First comparison between multicolor flow cytometry and droplet digital PCR for tumor burden quantification at baseline in mantle cell lymphoma

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Introduction

Quantification of tumor load at diagnosis has been shown to provide an additional prognostic tool, in mature lymphoproliferative disorders.[1] Multiparameter Flow Cytometry (MFC) is the most commonly used method to assess the degree of tumor infiltration at baseline. However, inter-laboratory standardization still needs to be fulfilled before MFC can be implemented in multicenter trials. Droplet digital PCR (ddPCR) represents a feasible alternative, effortless to standardize and potentially able to overcome some MFC drawbacks.

Aims

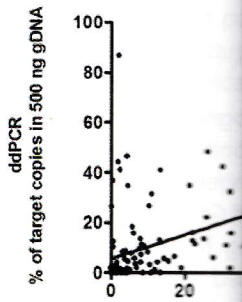
To compare the reliability of ddPCR versus MFC for tumor quantification at baseline, in a phase III, multicenter clinical trial for mantle cell lymphoma (MCL) patients.

Methods

ddPCR was performed in MCL patients enrolled in the "MCL0208" trial (EUdract:2009-012807-25) by FIL (Fondazione-Italiana-Linfomi). Quantification of IGH-VDJ by ddPCR was done (with the same allele-specific primers and consensus probes used in qPCR), using the QX100 ddPCR system (Bio-Rad) as described.[2] 500 ng of gDNA were loaded in triplicate, a negative control (gDNA pooled from 10 healthy donors) and NTC were included. The final tumor load was calculated as the merge of replicates. MFC was performed by a 6-color panel for BM (K/L/CD19/CD23/CD5) and PB (also CD22/CD20/CD43/CD200) on FACSCantoII (Becton Dickinson). qPCR was based on serial 10-fold dilution standard curves, starting from 500ng gDNA, using a AbiPrism7900HT (Life Technologies), according to Euro-MRD guidelines.[3] Methods comparison was assessed using bivariate Pearson's correlation and results were considered discordant when difference in clonal cells quantification was ≥1 log.

Results

The comparison, MF
64 MCL patients, 64 Bone
moderate correlation bet
the samples based on MF
100%), superimposable re
target copies, respectivel
Moreover, discordances we
patients or tissues. Notabl
> MFC, were confirmed by
shipment modalities in a
(8/33: 1 mid, 7 high) qPCR
cases (17/33, 52%, 7 low, 9
we cannot rely on qPCR da



Conclusion

This study represents
load quantification in a r
methods. Accurate guideli
can be implemented in th
simplicity and the potentia
load quantification in thos

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