

Treatment with dasatinib or nilotinib in chronic myeloid leukemia patients who failed to respond to two previously administered tyrosine kinase inhibitors – a single center experience

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OBJECTIVE: To evaluate hematological, cytogenetic and molecular responses as well as the overall, progression-free and event-free survivals of chronic myeloid leukemia patients treated with a third tyrosine kinase inhibitor after failing to respond to imatinib and nilotinib/dasatinib.

METHODS: Bone marrow karyotyping and real-time quantitative polymerase chain reaction were performed at baseline and at 3, 6, 12 and 18 months after the initiation of treatment with a third tyrosine kinase inhibitor. Hematologic, cytogenetic and molecular responses were defined according to the European LeukemiaNet recommendations. BCR-ABL1 mutations were analyzed by Sanger sequencing.

RESULTS: We evaluated 25 chronic myeloid leukemia patients who had been previously treated with imatinib and a second tyrosine kinase inhibitor. Nine patients were switched to dasatinib, and 16 patients were switched to nilotinib as a third-line therapy. Of the chronic phase patients (n=18), 89% achieved a complete hematologic response, 13% achieved a complete cytogenetic response and 24% achieved a major molecular response. The following BCR-ABL1 mutations were detected in 6/14 (43%) chronic phase patients: E255V, Y253H, M244V, F317L (2) and F359V. M351T mutation was found in one patient in the accelerated phase of the disease. The five-year overall, progression-free and event-free survivals were 86, 54 and 22% ($p < 0.0001$), respectively, for chronic phase patients and 66%, 66% and 0% ($p < 0.0001$), respectively, for accelerated phase patients. All blast crisis patients died within 6 months of treatment. Fifty-six percent of the chronic phase patients lost their hematologic response within a median of 23 months.

CONCLUSIONS: Although the responses achieved by the third tyrosine kinase inhibitor were not sustainable, a third tyrosine kinase inhibitor may be an option for improving patient status until a donor becomes available for transplant. Because the long-term outcome for these patients is poor, the development of new therapies for resistant chronic myeloid leukemia patients is necessary.

KEYWORDS: CML; Dasatinib; Nilotinib; Third-line TKI treatment.

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INTRODUCTION

Second-generation tyrosine kinase inhibitors (TKIs), such as nilotinib and dasatinib, are effective therapeutic options for chronic myeloid leukemia (CML) patients who

have failed to respond to imatinib as a first-line therapy. Indeed, approximately 50% of chronic phase (CP) patients achieve a complete cytogenetic response (CCyR) when treated with second-generation TKIs (1,2). Nevertheless, approximately 52% of patients must discontinue second-line TKI therapy, most often due to resistance or intolerance (3).

Allogeneic transplantation is the treatment of choice for patients who fail to respond to at least one second-generation TKI (2nd TKI). However, transplantation is not feasible for many older patients, for patients with poor performance status and for patients who do not have an available donor.

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These patients may be switched to a different TKI that was not used previously or switched to other drugs, such as interferon (INF) or hydroxyurea (HU) (4). Patients treated with a 3rd TKI should be closely monitored because novel mutations can occur with sequential TKI therapy, increasing the risk of resistance (5,6).

In Brazil, fewer treatment options are available for resistant cases as other TKIs, such as bosutinib and ponatinib, are not available. In this study, we present our experience with CML patients treated with dasatinib or nilotinib as a third-line (3rd TKI) therapy and emphasize the importance of developing other therapeutic options for these patients.

■ PATIENTS AND METHODS

Between July 2008 and December 2014, 213 CML patients were treated at the Hematology and Hemotherapy Center at the University of Campinas according to the 2006 and 2009 European LeukemiaNet recommendations (7,8). The first-line treatment for CML in Brazil is imatinib. The second-line TKI is chosen based on clinical factors, BCR-ABL1 mutation status and drug availability. Dasatinib was approved in 2008 and nilotinib was approved in 2009 in Brazil; before 2008, these drugs were available only through clinical trials. A total of 25 consecutive adult CML patients, 18 (72%) of whom were in the CP stage, 3 (12%) of whom were in the AP stage and 4 (16%) of whom were in the BC stage, who were resistant (n=23) or intolerant (n=2) to two prior TKIs and were switched to a 3rd TKI, were included in our analysis. Most of the patients were treated at our center since their initial diagnosis; however, three patients were referred from other treatment centers at the time of initiation of the 2nd TKI treatment and were followed at our center after discontinuation of the 2nd TKI. Patients were treated with 100-140 mg dasatinib daily (n=9) (after failure with imatinib and nilotinib) or 400-800 mg nilotinib daily (n=16) (after failure with imatinib and dasatinib). Doses were adjusted according to tolerance. Hematologic, cytogenetic and molecular responses as well as the CML phases were defined according to the European LeukemiaNet recommendations (8,9). Bone marrow karyotyping was performed using the Giemsa-Trypsin-Wright stain banding technique at baseline and at 3, 6, 12 and 18 months after the initiation of therapy with the 3rd TKI. Twenty metaphase cells were analyzed for each sample (10).

Detection of BCR-ABL1 transcripts

BCR-ABL1 transcripts were measured in the peripheral blood by real-time quantitative polymerase chain reaction (RQ-PCR) at baseline and then every 3 months using procedures described elsewhere with some modifications (11). First, cDNA was amplified using the ABI 7300 sequence detection system (Applied Biosystems) and TAQMAN Universal Master Mix in a final reaction volume of 25 μ L according to the instructions recommended by the manufacturer. *ABL1* was used for normalization. BCR-ABL1 transcripts were measured in duplicate. The copy numbers were calculated by comparison with a standard curve generated from serial dilutions (4-6 dilutions) of a linearized plasmid containing a BCR-ABL1 insert, which has been described previously (12). The results were reported as BCR-ABL1/*ABL1* ratio (%) after conversion to the international

scale (IS). Major molecular response (MMR) was defined as a transcript level $\leq 0.1\%$ (IS).

Detection of BCR-ABL1 kinase domain mutations

Mutations were detected by direct sequencing of DNA from peripheral blood samples collected from TKI-resistant CML patients who failed or displayed a sub-optimal response to IM or a 2nd TKI, according to methods that were described previously (13,14). Briefly, total RNA was transcribed to cDNA and then was amplified using *Taq* platinum high fidelity and primers; the forward primer annealed to BCR exon 2, and the reverse primer annealed to ABL exon 10. The PCR product was amplified in a semi-nested reaction, resulting in a 863-base pair fragment that was sequenced in both directions. The sample nucleotide sequences were compared to the GenBank accession no. X16416.

Statistical methods

Probabilities of overall survival (OS), progression-free survival (PFS) and event-free survival (EFS) were calculated using the Kaplan-Meier method. OS was calculated at the initiation of therapy with the 3rd TKI until the final follow-up or death for any reason. PFS was defined as survival without transformation to the accelerated or blastic phase after starting the 3rd TKI and was judged based on an event of progression or death. EFS was defined as loss of complete hematological response (CHR), CCyR, MMR, progression to advanced phases, death or 3rd TKI discontinuation for any reason (toxicity, resistance, transplant or patient lost to follow-up). $P < 0.05$ was considered statistically significant. The cut-off for the data analysis was March 2015.

Ethics

The study protocol was approved and was conducted in accordance with the ethical standards of the local Research Ethics Committee on human experimentation and the Helsinki Declaration of 1975, which was revised in 1983. Patients provided written informed consent for their participation.

■ RESULTS

Clinical and laboratory characteristics of the 25 CML patients at the time of diagnosis and before the initiation of the 3rd TKI are presented in Tables 1 and 2, respectively.

Chronic-phase CML patients (CP-CML) (n=18) were analyzed separately. Thirteen CP-CML patients were resistant to imatinib (72%), and 5 were intolerant to imatinib (28%). Five patients were treated with dasatinib (28%), and 13 patients were treated with nilotinib (72%). Sixteen patients (89%) were resistant to the 2nd TKI, and 2 patients (11%) were intolerant to the 2nd TKI. The resistant patients never achieved a previous CCyR with imatinib or with the 2nd TKI. The median follow-up duration was 52 (7-75) months, and 16/18 patients (89%) achieved or maintained a complete hematologic response during this period. Of 15 patients who were subjected to cytogenetic analysis, 2 (13%) achieved CCyR. Of 17 CP-CML patients with available molecular analysis data, 4 (24%) achieved a major molecular response (MMR), and 2 achieved a complete molecular response (CMR). For CP-CML patients, the frequencies of the transcript levels at baseline and at 3 and 6 months after the initiation of the 3rd TKI are shown in Table 3.



Table 1 - Characteristics of chronic myeloid leukemia patients at diagnosis (n=25).

Variables	n.	%
Median age (range) years	45 (14-72)	
Gender: male	13	52
Sokal risk group		
Low	5	20
Intermediate	1	4
High	9	36
Missing	10	40
Additional chromosomal abnormalities*	01/09	11.1
Splenomegaly	11/16	68.7
Spleen size > 10 cm below the costal margin	06/11	54.4
White cell count x 10 ⁹ /L (median, range)	137.10 (17.1 - 494.4)	
Platelet count x 10 ⁹ /L (median, range)	352.0 (141.0 - 2,901.0)	
Hemoglobin, g/L (median, range)	10.2 (5.1 - 13.7)	
Blasts PB, % (median, range)	3.5 (0 - 17)	
Basophils PB, % (median, range)	4 (0 - 34)	

* 47, XX, t (9;22) (q34;q11), +der(22)

Mutation analysis

BCR-ABL1 mutations were evaluated in 14 of 18 CP-CML patients, and mutations were detected in 6/14 patients (43%). One patient in the AP stage presented with the mutation M351T. The mutation F317L was found in 3 patients before the initiation of the 3rd TKI (during second-line dasatinib therapy), and the mutation F359V was found in one patient, who displayed imatinib resistance, before the initiation of dasatinib as a 2nd-line therapy. Five mutations were found during 3rd-line TKI therapy: E255V (dasatinib), Y253H (dasatinib), M244V (dasatinib), and F317L (nilotinib). The patient with the F359V mutation presented with a long history of disease and had been treated previously for 12 years at another center with busulfan and hydrea before imatinib treatment. The F359V mutation was detected for the first time when a patient developed imatinib resistance, but at the time there were no 2nd-line inhibitors available in Brazil. The patient underwent hematopoietic stem cell transplantation (HSCT) and relapsed one and a half years later with persistence of the F359V mutation. The patient was treated with dasatinib and achieved CHR but never achieved a major cytogenetic response. After 4 years of dasatinib treatment, the patient progressed to the AP stage. At this time, a new mutation analysis was performed, which revealed no evidence of the F359V mutation, but a new mutation, F317L, was identified. The patient was treated with nilotinib and achieved CHR but relapsed after 5 months; at the time of relapse, the patient maintained the

F317L mutation. A second HSCT was performed, which resulted in the achievement of a complete molecular response, but the patient died due to graft-versus-host disease.

Survival analysis

One patient in the CP stage died during 3rd TKI therapy. CP-CML patients had 5-year OS, PFS and EFS values of 86, 54 and 22% (*p*<0.0001), respectively, whereas AP-CML patients had 5-year OS, PFS and EFS values of 66, 66 and 0%, respectively (*p*<0.0001). BC-CML patients showed no response in the first year after treatment (Figures 1, 2 and 3).

Long-term outcome

During treatment, 9/16 (56%) CP-CML patients lost CHR within a median of 23 (3-37) months. Two patients lost CCyR after 12 and 13 months. One patient lost MMR after 7 months. Six (34%) patients are currently taking their 3rd TKI, although 3 of these patients lost their response (1 MMR, 1 CCyR and 1 CHR). Three CP-CML patients (17%) progressed to the BC (blast crisis) stage, and 2 CP-CML patients subsequently died. Discontinuation of the 3rd TKI occurred in 16 (89%) cases due to resistance (8); intolerance (3); loss to follow-up (3); and death (2) during the treatment.

Three AP-CML patients reached CHR, but one of these patients lost their response. Only one patient achieved CCyR and MMR, but those responses were lost. One patient discontinued treatment due to intolerance in the 4th month.

Table 2 - Clinical and laboratory characteristics of chronic myeloid leukemia patients at the initiation of the 3rd tyrosine kinase inhibitor (n=25).

Variables	n= 25
Median age (range) years	56 (22-75)
Median time of imatinib therapy (range) months	30 (1-66)
Achievement of CCyR with imatinib treatment n (%)	3 (12%)
Interval diagnosis - 3 rd TKI (range) months	98 (12-404)
Treated with dasatinib 100-140 mg once daily n (%)	16 (64%)
Treated with nilotinib 400 mg BID n (%)	09 (36%)
Disease status before 3 rd TKI n (%)	
CP	18 (72%)
AP	03 (12%)
BC	04 (16%)

Table 3 - Molecular responses of chronic phase-chronic myeloid leukemia patients treated with a 3rd tyrosine kinase inhibitor.

Time	RQ-PCR (IS)%	N	%
Baseline	> 10	11/18	61
	1 - 10	04/18	22
	0.1 - 1 <	03/18	17
3 months	≤ 0.1	0	0
	> 10	09/12	75
	1 - 10	02/12	17
6 months	0.1 - 1 <	0	0
	≤ 0.1	1/12	08
	> 10	04/08	50
	1 - 10	01/08	12.5
	0.1 - 1 <	01/08	12.5
	≤ 0.1	02/08	25

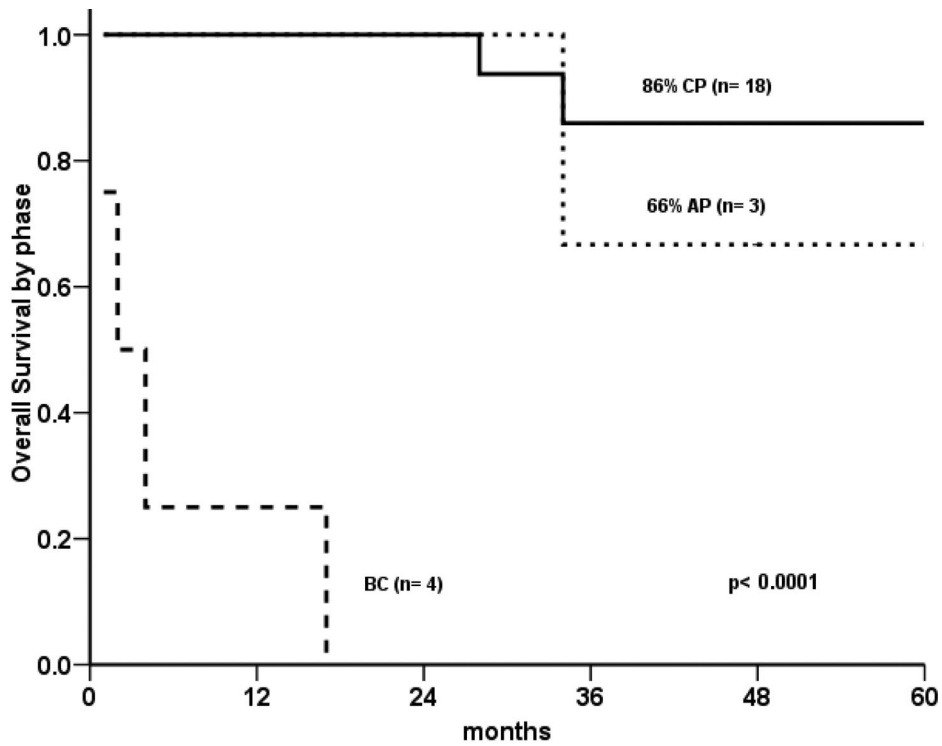


Figure 1 - Kaplan-Meier survival analysis. Five-year OS of chronic myeloid leukemia patients treated with a 3rd tyrosine kinase inhibitor according to disease phase.

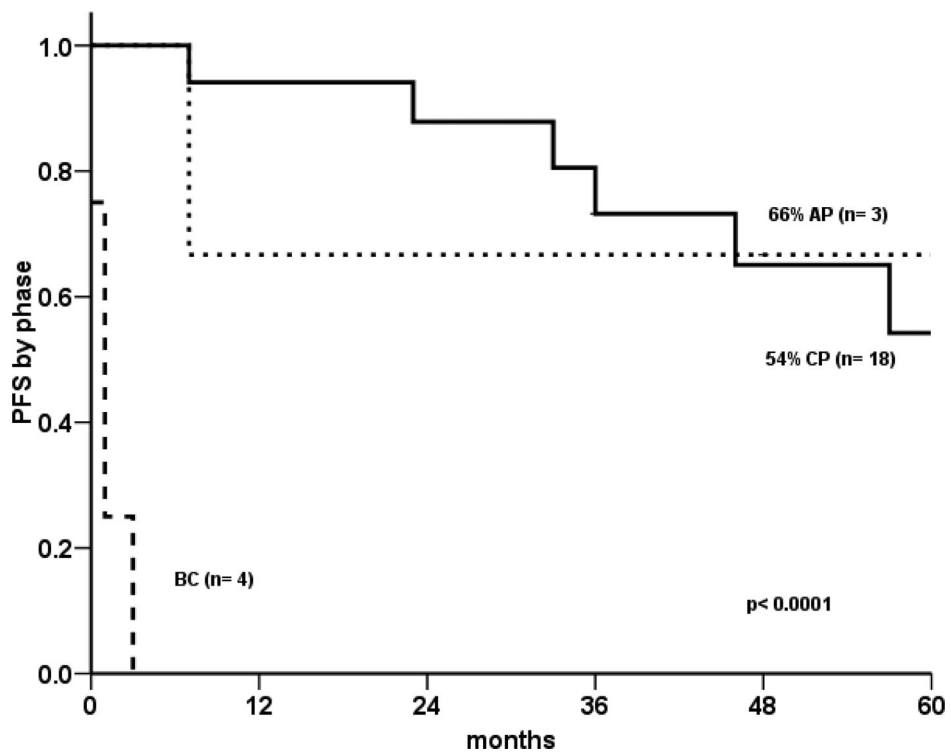


Figure 2 - Kaplan-Meier survival analysis. Five-year PFS of chronic myeloid leukemia patients treated with a 3rd tyrosine kinase inhibitor according to disease phase.

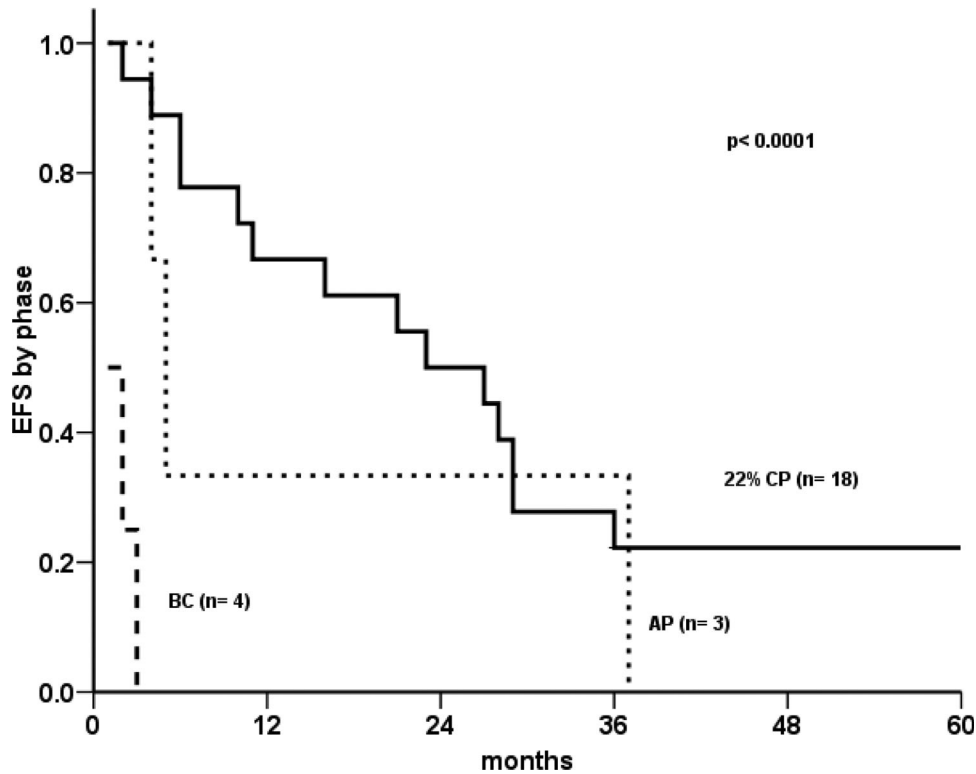


Figure 3 - Kaplan-Meier survival analysis. Five-year event-free survival of chronic myeloid leukemia patients treated with a 3rd tyrosine kinase inhibitor according to disease phase.

Four BC-CML patients did not reach hematological or cytogenetic responses and died within four months of the initiation of the 3rd TKI.

Regarding other treatments after discontinuation of the 3rd TKI, 14 patients were treated with the following drugs: hydrea (8), hydrea followed by HSCT (2), hydrea followed by low dose ARA-C and imatinib (1), interferon followed by hydrea (1), imatinib (1), and conventional chemotherapy followed by hydrea (1).

DISCUSSION

Our data show that only 22% of patients in the CP stage showed long-term benefits from the administration of a 3rd TKI after imatinib and a 2nd TKI failure. We found that 89% of our patients in the CP stage achieved CHR, 13% achieved CCyR, and 24% achieved MMR; however, 50% of those patients lost CHR within a median of 23 months. All patients with CCyR lost their response after 12 months, and 25% of patients lost MMR after 7 months.

Our results are in agreement with prior reports. Quintas-Cardama et al. (15) performed a study on 23 CML patients treated with dasatinib after imatinib and nilotinib failure and found that 43% of these patients achieved CHR and 30% achieved a cytogenetic response. Giles et al. (16), performed a study analyzing 60 patients treated with nilotinib after imatinib and dasatinib failure and found that 70% of CP-CML patients achieved CHR and 43% of CP-CML patients achieved a major cytogenetic response (MCyR). The authors also found that after 18 months, 59% of CP-CML patients were progression-free, and their estimated survival was 86%.

Regarding molecular responses, most of our patients had BCR-ABL1 transcript levels >10% at 3 months (75%) and >1% at 6 months (62.5%). The achievement of early responses to first- and second-line therapies, such as BCR-ABL1 transcript levels <10% at 3 months and <1% at 6 months, has been associated with long-term cytogenetic and molecular responses and better clinical outcomes (3,17–22). Only one patient in the CP stage achieved the optimal response criteria within 3 months and 6 months, respectively.

In our study, the EFS was 44% at 27 months for CP-CML patients, which is similar to the findings reported by Ibrahim et al. (23), where 26 CP-CML patients who failed to respond to two prior TKIs had 45.7% EFS at 30 months after the initiation of a 3rd TKI. These results show that although patients can achieve hematological and cytogenetic responses with a 3rd TKI, those responses are not sustainable. Similar observations were made by Garg et al.(24). The authors evaluated 48 CML patients, 25 of whom were in the CP stage, treated sequentially with three TKIs. Three patients in the CP stage and one in the AP stage achieved CCyR; the median duration of the response was 16.3 months. The median failure-free survival was 20 months for patients in the CP stage, 5 months for patients in the AP stage, and 3 months for patients in the BP stage.

Patients treated with sequential TKIs also have a higher risk of developing resistance and novel mutations (5,6). In fact, 76% of our patients discontinued the 3rd TKI, and 42% of those discontinuations were due to resistance. We found 5 BCR-ABL1 mutations in 14 CP patients during the 3rd TKI therapy. One patient harbored a F359V mutation and responded to dasatinib, however, another mutation



was selected in this patient when the disease progressed (F317L).

Although the responses to 3rd-line TKI therapy are not sustainable, 3rd-line TKIs may be an alternative for patients with CML who failed to respond to imatinib and a second generation TKI and are not eligible for HSCT (4). A 3rd-line TKI can improve the patient's condition until an alternative transplant donor is available. Nevertheless, because the long-term outcome of these patients is poor, it is important to emphasize the importance of developing new therapies for CML resistant patients.

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■ AUTHOR CONTRIBUTIONS

Ribeiro BF and Pagnano KB conceived and designed the study. Ribeiro BF, Duarte VO, Miranda EC, Almeida MH, and Pagnano KB performed the data collection. Delamain MT, Oliveira-Duarte G, and Pagnano KB treated the patients. Lorand-Metze I, Souza CA, Pagnano KB, Ribeiro BF, Vergílio B, Silveira RA, and Albuquerque DM performed the BCR-ABL1 mutation analysis and quantitative PCR experiments. Miranda ECM managed, analyzed, and interpreted the data. Ribeiro BF, Miranda ECM, Albuquerque DM, Delamain MT, Oliveira-Duarte G, Almeida MH, Vergílio B, Silveira RA, Oliveira-Duarte V, Lorand-Metze I, Souza CA, and Pagnano KB approved the final manuscript. All authors contributed to the collection, analysis and interpretation of the data and contributed to the critical revision of the article for intellectual content.

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