SERS for Acute Promyelocytic Leukemia Submission

A. BANCOS [1,5], S.IANCU [2], V.MOISOIU [3], A.GHIAUR [4], AB. TIGU [5], C.BURAN [5], G.ION [1], C.STANCIOAICA [5], B.IONESCU [4], M.DRAGOMIR [4], C.BULDUS [2], D.CENARIU [5], M.NISTOR [5], D.KEGYES [5], D.GULEI [5], N.LEOPOLD [2], D.CORIU [6] and C. TOMULEASA [1,5]

1. Oncology Institute Prof. Dr. Ion Chiricuta, Cluj Napoca, Romania; 2. Babes-Bolyai University, Cluj Napoca, Romania; 3. University of Zurich, Zurich, Switzerland; 4. Clinical Institute Fundeni, Bucharesti, Romania; 5. Iuliu Hatieganu University of Medicine and Pharmacy; 6. University of Medicine of Pharmacy Carol Davila

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Introduction

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia, accounting for approximately 10–15% of adult AML cases. It is characterized by the t(15;17) chromosomal translocation, which generates the PML-RARA fusion gene-a reliable molecular marker for measurable residual disease (MRD) detection using quantitative real-time PCR (qRT-PCR). Alongside other molecular alterations, such as FLT3 internal tandem duplications (FLT3-ITD) with known prognostic impact, MRD assessment plays a central role in APL management. Surface enhanced Raman scattering (SERS) is a highly sensitive technique that enhances Raman signals through metal nanoparticles such as silver or gold. Recent studies have shown that cancer-related DNA methylation changes affect how DNA interacts with these nanoparticles, highlighting the potential of SERS as a novel tool for MRD monitoring in hematologic malignancies, including APL.

Aim

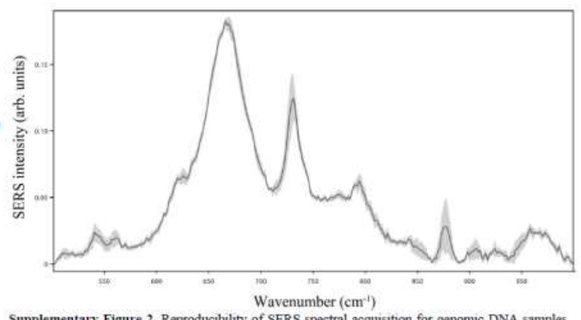
In this study, we used SERS to analyze the DNA from the bone marrow of patients with PML, with the aim of delineating the SERS spectral features associated with MRD positivity and to benchmark SERS against qRT-PCR, which is the gold standard for MRD detection in APL (7–9).

Methods

Supplementary Table 1. Demographic data of the patients and samples.

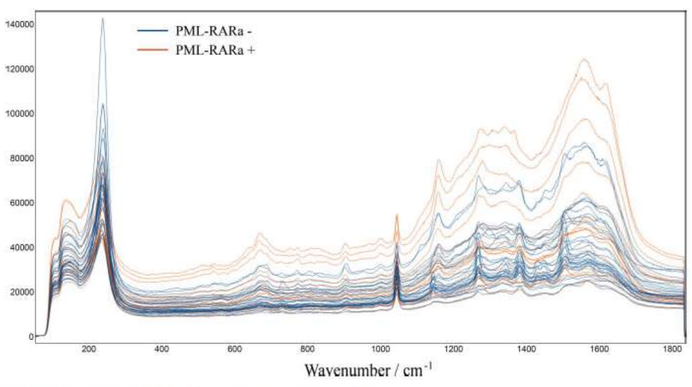
Characteristic	N = 30[1]
Age	46 (36, 57)
Sex	
F	10 (33%)
M	20 (67%)
WBC (mm^{-3})	1,790 (1,085, 3,542)
Unknown	4
Platelets (mm^{-3})	24,000 (16,500, 34,250)
Unknown	4
Hb (g/dL)	8.85 (7.93, 9.97)
Unknown	4
LDH (U/L)	313 (245, 461)
Unknown	5
Creatinine (mg/dL)	0.92 (0.83, 1.00)
Unknown	5
Blasts (%)	0.87 (0.71, 0.95)
Unknown	4

[1] Median (IQR); n (%)

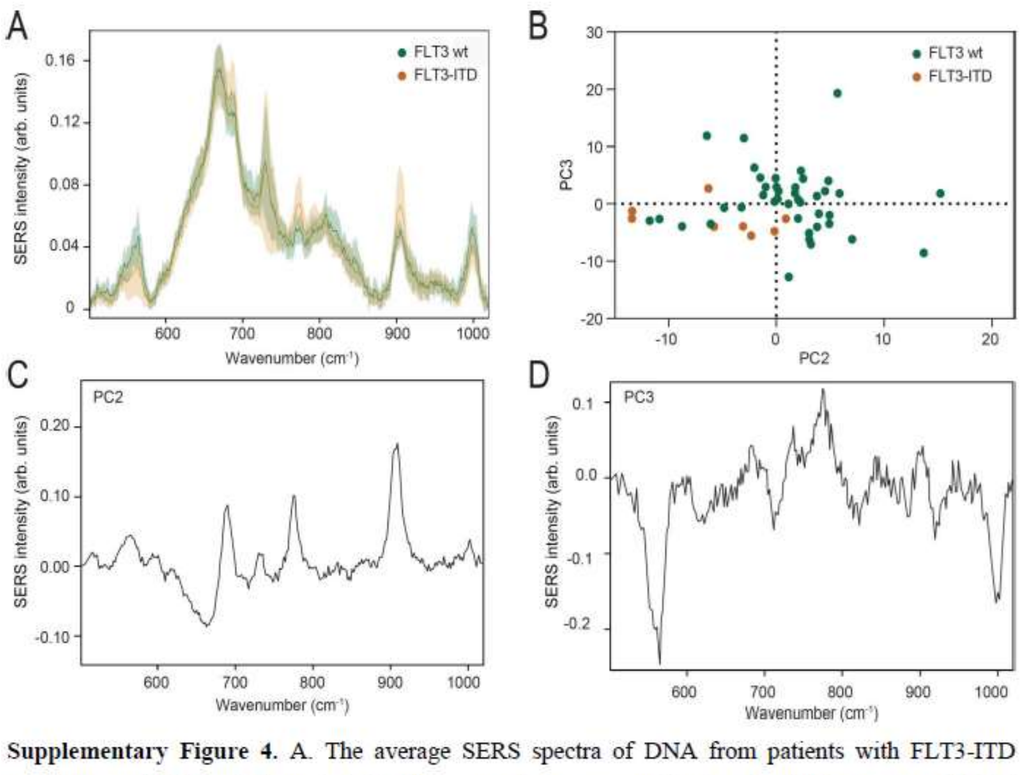


Supplementary Figure 2. Reproducibility of SERS spectral acquisition for genomic DNA samples. The figure shows the mean SERS spectrum and the standard deviation across four repetitions.

In this study, we analyzed 49 DNA samples extracted from bone marrow aspirations from 30 patients with APL, of which 15 samples exhibited MRD positivity for the *PML-RARA* transcript by qRT-PCR. The demographics of the patients included in this study are shown in Supplementary Table 1. The SERS amplification was performed using silver nanoparticles synthesized by reducing Ag+ with hydroxylamine hydrochloride. A detailed account of the materials and methods, including the characterization of the SERS substrate, is found in the Supplementary Information (Supplementary Figure 1 and Supplementary Figure 2). The study was approved by the Review Board of the Ion Chiricuta Cancer Center, Cluj-Napoca, Romania.



Supplementary Figure 3. The raw SERS spectra of DNA.

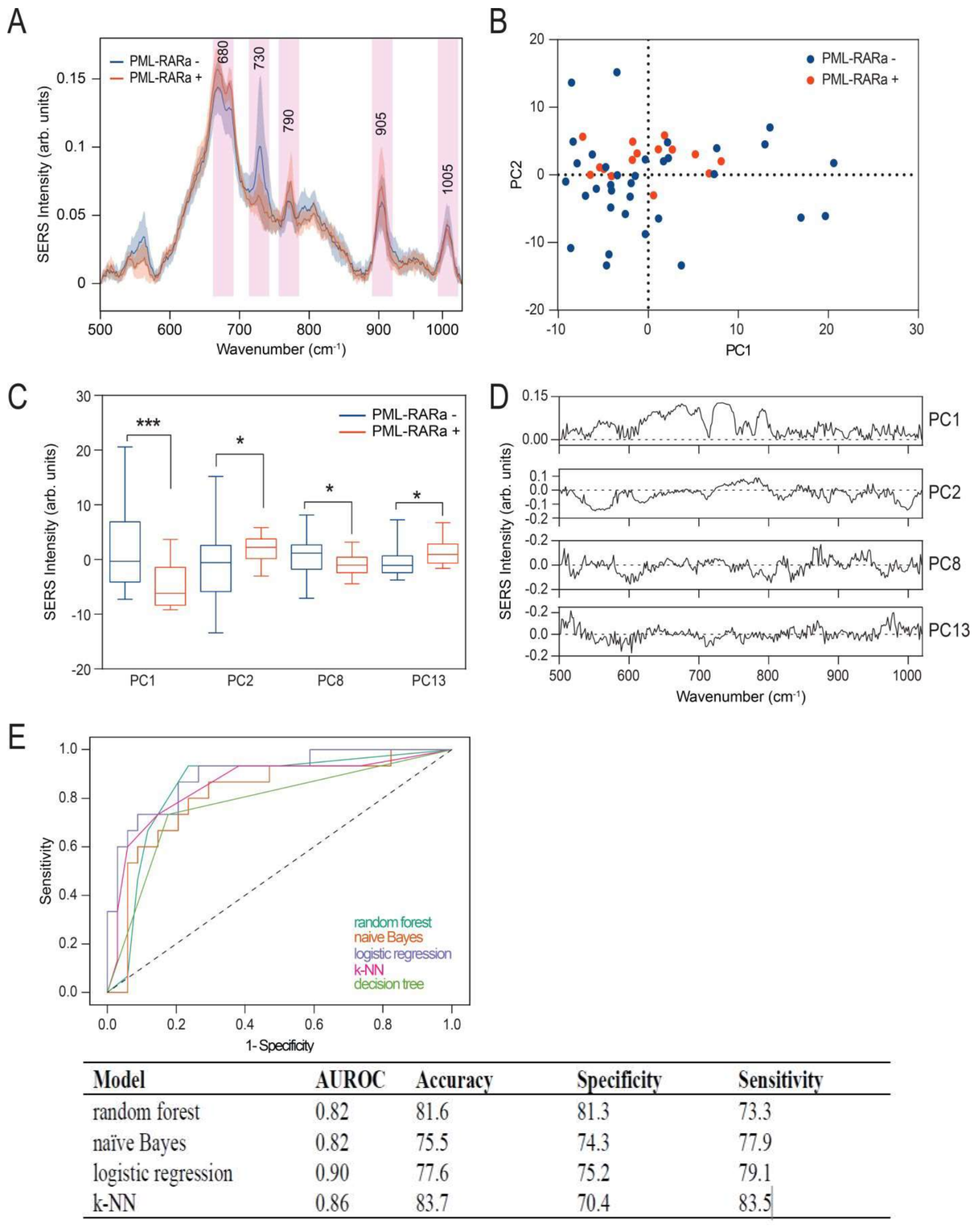


Supplementary Figure 4. A. The average SERS spectra of DNA from patients with FLT3-ITD mutations and FLT3 wildtype (wt). B. The score plots corresponding to principal component (PC) 2 and PC3. C, D. The loading plots of PC3 and PC3, respectively. The dataset consisted of 41 samples in the FLT3 wildtype group and eight samples in the FLT3-ITD group.

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Results



Model	AUROC	Accuracy	Specificity	Sensitivity
random forest	0.82	81.6	81.3	73.3
naïve Bayes	0.82	75.5	74.3	77.9
logistic regression	0.90	77.6	75.2	79.1
k-NN	0.86	83.7	70.4	83.5

Summary

Surface-enhanced Raman spectroscopy (SERS) analysis of genomic DNA from bone marrow aspirates of APL patients stratified by MRD status revealed distinct spectral features. Prominent Raman bands were observed at 680 cm^{-1} (guanine), 730 cm^{-1} (adenine), 790 cm^{-1} (cytosine), 905 cm^{-1} (phosphate backbone), and 1005 cm^{-1} (5-methylcytosine). MRD-positive samples exhibited a consistent reduction in the intensity of the 730 cm^{-1} band, associated with adenine ring-breathing modes. Principal component analysis (PCA) demonstrated a trend toward unsupervised separation of spectra based on MRD status, with the first 20 principal components accounting for 93% of the total variance. Among these, four PCs displayed statistically significant differences between MRD-positive and MRD-negative samples (Student's t-test, $p < 0.05$).

To evaluate the diagnostic utility of SERS, scores from these four PCs were used as input features for machine learning classification. Logistic regression achieved the highest diagnostic performance, with an area under the ROC curve (AUROC) of 0.90.

Additionally, PCA of a subset of samples with available FLT3 mutation status revealed a clustering tendency among FLT3-ITD–positive cases, suggesting that this mutation may influence the SERS spectral profile of DNA. However, the limited sample size precluded robust statistical analysis.

Discussion

We analyzed SERS spectra of DNA from MRD-positive acute promyelocytic leukemia (APL) patients and observed a distinct spectral signature characterized by decreased intensity at 730 cm^{-1} (adenine) and increased intensity at 770 cm^{-1} (5-methylcytosine). This contrasts with prior findings in other hematologic malignancies, where increased adenine and decreased 5-methylcytosine signals were typical—reflecting global hypomethylation in cancer DNA. The deviation in APL may stem from its unique DNA hypermethylation profile.

Using principal components from the SERS spectra, we trained four machine learning models; logistic regression achieved the best performance (AUROC = 0.90). Notably, cases with the FLT3-ITD mutation exhibited unique spectral patterns, possibly linked to their distinct clinical and molecular features, including elevated WBC counts and M3v morphology.

These findings suggest that SERS can detect DNA methylation-related changes and may support molecular classification and risk stratification in APL.

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