W1: MYD88 p.(L265P) detection on cell-free DNA in plasma and cerebrospinal fluid

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Patients with Waldenström Macroglobulinemia (WM) carry a specific hotspot mutation of the MYD88 gene, the p.(L265P) mutation, in the majority of cases (~90%)[1]. Detection of this mutation is already used for diagnosis of WM, as well as for targeted treatment (e.g. BTK inhibitors)[2]. In Bing Neel syndrome, WM cells enter the cerebrospinal fluid (CSF) and central nervous system (CNS). This is a rare but potentially fatal disease for which early diagnosis is paramount. Lumbar puncture is a less burdensome procedure than a brain biopsy, but current CSF parameters such as cytomorphology and flow cytometry are lacking in both sensitivity and specificity. We have already shown that MYD88 p.(L265P) can be detected on *cellular DNA* in CSF to diagnose patients with Bing Neel syndrome[3, 4]. In cases with little shedding of tumor cells in CSF, analysis of *cell-free DNA* (*cfDNA*) can increase sensitivity of mutation analysis. Furthermore, it would be very interesting to see if MYD88 p.(L265P) can be detected on *cfDNA* in plasma of WM and Bing Neel patients.

Therefore we have studied the detection of MYD88 p.(L265P) on both *cellular* as well as *cfDNA* in patients with primary central nervous system lymphoma (PCNSL), which carry the MYD88 mutation in ~85% of cases, to investigate if we can improve the diagnostic yield of CSF.

For this study we selected a total of 9 fresh CSF samples of 7 patients with PCNSL of which molecular analysis results were compared with routine parameters measured in CSF, such as cytomorphology and flow cytometry.

In addition, we collected 29 samples of frozen CSF material (20 PCNSL patients; 29 samples), which we analyzed for MYD88 p.(L265P) on *cfDNA* only.

Of the 9 fresh CSF samples, biopsy material was available in 7 cases, all positive for MYD88 p.(L265P). Six (67%) CSF samples tested positive for MYD88 p.(L265P) on *cfDNA*, compared to only 1 positive cases on *cellular* DNA. In none of the cases lymphoma cells were detected by cytomorphology and only 1 (11%) case displayed a clonal population by flow cytometry. This case was also positive for MYD88 p.(L265P) on *cellular* DNA and *cfDNA*.

Of the 29 frozen CSF samples, MYD88 p.(L265P) was detected on *cfDNA* in 5 out of 17 (38%) samples at diagnosis, in contrast to 2 out of 12 (17%) samples during treatment. In these CSF samples, amount of DNA was clearly less compared to the fresh CSF samples, which has probably been a limitation for MYD88 p.(L265P) detection.

Finally, we were able to detect MYD88 p.(L265P) in 3 plasma samples of PCNSL patients, collected during treatment, but this was too few for response assessment.

In conclusion, MYD88 mutation analysis is feasible on *cfDNA* in CSF and plasma. This type of liquid biopsy analysis could prevent an invasive tissue biopsy, is of added value compared to routine parameters and might be used for follow-up in the near future.

References

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