© Ferrata Storti Foundation

Positron emission tomography response and minimal residual disease impact on progression-free survival in patients with follicular lymphoma. A subset analysis from the FOLL05 trial of the Fondazione Italiana Linfomi

Follicular lymphoma (FL) is the most common indolent B-cell lymphoma in western countries. Overall, 70% of patients achieve complete remission after first treatment.[1] However, it is characterized by a pattern of relapsing and remitting disease. The outcome of patients with FL has clearly improved,[2] but heterogeneity in patients' survival still remains, making the quest for reliable prognostic factors a relevant issue.

Response assessment of patients with FL can be performed with CT scan and ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET) scan. FDG-PET has been confirmed to have the highest accuracy and was shown to be independent of CT scan and to be a stronger predictor of outcome.[3] Recently, PET has been acknowledged as a recommended procedure for FL staging and response assessment.[4] Moreover, the assessment of MRD by qualitative and quantitative polymerase chain reaction (PCR) for *BCL2/IGH* has been evaluated as a prognostic tool in FL.[5] Nevertheless, the impact of both end-of-treatment (EOT) PET and MRD in prognostic assessment remains to be determined.

The aim of the present study was to analyze the prognostic role of combined PET and *BCL2/IGH* analysis, performed at the EOT, in a subset study of the phase III trial FOLL05 (NCT00774826), in which patients with FL were randomized to R-CVP (rituximab plus cyclophosphamide, vincristine and prednisone), R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone) or R-FM (rituximab plus fludarabine and mitoxantrone).[6] This study was conducted in compliance with the Declaration of Helsinki, was approved by the appropriate research ethics committee, and required each patient to provide written informed consent.

In order to be considered for the current study, patients were required to have been enrolled in the FOLL05 trial that included previously untreated high tumor burden Ann Arbor stage II to IV with grade 1,2 or 3a FL.[6] Of note, the FOLL05 study included MRD evaluation at the EOT among planned study procedures.[5] In addition, for the purpose of this study patients should also have had data available on EOT PET, performed up to three months after the last dose of induction rituximab (+/- chemotherapy) and have been assessed for the *BCL2/IGH* at diagnosis and at the EOT within 2 months from last dose. Data on clinical presentation, treatment, response and follow-up were retrieved from the existing and published dataset of the randomized protocol.

PET was centrally reviewed by three independent nuclear medicine physicians applying the Deauville scale. Positive scans (PET+) were defined by residual FDG uptake ≥ score 4 (i.e. moderately increased uptake > liver uptake). The final result was selected by agreement between at least two of three reviewers.

Regarding MRD analysis, patients underwent bone marrow (BM) aspirate for qualitative and quantitative assessment of the *BCL2/IGH* fusion gene. DNAs from the patients were assessed for the *BCL2/IGH* at diagnosis, and if positive, at the EOT. All qualitative molecular analyses were centralized in the molecular laboratory of the Division of Hematology at the University of Pisa, Italy. DNA was extracted from BM mononuclear cells by the Wizard Genomic DNA Purification Kit (Promega). To amplify *BCL2/IGH* rearrangement, nested qualitative PCR reactions were performed.[7] The sensitivity of the qualitative PCR assays was confirmed by testing serial dilutions of DNA derived from the *BCL2/IGH*-positive DOHH-2 cell line, achieving a limiting dilution of 1:10^{-5}. As already reported, a second reaction for mcr breakpoint was also performed.[8]

The primary endpoint was progression-free survival (PFS), that was calculated as the time from the date of treatment initiation until the date of lymphoma progression, relapse, death from any cause or last follow-up visit. Standard descriptive analyses were carried out. For a crude association analysis, categorical data were analyzed using the chi-square test or Fisher's exact test (two-sided). Cohen's kappa statistic was used to verify agreement between PET and MRD results. The level of agreement was defined by Koch Landis scale. Survival curves were estimated using the Kaplan–Meier method and compared using the log-rank test. Univariate Cox regression analyses were conducted to verify the prognostic role of final PET and MRD regarding PFS. Two-tailed *P* values <0.05 were considered statistically significant. Statistical analysis was carried out using SPSS software (version 18.0, Chicago, IL, USA).

A total of 41 patients had available data on both PET and *BCL2/IGH* at the EOT. The median age was 54 years (39-71). Baseline characteristics of the study population did not differ from that of the FOLL05 study (Table 1). The distribution of cases according to EOT PET and MRD is shown in Table 2. PET/MRD concordance was 76%, with Kappa=0.249, suggesting that PET and MRD when done at the end of induction therapy are not strongly correlated.

With a median follow-up of 53 months (from 13 to 77 months), 5-year PFS was 62% (95% CI 45 to 75). By univariate analysis, EOT PET+ was associated to a poorer PFS (HR 3.61, 95%CI 1.15-11.4, *P*=0.028), while the EOT

Table 1. Comparison of baseline characteristics of study population and FOLL05 patients.

	Present study N=41		Remaining patients from FOLL05 N=463		*P*
	n	%	n	%	
Age > 60	11	27	156	34	0.39
Male	19	46	245	53	0.42
Ann Arbor stage III-IV	38	93	423	91	1.0
Bulky disease (> 6 cm)	16	39	118	25	0.07
BM involvement	23	56	251	54	0.47
FLIPI 3-5	16	39	172	37	0.59
First treatment					
R-CVP	12	29	156	34	
R-CHOP	13	32	152	33	0.7
R-FM	16	39	155	33	

Table 2. Distribution of patients according to PET response and MRD at the end-of-treatment.

	PET negative	PET positive
MRD negative	28 (68%)	2 (5%)
MRD positive	8 (20%)	3 (7%)

© Ferrata Storti Foundation

positive molecular status showed a trend towards a shorter PFS (HR 2.54, 95%CI 0.96-6.72, *P*=0.060) (Figure 1).

In a stratified analysis combining the information on PET and MRD, the 3-yr PFS were 78%, 50% and 27% in PET/MRD -/-, PET/MRD -/+ and PET+ groups, respectively (P=0.015 for all groups, and P=0.067 between PET/MRD -/- and PET/MRD -/+). We also stratified the patients into 2 groups (PET-/MRD- *vs.* PET+ or MRD+), and the achievement of both PET and MRD negativity was associated to a better outcome (HR 3.42, 95%CI 1.31-8.95, *P*=.012), with a 5-yr PFS of 75% (95% CI 54 to 87%) and 35% (95% CI 11 to 60%) for PET/MDR -/- and PET+, respectively (Figure 2).

To the best of our knowledge, this is the first report combining the information of PET and MRD at the end of the induction treatment in FL patients. Although this is a small subset of a large trial, the present results can provide some insights for future prospective trials.

The results showed that PET and MRD are not strongly correlated with each other, and can be used as complementary techniques at the end of therapy. PET is more accurate in assessing nodal disease, but has important limitations in bone marrow analysis because BM involvement in FL is usually diffuse and low volume. In contrast, MRD analysis describes disease at BM level and can reach a very high sensitivity of up to 10^{-5}. The small study sample represents a major limitation of this research, and is due to its retrospective nature and the established inclusion criteria; MRD analysis was a planned procedure in the FOLL05 trial, but a molecular marker was only available in about 60% of patients.[5] When FOLL05 was designed, PET was not acknowledged as a recommended procedure for staging and response assessment in FL, thus it was not included among the planned study procedures; however, it was performed at physician discretion in a substantial proportion of cases.[9] In addition while FDG avidity is almost universally present in FL, with current PCR techniques using both major and minor breakpoint sites for *BCL2/IGH* MRD analysis, as done in the present study, only around 50-60% of patients can be studied. This rate could be improved with better methods and technologies (VDJ region analysis or rarer breakpoint regions of *BCL2/IGH* chromosomal translocation). Although conducted on a small set of patients, the strength of this study is the use of a blinded central review of FDG-PET scans, the use of Deauville criteria and of a dedicated central lab for MRD analysis.

Over the last number of years, the concept that tumor cells undergoing apoptosis or necrosis release cell-free circulating DNA (cfDNA) into the blood, enabled the use of whole exome sequencing ("next-generation sequencing technologies" – NGS) to detect tumor presence from blood samples. Recently Roschewski *et al*. used this technology to monitor response in 126 patients with diffuse large B-cell lymphoma, and showed that the presence of detectable cfDNA during surveillance was associated with a higher risk of lymphoma progression compared with that of patients with undetectable circulating tumor DNA.[10] This new tool, named "liquid biopsy", and the use of peripheral blood might further improve MRD studies in FL.

In conclusion, although conducted on a small series of patients, this study shows that combining both EOT FDG-PET and MRD analysis in patients with FL may improve our ability to predict the risk of progression, and provide the rationale to design response adapted trials in FL to tailor post-induction therapy to the real risk of

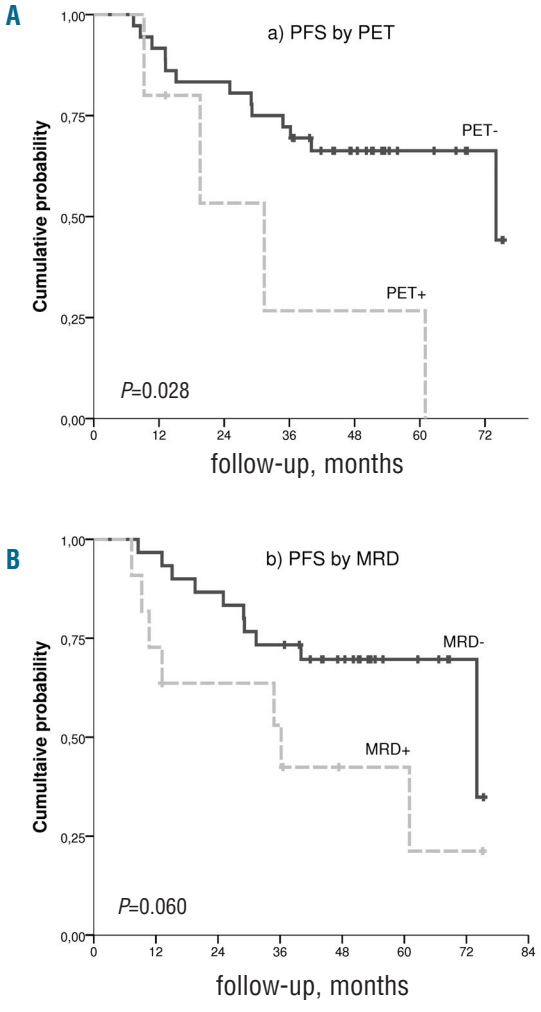

Figure 1. (A) PFS by PET. (B) PFS by MRD.

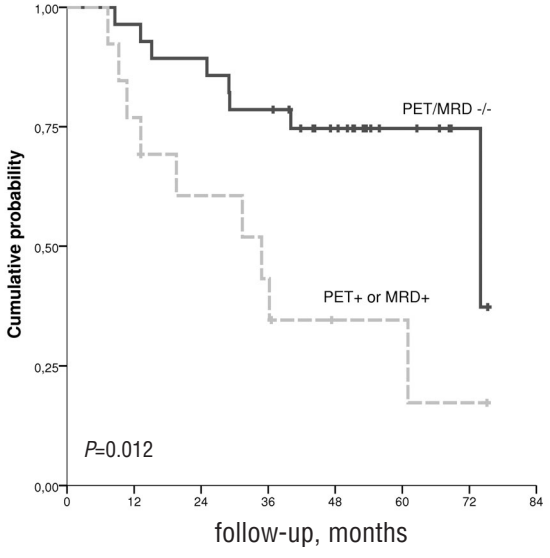

Figure 2. PFS according to combination of PET and MRD results.

relapse. Based on these results, the Fondazione Italiana Linfomi (FIL) planned the FOLL12 trial to investigate the efficacy of a response-adapted strategy, using EOT PET and MRD studies in patients with FL (*ClinicalTrials.gov Identifier: NCT02063685*). In the trial all patients receive 6 cycles of R-CHOP or R-bendamustine followed by 2 additional doses of rituximab. All responsive patients in the standard arm are treated with standard 2 years of maintenance with rituximab. Responding patients in the experimental arm receive post-induction therapy based on PET and MRD results: PET- patients do not receive maintenance, but are treated with pre-emptive rituximab therapy if MRD+; PET+ positive patients receive as consolidation treatment a ^{90}Y-ibritumomab tiuxetan dose prior to conventional rituximab maintenance.

Stefano Luminari,[1] Sara Galimberti,[2] Annibale Versari,[3] Irene Biasoli,[4] Antonella Anastasia,[5] Chiara Rusconi,[6] Angela Ferrari,[7] Mario Petrini,[2] Martina Manni[1] and Massimo Federico[1]

[1]Department of Diagnostics, Clinical and Public Health Medicine, University of Modena and Reggio Emilia, Italy; [2]Department of Internal and Experimental Medicine, University of Pisa, UO Hematology, Italy; [3]Department of Nuclear Medicine, Arcispedale S. Maria Nuova - IRCCS of Reggio Emilia, Italy; [4]Department of Medicine, University Hospital and School of Medicine, Universidade Federal do Rio de Janeiro, Brazil; [5]Hematology Unit, Spedali Civili di Brescia, Italy; [6]Division of Hematology, Department of Hematology and Oncology, Niguarda Hospital, Milan, Italy; and [7]Hematology Unit, Arcispedale Santa Maria Nuova, IRCCS of Reggio Emilia, Italy

Correspondence: sluminari@unimore.it
doi:10.3324/haematol.2015.132811

Key words: follicular lymphoma, minimal residual disease, nuclear medicine, FDG-PET, progression-free survival.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Freedman A. Follicular lymphoma: 2014 update on diagnosis and management. Am J Hematol. 2014;89(4):429-436.
2. Tan D, Horning SJ, Hoppe RT, et al. Improvements in observed and relative survival in follicular grade 1-2 lymphoma during 4 decades: the Stanford University experience. Blood. 2013;122(6):981-987.
3. Trotman J, Luminari S, Boussetta S, et al. Prognostic value of PET-CT after first-line therapy in patients with follicular lymphoma: a pooled analysis of central scan review in three multicentre studies. The Lancet Haematology. 2014;1(1):e17-e27.
4. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. J Clin Oncol. 2007;25(5):579-586.
5. Galimberti S, Luminari S, Ciabatti E, et al. Minimal residual disease after conventional treatment significantly impacts on progression-free survival of patients with follicular lymphoma: the FIL FOLL05 trial. Clin Cancer Res. 2014;20(24):6398-6405.
6. Federico M, Luminari S, Dondi A, et al. R-CVP versus R-CHOP versus R-FM for the initial treatment of patients with advanced-stage follicular lymphoma: results of the FOLL05 trial conducted by the Fondazione Italiana Linfomi. J Clin Oncol. 2013;31(12):1506-1513.
7. Gribben JG, Neuberg D, Freedman AS, et al. Detection by polymerase chain reaction of residual cells with the bcl-2 translocation is associated with increased risk of relapse after autologous bone marrow transplantation for B-cell lymphoma. Blood. 1993;81(12):3449-3457.
8. Buchonnet G, Lenain P, Ruminy P, et al. Characterisation of BCL2-JH rearrangements in follicular lymphoma: PCR detection of 3' BCL2 breakpoints and evidence of a new cluster. Leukemia. 2000;14(9):1563-1569.
9. Luminari S, Biasoli I, Arcaini L, et al. The use of FDG-PET in the initial staging of 142 patients with follicular lymphoma: a retrospective study from the FOLL05 randomized trial of the Fondazione Italiana Linfomi. Ann Oncol. 2013;24(8):2108-2112.
10. Roschewski M, Dunleavy K, Pittaluga S, et al. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. Lancet Oncol 2015; 16:541-549.

© Ferrata Storti Foundation