

A randomized controlled trial comparing peginterferon- α -2a versus observation after stopping tyrosine kinase inhibitor in chronic myeloid leukemia patients with deep molecular response for at least two years

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Abstract

Background: Interferon (IFN) is a logical possibility to increase treatment free remission (TFR) rate in chronic myeloid leukemia (CML). We conducted the first randomized controlled trial comparing the use of pegIFN versus observation in CML patients attempting TFR.

Methods: Adult CML patients with stable deep molecular response for ≥ 2 years with ≥ 2 readings of MR4.5 were randomized into two arms – subcutaneous pegIFN- α -2a starting at 180 μ g weekly for a year, followed by observation, or observation.

Results: A total of 30 patients were recruited (pegIFN n=15, observation n=15). Median follow-up was 38.1 months (range 15.9-49.4) and 23.8 (1.5-51.0) in pegIFN and observation arm, respectively. A total of 11 patients relapsed (pegIFN n=4, observation n=7). The median time of relapse was 13.1 months (range 9.2 to 25.5) and 4.4 (1.2 to 13.6) in pegIFN and observation arm, respectively. Only 8 out of 15 (53%) patients completed 52 doses of pegIFN with mean dose of 43 out of 52 doses (range 20 to 52). Dose of tolerable pegIFN was age dependent.

Conclusion: PegIFN is a potential consolidative therapy to increase TFR.

Trial registration:

(1) Malaysia National Medical Research Register (NMRR): NMRR-13-1186-15491. Approved 23rd September 2014, <https://www.nmrr.gov.my/fwLoginPage.jsp>

(2) ClinicalTrials.gov: NCT02381379. Registered on 24th Feb 2015, <https://clinicaltrials.gov/ct2/results?cond=&term=NCT02381379&cntry=&state=&city=&dist=>

Background

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm that originated from an abnormal pluripotent bone marrow stem cell and is consistently associated with *BCR-ABL 1* fusion gene.(1) The level of *BCR-ABL 1* fusion gene can be quantitated using real-time quantitative polymerase chain reaction (qPCR) and is standardized using International Scale (IS) (qPCR^{IS}).(2) *BCR-ABL 1* fusion gene is translated into an abnormal tyrosine kinase, a key player in pathogenesis of CML.

The first tyrosine kinase inhibitor (TKI) is imatinib (Glivec® or Gleevec®, Novartis Pharmaceuticals Corporation). TKI changes the paradigm of CML from a doomed incurable disease unless allogeneic hematopoietic stem cell transplantation was done, to a disease that most patients can have a near normal life span. It also revolutionizes the approach in oncology from chemotherapy/radiotherapy to targeted therapy. Since imatinib was approved by United State Food and Drug Administration in 2001(3), there were many advancement in the management of CML. One of them is the concept of treatment free remission (TFR), arguing the necessity of life long TKI. Stopping TKI treatment was reported previously(4–12), but it was not landscape changing until the landmark stop TKI trials from French(13)

and Australia(14) reported about 40% of CML patients, who had achieved complete molecular response (CMR), i.e. undetectable *BCR-ABL 1* gene from qPCR, for at least two years, were able to stop TKI safely and remain in TFR.

Interferon (IFN)- α , either IFN- α -2a or IFN- α -2b, is the standard treatment of CML before the era of TKI. Depending on the duration of administration, only about 10 to 30% of patients on IFN- α (15) achieved complete cytogenetic response compared to about 70 to 90% on imatinib(16). However, IFN acts differently from TKI. It might be able to target leukemic stem cells.(17) Case reports and case series show that IFN-responded patients could achieve long term TFR.(18–24) In the largest case series, 78 to 95% of IFN-responded CMR patients remained in TFR once IFN was withdrawn(25, 26), probably via IFN-induced immunity towards the leukemic stem cell clone. The role of IFN in TFR is further supported by studies incorporating IFN or its pegylated form (pegIFN) as upfront treatment together with TKI(27), upfront treatment together with TKI and consolidation(28, 29) and consolidation after stopping TKI(30–33). These studies showed higher TFR rate compared to the landmark stop TKI trials(13, 14). However, definitive conclusion that IFN/pegIFN can increase TFR could not be drawn from these studies because they were all single arm study.

We conducted a randomized controlled trial to compare the outcomes of pegIFN- α -2a for a year followed by observation versus observation after stopping TKI in CML patients with deep molecular response (DMR) (molecular response (MR) of 4-log reduction (MR4) (0.01%^{IS}) or deeper) for two years or more. To our knowledge, this is the first randomized controlled trial to study the effect of IFN- α to TFR. We reported the result of this pilot study here.

Methods

Study design and patient eligibility

This was a multi-center randomized open-label controlled trial conducted in Malaysia. This study was approved by Medical Research and Ethics Committee, Ministry of Health Malaysia. Written informed consent was obtained for each participant prior to the enrollment.

Inclusion criteria were 1) 18 years old or above, 2) chronic phase during diagnosis of CML, 3) treated with ongoing TKI at any dose for at least 3 years, 4) achieved stable DMR for two years or more by any TKI, and 5) the date of the latest qPCR^{IS} result must compel with Intervention Start Date (ISD). Stable DMR was defined as DMR with at least two results, including the latest, of MR4.5 (0.0032%^{IS})(2) or deeper over the last two years. Study Entry Date was defined as the date patient signed consent form. ISD was defined as the day after the last dose of TKI and is within four weeks of Study Entry Date or 17 weeks from the latest qPCR^{IS} test. Exclusion criteria were elaborated in Supplementary Data.

Randomization and intervention

Randomization process, allocation concealment and pegIFN- α -2a are elaborated in Supplementary Data. Patients were randomized into two arms, one was subcutaneous pegIFN- α -2a, starting at 180 μ g weekly, for one year followed by observation and one was observation throughout. The next day after the last dose of TKI, patients would receive either one of the two arms according to the randomization result.

Study outcomes

The primary outcome was relapse rate and relapse-free survival (RFS). Relapse was defined as: 1) one reading of loss of major molecular response (MMR) (0.1%^{IS}), or 2) positivity of *BCR-ABL 1* transcripts in qPCR^{IS}, as confirmed by a second analysis point, indicating the increase (≥ 1 log) in relation to the first analysis point at two successive assessments. Time of Relapse (ToR) is defined as the time of loss of MMR (relapse criteria no.1) or of the first analysis point (relapse criteria no.2), whichever is earlier. After relapse, the treating physician was recommended to restart the patient on TKI and required to monitor patient monthly until regain DMR (DMR₂) consecutively for two months, following which patient will return to the regular monitoring every 3 to 6 months.

The secondary outcomes were 1) Time to Relapse (TTR) (time from ISD to ToR), 2) DMR₂ rate, 3) Time to DMR₂ (ToR to time of first analysis point of DMR₂), 4) adverse side-effects of peginterferon and 5) quality of life (QoL) assessment.

Data collection, monitoring and safety precautions

Peripheral blood for qPCR^{IS} for *BCR-ABL 1* test (see Supplementary Data) was centralized and performed following the standard operating procedures in Molecular Laboratory, Ampang Hospital. Patients came for monthly qPCR^{IS} monitoring for the first 12 months, 2-monthly for subsequent 12 months, and 3-monthly thereafter (see Supplementary Data for other investigations and QoL assessment). Repeating the tests before the scheduled time interval was not allowed unless it is deemed indicated after discussed with the Principle Investigator.

Statistical analysis

Comparison of characteristics of the patients were performed using Chi-square test for categorical data and ANOVA or Kruskal Wallis test for normally and non-normally distributed continuous data, respectively. RFS was analyzed using log rank test. Rate were analyzed using Chi-square test (Fisher's Exact Test if the count in the cell is less than expected) and risk estimation. Comparison of duration was analyzed using independent T test. Descriptive analysis was used to analysis number of patients who developed side effect. Parametric tests for normally distributed data and non-parametric tests for skewed distribution were used to analyze QoL. Significant level was set at 0.05. Statistical Package for Social Science (SPSS) 24 software was used for statistical analysis of this study.

Results

The recruitment was started in March 2015 and closed in Sep 2018. ISD of the first and last recruited patient was 8th July 2015 and 7th October 2018, respectively. Analysis was done as per status on 7th October 2019. Data from a total of 30 patients was analyzed. Median follow-up was 33.0 months (range 1.5–51.0). The overall study flow diagram is shown in Fig. 1.

All patients were on imatinib before starting intervention, except one patient (pegIFN arm) was initially on imatinib but later changed to nilotinib due to imatinib-induced diarrhea. The baseline characteristics of the 30 patients is shown in Table 1. There is a difference in gender distribution between the two arms.

Table 1
Baseline characteristics of 30 patients

Characteristics	Peginterferon (n = 15)	Observation (n = 15)	p value
Age at diagnosis (year)	34.2 (14.2)	40.9 (15.7)	0.23
Age at ISD (year)	44.8 (13.0)	51.27 (16.9)	0.21
Gender ratio (M:F)	2.75 (11:4)	0.5 (5:10)	0.06
Ethnic (M:C:I:S/S ratio)	7:7:0:1	4:9:1:1	0.56
Time from diagnosis to ISD (year)	8.1 (3.4)	8.6 (3.8)	0.71
Time from TKI to ICS (year)	7.7 (2.8)	8.2 (3.2)	0.67
Time from 1st MMR to ISD (year)	4.9 (1.7)	4.9 (1.4)	0.88
Time from 1st MR4 to ISD (year)	4.4 (1.5)	4.5 (1.2)	0.76
Time from 1st MR4.5 to ISD (year)	3.9 (1.7)	3.7 (1.3)	0.67
History of IFN usage	2 (13.3)	1 (6.7)	1
History of transplantation	1 (6.7)	0 (0)	1
Sokal score (n = 19)			0.66
Low	6 (75.0)	7 (63.6)	
Intermediate	0 (0)	1 (9.1)	
High	2 (25)	3 (27.3)	
Hasford score (n = 18)			0.41
Low	4 (50.0)	4 (40.0)	
Intermediate	3 (37.5)	6 (60.0)	
High	1 (12.5)	0 (0)	
EUTOS score (n = 19)			0.07
Low	9 (100.0)	7 (70.0)	
High	0 (0)	3 (30.0)	
Data in mean (standard deviation) and n (%)			
EUTOS, European Treatment and Outcome Study; IFN, interferon; ISD, Intervention Start Date; M:C:I:S/S, Malay:Chinese:Indian:Sabah/Sarawak natives; M:F, male:female; TKI, tyrosine kinase inhibitor			

Primary and secondary outcomes

RFS is shown in Fig. 2. The median TTR was 13.1 months (range 9.2 to 25.5) and 4.4 (1.2 to 13.6) in pegIFN and observation arm, respectively. DMR₂ rate was 10 (91%) and regained at median of 2.7 (1.1–3.84) and 2.2 (2.1 – not reached yet) in pegIFN and observation arm, respectively. No patient had disease progression during the study. There were differences between the non-relapse and relapse patients in pegIFN arm, in which the relapse patients are female predominant ($p = 0.03$) and had shorter duration of TKI ($p = 0.12$) with shorter duration of MR3 (0.1%^{IS}) or deeper (see Fig. 2). In comparison to relapse patients in observation arm, relapse patients in pegIFN arm also had shorter duration of TKI ($p = 0.28$) and shorter duration of MR3 (0.1%^{IS}) or deeper (see Fig. 2).

In general, QoL (see Table 2) in both arms were similar at baseline and after two months stopping TKI. Symptoms related to TKI side effects like nausea and vomiting and diarrhea were reduced after stopping TKI in both arms.

Table 2
Comparison of changes in quality of life between the two arms

Aspect, mean (SD)		Peginterferon (n = 15)			Observation (n = 13 ¹)		
		Baseline	2-month	Δ	Baseline	2-month	Δ
Global health status / QoL		83.9 (21.0)	81.1 (14.2)	-2.8	73.7 (18.9)	71.8 (24.2)	-1.9
Functional scale	Physical	90.2 (17.6)	91.6 (9.2)	1.4	88.2 (11.2)	86.1 (14.0)	-2.1
	Role	91.1 (19.8)	90.0 (15.2)	-1.1	91.0 (14.6)	91.0 (16.1)	0
	Emotional	86.7 (18.8)	83.3 (18.9)	-3.4	82.1 (18.3)	86.5 (22.2)	4.4
	Cognitive	85.6 (17.7)	84.4 (14.7)	-1.2	82.5 (17.3)	84.6 (25.9)	2.1
	Social	93.3 (12.3)	95.6 (11.7)	2.3	95.2 (7.3)	84.6 (20.9)	-10.6
Symptom scales / items	Fatigue	24.4 (21.9)	25.9 (19.1)	1.5	26.5 (18.4)	32.5 (16.0)	6.0
	Nausea & vomiting	12.2 (16.0)	2.2 (5.9)	-10.0	23.1 (28.5)	3.8 (10.0)	-19.3
	Pain	16.7 (27.5)	16.7 (17.8)	0	10.3 (14.5)	25.6 (30.1)	15.3
	Dyspnea	15.6 (21.3)	11.1 (20.6)	-4.5	12.8 (16.9)	7.7 (14.6)	-5.1
	Insomnia	11.1 (27.2)	13.3 (16.9)	2.2	20.5 (21.7)	20.5 (21.7)	0
	Appetite loss	11.1 (20.6)	11.1 (20.6)	0	12.8 (21.7)	10.3 (28.5)	-2.5
	Constipation	15.6 (24.8)	11.1 (16.3)	-4.5	10.2 (16.1)	10.3 (21.0)	0.1
	Diarrhea	13.3 (27.6)	11.1 (16.3)	-2.2	17.9 (17.3)	2.6 (9.2)	-15.3
Financial difficulties	11.1 (16.3)	2.2 (8.6)	-8.9	10.3 (21.0)	7.7 (14.6)	-2.6	

¹2 patients did not fill QoL questionnaire because 1 patient died at 1.5 month and 1 patient forgot to fill.

Safety and side effects

Among the 23 reasons of pegIFN dose reduction/withholding, 12 (52%) were due to liver transaminitis, followed by blood count changes 4 (17%), intolerance like giddiness and pruritus 3 (13%), symptoms of carpal tunnel syndrome 2 (9%), raised blood pressure 1 (4%) and thyroid dysfunction 1 (4%). Among the 15 patients on pegIFN arm, the highest alanine transaminase (ALT) rise was 7 times upper limit of normal (ULN) (n = 1), followed by 6 times (n = 2), 5 times (n = 1), 2 times (n = 6), 1 time (n = 2) and never raise more than 1 time (n = 3). Five patients needed to withhold pegIFN due to ALT \geq 5 times ULN (n = 4) and platelet $< 20 \times 10^9/L$ (n = 1). The ALT rise of \geq 5 times ULN in three out the four patients could have been avoided if the starting dose of pegIFN followed the age of the patients (see Discussion). All the transaminitis resolved after dose reduction/withholding.

TKI withdrawal syndrome (reported myalgia and arthralgia) occurred more in observation arm 8 (53%) compared to pegIFN arm 5 (33%), which is also reflected in QoL (see Table 2). The reported myalgia and arthralgia were grade 1 except one patient had grade 2 and one patient was severe requiring withdrawal from the study (see Supplementary Data). One death but was unrelated to CML or the study (see Supplementary Data).

Discussion

General limitation of the study

The main limitation is the small sample size. However, if a significant finding was found in a small sample, like our pilot study, it encourages further and larger study. The lower relapse rate 4 (27%) versus 7 (47%) and later TTR 12.9 versus 1.8 months in pegIFN arm versus observation arm, respectively, might provoke argument that the finding merely because, as a form of CML treatment, pegIFN delays the relapse. Thus, the duration of follow-up must be long enough to overcome this potential confounder. We reported our study after the last recruited patient completed a year of follow-up and by chance, the pegIFN arm had longer follow-up 38.1 months (range 15.9–49.4) compared to observation arm 23.8 months (1.5–51.0). Hence, this could likely eliminate the confounder. Of course, a longer follow-up of the study is needed.

Special note is needed during reading data on the first documented and duration of MMR, MR4 and MR4.5 in Table 1 and Fig. 2 because of the timing starting qPCR^{IS} in the two centers where our patients had their qPCR^{IS} monitoring prior to enrollment (Ampang Hospital in March 2010, while Singapore General Hospital from 15th May 2012 (personal communication with Dr. Charles Chuah)). Ampang Hospital issued the qPCR^{IS} reports according to a new guideline(2) starting from 15th January 2016. All enrolled patients' reports were carefully checked and primary data on the RNA replicates was checked if needed to ensure inclusion criteria was fulfilled.

PegIFN

Three important questions on pegIFN during the design of the study are which pegIFN to be used, the starting dose, and the duration. There is no comparison study of pegIFN- α -2a and 2b in CML. We picked

pegIFN- α -2a because the stock flow was steadier in Malaysia. The starting dose of 180 μ g was selected based on the available literature, as single agent at 180 μ g(31), 270 μ g(34), and 450 μ g(35), or combined with TKI as first line treatment in CML at 45 to 90 μ g(36–38) and 180 μ g(31). We do not know the appropriate duration of pegIFN- α -2a. Previous history of IFN usage, not reported it was either conventional IFN or pegIFN, for > 1.5 years was associated with higher TFR.(39) Usuki et al. used conventional IFN 3 MU 2–5 times a week for two years as consolidation.(32) Hardan et al. used pegIFN- α -2a for a year as consolidation.(31) Looking at these studies, we decided 1-year duration of pegIFN- α -2a in this pilot study, but the question of an adequate duration of pegIFN to increase TFR would continue until further study is available.

In this study, pegIFN was given for a year, regardless pegIFN was withheld in between. Only 8 out of 15 (53%) patients completed 52 doses of pegIFN with mean dose of 43 out of 52 doses (range 20 to 52). Seven patients did not complete 52 doses because of transaminitis (n = 5), missed dose (n = 1) and relapse (n = 1). Two of the four patients with transaminitis \geq 5 times ULN refused to restart pegIFN when transaminitis resolved. This factor of incomplete pegIFN course should be considered during interpretation of the study's finding, too. We would suggest at least 52 completed tolerable doses of pegIFN instead of the 1-year bound in future similar study.

From this study, we found the likely tolerable pegIFN- α -2a dose is correlated with age (see Supplementary Fig. 1). Among 13 patients who required pegIFN dose reduction, 9 (69%) patients could have avoided/reduced the need of dose reduction if the starting dose was lower. This would certainly help future similar study to achieve the target accumulative dose of pegIFN- α -2a, reduce unnecessary side effects, and improve compliance. We would suggest the starting pegIFN- α -2a dose as 180 μ g weekly in patients age below 40 years old, 135 μ g weekly in patients age 40 to 60 years old and 90 μ g weekly in patients age more than 60 years old in future similar study.

Fluctuation of qPCR^{IS} level

Fluctuation of qPCR level below MMR (0.1%^{IS}) is a known phenomenon after stopping TKI.(40) To our knowledge, there was no report on fluctuation that exceeding MMR, which is probably the reason it is recommended as a criterion of relapse(41) and used in most of the stop TKI trials(42). We would like to report two cases of fluctuation exceeding MMR here.

Two patients in the observation arm, both from the same study site, relapsed at different time point (one at 12 months (Dec 2017) and one at 14 months (Feb 2018) after stopping TKI) according to the relapse criteria no.1, i.e. loss of MMR. TKI was reinitiated as per protocol. However, a repeated qPCR^{IS}, which was not prohibited in the study protocol, was done prior to the initiation of TKI, which showed DMR. Investigations showed no evidence of wrong sampling or laboratory error. After discussion, investigators decided to stop their TKI after two months of TKI intake. These two patients remained in TFR up to the time of writing. Their qPCR^{IS} trend and blood count changes compared to the other two relapsed patients at the same study site and other study sites are shown in Supplementary Fig. 2 and Supplementary Fig. 3, respectively.

These two “relapse” cases challenge MMR as a relapse criterion and raise doubt on the four relapse cases prior to the incidence. We re-examined these four cases and could only truly confirm relapse in one case (observation arm), in which the previous two readings already showed 1-log increment from 0.0059%^{IS} to 0.0896%^{IS}, fulfilled relapse criterion no.2, before loss MMR with reading of 0.2594%^{IS}. We could not rule out the possibility of fluctuation in other three cases confidently because no repeat qPCR^{IS} was done before restarting TKI. One patient (observation arm) had two prior readings of 0.0117%^{IS} and 0.0774%^{IS} before loss of MMR 0.1931%^{IS}. One patient (pegIFN arm) had two prior readings of 0.0225%^{IS} and 0.0680%^{IS} before loss of MMR 0.1085%^{IS}. One patient (pegIFN arm) had two prior readings of 0.0203%^{IS} and 0.0664%^{IS} prior to loss of MMR 0.1653%^{IS}. Following the incidence, study protocol was amended to include a repeated qPCR^{IS} on the time of restarting TKI after loss of MMR, which means relapse criteria no. 1 – loss of MMR – must be confirmed by two successive readings, and re-stopping TKI if the repeated qPCR^{IS} does not confirm the loss of MMR. In fact, at the time of writing (Apr 2020), there was another patient (pegIFN arm), who had such fluctuation, with two prior reading of 0.0108%^{IS} and 0.0337%^{IS} before loss of MMR 0.1046%^{IS} and the repeated reading of 0.0232%^{IS}. He was restarted and planned on TKI for two months, just like the previous two cases of fluctuation, but had not come back for review due to Malaysia and Singapore lock-down during COVID-19.

Gender effect on the efficacy of pegIFN?

The limitation of this pilot study is small sample size, which caused the significant difference in the gender ratio between the two arms. PegIFN arm had more male patients whereas observation arm had more female patients. Earlier single arm observation study (13) suggested female is less likely to maintain TFR, but the finding was disputed after a longer follow-up(43). Other single arm observation studies also did not find gender as a predictor of TFR.(39, 41, 44–46) Thus, it is unlikely gender distribution affects the outcome in the observation arm, but we cannot extrapolate the same assumption to pegIFN arm. In fact, there are data to suggest pegIFN is less effective in female compared to male in the treatment of Hepatitis C using pegIFN and ribavirin.(47–49) A significant more female in relapse pegIFN arm compared to non-relapse pegIFN arm ($p = 0.03$) in our small sample size warrants careful interpretation of the outcomes.

TKI and DMR duration

Relapse patients in pegIFN arm had shorter duration of TKI and shorter duration of DMR compared to non-relapse patients in pegIFN arm and either relapse or non-relapse patients in observation arm (see Fig. 2). The clinical predictors of TFR found in many single arm observation studies are longer duration of TKI and/or longer duration of DMR.(39, 43, 45, 46) This factor should be considered during interpretation of the outcomes.

Other findings

First, the increment of the blood counts during the first two visits and plateau off after that period in the observation arm (see Supplementary Fig. 4) provides further evidence for the myelosuppression effect of

TKI. It also explains partly the improvement in QoL (see Table 2) and justifies the need of TFR. Second, there was a groove in the trend of the blood counts in the pegIFN arm during the six months stopping TKI corresponding to the adjustment of pegIFN dosage during that period (see Supplementary Fig. 4). However, the blood count trend and the use of pegIFN did not affect the QoL (see Table 2). Third, absolute basophil count (ABC), but not total white cell count (TWC), was shown to correlate with percentage of Philadelphia chromosome in marrow metaphases of pre-clinical CML.(50) However, it seems that ABC also correlate with qPCR^{IS} (see Supplementary Fig. 3 (c)) at lower disease burden as in pre-clinical CML. TWC seems to correlate with qPCR^{IS}, too. These findings were unexpected. Thus, we examined whether these correlations could be used as surrogate marker of the fluctuation. The data is immature, but TWC and ABC might be the surrogate marker of the fluctuation (see Supplementary Fig. 3 (a)).

Conclusion

In conclusion, this is the first randomized controlled trial studying whether pegIFN could increase TFR rate compared to observation. PegIFN is a potential consolidative therapy to increase TFR. Tolerable dose of pegIFN is age dependent. Study with larger sample size is needed.

Abbreviations

ABC, absolute basophil count

CML, chronic myeloid leukemia

CMR, complete molecular response

DMR, deep molecular response

DMR₂, regain deep molecular response after relapse

IFN, interferon

IS, International Scale

ISD, Intervention Start Date

MMR, major molecular response

MR^X, molecular response of X-log reduction

pegIFN, pegylated form of interferon

QoL, quality of life

qPCR, real-time quantitative polymerase chain reaction

qPCR^{IS}, real-time quantitative polymerase chain reaction which is standardized using International Scale

RFS, relapse-free survival

TFR, treatment free remission

ToR, time of relapse

TTR, time to relapse

TKI, tyrosine kinase inhibitor

TWC, total white cell count

ULN, upper limit of normal

Declarations

Ethics approval and consent to participate

this study was approved by Medical Research and Ethics Committee, Ministry of Health Malaysia. Written informed consent was obtained for each participant prior to the enrollment.

Consent for publication

not applicable.

Availability of data and materials

the datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

the authors declare that they have no competing interests.

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Authors' contributions

JW Kuan initiated and conducted the study, recruited and monitored patients, applied and managed grants, collected and analyzed data, wrote and submitted manuscript; KM Chang initiated and conducted

the study, recruited and monitored patients, applied and managed grants; CL Phan conducted molecular monitoring part of the study; SP Wong conducted peginterferon part of the study; SM Lim assisted in conduct of the study, recruited and monitored patients; SG Toh, WK Loh, HN Begum, SL Hon, SK Chiang and YK Guan recruited and monitored patients; AJM Chai, SY Ng, KY Yong, G Welfred, LLL Wong, KW Ho, HG Teo and ASO Tang monitored patients.

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Footnotes

Supplementary data is attached in a separate file, which includes Supplementary Figure 1, 2, 3 and 4.

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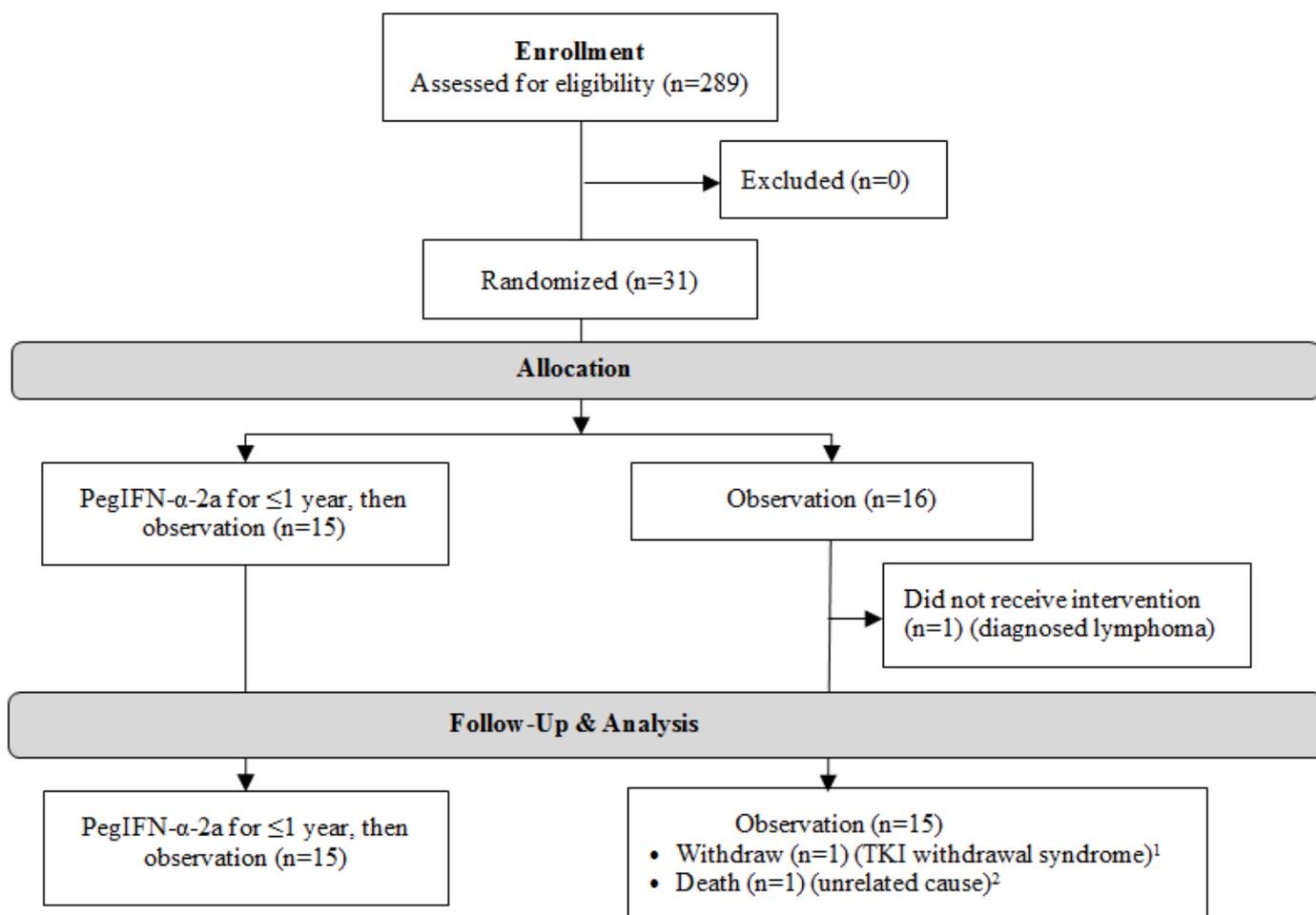
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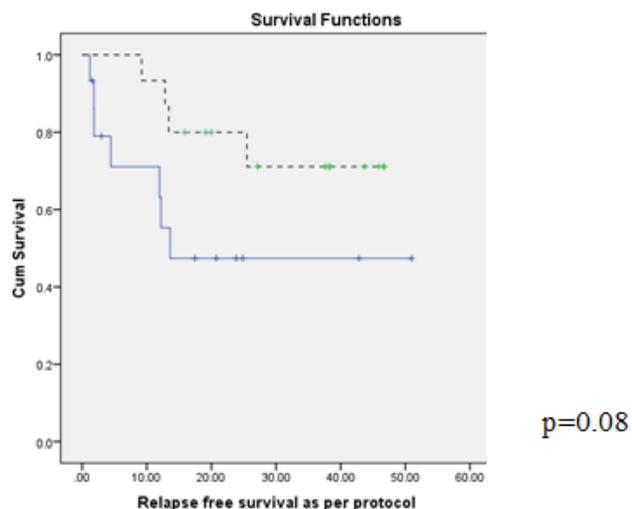
Figures



^{1,2}see Supplementary Data

Figure 1

Overall study flow



Characteristics	Observation (n = 15)			Peginterferon (n = 15)		
	Non-relapse (n = 8)	Relapse (n = 7)	p value	Non-relapse (n = 11)	Relapse (n = 4)	p value
Time to Relapse, month	1.2, 1.8, 1.8, 4.4, 12.0, 12.2, 13.6			9.2, 12.8, 13.4, 25.5		
Median f/up, month (range)	23.8 (1.5 – 51.0)			38.1 (15.9 – 49.4)		
History of IFN usage	0	1	0.33	2	0	1.00
M:F ratio	0.6 (3:5)	0.3 (2:5)	1.00	10 (10:1)	0.3 (1:3)	0.03
Duration of TKI	8.0 (3.2)	8.3 (3.4)	0.88	8.4 (2.8)	5.9 (1.9)	0.12
Duration of MR3	4.5 (0.9)	5.2 (1.7)	0.28	5.1 (1.8)	4.3 (1.4)	0.41
Duration of MR4	4.3 (1.0)	4.9 (1.4)	0.40	4.7 (1.6)	3.5 (0.9)	0.21
Duration of MR4.5	3.3 (0.9)	4.1 (1.5)	0.25	4.1 (1.9)	3.4 (1.0)	0.53
Mean (standard deviation) % no. of peginterferon dose/52 doses				83.8 (25.0)	84.6 (15.8)	0.95

Duration in mean (standard deviation) with unit in year unless specified otherwise. f/up; follow-up; M:F, male:female.

Figure 2

Relapse free survival and characteristics of relapse and non-relapse patients in the two arms.

Supplementary Files

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- [Suppdata.pdf](#)