W32: Assessment of molecular response using real-time quantitative polymerase chain reaction (RT-qPCR) for MYD88 (L265P) in patients with Waldenström's macroglobulinemia treated with immunochemotherapy

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Background. Several studies have shown that the attainment of minimal residual disease (MRD) negativity, evaluated either by molecular methods or flow citometry, is associated with a longer progression-free survival (PFS) in many lymphoproliferative disorders and in multiple myeloma. The high prevalence of the MYD88 (L265P) somatic mutation in patients with Waldenström's Macroglobulinemia (WM) makes it a suitable marker for the assessment of molecular response after treatment.

Aims. The aims of the study were: i) to evaluate the rate of complete molecular responses after treatment with Rituximab in combination with nucleoside-based or alkylating-based chemotherapy; ii) to evaluate whether the rate of MRD negativity is different according to the type of treatment; iii) to evaluate whether the molecular response correlates with clinical response to treatment; iv) to evaluate whether the attainment of a complete molecular response MRD is associated with a longer progression-free survival (PFS).

Methods. All WM patients treated with immunochemotherapy at the Division of Hematology of Policlinico San Matteo, for whom baseline and post-treatment bone marrow samples were available, were included in this study. The MYD88 (L265P) mutation was assessed in CD19+ bone marrow mononuclear cells using real-time quantitative polymerase chain reaction (RT-qPCR). Cell lines OCI-LY19 (MYD88 wt) and OCI-LY3 (MYD88 mutated, L265P) were used to construct two different standard curves by dilution series of 7 different concentrations ranging from 40 ng/µl to 0.08 ng/µl corresponding to allele burdens ranging from 100% to 0.5%. Allele burden quantification was performed by the ratio MYD88 L265P mutant/MYD88 mutant and wild-type alleles. Clinical response was assessed according to the VI International Workshop on Waldenström's Macroglobulinemia (IWWM).

Results. Of 35 patients with BM samples collected before and after immunochemotherapy, 33 harbored the MYD88 (L265P) mutation at baseline, with a median allele burden of 36.6% (range: 1.0 - 97.03). The remaining 2 patients were MYD88 wild-type before treatment and therefore were excluded from further analysis. Of the 33 MYD88-mutated patients, 19 were treated with Rituximab (R) plus nucleoside analogs (R-Bendamustine, n=17 or FCR, n=2) and 14 with Rituximab combined with alkylating agents (R-CP, n=10, R-CVP n=3, R-CHOP n=1). Immunochemotherapy was administered as first line treatment in 27/33 (82%) patients, and as second or further line of treatment in 6/33 (18%). The median reduction of MYD88 allele burden with respect to baseline was - 99,5% (interquartile range, IQR -100 to -88.9). In 19/33 MYD88-mutated patients (58%) the mutation was undetectable after treatment. The rate of MRD negativity was similar in patients treated with Rituximab plus nucleoside-based chemotherapy and in patients treated with Rituximab plus alkylating agents (63% versus 50%, P = 0.5). Undetectable MRD was more commonly found in patients achieving a clinical complete remission (CR) or a very good partial response (VGPR) as compared with patients in partial response (PR) or stable disease (SD) after treatment (P = 0.003). With a median follow-up of 27 months (range: 6.2 – 155.5) from the start of immunochemotherapy, there was a trend toward a better PFS for patients attaining a complete molecular response after treatment (P= 0.07).

Conclusions. This study shows that more than half of the WM patients treated with immunochemotherapy attains a complete molecular response, which strongly correlates with a deep clinical response. Patients attaining a complete molecular response seem to have a better progression-free survival, but longer follow-up is needed to confirm this trend.