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# 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.<sup>1</sup> 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

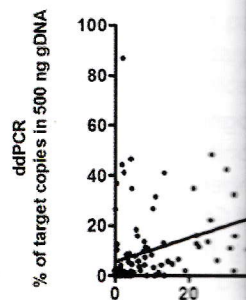
To compare the reliability of ddPCR versus MFC for tumor quantification at baseline, in a phase III, multicenter clinical trial for mantle cell lymphoma (MCL) patients.

## 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.<sup>2</sup> 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 FACSCantoll (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.<sup>3</sup> Methods comparison was assessed using bivariate Pearson's correlation and results were considered discordant when difference in clonal cells quantification was  $\geq 1$  log.

## Results

The comparison, MFC vs ddPCR, in 64 MCL patients, 64 Bone Marrow (BM) samples, showed a moderate correlation between the samples based on MFC (r=0.45, p<0.001, 100%), superimposable results for target copies, respectively. Moreover, discordances were observed in 10 patients or tissues. Notably, 8/33 (> MFC), were confirmed by MFC. Shipment modalities in a phase III trial (8/33: 1 mid, 7 high) qPCR cases (17/33, 52%, 7 low, 9 high) we cannot rely on qPCR data.



## Conclusion

This study represents a comparison of tumor load quantification in a phase III trial. Accurate guidelines can be implemented in the future, given the simplicity and the potential of ddPCR for tumor load quantification in those patients.

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