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Capillary Sampling Enables Venetoclax Concentration Measurement in Acute Myeloid Leukaemia Within Academic Multicentre Trial

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ABSTRACT

Venetoclax has improved outcomes for acute myeloid leukaemia (AML) patients unfit for intensive chemotherapy. Managing cytopenias and infections remains challenging. Previous pharmacokinetic studies have shown considerable variability in venetoclax concentrations between individuals; however, data regarding whether higher levels increase toxicity or impact efficacy are limited. This study assessed the feasibility of using fingertip capillary blood plasma, collected via microsampling, to measure venetoclax trough concentrations and explored their association with toxicity and treatment outcomes. Concentrations were measured during the first two therapy cycles in 89 patients with newly diagnosed or relapsed or refractory AML receiving azacitidine and venetoclax. Validation with 37 parallel venipuncture and capillary samples showed excellent correlation (R^2 of 0.835, $p < 0.0001$). No significant associations were found between venetoclax concentrations and patient characteristics such as gender, age and weight. While no statistically significant effects on therapy outcomes or adverse events were identified, trends suggested lower concentrations in refractory patients and higher in those with morphologic leukaemia free state or extended cycle length. Additionally, three separate *CYP3A4* and *CYP3A5* single-nucleotide polymorphisms were analysed in 81 patients for their potential impact on venetoclax concentrations. This study demonstrates that the capillary blood plasma method is viable for measuring venetoclax levels.

1 | Introduction and Background

Chemotherapy, often followed by allogeneic stem cell transplantation, is the standard treatment for acute myeloid leukaemia (AML). However, many patients are ineligible for intensive therapy due to comorbidities or advanced age, resulting in poor survival. The proapoptotic, selective BCL-2 inhibitor venetoclax, when combined with a hypomethylating agent, improves outcomes in previously untreated AML. In VIALE-A trial, combining venetoclax with azacitidine increased the median overall survival (OS) to 14.7 months compared to 9.6 months with azacitidine monotherapy. [1]

Summary

We assessed the capillary plasma method for measuring venetoclax trough concentrations in 89 patients receiving azacitidine and venetoclax for acute myeloid leukaemia in our prospective multicentre clinical trial. The capillary method correlated excellently with the standard approach in 37 parallel venous and capillary blood samples. Demographic factors had no significant impact on concentration. We also analysed three separate *CYP3A4 and CYP3A5* single-nucleotide polymorphisms in 81 patients to evaluate their effect on concentrations. A trend of lower concentrations in therapy-refractory patients and higher concentrations for those with low cellularity were observed, but no statistically significant effects were found.

Although previous trials have established venetoclax as a well-tolerated therapy in AML, it often causes prolonged neutropenia, leading to infections and delays in subsequent treatment cycles. [1] Other frequently reported adverse events include thrombocytopenia, nausea, diarrhoea and fatigue. In early venetoclax trials for chronic lymphocytic leukaemia (CLL), severe tumour lysis syndrome (TLS) was occasionally observed, but preventive strategies in later studies significantly reduced its occurrence. [2–4] In AML, venetoclax is commonly started with a 3-day dose escalation of 100–200–400 mg with concomitant pre-emptive administration of hypouricemic agents. Significant TLS has been reported rarely in AML when these prophylactic measures have been implemented. [1, 5]

In plasma, venetoclax is highly bound to plasma proteins (> 99%) with a large volume distribution. The concentration peaks at 4–8 h, and terminal half-life is 16–19 h. [6–11] Concomitant high-fat meal delays the peak concentration by 2 h. [12] Venetoclax steady-state exposures are achieved approximately after 4 days of daily venetoclax administration. The urinary excretion of venetoclax is minimal, and it is cleared from the body mainly by hepatic metabolism through CYP3A4 and CYP3A5. When using moderate or strong CYP3A4 inhibitors, e.g. triazoles, venetoclax exposure increases three- to eightfold. [13–16] In previous studies, moderate impairment of liver or kidney function did not affect venetoclax metabolism significantly. Single-nucleotide polymorphisms (SNPs) in *CYP3A4* and *CYP3A5* can potentially impact the enzyme function and affect the venetoclax concentration. However, to our knowledge, data of the *CYP3A4* or *CYP3A5* SNPs are lacking in AML patients undergoing treatment with azacitidine and venetoclax.

Drug concentrations are routinely measured from plasma obtained through venipuncture. The blood is then centrifuged to separate the plasma, which is subsequently frozen for later analysis. Although the traditional methods are well-established and validated, they necessitate prompt sample handling and cold chain storage. These requirements can pose challenges in academic multicentre clinical trials. Capillary blood sampling using volumetric absorptive microsampling (VAMS) devices has been effectively employed for analysing drug concentrations in various biological matrices, including blood, urine and saliva. [17] This method reduces the need for specialized laboratory personnel or facilities for sampling, immediate processing and freezer storage, making it a more convenient option for drug concentration analysis. Additionally, capillary devices enable self-sampling at home when appropriate. Dried capillary samples can generally be stored at room temperature without significantly affecting analyte stability. Using dried microsamples also helps eliminate the costs associated with cold chain shipping and storage. However, there is limited data on the use of capillary plasma methods for evaluating venetoclax concentrations.

Venetoclax plasma concentrations have been evaluated in Phase I–III trials in CLL and non-Hodgkin lymphoma. [7–9, 11, 18–23] Marked variability of concentrations exists between patients. While some clinical trials have assessed the impact of patient demographics on the venetoclax plasma concentrations, no significant correlations with age, weight or gender have been found. In AML, studies on venetoclax therapeutic drug monitoring remain limited. Zappasodi et al. examined venetoclax concentrations of seven AML patients receiving concomitant posaconazole during both the ramp-up and steady-state phases, finding similar variability in trough concentrations to what has been reported in other trials [24]. Yang et al. evaluated venipuncture blood peak and trough concentrations in 62 AML patients and reported a median trough concentration of 836.6 (74.2–3257.0) ng/mL and noted an association between higher maximum concentration and older age. [25]

We evaluated the feasibility of capillary plasma sampling for venetoclax concentration analysis within our academic VenEx trial. For validation, we compared venetoclax trough concentrations obtained from capillary samples with parallel venipuncture plasma samples in a subset of participants. Furthermore, we determined the correlation between venetoclax capillary plasma trough concentrations, patient demographics, adverse events and therapy outcome. Finally, we analysed whether the selected SNPs *CYP3A4*2* c.666T > C, *CYP3A4*22* c.522-191G > A or *CYP3A5*3* c.219-237A > G have impact on the venetoclax concentrations.

2 | Materials and Methods

2.1 | VenEx Trial and Patients

VenEx (NCT04267081) was an open-label, Phase 2 prospective multicentre trial conducted by the Finnish Leukaemia Group in five university hospitals in Finland. [26, 27] The primary aim of the study was to evaluate whether ex vivo venetoclax sensitivity predicts the therapy outcome. Between February 12, 2020, and April 13, 2022, the trial recruited 104 participants with AML, including previously untreated ($n = 48$), previously treated secondary ($n = 17$) and relapsed/refractory (R/R) AML ($n = 39$). The main exclusion criteria were acute promyelocytic leukaemia, prior venetoclax therapy for myeloid malignancy, ECOG performance status > 3, known central nervous system involvement with AML, uncontrolled infection and cardiovascular disability status of New York Heart Association more than class two. Participants were required to have adequate renal function defined by calculated creatinine clearance ≥ 30 mL/min determined by the Cockcroft Gault formula and adequate liver function defined by plasma alanine aminotransferase (ALT) ≤ 4.0x

ULN and bilirubin ≤1.5x ULN. To reduce the risk of TLS, only participants with peripheral blood leukocyte count < 25x 10E9/L were permitted to start the study therapy. Hydroxyurea was allowed prior to the study entry to meet this criterion.

Altogether 12 participants with R/R or secondary AML were excluded from study therapy based on the ex vivo venetoclax resistance. Additionally, two early deaths occurred during the first therapy cycle before the first capillary sampling, first at Day 4 due to uncontrolled infection combined with tumour lysis leading to multiple organ failure and second at Day 14 due to uncontrolled infection. For one participant, no capillary samples for the venetoclax concentration analysis were obtained. The remaining 89 participants were included in pharmacokinetic analysis.

2.2 | Study Therapy

Therapy consisted of 28-day cycles of azacitidine and venetoclax. For responsive patients, extending the cycle length up to 42 days was allowed to recover from cytopenia. Azacitidine was administered subcutaneously 75 mg/m2 daily for 7 days in each cycle and after six cycles dosing was reduced to 5 days for responding patients. Venetoclax was started with a dose escalation of 100–200–400 mg during the first 3 days of the therapy and with further dose of 400 mg daily up to 28 days in each cycle until CR/CRi/MLFS response was achieved. For responding patients, the duration of venetoclax was reduced to 21 days per cycle and further to 14 days after three cycles if the response persisted. Venetoclax was recommended to be taken at noon, 30 min after a light meal.

The study protocol prohibited the use of moderate or strong CYP3A4 inducers, such as carbamazepine, phenytoin and rifampin, during the study therapy. Instead of strong CYP3A4 inhibitors such as voriconazole, posaconazole and clarithromycin, alternative medications were recommended. If a strong CYP3A4 inhibitor was unavoidable, venetoclax dosing was reduced to 10–20–50 mg during the ramp-up phase and maintained at 50 mg per day during the steady dosing phase. Similarly, if a moderate CYP3A4 inhibitor, such as ciprofloxacin, cyclosporine, fluconazole or isavuconazole, was required, venetoclax dosing was reduced to 50–100–200 mg during ramp-up and 200 mg per day thereafter.

The study protocol required the reporting of all severe adverse events (SAEs) and unexpected adverse events. Adverse events listed in the prescribing information for azacitidine or venetoclax were considered expected and needed to be reported only if they reached Grade 3 or higher. Furthermore, grade 2 adverse events had to be reported if they caused a delay in starting the next therapy cycle beyond Day 42 or resulted in a dose modification of the study therapy. The protocol also allowed cytopenia to be recorded as part of a related adverse reaction, such as infection, rather than as a separate adverse event.

2.3 | Sampling

For venetoclax trough concentration, two 20-μL fingertip capillary blood samples were collected immediately before the next venetoclax dose. Capillary sampling was required by the protocol for all participants on Day 15 of the first therapy cycle (C1D15). Additional samples on Day 15 of the second cycle (C2D15) were optional. Samples on Day 2 of the first and second therapy cycles (C1D2 and C2D2) were taken in one study centre. Capillary blood was collected via rapid capillary action using the volumetric absorptive micro sampling devices (VAMS, Mitra, Neoteryx, Torrance, CA, USA). Capillary samples for Day 15 were accepted if taken within one to 3 days before or after the scheduled time point, provided there were no changes in venetoclax dosing or in other relevant medications, or the patient's clinical condition during the steady-state period. Capillary plasma samples were stored at room temperature in the study centres until shipment to the central laboratory.

To validate the capillary concentration measurements, we collected 37 venipuncture 4-mL blood samples concurrently with capillary samples on C1D15 or C2D15, using potassium ethylenediaminetetraacetic acid (K2-EDTA) tubes. The peripheral blood samples were immediately centrifuged, and the plasma was aliquoted into two tubes and stored at −80°C until analysis (Figure 1A).

2.4 | Determination of Venetoclax Concentrations

Venetoclax analyte and the deuterated internal standard (IS), venetoclax-d7, were obtained from Alsachim (Strasbourg, France). Stock solutions were prepared in acetonitrile, with concentrations of 2 mg/mL for venetoclax and 0.5 mg/mL for the IS. Working standard solutions were prepared by diluting the stock solution in acetonitrile. Calibration standards (STD) and quality controls (QC) were created by spiking plasma and EDTA whole blood with diluted working standards. The spiked plasma samples were then aliquoted and stored at −20°C until analysis.

The spiked whole blood samples were used fresh to prepare STDs and QCs for the VAMS devices. The VAMS devices were loaded by touching the spiked whole blood with the sampling tip until fully saturated, then held for an additional 2 s. Afterward, the devices were dried and stored at room temperature until analysis.

For plasma sample analysis, 100-μL aliquots of both patient and spiked plasma samples were transferred into Phree phospholipid removal plates (Phenomenex, Torrance, CA, USA). Next, 300 μL of acetonitrile containing 1% formic acid and the IS was added to each well using the Tecan Freedom EVO 150 automated liquid handler (Tecan Group, Männedorf, Switzerland). The plate was vortex-mixed and incubated at room temperature for 10 min to precipitate proteins, after which a vacuum was applied to collect the extracts into a new plate.

To extract venetoclax from the VAMS devices, the tips were placed into microwell plates, and 200 μL of methanol containing 1% formic acid and the IS was added to each well. The plates were shaken at 450 rpm using an Eppendorf ThermoMixer C for 60 min at room temperature, followed by 15 min of sonication; 50-μL aliquots of the extracts were diluted with 200 μL of 50% methanol prior to analysis.

The extracted analytes were analysed using an LC–MS/MS system, comprising a Nexera X2 UHPLC system (Shimadzu, Kyoto,

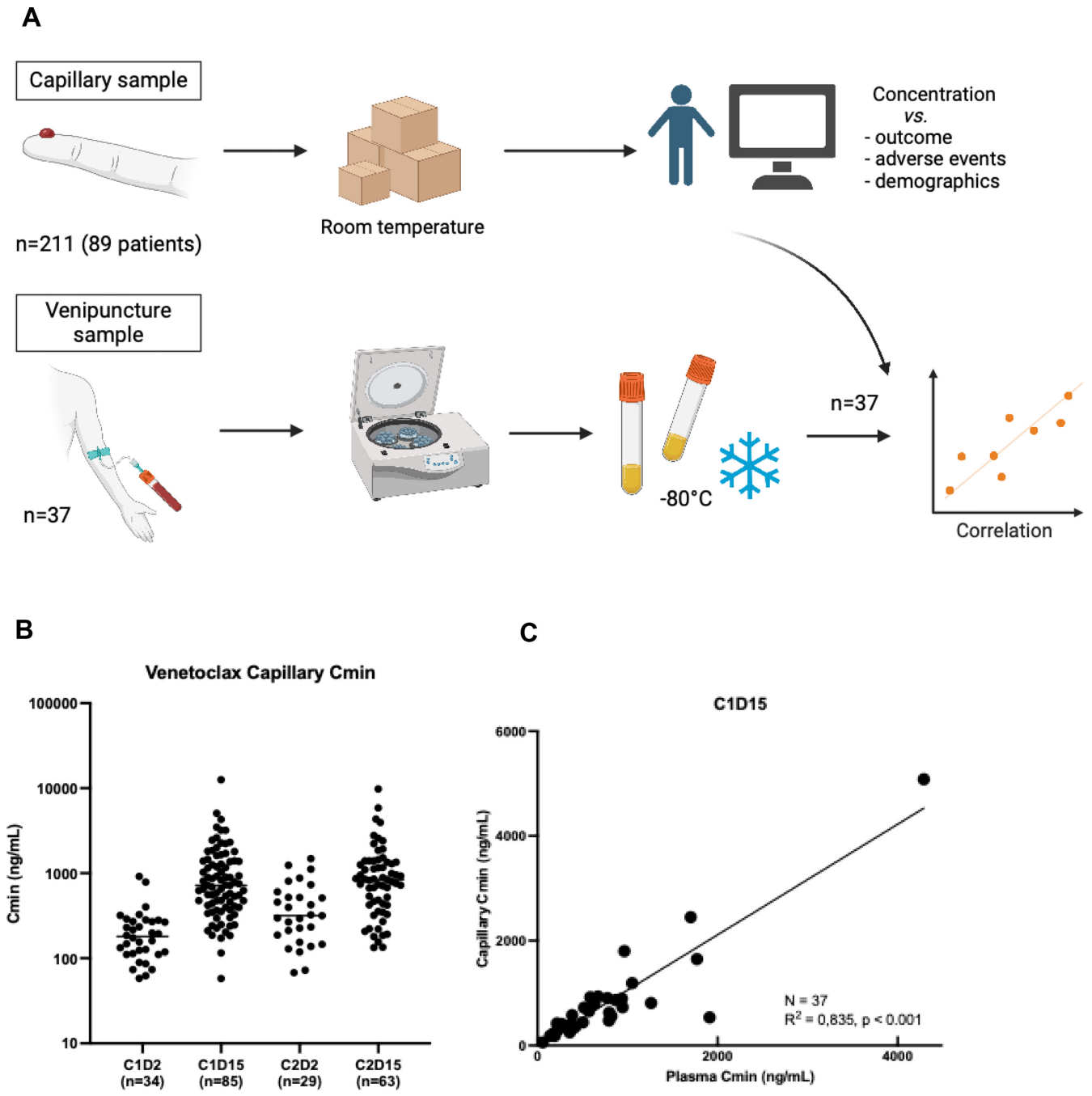

FIGURE 1 | **Study design and validation of capillary sampling method.** (A) Capillary blood samples ($N = 211$) from 89 participants were stored at room temperature. Venous blood samples were collected concomitantly with the Day 15 capillary sample from 37 patients and stored at freezing temperature as per standard practice. The correlation of venetoclax trough concentration obtained with the two methods was analysed using 37 parallel Day 15 samples. Next, capillary concentrations were employed to examine their correlation with demographic factors, adverse reactions and treatment outcomes. (B) Venetoclax capillary trough concentrations at four time points. (C) The correlation between venetoclax trough concentrations in capillary and venous blood. C1D2, Day 2 of the first therapy cycle; C1D15, Day 15 of the first therapy cycle; C2D2, Day 2 of the second therapy cycle; C2D15, Day 15 of the second therapy cycle; Cmin, venetoclax trough concentration.

Japan) and an API3000 mass spectrometer (Sciex, Toronto, ON) with a turbo ion spray source. The analytes were separated on an Atlantis T3 column (2.1 × 100 mm, 3 μm, Waters, Milford, MA) at eluent flow rate 200 μL/min using 5-mM ammonium acetate and methanol as mobile phases. Methanol gradient started from 60% and was increased to 95% between 0.5 and 5 min and kept constant for 4 min before balancing at 60% before the next injection. The multiple reaction monitoring transitions of the analytes $[M+H]^+$ were m/z 868.6–321.4 for the venetoclax and m/z 875.6–321.4 for the IS. The lower limit of quantification was 10 ng/mL for plasma analysis and 50 ng/mL for whole blood samples. The between-day coefficients of variation for the QC samples ($n = 4$) were ≤ 3.1% for plasma and ≤ 5.7% for whole blood, at the relevant concentrations.

2.5 | *CYP3A4* and *CYP3A5* Variant Analysis

Three single-nucleotide variants, *CYP3A4**2 c.664T > C (rs55785340), *CYP3A4**22 c.522-191G > A (rs35599367) and *CYP3A5**3 c.219-237A > G (rs776746), were genotyped using TaqMan SNV Genotyping assays on QuantStudio k12 Flex Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). All genotyping runs included positive and negative controls, and samples with inconclusive results were regenotyped.

2.6 | Statistical Methods

The data cut-off date was October 23, 2023. Median OS was assessed using the Kaplan–Meier method, and the difference between groups was compared with a log-rank test. For the participants alive at the time of analysis, the data were censored on the date of the last contact. Median follow-up time was calculated for censored events. Medians of non-normally distributed concentration values between two subgroups were compared using the Mann–Whitney *U* test (with Bonferroni correction) and across multiple subgroups using the Kruskal–Wallis test. Univariate linear regression was used to estimate the odds ratios (ORs) for the associations between venetoclax concentrations in each timepoint and patient characteristics including age, gender, weight, *CYP3A4* and *CYP3A5* SNPs and the amount of Grades 3–4 adverse events. A model including age, gender and weight was also evaluated through a multivariate linear regression model using venetoclax trough concentration at C1D15 as the dependent variable. Statistical analyses and graphical illustrations were performed using IBM SPSS Statistics version 28.0.0.0 (190) and Prism version 9.5.1 (528).

The study was conducted in accordance with the Basic and Clinical Pharmacology and Toxicology policy for experimental and clinical studies. [28]

3 | Results

3.1 | Patients and Samples

A total of 89 participants from the VenEx trial were selected for pharmacokinetic analysis, including 39 females (44%) and 50 males (56%). Of these, 68 participants (76%) had an ECOG performance status of 0–1, and 21 (24%) had a status of 2–3. At screening, the median age was 73.3 years (range 41.9–84.6), median weight was 77.4 kg (range 49.0–149.0), and median BMI was 26.6 (range 20–45) (Table 1A). Due to the trial exclusion criteria, participants did not have significant kidney or liver dysfunction.

Capillary plasma samples were collected at four specific timepoints: C1D2 (*N* = 34), C1D15 (*N* = 85), C2D2 (*N* = 29) and C2D15 (*N* = 63) (Table 1B). Samples were collected from 62 participants (70%) at both the C1D15 and C2D15 timepoints. For 24 participants (27%), only a C1D15 (23/24) or C2D15 (1/24) capillary sample was obtained. In three participants (3%), only a C1D2 or C2D2 capillary sample was available. In some cases, the tip was only partially filled, resulting in a moderately lower capillary blood volume than the intended 20 μL. Of the samples collected, 14 (16%) of the C1D15 and 17 (27%) of the C2D15 capillary samples were underfilled.

TABLE 1 | (A) Patient demographics. (B) Median capillary venetoclax trough concentrations and sample counts across four time points for all 89 participants.

A

	All (*N* = 89)	
	N	**%**
Female>	39	43.8
ECOG 0–1	68	76.4
ECOG 2–3	21	23.6
ND AML	45	50.6
sAML	12	13.4
R/R AML	32	36
Prior alloHSCT	16	18

B

		All (*N* = 89)			
		Median	**95% CI**	**Min**	**Max**
Age	years	73.3	71.3-74.6	41.9	84.6
Weight	kg	77.4	76.0-80.1	49	147
Height	cm	170	168–173	146	192
BMI	kg/m2	27	26–27	20	45
		VEN Cmin (ng/mL), all (*N* = 89)			
	N	**Median**	**95% CI**	**Min**	**Max**
C1D2	34	180	127–235	58	917
C1D15	85	720	561–895	58	12 600
C2D2	29	318	227–518	68	1490
C2D15	63	846	713–992	134	9790

Abbreviations: alloHSCT, allogeneic stem cell transplantation; BMI, body mass index; C1D2, Day 2 of the first cycle; C1D15, Day 15 of the first cycle; C2D2, Day 2 of the second cycle; C2D15, Day 15 of the second cycle; ECOG, Eastern Cooperative Oncology Group performance status; ND AML, newly diagnosed AML; R/R AML, relapsed or refractory AML; sAML, secondary AML; VEN Cmin, venetoclax capillary trough concentration.

The median venetoclax trough concentrations (Cmin) demonstrated wide variability between patients and were at specific timepoints as follows: C1D2, 180 ng/L (range 58–917); C1D15, 720 ng/L (range 58–12 600); C2D2, 318 ng/L (range 68–1490); and C2D15, 846 ng/L (range 134–9790). (Figure 1B and Table 1B).

3.2 | Correlation Between Venipuncture and Capillary Methods

To analyse the correlation between capillary and venous blood venetoclax trough concentrations and to validate the capillary method, 37 parallel venipuncture blood plasma samples were

collected at C1D15 or C2D15. Among these paired samples, 14 (38%) had a low-fill capillary counterpart. Venetoclax trough concentrations measured with these two methods showed excellent correlation ($R^2 = 0.835$, $p < 0.0001$), demonstrating the capillary assay's feasibility (Figure 1C).

3.3 | Correlation Between Venetoclax Concentration and Demographic Factors

No significant correlation was observed between venetoclax Cmin and demographic factors including age (C1D15:

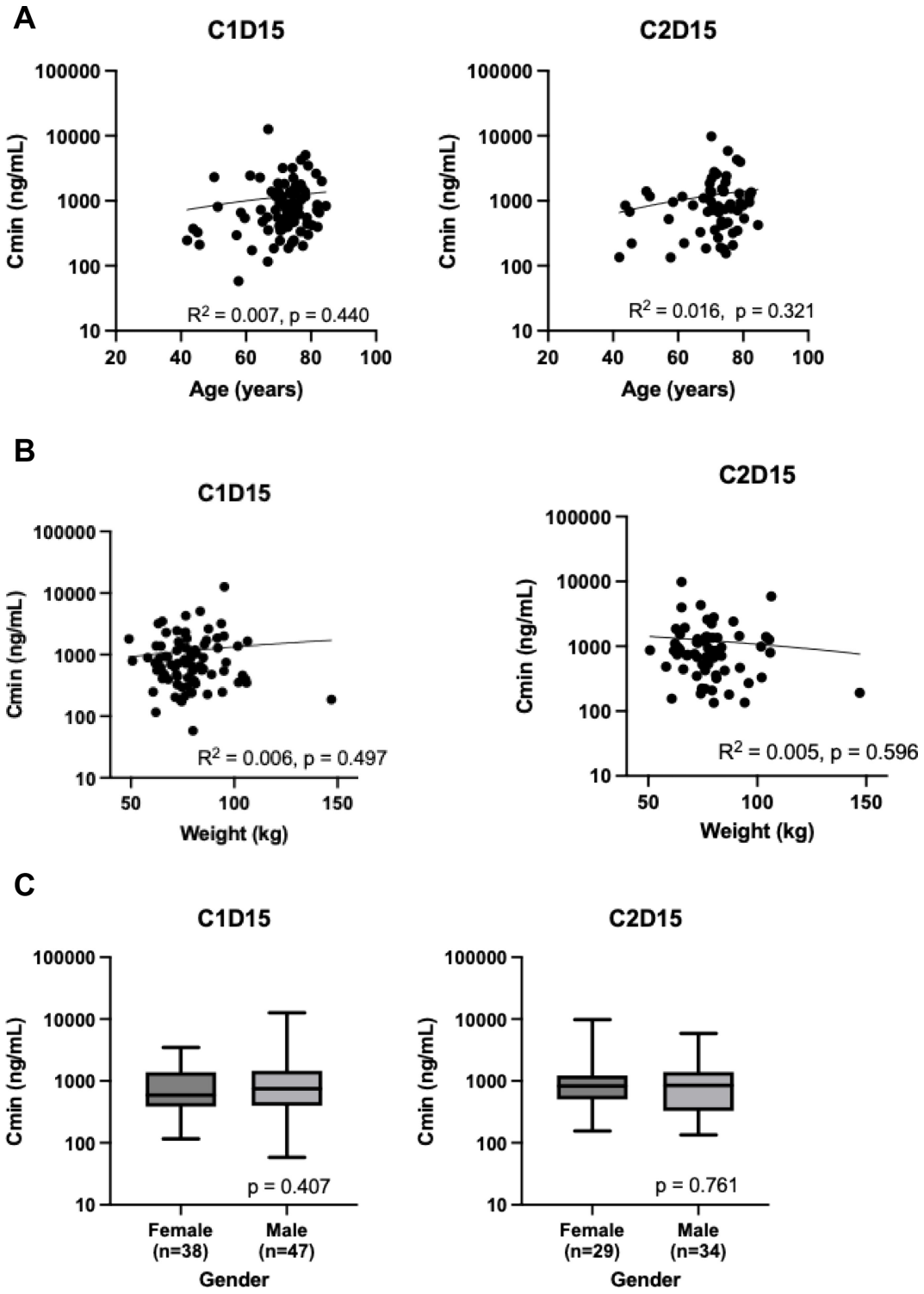

FIGURE 2 | **Associations between demographic factors and venetoclax trough concentrations.** (A) Correlation between age and venetoclax capillary trough concentrations on Day 15 of the first and second therapy cycles. (B) Correlation between weight and venetoclax capillary trough concentrations on Day 15 of the first and second therapy cycles (C) Comparison of median venetoclax capillary trough concentration between genders in the first and second therapy cycles. The difference between medians was assessed using Mann–Whitney *U* test. Cmin, venetoclax capillary trough concentration; C1D15, Day 15 of the first therapy cycle; C2D15, Day 15 of the second therapy cycle; *n*, number of capillary samples.

$R^2 = 0.007$, $p = 0.440$, C2D15: $R^2 = 0.016$, $p = 0.321$) and weight (C1D15: $R^2 = 0.006$, $p = 0.497$ and C2D15: $R^2 = 0.005$, $p = 0.596$) (Figure 2A–B). Median Cmin also did not differ by weight quartiles, age groups (<75 vs. ≥75) (Figures S1–S2), or gender (Figure 2C).

Additionally, we assessed the predictive relationship between three demographic factors—gender, age and weight—and capillary blood venetoclax Cmin at C1D15 using a linear regression model. The linear regression analysis was not statistically significant (R^2 of 0.02, $p = 0.640$). Results indicate that gender, age and weight do not substantially contribute to the variability in venetoclax trough concentrations.

3.4 | Association Between Venetoclax Concentration and Adverse Events

We then assessed whether venetoclax trough concentrations were associated with the frequency of adverse events. A total of 157 adverse events were recorded across the first two therapy cycles, with 96 (61%) occurring during the first cycle and 61 (39%) during the second. In cycle one, 34 participants (38.2%) had no adverse events, while 55 (61.8%) experienced at least one. In Cycle two, 47 participants (54.7%) had no adverse events, while 39 (45.3%) experienced at least one. The majority of events (126, 80.3%) were classified as grade 3. The most common adverse events were febrile neutropenia ($n = 46$, 29%) and infections ($n = 45$, 29%), with respiratory, skin and gastrointestinal infections being the most frequent. Infections frequently occurred during neutropenia; however, per protocol, neutropenia was recorded as part of infection events rather than separately. All adverse events were attributed to either venetoclax or azacitidine, but their individual contributions could not be determined due to concurrent administration. Median venetoclax Cmin on C1D15 and C2D15 showed no clear differences based on the number of adverse events (0, 1–2, or more than 2) or between patients with infection/neutropenia versus those with other or no reported adverse events (Figure S3).

Next, we evaluated the impact of Cmin on neutropenia. Since laboratory results were available only on Days 1, 15 and 28 of each cycle, the exact duration of neutropenia could not be determined. Given that cytopenia and infections were the primary reasons for prolongation of therapy cycles, we used cycle length as a surrogate marker for neutropenia. We compared the median Cmin between patients with cycle lengths within targeted 28–35 days to those with prolonged cycles of 36–49 days or more than 49 days. A trend towards higher median trough concentrations was observed for patients with prolonged cycles, reaching statistical significance only when both cycles were analysed together ($p = 0.032$) (Figure 3A).

Furthermore, we assessed separately the rare adverse events in this cohort. Two cardiac events were reported during the first cycle—first with moderate cardiac failure related to *Staphylococcus aureus* septicaemia, pulmonary infection and Grade 4 cytopenia and second with non-ST elevation myocardial infarction related to infectious fever. Respective Cmin on C1D15 were 4290 and 2000 ng/mL. However, it remains uncertain if cardiac events were related to therapy. One transverse myelitis was reported during the second cycle. Cmin at C1D15 was 1710 ng/mL. Unfortunately, the Cmin on C2D15 was not available.

3.5 | Effect of Venetoclax Trough Concentration on Therapy Outcome

To evaluate whether venetoclax trough concentrations influenced therapy outcomes, we classified participants based on their responses during the first three therapy cycles into three groups: CR/CRi, MLFS and refractory disease. On C2D15, venetoclax trough concentrations were higher in the MLFS group and lower in the refractory group, although no significant differences were observed on C1D15 or when both cycles were combined (Figure 3B). Furthermore, when participants were divided into quartiles based on their venetoclax capillary trough concentration at C1D15, the median OS did not differ significantly between the groups ($p = 0.599$) (Figure 3C). These findings suggest a potential association between drug concentration and therapy response, but the limited sample size precludes definitive conclusions.

3.6 | *CYP3A4* and *CYP3A5*

Next, we assessed the effect of moderate and strong CYP3A4 inhibitors (CYP3A4i) on venetoclax trough concentrations. During the first cycle, only two participants received moderate CYP3A4i (fluconazole). In the second cycle, five participants received moderate CYP3A4i (fluconazole), and three received strong CYP3A4i (posaconazole). As expected, with protocol-guided venetoclax dose reductions, we found no statistically significant differences compared to patients not receiving CYP3A4i (Figure S4 A–B).

Finally, to assess whether SNPs in three selected *CYP3A* genes affect venetoclax concentrations through altered CYP3A enzyme function, we genotyped 81 patients (91%) with measured venetoclax Cmin for three specific SNP variants of *CYP3A4* and *CYP3A5*. For the *CYP3A4*2* c.664T > C (rs55785340) variant, only one patient (1.2%) was heterozygous (T/C), with no homozygous variants observed. For *CYP3A4*22* c.522-191G > A (rs35599367), four patients (4.7%) were heterozygous (G/A), and one patient (1.2%) was homozygous (A/A). For *CYP3A5*3* c.219-237A > G (rs776746), 10 patients (11.8%) were heterozygous (A/G), and no homozygous variants were found. Venetoclax trough concentration did not differ from the median in patients with heterozygous SNPs. Notably, the only patient homozygous for *CYP3A4*22* (c.522-191G > A) had an exceptionally high Cmin of 12 600 ng/L on C1D15.

4 | Discussion

Drug concentrations are typically measured from peripheral blood plasma, requiring immediate processing and freezing on the same day—posing logistical challenges in academic clinical trials. In contrast, the capillary blood method allows for room temperature storage, offering a more practical solution. We assessed the usability of capillary blood sampling to measure venetoclax trough concentrations in our academic clinical trial. Capillary blood plasma concentrations showed a

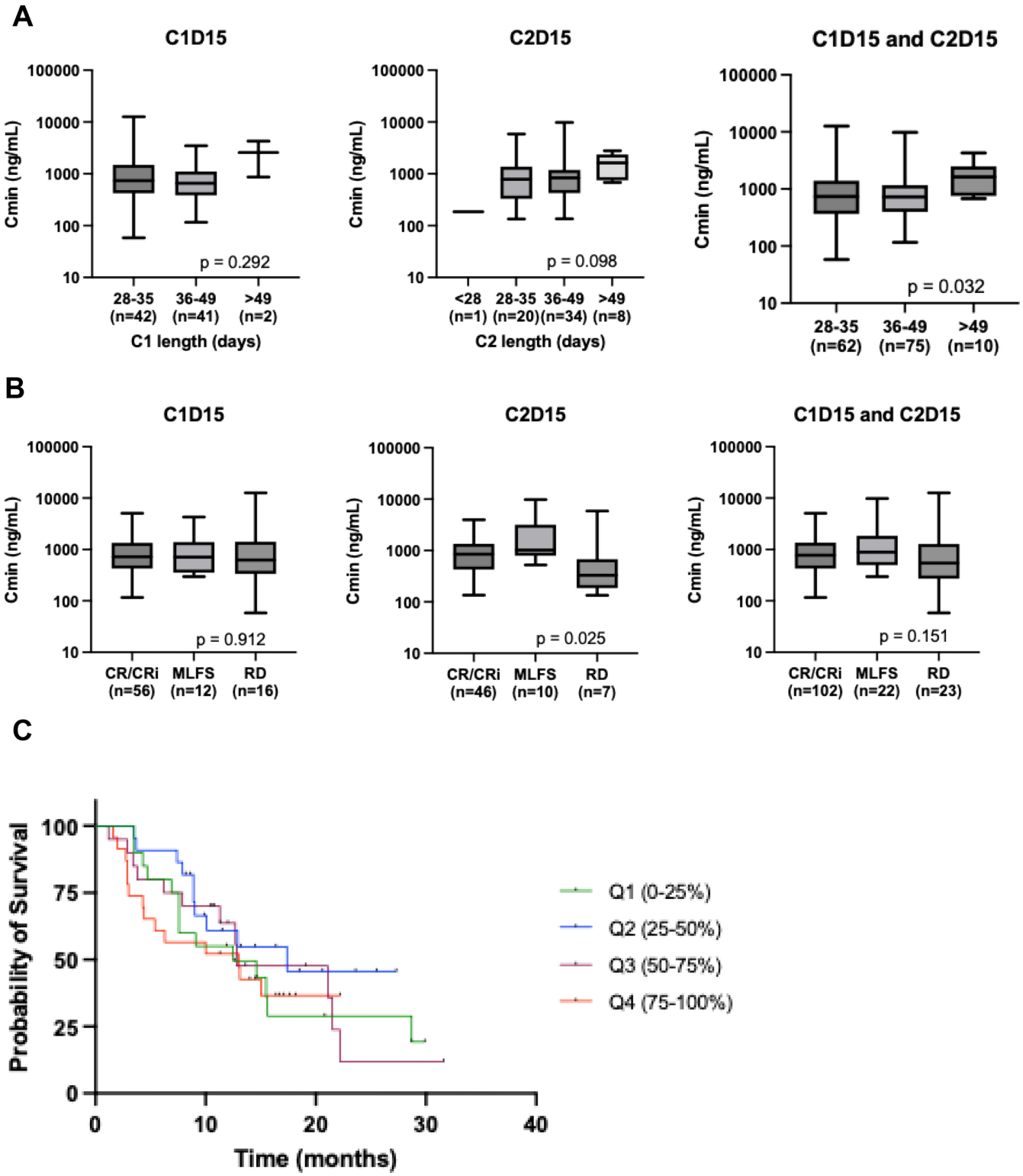

FIGURE 3 | **Associations between venetoclax trough concentrations, cycle length and responses.** (A) Venetoclax trough concentrations in patients whose therapy cycle was extended beyond the intended 28–35 days due to cytopenias and/or infections. Additionally, for one patient shown only in C2D15 figure, the therapy ended before Day 28 of the second cycle due to infection and leukaemia progression. (B) Median venetoclax trough concentrations in patients categorized by their best response during the first three therapy cycles. Concentrations are shown for C1D15, C2D15 and the combined measurements from both cycles. The response was not evaluable for one participant on C1D15. (C) Stratification by venetoclax median trough concentration in C1D15 quartiles and its association with overall survival. C1D15, Day 15 of the first cycle; C2D15, Day 15 of the second cycle; Cmin, venetoclax capillary trough concentration; CR, complete remission; CRi, complete remission with incomplete blood count recovery; MLFS, morphological leukaemia-free state; *n*, number of capillary samples; Q1, lowest Cmin quartile (0%–25%); Q2, lower middle Cmin quartile (25%–50%); Q3, upper middle Cmin quartile (50%–75%); and Q4, upper Cmin quartile (75%–100%). The difference between medians was assessed using Kruskal–Wallis test.

strong correlation with venipuncture concentrations ($R^2 = 0.835$, $p < 0.0001$), confirming the viability of the capillary method for venetoclax measurement.

We identified a subset of visually underfilled capillary samples, where the target volume of 20 μL was likely not achieved. Underfilled samples can yield slightly lower concentration values than expected. As the actual volume of these samples cannot accurately be determined, their inclusion may have contributed to a reduced overall correlation between venipuncture and capillary plasma methods. However, we chose to include them to better represent real-world clinical conditions. This underscores the importance of proper sampling techniques when using capillary methods, highlighting the need for comprehensive staff training and validation of collection procedures.

Our finding of wide variability in steady-state venetoclax trough concentrations, independent of demographic factors, aligns with previous studies. [23, 25] The underlying cause of this variability remains unclear. In previous studies, no consistent correlation with demographic factors has been established and similarly, we did not observe any statistically significant correlations between venetoclax trough concentrations and demographic factors such as age, weight or gender. In our patient cohort, we were unable to draw conclusions regarding the impact of kidney or liver function on venetoclax concentrations, as there were no patients with significant renal or hepatic dysfunction.

Our finding of exceptionally high venetoclax concentration in a patient with a homozygous CYP3A4*22 c.522-191G > A variant suggests that SNPs may in rare cases impact venetoclax pharmacokinetics. However, this participant did not experience a significantly higher number of adverse events or more severe toxicities compared to other patients in the trial. SNPs in drug-metabolizing CYP genes vary across different populations, and it is important to note that the population in this study consisted solely of individuals of Finnish background. Therefore, the data on SNPs may not be directly generalisable to other populations. Given the rarity of homozygous SNPs, the available data are extremely limited and warrant further evaluation in larger patient cohorts.

We found no statistically significant association between venetoclax trough concentrations and the number of reported adverse events. Notably, reported infections in this cohort were commonly neutropenic, with neutropenia recorded within the infection event, causing neutropenic events to appear less frequent. There was no statistically significant difference between median trough concentrations in patients with infectious/neutropenic adverse event and those with other type of event or no reported adverse reactions. However, when using therapy cycle length as a surrogate marker for prolonged neutropenia, we observed a trend towards higher concentrations in patients with cycle lengths significantly longer than targeted (> 49 days). Although we observed a *p*-value that reached statistical significance, the limited number of patients, especially with longest cycle lengths, makes it difficult to draw definitive conclusions. Similarly, Kobayashi et al. reported higher area under the concentration–time curve (AUC_{0-24}) in Japanese patients ($n = 33$) with prolonged Grade 3 neutropenia [29]. These findings suggest that higher venetoclax trough concentrations may contribute to prolonged neutropenia.

The analysis of venetoclax trough concentrations and best response during the first three therapy cycles revealed a trend towards higher Cmin concentrations in the second cycle among cytopenic patients who achieved a morphologic leukaemia-free state (MLFS) and lower concentrations in refractory patients. Although the small sample size limits definitive conclusions, these findings suggest a potential relationship between venetoclax levels and treatment response, warranting further investigation in a larger cohort.

In our study, we opted to use trough concentrations, as the simpler protocol allowed us to achieve a larger cohort with reasonable effort and time, without the challenges with resources associated with more intricate protocols and repeated sampling. Nonetheless, assessing drug exposure—such as peak concentrations and AUC—could provide deeper insights into the association between concentration and adverse events, such as prolonged neutropenia, as well as its relationship with therapy outcomes.

Measuring drug concentrations from capillary plasma offers a viable approach for therapeutic drug monitoring. For venetoclax, reduced dosing—whether through shorter courses or lower daily doses—can still yield effective responses in patients predicted to be highly sensitive, such as those with certain mutational profiles like *IDH2*. Ongoing studies are evaluating dose adjustments in elderly AML patients. Furthermore, trials are investigating whether lower dosing of venetoclax, alongside CYP3A4 inhibitor like posaconazole, can achieve comparable plasma concentrations and clinical efficacy in AML to standard regimens. Monitoring venetoclax concentrations is valuable in these settings.

Author Contributions

Mikko Niemi, Janne T. Backman, Heikki Kuusanmäki and Mika Kontro: conceptualization, study design, funding acquisition and supervision. **Sari Kytölä, Mika Kontro and Heikki Kuusanmäki**: project administration and coordination. **Pia Ettala, Johanna Rimpiläinen, Timo Siitonen, Marja Pyörälä and Mika Kontro**: principal investigators, administration, coordination, supervision and resource management at sites. **Pia Ettala, Johanna Rimpiläinen, Timo Siitonen, Marja Pyörälä, Mika Kontro, Sari Kytölä, Annasofia Holopainen, Anu Partanen, Milla E.L. Kuusisto and Sirpa Koskela**: patient recruitment, patient care and data collection. **Mika Kurkela and Johanna I. Kiiski**: sample processing and investigation. **Sari Kytölä, Ida Vänttinen, Mika Kontro, Heikki Kuusanmäki and Tanja Ruokoranta**: methodology, data curation, formal analysis and visualization. **Sari Kytölä, Ida Vänttinen, Tanja Ruokoranta, Heikki Kuusanmäki and Mika Kontro**: wrote the original manuscript. All authors contributed to the reviewing and editing and approved the final manuscript.

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Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Helsinki University Hospital.

Conflicts of Interest

AP reports personal fees (AbbVie, Astra Zeneca, Behring, Janssen-Cilag, Novartis, Sanofi, Takeda); PE reports personal fees (Novartis, Pfizer, Amgen, Sanofi, AbbVie, BeiGene); MP reports personal fees (Pfizer, Novartis, AbbVie, Bristol-Myers Squibb, Servier); JR reports personal fees (Astellas Pharma, AbbVie, Bristol-Myers Squibb, Pfizer, Sanofi) TS reports personal fees (Amgen, AbbVie, Bristol-Myers Squibb, Otsuka Pharma, Janssen-Cilag, GSK); HK reports research funding from (AbbVie, outside the submitted work) and personal fees (Faron Pharmaceuticals); MK reports personal fees (Astellas Pharma, AbbVie, Bristol-Myers Squibb, Faron Pharmaceuticals, Novartis and Pfizer) and research funding (AbbVie, outside the submitted work). All other authors report no competing interests.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.