editing. TS: Data selection and analysis, visualisation, methodology, writing–original draft, writing–review and editing. CL: Methodology, writing–review and editing. PB: Conceptualization, resources, data curation, formal analysis, supervision, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing.

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We present the clinical implementation of a targeted Oxford Nanopore Technologies (ONT) long-read sequencing (LRS) panel enabling analysis of actionable mutations in acute myeloid leukemia (AML) within 1-2 days of diagnosis. Our approach offers rapid and simultaneous analysis of *NPM1, FLT3, IDH1, IDH2* and *TP53*, enabling flexible sequencing of individual samples, without batching, at a relatively low cost per sample (Figure 1).

The introduction of next-generation sequencing (NGS) has substantially improved our understanding of the pathophysiology of AML, facilitating the development of targeted therapies. These advances, however, may be challenging, especially for non-centralized laboratories that operate locally, as rapid, cost-effective, and reliable molecular testing pipelines are required. European Leukemia Net (ELN) risk-stratification guidelines recommend short turnaround-time (TAT) from sampling to clinical reporting for mutations in *FLT3, NPM1, IDH1*, and *IDH2*, in newly diagnosed AML patients. Other mutations, i.e. *TP53*, demand early detection, as they influence therapeutic decision making and planning [1-3].

To meet these challenges, and due to limitations of most short-read sequencing (SRS) platforms, current AML diagnostics rely mainly on multiple parallel assays including fragment-length-analysis for *FLT3* internal tandem duplications (ITDs), *NPM1* and tyrosine kinase domain (TKD) mutations, as well as droplet-digital PCR (ddPCR) or other PCR-based protocols for *IDH1/2* hotspot mutations [4]. These targeted assays are typically performed in parallel with the standard-of-care (SoC) investigations (cytogenetics, fluorescence in situ hybridization (FISH), and SRS targeted somatic panels).

LRS is a promising technology that captures longer sequences than SRS, offering a more complete and continuous view of the genome. Moreover, LRS on the ONT platform enables rapid sequencing of individual samples. To date, LRS has been applied predominantly in rare disease diagnostics, while in oncology it is primarily used in solid tumors [5, 6]. The potential

applications of LRS in hematology have been intensively explored with several studies reporting promising results on the detection of fusion genes, structural variants, and epigenetic signatures [7-11].

Three patient cohorts were included in the study, analyzed in 3 consecutive steps A, B and C (Figure 1, Supplemental Table 1). A: analytical performance: Thirty-six patients with AML or other hematological malignancies, carrying 64 previously identified variants and/or fragment analysis [*FLT3*-ITD: n=8 range: 18-176bp, TKD: n=3), *NPM1:* n=5, *IDH1*: n=5, *IDH2*: n=3, and *TP53*: n=40, variant allele frequencies (VAFs): 0.01-0.83]. Fifteen of the 64 variants were sequenced on both Flongle and MinION platforms. B: Enrichment for *TP53* variants-Phasing: Eighteen patients [AML: n=12; chronic lymphocytic leukemia (CLL): n=6] carrying 39 *TP53* variants (VAF: 0.02-0.62) with each patient carrying ≥1 variant. C: prospective comparison with SoC, MinION: Twenty-six patients recruited from January 2026 to April 2026. SoC testing included classical cytogenetics, FISH, fragment analysis for *FLT3*-ITD/TKD, and a somatic myeloid panel [12]. The limit of detection for hotspots in *IDH1* and *IDH2* was set to a VAF of 0.05 based on median noise for samples without mutations at the positions of interest (0.12%-0.33% with standard deviations of 0.06%-0.17%, Supplemental Figure 1).

DNA was extracted from whole blood, bone marrow, or cryopreserved viable cells according to local protocols, as described previously [12]. The study was approved by the Swedish Ethical Review Authority (DNR 2025-01156-01), in accordance with the Declaration of Helsinki, including written consent when applicable. The design of the ONT-amplicon panel (Figure 2) including the protocol and the bioinformatics analysis is publicly available online [13, 14].

In step A, all variants except one *FLT3*-ITD with an allelic ratio of 0.009 (VAF:0.009) were detected by ONT. The vast majority (60/64, 93.8%) were identified directly by the variant callers, while the remaining four variants, a *FLT3*-ITD with an allelic ratio of 0.012 (VAF: 0.012)

and three complex *TP53* variants, were detected through targeted manual evaluation (Supplemental Figure 2). The concordance between the ONT-amplicon panel and the SRS-based methodology was high both on Flongle (R^2=0.97) and MinION (R^2 =0.994) (Figure 2), while the overall sensitivity and specificity was 98.4% (63/64 variants) and 100% (variants with VAF >3%), respectively.

In the enriched *TP53* cohort, all variants were successfully detected with the ONT-amplicon panel (Sensitivity:100%) with high concordance compared to the SRS-methodology (R^2=0.936) (Figure 2) and 100% specificity for variants with VAF >0.03. In 15/18 patients with multiple *TP53* variants, we performed phasing confirming the positioning of the variants in different alleles (Figure 2, Supplemental Figure 1).

In step C, we evaluated the performance of the ONT-amplicon panel versus SoC, focusing on the evaluation of hands-on-time, cost for each step of the analysis and performance of the flow-cells between the different runs. The overall sensitivity and specificity were both 100% using reporting threshold of 0.05 VAF for common variants and 0.05 allelic ratio for *FLT3*-ITDs (Supplemental Table 1).

Among the included patients, two carried *FLT3*-ITDs with an allelic ratio of ~0.01, below the limit of detection of the automated ONT-pipeline. One of them was detected by the NGS panel performed a week later according to the SoC, while the second was not identified by the NGS myeloid panel either. The patient, however, carried two additional ITDs with high VAFs and therefore the clinical management was not affected (Supplemental Figure 2).

TAT was evaluated as time elapsed from the initiation of sample handling for PCR to completed sequencing and interpretation (Figure 3). Sequencing took 55 minutes per sample on average (depending on the number of samples multiplexed) and was terminated when all samples in the run had achieved at least 100 000 reads (Supplemental Figure 2). Overall, the

workflow (from PCR to sequencing) required 9–13 hours of laboratory work and yielded clinically interpretable results within approximately two working days. To ensure reduced cost per sample, we validated the use of the same MinION flow-cell up to 5 uses within 1 month. Following this approach, the cost of the ONT-pipeline was comparable to that of *FLT3*-fragment analysis at our laboratory (~500 Euros per sample) while also providing information on the mutational status of *NPM1*, *IDH1/2*, and *TP53* at no additional cost.

We present a real-world clinical implementation of LRS technology in a diagnostic lab operating on a single-shift basis, to accelerate the diagnostic workflow in AML, offering rapid reporting and timely treatment management. Our approach provides the advantage of a single assay reducing the need for multiple parallel testing, enabling the analysis of individual samples without batching, a known limitation of conventional NGS. Importantly, the reusage of the same flow-cell ensures a limited cost per run which resembles the cost of the fragment analysis for *FLT3* ITD/TKD at our institution.

Such pipelines can be particularly suitable for laboratories with relatively limited sample flow and resources. A caveat can be the need for bioinformatic analysis which can be overcome by implementing the publicly available bioinformatic protocol within the sequencing platform. Such rapid sequencing approaches may also be valuable in the context of clinical trials, where timely and accurate molecular characterization is essential. Of note, the content of the panel can be easily expanded depending on the underlying diagnosis, making such sequencing assays attractive even for other hematological malignancies, where rapid evaluation of certain genes may be relevant. Accordingly, this approach should be viewed as one of several possible alternatives within a rapidly evolving field. Sequencing technologies continue to improve at an enormous pace; therefore, other alternative or complementary solutions may emerge.

Certain limitations should be acknowledged. In *FLT3*-ITDs, we observed reduced sensitivity for *FLT3*-ITDs at very low allelic burdens (~0.01 VAF), which according to recent reports are of clinical importance especially in the context of allogeneic stem cell transplantation [15]. We therefore recommend our ONT-LRS panel only in the diagnostic setting and not as an assay suitable for measurable residual disease (MRD), where SRS-based NGS is the current gold standard. Further optimization of the method is also indicated due to high error sequencing rate especially for small indels across all sequenced regions. This limitation in our case refers mostly to *TP53* and is overcome by setting the reportable VAF limit to 0.05.

A major advantage of LRS is the ability to sequence larger DNA molecules which enables phasing of co-occurring variants as in the case of *TP53*, where the allelic configuration of multiple variants may carry important biological and clinical implications [1, 11]. At the same time, the current assay design does not reliably detect copy number alterations (CNA), including loss of heterozygosity or deletion of the second *TP53* allele. Further optimization of the panel with targeted CNA backbone and normal SNPs is an option that needs further investigation. Finally, a unique feature of our study is that the assay was applied in a routine diagnostic setting, an essential aspect given that many previous studies of LRS in hematological malignancies have been limited to selected cohorts or individual targets.

In summary, targeted ONT-based LRS provides a rapid, reliable, and cost-effective complement for molecular profiling in AML for variants with VAF down to 0.05. Further optimization of the methodology is needed for the detection of low-VAF variants. Implementation of such diagnostics pipelines in clinical routine can enhance diagnostic accuracy, support more personalized treatment strategies and ultimately contribute to the improvement of patient outcomes in hematology.

References

1. Shahzad M, Amin MK, Daver NG, et al. What have we learned about TP53-mutated acute myeloid leukemia? Blood Cancer J. 2024;14(1):202.
2. Yu J, Li Y, Zhang D, Wan D, Jiang Z. Clinical implications of recurrent gene mutations in acute myeloid leukemia. Exp Hematol Oncol. 2020;9:4.
3. Erba HP, Montesinos P, Kim HJ, et al. Quizartinib plus chemotherapy in newly diagnosed patients with FLT3-internal-tandem-duplication-positive acute myeloid leukaemia (QuANTUM-First): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2023;401(10388):1571-1583.
4. Dohner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377.
5. Patel A, Gobel K, Ille S, et al. Prospective, multicenter validation of a platform for rapid molecular profiling of central nervous system tumors. Nat Med. 2025;31(5):1567-1577.
6. Eisfeldt J, Ek M, Nordenskjold M, Lindstrand A. Toward clinical long-read genome sequencing for rare diseases. Nat Genet. 2025;57(6):1334-1343.
7. Geyer J, Opoku KB, Lin J, et al. Real-time genomic characterization of pediatric acute leukemia using adaptive sampling. Leukemia. 2025;39(5):1069-1077.
8. Shi X, Wang S, Yu S, et al. Transcript isoform diversity defines molecular subtypes and prognosis in acute myeloid leukemia through long-read sequencing. Cell Rep. 2025;44(9):116216.
9. Cumbo C, Minervini CF, Orsini P, et al. Nanopore targeted sequencing for rapid gene mutations detection in acute myeloid leukemia. Genes (Basel). 2019;10(12):1026.
10. Abel HJ, Mahgoub M, Davarapalli N, et al. Evaluation of long-read genome sequencing for genomic profiling of myeloid cancers. J Mol Diagn. 2025;27(12):1242-1254.
11. Zeuthen M, Christoffersen LS, Hildonen M, et al. Long-read sequencing resolves TP53 allelic status in myeloid neoplasms. Haematologica. 2026;111(9):3148-3153.
12. Al-Djaber A, Munters A, Cammenga J, et al. Low rate of germline investigation for variants of suspected germline origin detected during the diagnostic work-up of myeloid neoplasms. EJHaem. 2025;6(6):e70206.
13. Clouard C. neville_mx_amplicon: Bioinformatics workflow for ONT amplicon sequencing analysis. 2026. Available from: https://github.com/clinical-genomics-uppsala/neville_mx_amplicon Accessed on 2026, Nov 16.
14. Östlund R, Clouard C, Lindholm Carlström E, et al. ONT AML Multiplex Amplicon Sequencing. 2026. Available from: https://dx.doi.org/10.17504/protocols.io.m7vznm2b2vx1/v1 Accessed on 2026, Nov 16.
15. Duployez N, Joudinaud R, Boudry A, et al. Prognostic impact of FLT3-ITD microclones in young adults with acute myeloid leukemia treated with intensive chemotherapy. Blood. 2026;148(5):598-609.

Figure Legends

Figure 1. Study design. A) Conventional genomic profiling of AML requires multiple parallel testing, including fragment analysis, ddPCR/qPCR and sequencing approaches to provide diagnostic information within the required turn-around time (TAT). Targeted ONT-LRS integrates detection of all target alterations into a single assay, reducing laboratory workload, reagent consumption, and resource requirements while maintaining a short TAT. x2: two assays, xn: multiple testing.

B) Flowchart of the study cohorts. (A): assessment of analytical performance; (B): validation of the results and extension to variant phasing, and; (C): benchmarking against standard of care (SoC). AML: acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL).

Figure 2. Targeted panel design, performance and phasing potential of the assay. A) Schematic overview of the targeted regions in *TP53, NPM1, FLT3, IDH1,* and *IDH2*. Amplicons covering the regions of interest are shown in blue, orange, or purple, corresponding to their respective primer pools.

B) Concordance of VAFs between the ONT-LRS amplicon sequencing pipeline and the SoC SRS panel in cohort A. The X-axis shows VAFs called by the SRS bioinformatic pipeline, and the Y-axis shows corresponding VAFs called by the ONT-amplicon pipeline. Linear regression analysis demonstrated high concordance between the two approaches for both Flongle- (R^2=0.97) and MinION sequencing (R^2=0.994). Color of data points represents the magnitude of the difference between the two approaches, with increasing color intensity indicating greater discrepancy.

C) Concordance of VAFs between the ONT-LRS sequencing pipeline and SoC SRS panels in cohort B. AVC, UPP and ANT are the coding names for the patient groups included in cohort

B. Two patients (red circle) show a non-significant difference between short-read and long-read methods.

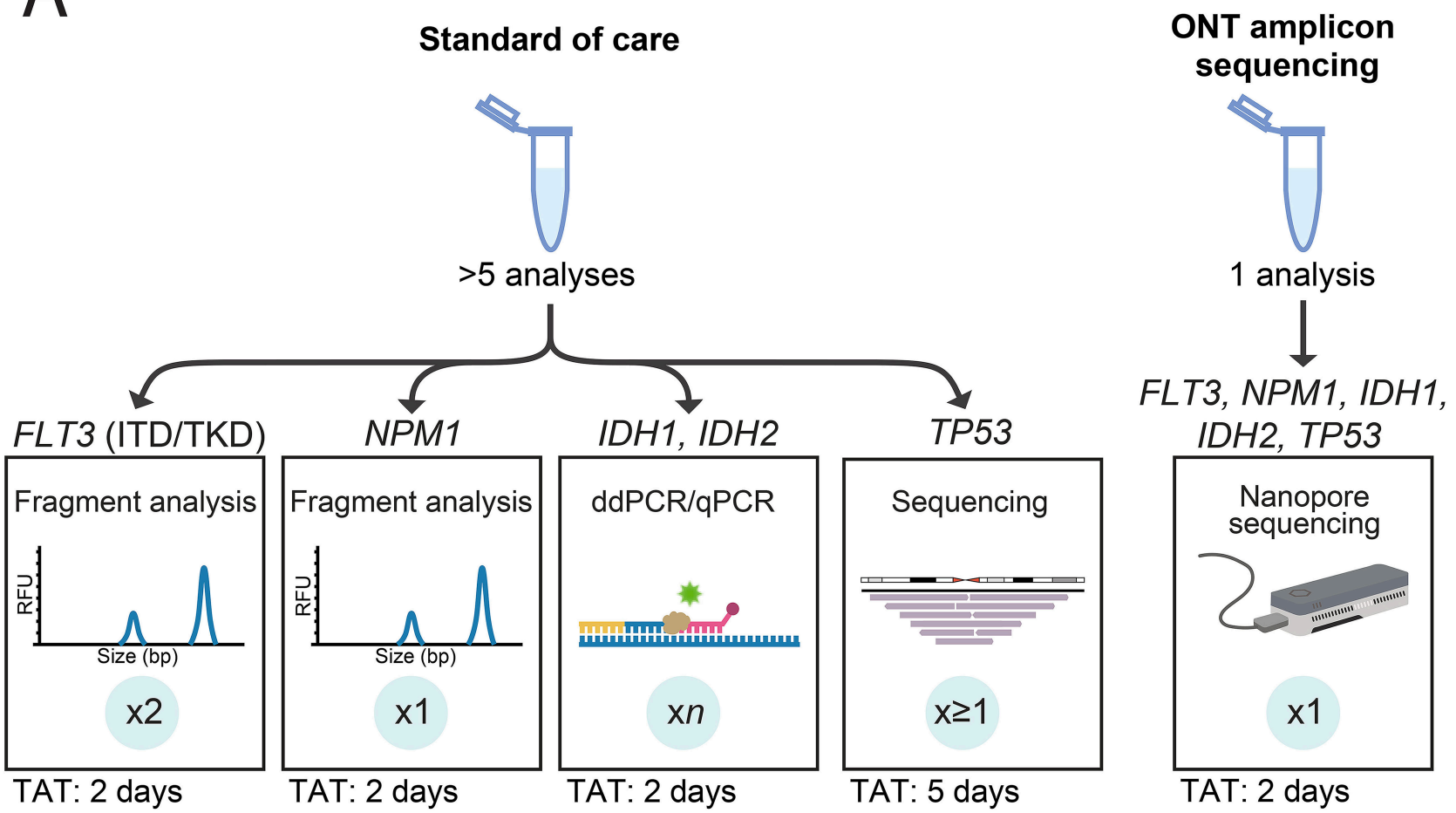
D) Phasing analysis of two pathogenic *TP53* variants in sample CLL_BOR_94. LRS-reads were used to phase two variants: NM_000546.6:c.995A>T and NM_000546.6:c.701A>G. The variants were observed on different sequencing reads/haplotypes, indicating that they are present on different alleles and therefore occur in a trans configuration.

E) Phasing analysis of two *TP53* SNPs in sample AML_P4. Reads spanning the *TP53* locus were used to determine the allelic phase of two benign SNPs, c.74+38C>G (rs1642785) and c.215C>G (rs1042522), detected in an AML patient. Phasing analysis shows that the two variants are carried on the same alleles, indicating a cis configuration on the same haplotype.

F) Haplotype summary of pathogenic variants (red triangle in trans) and benign variant (green triangle in cis) for sample AML_P4.

Figure 3. Overview of turn-around time for the ONT-LRS panel from sample processing to clinical reporting, consisting of 5 steps: (1) DNA extraction, (2) target amplification and library preparation, (3) sequencing, (4) bioinformatic processing, and (5) interpretation and report generation.

A



B

AIM 1
Assess analytical performance

AIM 2
Validate and extend to variant phasing

AIM 3
Benchmark against standard of care

Patient samples - whole blood / bone marrow / cryopreserved cells

(A) Retrospective: n=36

AML & other myeloid neoplasms
64 variants

Mutations
FLT3(ITD n=8, TKD n=3), *NPM1*(n=5)
IDH1(n=5), *IDH2*(n=3), *TP53*(n=40)
VAF 1–83% · ITD 18–176 bp · AR 0.009–0.6

Outcome:
detection accuracy & limits

(B) *TP53* focused: n=18

AML (n=12) & CLL (n=6)
39 *TP53* variants

Variant profile
≥ 1 *TP53* variant / patient
VAF range: 2–62%

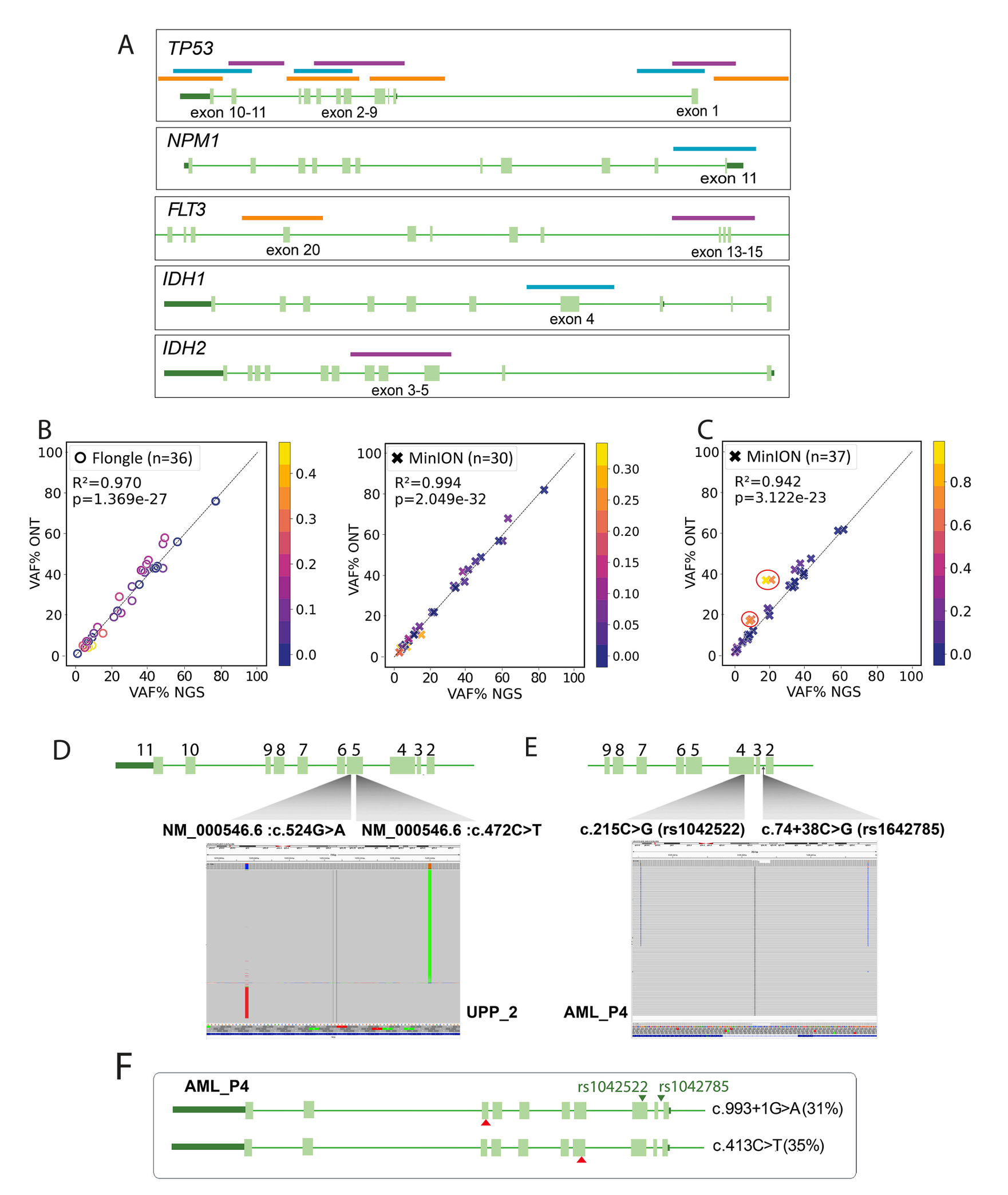
Outcome:
phasing of co-occurring *TP53* variants

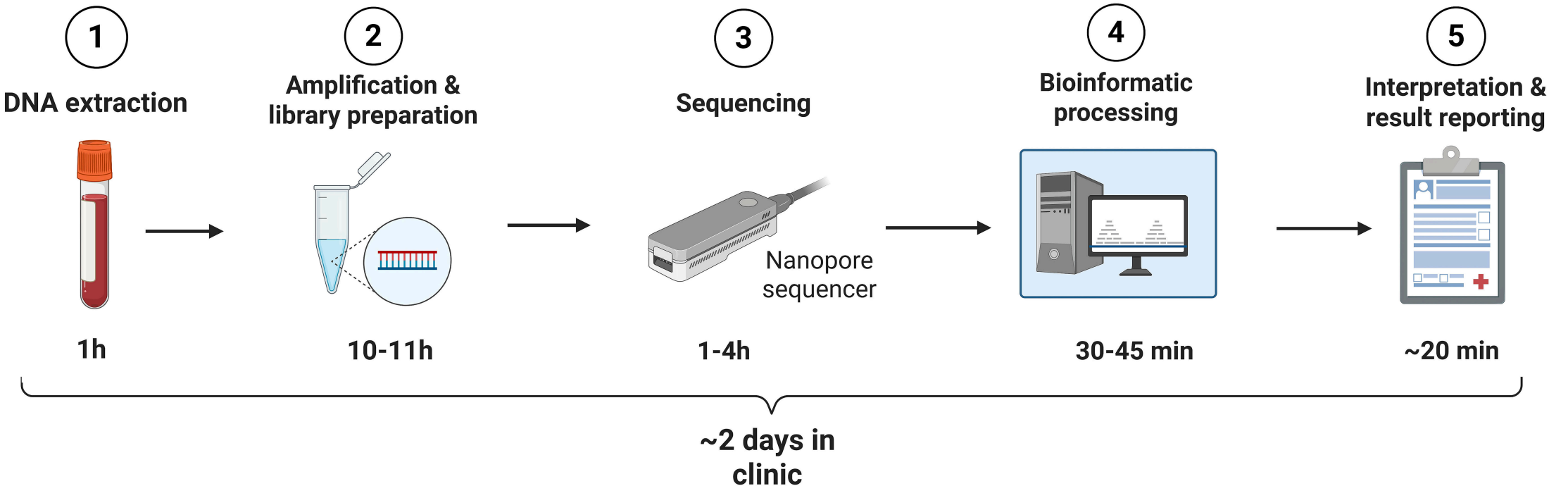
(C) Prospective : n=26

Confirmed / suspected AML
prospective enrollment

ONT panel analysis
Head-to-head comparison with SoC

Outcome:
concordance with SoC workflow


A
TP53
exon 10-11
exon 2-9
exon 1
NPM1
exon 11
FLT3
exon 20
exon 13-15
IDH1
exon 4
IDH2
exon 3-5
B
Flongle (n=36)
R²=0.970
p=1.369e-27
VAF% ONT
VAF% NGS
MinION (n=30)
R²=0.994
p=2.049e-32
VAF% ONT
VAF% NGS
C
MinION (n=37)
R²=0.942
p=3.122e-23
VAF% ONT
VAF% NGS
D
11 10 9 8 7 6 5 4 3 2
NM_000546.6 :c.524G>A
NM_000546.6 :c.472C>T
UPP_2
E
9 8 7 6 5 4 3 2
c.215C>G (rs1042522)
c.74+38C>G (rs1642785)
AML_P4
F
AML_P4
rs1042522
rs1042785
c.993+1G>A(31%)
c.413C>T(35%)

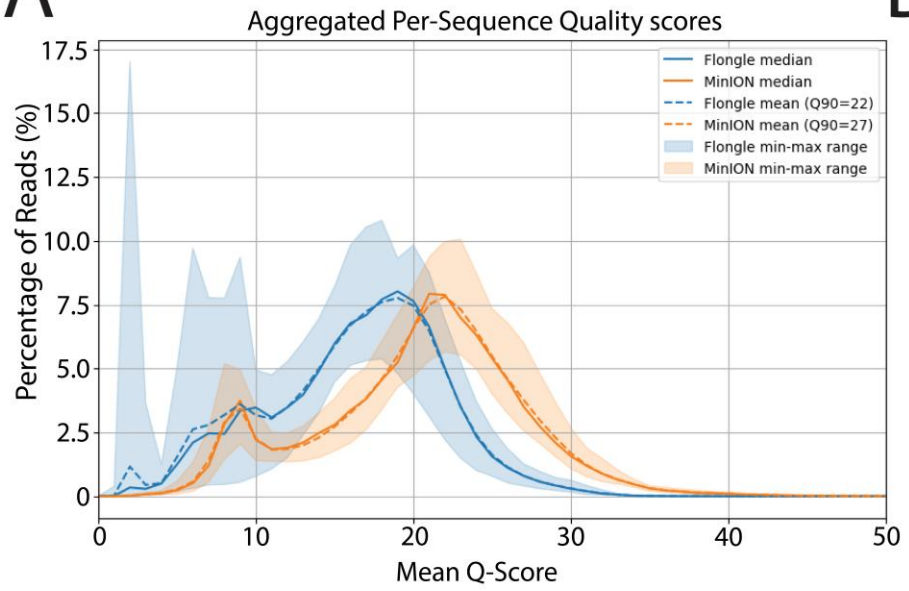

1
DNA extraction
1h
2
Amplification &
library preparation
10-11h
3
Sequencing
Nanopore
sequencer
1-4h
4
Bioinformatic
processing
30-45 min
5
Interpretation &
result reporting
~20 min
~2 days in
clinic

Supplementary Material

Rapid diagnostics of actionable mutations in acute myeloid leukemia using amplicon nanopore sequencing

Supplemental table 1. Patient and cohort variant information. (See Excel File)

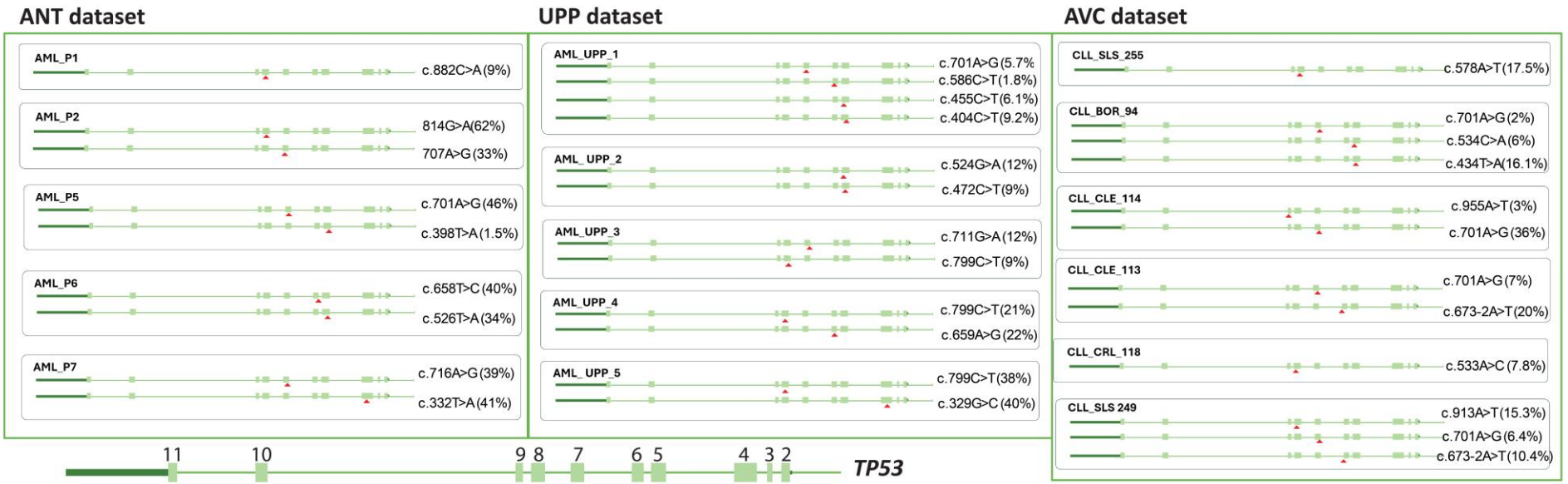
A



B

genomic_position_hg38	gene	transcripts	protein_changes	ref_allele	median_noise	sd_noise	threshold_5sd	nb_samples
chr13:28018497	FLT3-TKD	NM_004119.3:c.2508_2510del	p.I836del	C	0,001930813	0,001054191	0,007201768	65
chr13:28018503	FLT3-TKD	c.2505_2508delinsAATA	p.D835E	A	0,001196172	0,000627138	0,004331861	65
chr13:28018505	FLT3-TKD	NM_004119.3:c.2503G>T	p.D835Y	C	0,00146308	0,001440601	0,008666085	66
chr15:90088702	IDH2	NM_002168.4:c.419G>A	p.R140Q	C	0,001156556	0,00091173	0,005715205	62
chr2:208248388	IDH1	NM_005896.4:c.395G>A	p.R132H	C	0,001115398	0,000833034	0,005280568	54
chr2:208248389	IDH1	NM_005896.4:c.394C>?, NM_005896.4:c.394C>A, NM_005896.4:c.394C>T	p.R132?, p.R132C, p.R132S	G	0,003286418	0,00174873	0,01203007	60
chr5:171410539	NPM1	NM_002520.7:c.860_863dup,NM_002520.7:c.860_863dup?,NM_002520.7:c.860_863dupTCTG	p.W288fs*,p.W288fs*12, p.W288fs*?	C	0,001033993	0,000547731	0,003772648	63

C



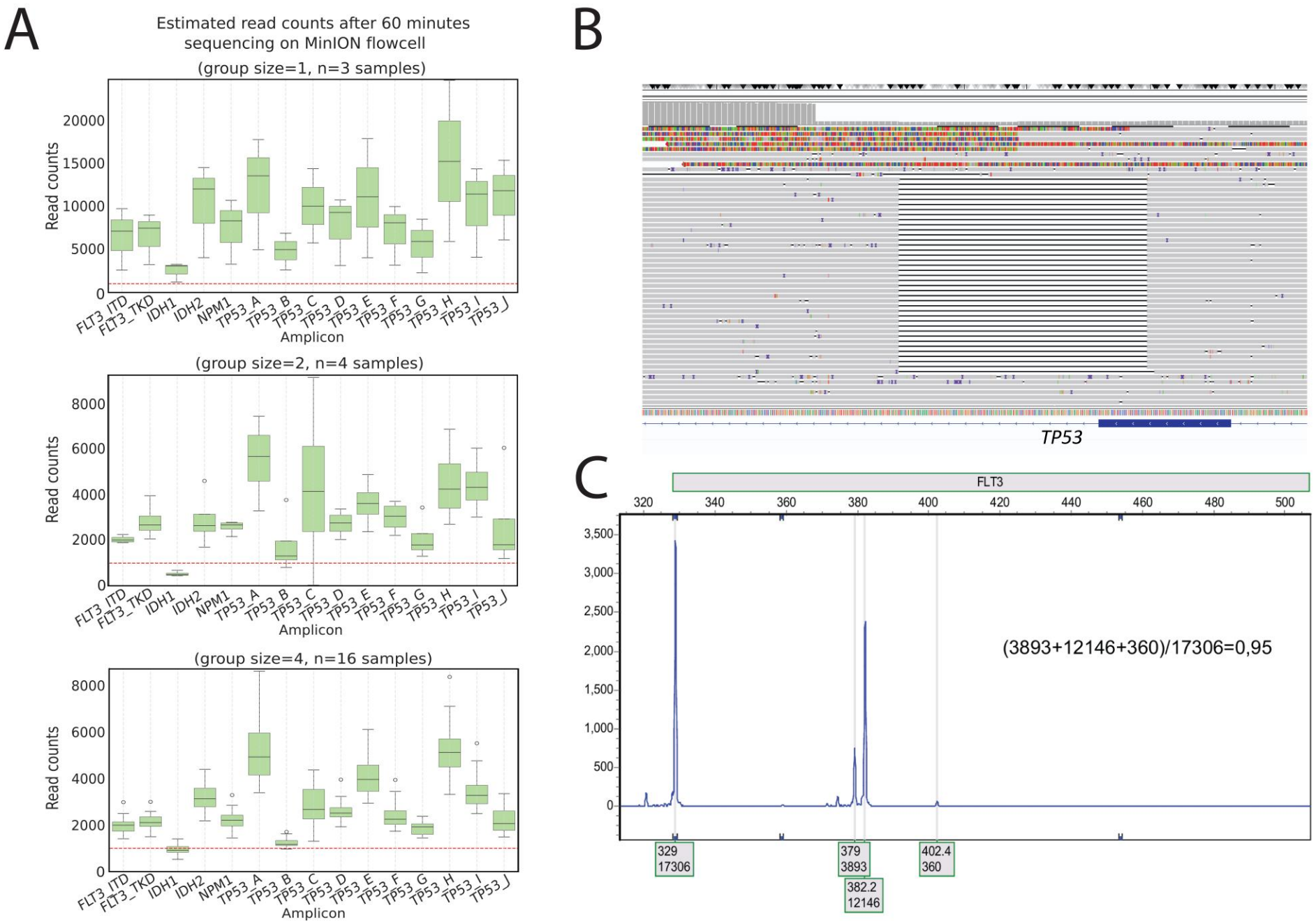
Supplementary figure 1. Sequencing performance and phasing haplotypes.

A) Of the 36 samples included in cohort A, 15 samples with 27 different genetic variants were sequenced on Flongle and 15 (22 different variants) on MinION. Six additional samples were sequenced on both instruments (15 variants). The sequencing runs on Flongle yielded a minimum of 1600x (1602x-80468x) coverage for regions of interest after 24h of sequencing and a mean N50 of 3.18kb. Sequencing on MinION generated a minimum of 580x coverage (580x-93421x) after an average time of 111 min/sample (47-284 min/sample) with a mean N50 of 3.07kb. The data produced with MinION had greater median Q-Score values (mean median Q-score=21.4 (19.5-23.4), n=8) than that produced by Flongle sequencing (mean median Q-score=17.5 (16.2-18.4), n=21). Both platforms had the possibility of obtaining real-time sequencing data for interim analysis every 10 minutes. All samples from cohort B were sequenced on the MinION platform, generating a minimum coverage of 1266x (1266x-71008x) of regions of interest after an average of 210 minutes/sample (33-327 minutes/sample).

B) To estimate sensitivity in variant detection on MinION flow cells, the background noise in quality-filtered and aligned BAM files in selected hotspot positions was analyzed. The background noise is defined per hotspot from the distribution of per-sample variant allele fractions (VAFs) across the samples in the background cohort that do not carry the hotspot mutation. Per hotspot and per sample, the VAF is computed as the ratio of non-reference allele to the read depth. The median VAF, the standard deviation (SD) in VAF and a 5*SD threshold in the background cohort is reported.

C) Phasing in cohort B. All patients with CLL diagnosis from Avicenne University Hospital (AVC) (HUPSSD, AP-HP) provided informed consent and were enrolled in a "B-cell lymphoproliferative disorders" cohort (DC 2009 936). CLL cells were isolated from peripheral blood using the Ficoll method, and flow cytometry analysis confirmed tumor-cell purity of >80% in all cases. For short-read sequencing, Ion Torrent technologies (Genexus system) were used with a VAF of 1% as recommended by the GBMHM. Raw data were aligned using different bioinformatic tools such as GATK (RRID:SCR_001876) or the Genexus software (V6.8.2.0). Minimal average base coverage depth was around 5000x, with a minimal coverage for variant calling at 1000x with at least 10 variant reads and a VAF cut-off of 1%. Additionally, DNA samples were obtained from seven patients at Saint-Antoine Hospital (ANT) (APHP-Paris) with secondary AML (3 post-polycythemia vera AMLs, 3 post-essential thrombocythemia and 1 post-primary myelofibrosis AML, with a median age of 74 years. Targeted NGS analysis was performed at diagnosis of AML, using DNA samples

extracted from bone marrow and Illumina MiSeq method according to the manufacturer's recommendations. Bioinformatic analysis for alignment and variant identification was carried out by Sophia Genetics. Minimal coverage depth was around 500x and VAF of 1-2%.


A
Estimated read counts after 60 minutes sequencing on MinION flowcell
(group size=1, n=3 samples)
Read counts
Amplicon
(group size=2, n=4 samples)
Read counts
Amplicon
(group size=4, n=16 samples)
Read counts
Amplicon
FLT3_ITD
FLT3_TKD
IDH1
IDH2
NPM1
TP53_A
TP53_B
TP53_C
TP53_D
TP53_E
TP53_F
TP53_G
TP53_H
TP53_I
TP53_J
B
TP53
C
FLT3
(3893+12146+360)/17306=0,95
329
17306
379
3893
382.2
12146
402.4
360

Supplementary figure 2. Coverage after 60 minutes and manual curation of variants.

A) Read count distribution for amplicon targets after 60 minutes of MinION sequencing. Samples were multiplexed at different levels (group sizes 1, 2, and 4), and boxplots show sequencing depth per sample for each multiplexing condition.

B) IGV-snapshot showcasing the variant NM_000546.6:c.1062_1100+161del in patient UU_LRS32 that was manually curated.

C) *FLT3*-ITD PCR capillary electrophoresis analysis of patient UU_SOC14 with clinically reported allele ratio of 0.95. Three separate ITDs were detected. The ITD with a peak at 379 was not detected by the LRS panel.