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Patched Homolog Gene (PTCHI) As a Reliable Marker for Predicting Imatinib Response in Chronic Phase Chronic Myeloid Leukemia Patients in Correlation with Early Response to First Line of Treatment Imatinib

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ABSTRACT

Background: Chronic myeloid leukaemia (CML) is a clonal disease of a hematopoietic stem cell. The incidence of this disease is about 15% of leukemias and may be present at any age. The presence of the Philadelphia (Ph) chromosome confirms the diagnosis of CML. The expression of the Patched homolog 1 gene (PTCH1) has been proposed as a prognostic marker of imatinib response in chronic phase chronic myeloid leukaemia (CP-CML) patients.

Aim: This study aimed to measure the level of PTCH1 protein in newly diagnosed CP-CML and to find correlation between its level in those patients and response to first line of treatment, imatinib and other prognostic factors.

Patients and Methods: Our study enrolled 50 patients of newly diagnosed CP-CML. We have measured the level of PTCH1 protein initially once the patient diagnosed and after 6 months of treatment with imatinib by ELISA test.

Results: There was highly significant difference between initial PTCH1 level and its level after 6 months of imatinib treatment *(P=0.000).* The level of PTCH 1 was correlated significantly with the molecular response both at the beginning of treatment with imatinib and six months later. *(P=0.002), (p=0.001)* respectively.

Conclusion: To the best of our knowledge that we are the first to report on PTCH1 protein as a new diagnostic and prognostic marker in CP-CML patients.

Keywords: Chronic Phase, Patched Homolog Gene (PTCHI), Chronic Myeloid Leukemia, ELISA, Imatinib.

INTRODUCTION

CML is a type of pluripotent hematopoietic stem cell tumor that is distinguished by the presence of the BCR-ABL fusion gene which is resulting from Philadelphia (Ph) chromosome. Ph chromosome is the result of a translocation between chromosomes 9 and 22 long arm, t (9;22) (q34; q11). CML accounts for 15% to 20% of adult leukemia cases **[1].** In the IRIS study, imatinib was tested as a first-generation TKI and reported higher rates of cytogenetic and molecular responses compared to recombinant interferon alpha (IFN) and low-dose cytarabine regimen with better overall survival (OS) and progression-free survival (PFS). As a result, imatinib is now considered the first-line option for CML treatment **[2].**

The determination of the optimal response to continue the treatment, the failure or resistant to change the drug, or warning sign to carefully continue or change the drug according to patient's risk factors like comorbidities and tolerability by monitoring of IS of BCR-ABL1 transcript level at 3rd, 6th, and 12th months. Additional quantitative PCR testing is indicated to detect accurate drug response. Achieving a Major Molecular Response (MMR) (BCR-ABL1 0.1%) is associated with improved CML-specific survival **[3].**

The PTCH1 gene, which is located on chromosome 9q22.3, is the human homolog of the Drosophila patched-1 gene. PTCH1 is a transmembrane glycoprotein with 1447 amino acids that is part of the hedgehog (Hh) pathway. The Hh pathway, which controls cell proliferation, tissue polarity, and cell differentiation, is essential for embryonic development and tumor development. PTCH1 protein is acting as an inhibitor of the transmembrane protein smoothened (SMO). Extracellular ligands interacting to the PTCH1 receptor released this inhibition, allowing SMO to communicate with downstream transcription factors and activate GLI transcription factors. PTCH1 is considered as a tumor suppressor gene based on its role in inhibiting uncontrolled cell proliferation. On the other hand, SMO is considered as an oncogene **[4].** Regarding the role of the Hh pathway in CML, numerous studies have shown that Hh signaling is activated in progenitor cells that are positive for BCR-ABL, and that Hh signaling is further elevated as the disease progresses **[5].**

Patched homolog 1 gene **(***PTCH1)* is marker for expecting first line of treatment imatinib response in CP-CML in correlation with response to imatinib. It is detected by PCR and proved to be a reliable marker to detect early imatinib response in CP-CML patients **[6].** In our study, we measured the level of PTCH1 protein in CP-CML patients by ELISA as a cost benefit in comparison to PCR in newly diagnosed CML patients on first line of treatment imatinib then follow up after 6 months of treatment.

PATIENTS

In our study there were 50 newly diagnosed CP-CML patients recruited from Hematology Outpatient Clinic in Ain Shams University Hospital within duration from August 2021 to May 2022. We have

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measured level of PTCH1 protein in newly diagnosed CP-CML and its follow up after 6 months of imatinib treatment. Patients involved in our study, ranged 18-65 years old, were using imatinib as first line of treatment.

Exclusion criteria:

Patients started imatinib more than 3 months, or those with other solid neoplasms or hematopoietic malignancies, or with autoimmune diseases, or on any TKIs other than imatinib, or show any form of resistance according to WHO or ELN criteria.

All patients were subjected to the following: Complete medical histories and full clinical examination were done. We have investigated our patients initially through laboratory measures (CBC, kidney functions, liver functions, serum electrolytes, radiologically by pelvi-abdominal ultrasound, echocardiography and bone marrow aspiration for cytomorphology and flow cytometry to confirm diagnosis), cytogenetic analysis (Karyotyping using G-banding, and PCR for BCR-ABL, fluorescence in situ hybridization (FISH) for genetic abnormalities). We have measured the level of PTCH1 by ELISA (Enzyme-Linked Immunosorbent Assay) pretreatment then follow up level after 6 months of imatinib treatment. Patients follow up after starting imatinib therapy was obtained by CBC and PCR for BCR-ABL after 3 and 6 months, monitoring hematological remission, which was achieved by normalization of CBC picture and decreasing size of spleen and searching for molecular response at 3- & 6-months post-treatment according to NCCN guidelines [7].

Methods

It is an ELISA kit. A human PTCH1 antibody precoat has been applied to the plate. The biotylinated human PTCH1 antibody was combined and attaches to the PTCH1 in the sample after PTCH1 is introduced and binds to antibodies on the wells. The biotylinated PTCH1 antibody was then combined with streptavidin-HRP and bound. Unbound streptavidin-HRP was sprayed during a washing stage following incubation. After adding the substrate solution, color develops in response to the amount of human PTCH1. By adding an acidic stop liquid, the reaction was completed, and absorbance was calculated at 450 nm.

Ethical approval:

An approval of the study was obtained from Ain Shams University Faculty of Medicine's Ethical Committee with number and date MS 13/2022. All procedures were carried out in our study involving human subjects according to the code of Ethics of the Word Medical Association (1964 Helsinki Declaration). Every patient signed an informed written consent for participation in the study.

Statistical analysis

Data were gathered, reviewed, coded, and put into IBM SPSS version 23 of the statistical software for social science. The quantitative data were presented as mean, standard deviations, and ranges if parametric, and median, inter-quartile range (IQR) if non-parametric. Numbers and percentages were used to represent qualitative variables. Reasonable statistical test was used according to type of data. To predict EMR+MMR in the studied patients, the receiver operating characteristic curve (ROC) was used in quantitative form to detect sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), area under curve (AUC), and best cut of point PTCH1. The confidence interval was set to 95%, and the acceptable margin of error was set to 5%. As a result, the p-value was considered significant as $P \leq 0.05$ and highly significant as $P \leq 0.01$ but non-significant when $P > 0.05$.

RESULTS

50 patients of newly diagnosed CML (chronic phase) were enrolled in our work: 22 males (44%), 28 (56%) females. Their median age was 40 ± 12 years. 39 patients (78%) were low risk and 11 patients (22%) were intermediate risk according to SOKAL score. According to ELT score, 33 patients (66%) were low risk and 17 (34%) patients were intermediate risk. The median level of initial PTCH1 protein was 6000 ng/L, the median level of it after 6 months was 10 ng/L. As regards molecular response, 32 (64%) patients developed DMR, 13 (26%) patients developed MMR and 5 (5%) patients developed EMR (Table 1).

Table (1): Demographic data analysis**,** laboratory, and clinical data of the studied patients

			No. = 50
Age (Years)		Mean ± SD range	41.64 ± 12.76 16 – 72
Gender		Male Female	22 28
PCR (polymerase chain reaction) on presentation		Median (IQR) Range	67.5 (55 – 89) 15 – 108
FISH (Fluorescence in situ hybridization) on presentation		Mean ± SD Range	80.08 ± 16.09 55 – 101
spleen size initial (cm)		Mean ± SD Range	20.04 ± 4.21 9.5 – 30
SOKAL score		Low Intermediate	39 (78.0%) 11 (22.0%)
ELT score		Low Intermediate	33 (66.0%) 17 (34.0%)
PTCH1 Initial (ng/L)		Median (IQR) Range	6000 (5400 – 8000) 2500 – 10000
Molecular response	DMR EMR+MMR	32 18	64.0% 36.0%

There was highly significant correlation between initial level of PTCH and molecular response (p=0.002) but there was no significant correlation between initial total leukocytic count, age and SOKAL & ELT scores, spleen size and initial PCR for BCR-ABL with initial PTCH1 protein level (Table 2 & figure 1).

Table (2): Comparison between initial PTCH1and clinical data of patients

		PTCH1 Initial (ng/L)		Test value	P-value	Sig.
		Median(IQR)	Range			
SOKAL score	Low Intermediate	6000 (5200 – 8000) 6000 (5800 – 7000)	2500 – 10000 3000 – 9000	-0.273‡	0.785	NS
ELT SCORE	Low Intermediate	6000 (5000 – 8000) 6000 (6000 – 7000)	2500 – 10000 3000 – 9000	0.000‡	1.000	NS
Molecular response	EMR MMR DMR	7000 (6000 – 8200) 7800 (7000 – 8000) 5900 (5000 – 6500)	6000 – 9000 6000 – 9000 2500 – 10000	12.506‡‡	0.002	HS

‡: Mann Whitney test; ‡‡: Kruskal Wallis test

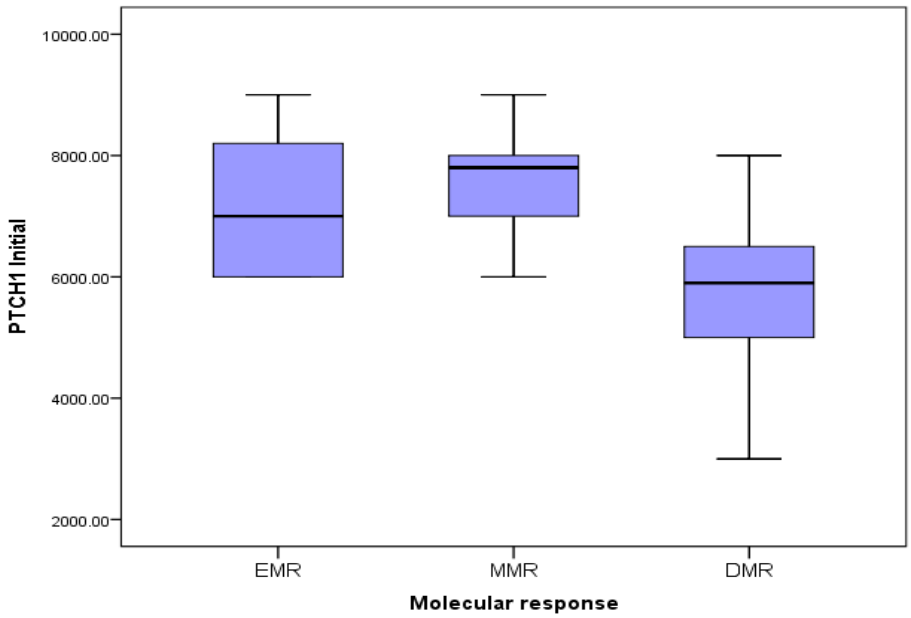

Figure (1): Difference between initial PTCH1 levels among different molecular response group

There was highly significant difference between initial PTCH1 level and its level after 6 months (P=0.000). The median initial level of PTCH1 was 7000 ng/L in patients who developed early molecular response, median = 7800 ng/L in patients with major molecular response and median = 5900 ng/L in patients with deep molecular response. Also, median level after 6 months was 20 ng/L in patients who developed early, and major molecular response and its median level was 10 ng/L in patients with deep molecular response (Table 3 & figure 2).

Table (3): comparison between PTCH1 initial and after 6 months

PTCH1 (ng/L)	Initial No. = 50	6 months No. = 50	Test value‡	P-value	Sig.
Median(IQR) Range	6000 (5400 – 8000) 2500 – 10000	20 (10 – 20) 10 – 30	-6.157	0.000	HS

‡: Wilcoxon Rank test

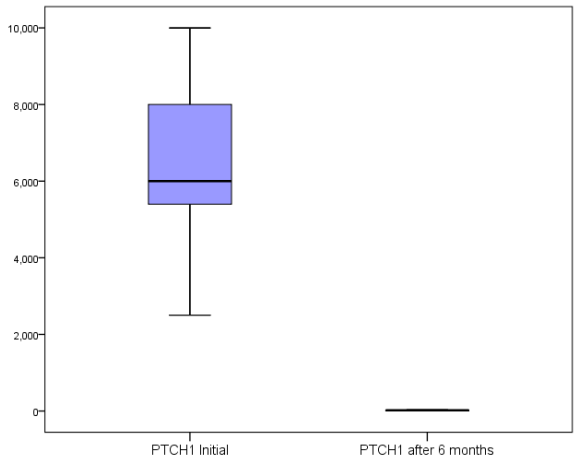

Figure (2): Difference between Level of PTCH1 initial and after 6 months

There was positive correlation between PTCH1after 6 months and spleen size (p=0.001) but there was no significant correlation between PTCH1 after 6 months and age, TLC, PCR for BCR-ABL and SOKAL & ELT scores. There was statistically significant difference between level of PTCH1 after 6 months and molecular response (p=0.001) (Tables 4 & 5 and figure 3).

Table (4): Correlation between PTCH1 after 6 months and CBC, PCR for BCR-ABL and spleen size after 6 months

	PTCH1 after 6 months (ng/L)	
	r	**P-value**
Age (years)	-0.196	0.173
Spleen size after 6 months (cm)	**0.456****	**0.001**
Total Leucocytic Count after 6 months	0.001	0.995
PCR 6 month	0.076	0.600

Table (5): Comparison between PTCH1 after 6 months and risk stratification, among molecular response groups.

		PTCH1 after 6 months (ng/L)		Test value	P-value	Sig.
		Median(IQR)	Range			
SOKAL score	Low	20 (10 – 20)	10 – 30	-0.380‡	0.704	NS
	Intermediate	20 (10 – 20)	10 – 30			
ELT SCORE	Low	20 (10 – 30)	10 – 30	-1.240‡	0.215	NS
	Intermediate	10 (10 – 20)	10 – 30			
Molecular response	EMR	20 (20 – 30)	10 – 30	14.142‡‡	0.001	HS
	MMR	20 (20 – 30)	10 – 30			
	DMR	10 (10 – 20)	10 – 30			

‡: Mann Whitney test; ‡‡: Kruskal Wallis test.

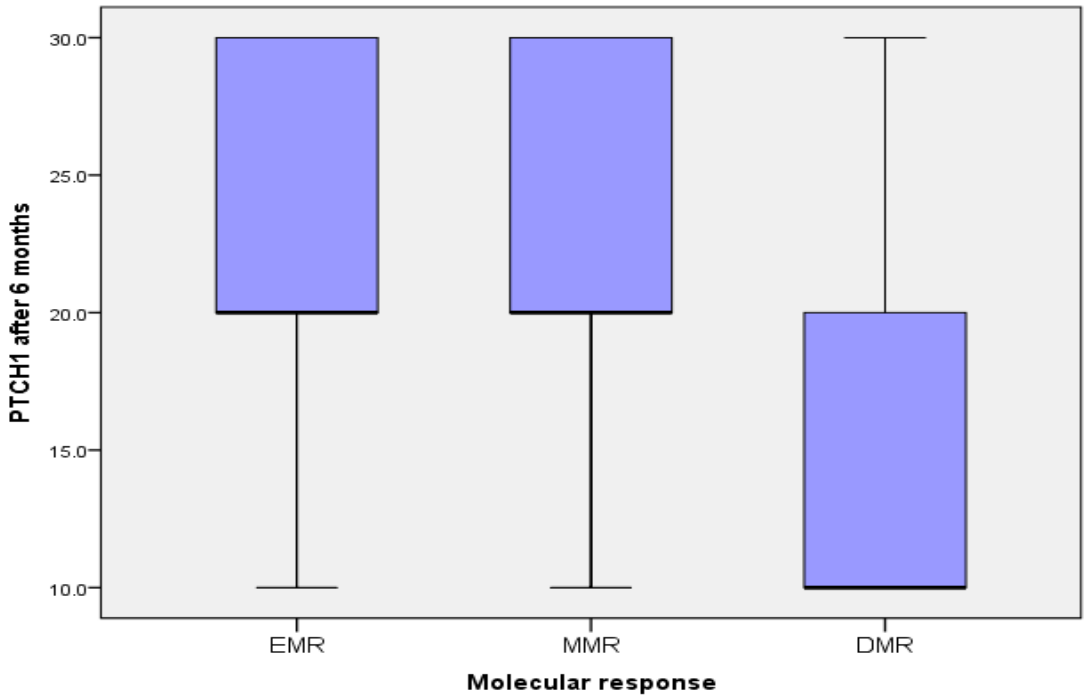

Figure (3): Difference between levels of PTCH 1 after 6 months among different response groups

Area under the receiver operating curve of initial PTCH1 for prediction of molecular response was 0.799 with estimating cut of point of equal or more than 6000 predicting molecular response. Sensitivity was 77.78%, specificity was 75%, positive predictive value was 63.6 % and negative predictive value was 85.7%. Area under the receiver operating curve of PTCH1 after 6 months for prediction of molecular response was 0.798 with estimating cut of point of equal or more than 10 predicting molecular response. Sensitivity was 88.89 %, specificity was 65.62 %, positive predictive value was 59.3 % and negative predictive value was 91.3 % (Table 6 & figure 5).

Table (6): Area under the receiver operating characteristic curve of initial PTCH1 and after 6 months for prediction of molecular response

PTCH1	Area under the curve	Cut of Point	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Initial	0.799	>6000	77.78	75.0	63.6	85.7
After 6 months	0.798	>10	88.89	65.62	59.3	91.3

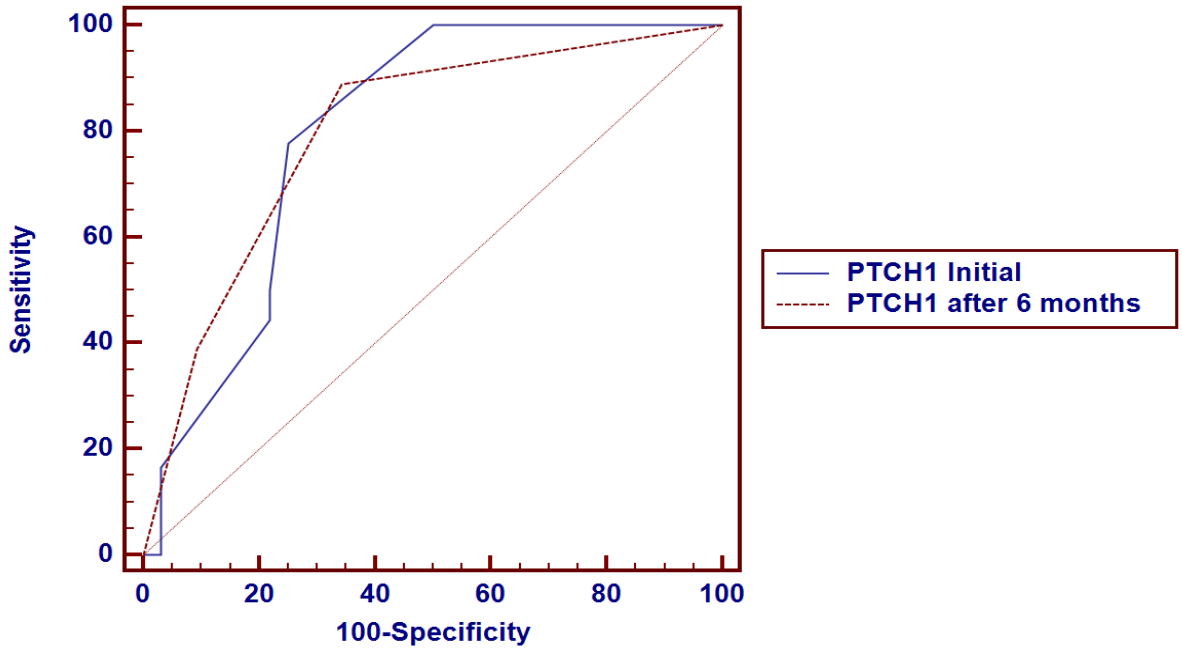

Figure (4): ROC curve of PTCH1 initial and after 6 months as a predictor of EMR+MMR

DISCUSSION

We are in need for a new easy, reliable, and cheaper marker to assess imatinib responsiveness in individuals with recently diagnosed CR-CML, also to be a prognostic marker. To the best of our knowledge about PTCH1 protein we are the first to report on PTCH1 protein as a new diagnostic and prognostic marker measured by ELISA on CML patients. Our study was conducted on 50 patients of newly diagnosed CP-CML, 22 (44%) males and 28 (56%) female. Their median age was 40 ± 12 years.39 patients (78%) were low risk and 11 patients (22%) were intermediate risk according to SOKAL score. While, 33 patients (66%) were low risk and 17 (34%) patients were intermediate risk according to ELT score.

We did not wait to assess deep molecular remission in our patients. However, we detected early trend changes as regards BCR-ABL during the first 3 months as an early indicator to shift to second line of tyrosine kinase inhibitors as far as detection of kinase domain variants is not feasible in most circumstances. Any patient who developed resistance to imatinib was shifted to second line tyrosine kinase inhibitors then omitted from the study and substituted by another who showed response to imatinib.

There was highly significant difference between initial PTCH1 level and its level after 6 months *(P=0.000)* in our study. The median initial level of PTCH1 was 6000 ng/L (range: 2500-10000) while its median after treatment was 20 ng/L (rang: 10-30) so we found that there was high expression of initial PTCH1 protein level.

Our finding agrees with **Moussa *et al.*** [8] who conducted prospective case-control study that included 55 subjects: 45 patients with CP-CML and 10 healthy age and sex-matched controls. PTCH1 mRNA level was detected by real time RT-PCR and found that PTCH1 gene was more expressed in CML cases than in control (P=0.024). Our finding disagrees with **Bing long *et al.*** [9] who conducted a prospective study involving 60 new cases of CML and 25 healthy donors and found that there was no significant difference in the PTCH1 gene's mRNA expression between the CML group and the control group ($p > 0.05$), but they did find higher PTCH1 transcript levels in patients with CML-BC (blastic crisis) and CML-AP (accelerated phase)

compared to the CML-CP group, despite the lack of significant differences ($p > 0.05$).

We discovered a significant correlation of initial PTCH1 levels and after 6 months level with molecular response (P=0.002) (p=0.001), respectively. Patients who attained deep molecular response had lower level of PTCH1 protein initially and after 6 months than patients with major and early molecular response. Our results agree with **Moussa *et al.*** [8] who reported that patients with good response to imatinib had lower PTCH1 gene expression than patients who did not achieve good response to imatinib (P=0.017). Also, **Abd Elrhman *et al.*** [10] to determine if the PTCH1 gene mutation exists or not, 50 CML patients who had recently diagnosed, and 10 healthy controls were tested. According to their findings, imatinib-responding CML patients had a lower rate of PTCH1 gene mutations than imatinib-failure patients (P=0.03). But this disagrees with **Alonso-Dominguez JM *et al.*** [6] who used a simplified qPCR method on 101 patients with CP-CML and reported that the low PTCH1 expression group had a non-ideal molecular response at 3 months follow up and that there were no significant differences in cytogenetic response between the high and low PTCH1gene expression groups., That debate may be brought on by the disparity in patient populations between studies and the many ways that PTCH1 has been identified as a protein or a gene expression.

CONCLUSION

We concluded that PTCH1 protein might be employed as a diagnostic and prognostic marker in CP-CML patients receiving imatinib as their first line treatment as well as to forecast the patients' type of response. Finally, we recommend studying the level of PTCH1protein by ELISA in large cohort studies and in different groups of CML patients, including chronic phase, accelerated phase, and blast crisis, as well as the molecular response to treatment with not only imatinib but also other types of tyrosine kinase inhibitors generations.

DECLARATIONS

Conflict of interest: no conflict of interest.

Availability of data and materials: The corresponding author will provide the materials obtained and/or processed during the current study upon reasonable request.

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