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Prognostic impact of meningeal dissemination in primary CNS lymphoma (PCNSL): experience from the G-PCNSL-SG1 trial

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Background: We evaluated the frequency and prognostic impact of meningeal dissemination (MD) in immunocompetent adult patients with primary central nervous system lymphoma treated in a randomized phase III trial.
Patients and methods: MD was evaluated at study entry and defined by lymphoma proof in the meningeal compartment detected by at least one of the following methods: cerebrospinal fluid (CSF) cytomorphology, detection of clonal B cells by IgH PCR in CSF or contrast enhancement of the leptomeninges on magnetic resonance imaging (MRI).
Results: Data on MD were available in 415 patients, of those, MD was detected in 65 (15.7%): in 44/361 (12.2%) by CSF cytomorphology, in 16/152 (10.5%) by PCR and in 17/415 (4.1%) by MRI. Major patients' characteristics and therapy did not significantly differ between patients with MD (MD+) versus those without MD (MD–). There was a significant correlation of MD with CSF pleocytosis (>5/μl; $P < 0.0001$), but no correlation with CSF protein elevation (>45 mg/dl). Median progression-free survival was 6.7 months [95% confidence interval (CI) 0–14.5] in MD+ and 8.3 months (5.7–10.8) in MD– patients ($P = 0.95$); median overall survival was 21.5 months (95% CI 16.8–26.1) and 24.9 months (17.5–32.3), respectively ($P = 0.98$).
Conclusion: MD was detected infrequently and had no impact on outcome in this trial.
Key words: cerebrospinal fluid, meningeal dissemination, primary CNS lymphoma

introduction

Primary central nervous system lymphoma (PCNSL) usually manifests as an intracerebral mass. The incidence of meningeal dissemination (MD) in PCNSL is not exactly known. A wide frequency range of 7%–42% has been reported, depending on diagnostic methods applied, number of cerebrospinal fluid (CSF) examinations and inclusion of both newly diagnosed and recurrent patients in the analysis [1–12]. An optimal diagnostic CSF work-up has not been established in PCNSL with CSF cytomorphologic evaluation still being regarded the gold standard. In studies comparing CSF cytomorphology with other diagnostic methods, a strikingly high rate of discordant results was observed [13, 14].

The prognostic impact of MD in PCNSL has not been sufficiently evaluated either. Whereas no impact of cytomorphologically confirmed MD has been reported in several trials [1, 2, 8, 10, 11], a negative impact was found by others [7, 14]. Due to the rarity of PCNSL and the relatively low frequency of MD diagnosed by routine methods, it seems probable that the series published thus far were too small to detect significant differences in outcome between MD+ and MD– patients. Moreover, the retrospective analyses including larger patients' numbers might have been hampered by heterogenous treatments frequently not containing high-dose methotrexate (HDMTX). The present analysis is the first to evaluate MD on a large prospective cohort treated within one protocol.

methods

patients and treatment

As previously reported [15], 526 eligible patients with newly diagnosed PCNSL were enrolled at 75 centers and treated between May 2000 and May 2009. Inclusion criteria were immunocompetent adult patients with histologically or cytologically (in CSF) confirmed PCNSL, Karnofsky performance score (KPS) >30 when due to PCNSL or >50 when due to other conditions, creatinine clearence ≥50 ml/min and written informed consent. Patients were randomly allocated to receive first-line chemotherapy based on HDMTX with or without subsequent whole-brain radiotherapy (WBRT), with stratification by age (<60 versus ≥60 years) and institution (Berlin versus Tübingen versus all other sites). Between May 2000 and August 2006, study therapy consisted of HDMTX (4 g/m^2 as a 4-h i.v. infusion with dose reduction according to creatinine clearence) on day 1 of six 14-day cycles; thereafter, patients were to receive HDMTX plus ifosfamide (1·5 g/m^2) on days 3–5 of six 14-day cycles. In those assigned to receive first-line chemotherapy followed by radiotherapy, WBRT was to be given at a total dose of 45 Gy. Patients allocated to first-line chemotherapy without WBRT who had not achieved complete response were given high-dose cytarabine (2 × 3 g/m^2 on days 1–2 of 22-day cycles). Intrathecal chemotherapy was not included in the treatment protocol.

The study protocol was approved by local institutional review boards or ethics committees. All participants gave written informed consent.

Preliminary data concerning CSF diagnostics in 116 [16] and 282 patients [13] included in the G-PCNSL-SG1 trial have been published before.

CSF analysis

CSF was obtained by single lumbar puncture before treatment and evaluated using standard methods for cell count, cytomorphology and protein immediately after sampling at the treating institution. Each sample was interpreted by an experienced hematopathologist or neuropathologist. Normal values were defined as follows: CSF cells ≤5/μl and CSF protein ≤45 mg/dl. CSF cytomorphology was termed positive only for conclusive detection of lymphoma cells. Patients with suspicious cytomorphology were regarded negative.

PCR was carried out in patients with B-cell lymphoma only. A sample of 1.5–5 ml (median 3.5 ml) of native CSF was sent over night at room temperature to a central molecular biological laboratory. Here, DNA was extracted from cell pellets after centrifugation at the day of arrival using commercially available kits (Qiagen; Qiagen, Hilden, Germany) and stored at room temperature until PCR analysis. All DNA extracts were controlled for their suitability as templates for IgH PCR by determination of quantity (NanoDrop; Thermo Fisher Scientific, Wilmington, DE) and quality (quality control PCR) [17]. Until March 2006, PCR was carried out as described previously [6]. Thereafter, PCR was carried out according to the BIOMED-2 protocol [17]. In each PCR run, positive (DNA from a B cell line) and negative controls (sterile water) and polyclonal (tonsilar DNA) controls were included. All specimens were amplified at least twice in independent PCR runs; the products were subjected to an automated fluorescent fragment analysis (GeneScan; ABI3130). A monoclonal pattern

was defined as single or dominating amplicon of identical size in repetitive experiments, whereas multiple peaks characterized polyclonality.

neuroimaging

Brain magnetic resonance imaging (MRI) was obtained before treatment and locally reviewed by an experienced neuroradiologist. All examinations included T1- and T2-weighted sequences and contrast-enhanced studies on various MR scanners with field strengths of 1.0–1.5 T. MD on MRI was defined as contrast enhancement of the leptomeninges.

definition of MD

Patients were regarded having MD (MD+) if at least one of the following conditions was fulfilled: positive CSF cytomorphology, monoclonal IgH PCR pattern or typical contrast enhancement on MRI. Patients without contrast enhancement and no data on CSF examination were classified as MD not determined.

statistics

For statistical analysis, patient pretherapeutic characteristics were grouped according to prognostic factors previously published: age, KPS, the Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic score (class 1 patients <50 years, class 2 patients ≥50 and KPS ≥ 70, class 3 patients ≥50 and KPS < 70) [11], lactate dehydrogenase (LDH) in serum, number of brain lesions (0–1 and ≥2) and steroids before diagnostics. Whereas in the descriptions always three groups (MD+, MD−, MD status unknown) are presented, the tests of significance were carried out only between MD+ and MD− patients. Progression-free survival (PFS) was defined as the time from study entry to first progression or death from any cause. Overall survival (OS) was defined as the time from study entry to death. PFS and OS were estimated by the Kaplan–Meier method. Group comparisons were carried out using the log-rank test. Distribution of patients characteristics to different groups was analyzed by the chi-square test. MD status and CSF pleocytosis or elevated CSF protein were compared by Fisher's exact test. Mean values of independent groups were compared with Student's *t*-test. The level of significance was 0.05 (two-sided). This was an exploratory analysis, thus, no correction for multiple testing was applied. Under this restriction and taking unequal group sizes into account, standardized differences (mean difference/standard deviation) of 0.38 and differences in proportions of 15%–20% could be shown with a power of 80%. With 247 events for OS and 293 events for PFS, hazard ratios of 1.60 and 1.53, respectively, could be detected with a power of 80%. Commercially available software was used (SPSS for Window, release 18.0).

results

patient characteristics

Data on MD were available from 71 centers: on CSF in 365 patients (69.4%) of whom 361 (68.6%) were evaluated by CSF cytomorphology, and 152 (28.9%) by PCR. Data on MD on MRI were available for 415 (78.9%) patients. Data on CSF protein levels were available in 304 (57.8%) patients and on CSF cell count in 290 (55.1%) patients.

Major patient characteristics and therapy are presented in Table 1. No significant difference between MD+ and MD− patients was observed for any parameter. When initial therapy was compared, MD+ patients were treated more frequently with HDMTX as monotherapy and significantly less frequently with HDMTX/IFO than MD− patients ($P = 0.05$).

detection of MD

CSF cytomorphology was positive in 44 of 361 (12.2%), suspicious in 23 (6.4%) and negative in 294 (81.4%) patients. PCR was monoclonal in 16 of 152 (10.5%) and polyclonal in 102 of 152 (67.1%) patients; no IgH-specific amplification products were found in 34 (22.4%) patients. Table 2 shows the comparison of PCR results with those of cytomorphologic CSF examination as a reference. Of 17 patients with positive CSF cytomorphology and PCR results available, a monoclonal PCR product was detected in only 5. Conversely, of 82 patients with inconsistent or negative CSF cytomorphology, a monoclonal IgH PCR product was found in 10. Of 15 patients with monoclonal B-cell populations and data on CSF cytomorphology, positive cytomorphology was found in only 5, whereas of 84 patients lacking B-cell clonality after IgH PCR, 12 were cytomorphologically positive.

Leptomeningeal enhancement was present in 17 patients (4.1%) and absent in 398 (95.9%). Table 3 shows the comparison of MRI with cytomorphologic findings. Of 14 patients with MD on MRI and data on CSF cytomorphology, only 7 were cytomophologically positive; of 34 patients with positive CSF cytomorphology and MRI results available, only 7 had MD in MRI.

Considering any of the three methods used, MD was detected in a total of 65 of 415 patients (15.7%).

Elevated CSF protein was found in 77.8% of MD+ patients and in 67% of MD− patients ($P = 0.22$). Elevated CSF cell count was significantly more frequent in MD+ patients: 69.6% versus 39.7% ($P < 0.0001$).

outcome

The median follow-up of all patients was 50.7 months.

Median PFS in the MD+ group was 6.7 months [95% confidence interval (CI) 0–14.5] versus 8.3 months (95% CI 5.7–10.8) in MD− patients ($P = 0.95$) and in those with undetermined MD status 4.5 months (95% CI 2.1–6.9) (Figure 1A). No significant differences were found in responders to HDMTX-based primary chemotherapy (complete and partial remission) for PFS when MD+ patients were compared with MD− within one treatment arm (WBRT and no WBRT). MD+ patients with WBRT had a median PFS of 33.8 months as compared with 20.2 months in patients without WBRT; this difference, however, was not significant ($P = 0.351$).

OS was not affected by treatment arm in the G-PCNSL-SG1 trial; thus, patients were combined for OS analysis. Median OS of MD+ and MD− patients was 21.5 months (95% CI 16.8–26.1) versus 24.9 (17.5–32.3) ($P = 0.98$) and that of undetermined MD status 15.9 months (95% CI 8.0–23.7), respectively (Figure 1B).

Patients with positive CSF cytomorphology had a median PFS of 5.9 months (1.4–10.4) and those without MD on cytomorphologic CSF examination 8.3 (5.6–10.99) ($P = 0.89$); the median OS was 20.5 months (14.8–26.3) and 24.1 months (17.4–30.8), respectively ($P = 0.64$) (supplemental Figure S1, available at *Annals of Oncology* online). PFS and OS were not significantly different in patients with elevated CSF cell count or elevated CSF protein as compared with those with normal CSF values.

Table 1 Patients' characteristics and therapy

Characteristics	Total no. of patients	MD undetermined	MD+	MD−	P (MD+ versus MD−)
No. of patients	526	161	65	300	
Age (years)					
Median/range		65/29–84	61/22–83	63/19–80	0.17
KPS	438	70	80	70	0.11
Male/female ratio	526	96/65	34/31	169/131	0.58
MSKCC score	452				
1	84	23 (19.8%)	17 (29.5%)	44 (15.8%)	0.13
2	218	57 (49.1%)	27 (46.6%)	134 (48.2%)	
3	150	36 (31%)	14 (24.1%)	100 (35.8%)	
LDH elevated	307	36 (37.9%)	13 (36.1%)	60 (29.1%)	0.31
No. of lesions	431				
0–1	262	80 (64%)	19 (54.3%)	163 (60%)	0.546
≥2 lesions	169	45 (36%)	16 (45.7%)	108 (40%)	
Symptoms					
None	51	14 (10.2%)	8 (13.6%)	29 (9.8%)	0.36
Headache	137	42 (33.6%)	13 (22.4%)	82 (28.7%)	0.42
Brain pressure	34	11 (9.2%)	2 (3.5%)	21 (7.4%)	0.39
Hemisymptoms	180	45 (36.6%)	16 (27.1%)	119 (41.2%)	0.06
Cognitive dysfunction[a]	242	67 (55.8%)	33 (55.9%)	142 (49.7%)	0.39
Cranial nerves	76	13 (11.1)	11 (19.6%)	52 (18.6%)	0.85
Cerebellar	75	18 (14.9%)	5 (8.6%)	52 (18.3%)	0.08
Epilepsia	53	16 (13.1%)	5 (8.6%)	32 (11.1%)	0.82
Organic psychosyndrome	198	51 (40.8%)	22 (37.3%)	125 (43.3%)	0.47
Steroids before diagnostics	526				
Unknown	130	39 (24.2%)	25 (38.5%)	66 (22%)	0.23
No	134	35 (19.9%)	17 (26.2%)	82 (27.3%)	
Yes	262	87 (54%)	23 (35.4%)	152 (50.7%)	
Therapy	526				
HDMTX versus	401	115 (71.4%)	57 (87.7%)	229 (76.3%)	0.05
HDMTX/IFO	125	46 (28.5%)	8 (12.3%)	71 (23.7%)	
Median total MTX dose (4 g per cycle = 100%, maximum 600%)		387%	492.5%	455%	0.71
					0.52
WBRT	245	77 (47.8%)	34 (52.3%)	134 (44.7%)	

[a]Clinically evident.

HDMTX, high-dose methotrexate; KPS, Karnofsky performance score; LDH, lactate dehydrogenase; MSKCC, Memorial Sloan-Kettering Cancer Center; MTX, methotrexate; WBRT, whole-brain radiotherapy.

Table 2 Comparison cerebrospinal fluid cytomorphology versus PCR

Cytomorphology	PCR monoclonal ($N = 16$)	PCR polyclonal ($N = 102$)	No amplification ($N = 34$)	No PCR ($N = 374$)
Positive ($N = 44$)	5	12	2	25
Negative ($N = 317$)	10	72	27	208
Unknown ($N = 165$)	1	18	5	141

discussion

The MD frequency in this analysis was relatively low and comparable to 7%–29% previously found by cytomorphologic CSF examination alone in smaller series [2–12, 18, 19]. However, in a study with repeated CSF examination and meningeal biopsy, a much higher MD frequency of 42% was detected [1]. In that study, all patients with pathological evidence of meningeal infiltration had negative CSF cytomorphology indicating that the absence of malignant lymphocytes on a CSF specimen does not exclude the presence of meningeal infiltration. Autopsy studies reveal that parenchymal lesions of PCNSL are always in contact with either leptomeninges or the ependymal surface [20, 21]. This data suggest that methods routinely used for MD detection in PCNSL may underestimate this condition. Pretreatment with steroids in many of our patients—similar to other studies—might additionally have hampered the MD diagnostics; however, the proportion of patients on steroids was not significantly different in MD+ and MD− patients (Table 1).

Diagnostic methods for MD detection used in this study are nowadays routinely used in lymphoma patients by many clinicians; however, their value has not been clearly defined. In accordance with our previous analyses [13, 14], a remarkably high rate of discordant findings between the three diagnostic methods used were found. In lymphoma, false-positive CSF

cytomorphology results might be caused by the misinterpretation of reactive lymphocytes as lymphoma cells, whereas false-negative results might be due to the paucity of tumor cells. PCR is frequently difficult to perform in PCNSL as compared with systemic lymphoma due to its high mutational frequency preventing annealing of primers [22, 23]. The relatively low sensitivity of neuroimaging for MD detection in malignant lymphoma is well known. Moreover, spinal neuroimaging has not routinely been carried out.

Immunocytologic CSF evaluation was not routinely carried out in this trial. The study was designed in 1999 when it was not considered feasible in the context of a nation-wide multicenter trial. CSF flow cytometry was reported to detect MD in systemic lymphoma more frequently than CSF cytomorphology; however, the prognostic impact of a positive finding is less clear [24–26]. In a monocenter cohort, MD was detected by CSF flow cytometry in only one of 32 PCNSL patients evaluated; however, >50% of these patients were on steroids at the time of analysis [27]. The diagnostic and prognostic value of CSF immunophenotyping in PCNSL still remains to be defined.

No significant association of MD with any clinical characteristic tested was found in this study. Moreover, clinical symptomatology did not differ between MD+ and MD− patients indicating that it is no guide to the presence of MD.

CSF protein elevation was not associated with MD in this trial. This is in contrast to a previous study where elevated CSF protein was significantly associated with leptomeningeal tumor [1]. We found a significant association with CSF pleocytosis, which may reflect the association of meningeal compartment involvement with higher tumor burden. This finding is in accordance with our previous results [13, 16].

We found impact neither of CSF protein elevation nor CSF pleocytosis on survival. This is in contrast to two retrospective analyses reporting CSF protein elevation as a negative prognostic factor in PCNSL [2, 28]. Moreover, no impact on outcome for MD defined by any of the methods used alone or in combination was found which is consistent with the literature [1, 2, 8, 10, 11] and apparently does not support CSF-directed treatment in PCNSL. This is also suggested by two retrospective analyses in which no difference in survival was found between patients who received intrathecal chemotherapy in addition to systemic treatment and those who did not [7, 28]. On the other hand, treatment against the leptomeninges was significantly associated with longer freedom from relapse in a prospective study with 96 patients [1]. Moreover, an excellent outcome with a median OS of 50 months and 57% of patients <60 years being alive after a median follow-up of 100 months was achieved with a chemotherapy-only regimen including intraventricular chemotherapy (Bonn protocol) [29]. These results could not be reproduced when intraventricular chemotherapy was omitted [30]. Intraventricular chemotherapy in the Bonn protocol was much more intensive than in the retrospective analyses reporting no impact of CSF treatment on outcome, suggesting that the different findings may be due to different dosing. All these data may indicate that MD can be present in more PCNSL patients than routinely identified and serve as a reservoir for lymphoma cells not adequately reached by systemic chemotherapy alone without intra-CSF treatment.

Table 3 Comparison CSF cytomorphology versus neuroimaging

Cytomorphology	Radiological MD, $N = 17$	No radiological MD, $N = 398$	No data, $N = 111$
Positive ($N = 44$)	7	27	10
Negative ($N = 317$)	7	264	46
Unknown ($N = 165$)	3	107	55

CSF, cerebrospinal fluid; MD, meningeal dissemination.

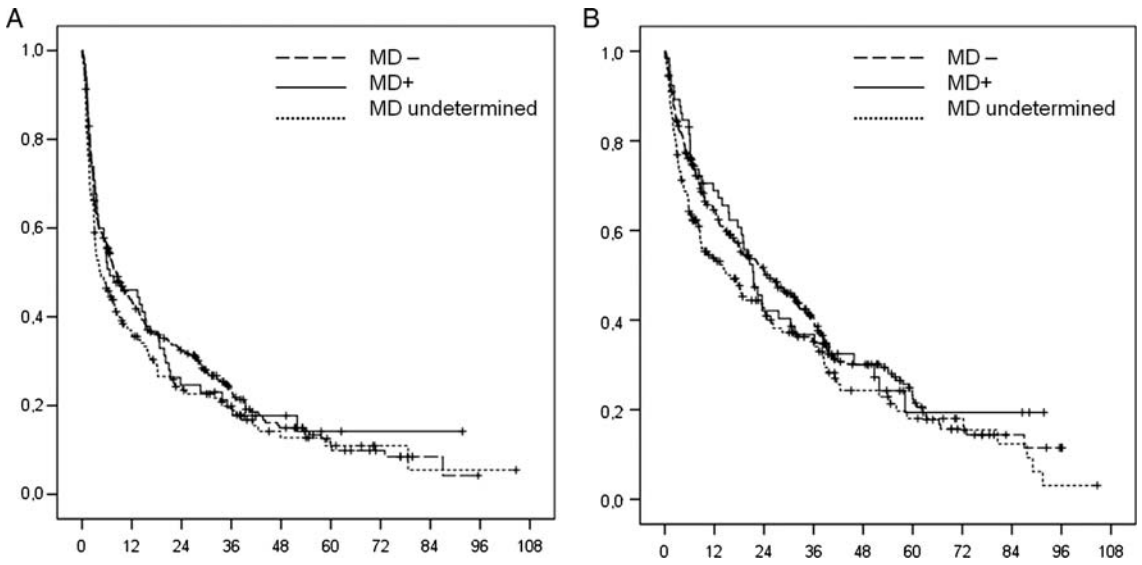

Figure 1. Progression-free survival (A) and overall survival (B) (in months) for patients without MD (MD−) versus those with MD (MD+) versus those with MD status undetermined. MD, meningeal dissemination.

Interestingly, in the present trial patients who did not have MD assessed had a relatively poor outcome. It may be speculated if this was due to their relatively high median age of 65 years or to the fact that the sickest patients were not tapped.

Lack of data on MD in a substantial proportion of our study patients represents a limitation of this analysis. Lumbar puncture was not carried out in this trial in patients with suspected elevated brain pressure and at patient's refusal. CSF examination by PCR was not obligatory and was not done in all centers. To minimize this bias, we compared major prognostic factors such as age, KPS, MSKCC, number of brain lesions and LDH in serum in MD−, MD+ and MD undetermined patients and found no significant differences between these groups, suggesting that our results are not biased by patients' selection. The lack of central MD diagnostics (except for IgH PCR) is a further limitation of this study. Lastly, pretreatment with steroids might have substantially impacted the outcome analyses in our study. Steroids are lymphotoxic and might have masked MD in some patients who might have been falsely classified negative and negatively impacted the outcome of the MD− group blurring the difference to MD+ patients. Nevertheless, no significant association between pretreatment with steroids and MD detection was found in this trial.

In summary, MD is infrequently detectable by current methods in PCNSL and did not impact survival in this trial. It can be speculated that better diagnostic tools and withholding steroids until the completion of the diagnostic work-up would help to diagnose this condition more accurately.

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disclosure

The authors declare no conflict of interest.

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Rituximab induction immunotherapy for first-line low-tumor-burden follicular lymphoma: survival analyses with 7-year follow-up

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Background: The purpose of this study was to report long-term results of rituximab induction monotherapy in patients with low-tumor-burden follicular lymphoma (LTBFL).
Patients and methods: Of 49 first-line LTBFL patients who received weekly doses of rituximab (375 mg/m^2), 46 have been followed with a long-term analysis of clinical and molecular responses.
Results: Best clinical response (at any staging within a year following treatment) was 80%, 24 (52%) patients had complete or unconfirmed complete response, 13 (28%) had partial response and 9 (20%) had stable or progressive disease. Of 31 patients having a positive bcl2-JH rearrangement, 15 (48%) became negative following treatment.After 83.9 months of follow-up (95% confidence interval 6.4–92.8 months), the median progression-free survival is 23.5 months and overall survival (OS) is 91.7%. Five patients died (one progression, one myelodysplasia, one diffuse large B-cell lymphoma and two solid tumors). Seven patients (15%) are progression-free including five who are bcl2 informative. No unexpected long-term adverse event has been observed.
Conclusion: A significant proportion of patients remain progression-free 7 years after a single 4-dose rituximab treatment in first-line LTBFL. The 7-year overall survivalOS is very high in this selected population of patients.
Key words: follicular lymphoma, induction monotherapy, low-burden, rituximab

introduction

Although a number of standard chemotherapeutic and immunotherapeutic agents are active in the treatment of patients with follicular lymphoma (FL), these treatments do not cure the patient. In patients with low-tumor-burden and without adverse prognostic factors, three randomized studies failed to show any survival benefit of chemotherapy compared with the observation [1–3]. Hence, watchful waiting is currently considered as the standard strategy, until clinical signs or symptoms warrant intervention [4, 5].

Rituximab (MabThera®) is a chimeric murine/human anti-CD20 monoclonal antibody capable of lysing CD20$^+$ lymphoma cells through multiple mechanisms of action,

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