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Short communication

Diffuse large B-cell lymphoma response to Rituximab-PMitCEBO regimen is not predicted by FcγRIIIa158 polymorphism

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Abstract. Introduction: The addition of rituximab to conventional chemotherapy has improved the treatment outcome in diffuse large B-cell lymphoma. Fc gamma receptor polymorphisms have been shown to influence rituximab mediated antibody dependent cellular cytotoxicity. The current study evaluated the impact of Fc gamma receptor IIIa (FcγRIIIa) polymorphism on the survival results of diffuse large B-cell lymphoma patients to Rituximab-PMitCEBO therapy. Material and methods: Thirty-nine patients, aged over 65 years, were involved, 11 (28%) men, 28 (72%) women. Their median age was 76,35 years. Genotyping of FcγRIIIa was performed using allele-specific polymerase chain reactions. Results: The FcγRIIIa polymorphism at the 158 aminoacid position, showed 14 (36%) carriers of VV and VF receptors and 25 (64%) of the homozygous FF receptor. There was no significant difference between the two groups in event free survival and overall survival. Conclusion: These results suggest that antibody dependent cellular cytotoxicity via FcγRIIIa may not be the main tumour cell killing mechanism when rituximab is associated to chemotherapy.

Key Words: diffuse large B-cell lymphoma, rituximab, Fc-gamma-receptor IIIa, polymorphism, survival.

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Introduction

Non-Hodgkin lymphomas (NHL) include an array of heterogeneous malignancies and the most common subset is diffuse large B-cell lymphoma (DLBCL), accounting for 30% to 40% of all NHL (Ahlgrimm et al 2011). DLBCL is a fast-growing and aggressive NHL, and its incidence has doubled over the past recent decades, with an increase that predominantly affects older patients (Fisher & Fisher 2004; Muller 2005).

However, treating elderly patients for DLBCL presents several constraints, particularly the inability of this group to tolerate chemotherapy. Dosage reductions and shortening of the period of treatment are important characteristics of the regimens developed for treating elderly patients, and this is the case for the PMitCEBO regimen (prednisolone, mitoxantrone, cyclophosphamide, etoposide, bleomycin and vincristine), which is an acceptable alternative to CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone), when the toxicity profile is preferable (Burton et al 2006). PMitCEBO has the advantage of containing about 30% less anthracycline than CHOP, an aspect relevant in elderly patients in whom the incidence of cardiac toxicity is higher. It is also given over a shorter treatment duration, induces less alopecia and less gastrointestinal disturbance (Burton et al 2006). In phase III trials, comparing CHOP, the standard treatment in DLBCL, with PMitCEBO, there were no significant differences in failure-free, progression-free and overall survival (OS) (Burton et al 2006). For the elderly patients, the three year OS for both chemotherapy regimens is around 45% (Burton et al 2006).

Rituximab (R), an anti-CD20 monoclonal antibody (mAb), in combination with chemotherapy, has improved the outcome of DLBCL patients (Coiffier et al 2002; Habermann et al 2006; Pfreundschuh et al 2006). However, there is only one trial of the Mabthera International Trial Group (MInT) that evaluated the impact of rituximab in combination with CHOP-like regimens (Pfreundschuh et al 2006). This trial assessed the role of rituximab in young patients, and the event-free survival (EFS) and OS were significantly increased. To our knowledge, there is no trial that evaluated the role of rituximab associated to PMitCEBO in older patients.

Among the different mechanisms of action assigned to rituximab, antibody dependent cellular cytotoxicity (ADCC) is believed to play the most important role in the B-cell killing effect of this anti-CD20 mAb (Renaudineau et al 2009). ADCC refers to the method used by effector cells (NK cells and monocytes) that express Fc gamma receptors (FcγR) to kill target cells coated by IgG. In rituximab's case, it could be an IgG1 Kappa mAb (Shields et al 2001). Expressed by NK cells and monocytes, FcγRIIIa has polymorphic alleles that correspond to phenotypic

expression of valine (V) or phenylalanine (F) at position 158 and can influence the binding of antibody (Dall'Ozzo et al 2004; Stevenson 2014). The therapeutic efficacy is improved when effector cells are homozygous for FcγRIIIa-158V as demonstrated in low grade NHL (Cartron et al 2002; Cornec et al 2012). Regarding DLBCL patients treated with rituximab plus CHOP, the prognostic significance of phenotypical characters of FcγRIIIa is subject of controversy (Fabisiewicz et al 2011; Kim et al 2006).The inconsistence is related to response to immunochemotherapy, but no study showed influence on survival (Kim et al 2006; Mitrovic et al 2007).

In this retrospective, single center study, our aim was to investigate if FcγRIIIa polymorphism has an impact on the therapeutic response and survival data in DLBCL patients, aged over 65 years and treated with R-PMitCEBO.

Material and methods

The cohort consisted of 39 patients, aged over 65 years, with newly diagnosed DLBCL according to the World Health Organization (WHO) classification (Swerdlow et al 2008) receiving R-PMitCEBO immunochemotherapy. Informed consent was obtained from all patients and the Institutional Review Board of Brest University Medical School Hospital approved the study. The clinical files were reviewed with particular reference to age, gender, Ann Arbor stage, International Prognostic Index (IPI), response to treatment and survival. OS was determined by consideration of death events due to any reasons and event free survival (EFS), by consideration of death events, relapses or disease progression that determined further treatment. Therapy response was evaluated by positron emission tomography (PET) according to Cheson et al (2007) revised response criteria. All patients underwent PET examination after 3 cycles and at completion, at six cycles. Complete remission (CR) was considered when all evidence of disease disappeared, partial remission (PR), when measurable disease regressed and no new PET positive sites appeared, progressive disease (PD), in case of new lesions or increase by $\geq$ 50% of previously involved sites and stable disease (SD), when CR, PR or PD were not attained. The patients were diagnosed between 2006 and 2011 and median follow-up was 23 months. Pre-treatment clinical features of patients according to FcγRIIIa polymorphisms are shown in Table 1. The patients were devised in two groups, VF/VV and FF, according to FcγRIIIa phenotype.

The BioSprint 15 DNA blood kit was used to extract genomic DNA from mononuclear cells isolated, according to manufacturer's instructions (Qiagen, Valencia, CA). Polymorphisms were determined as previously described (Tempescul et al 2015) using allele-specific polymerase chain reactions (PCRs) with one unmodified primer for FcγRIIIa-158V/F (ATATTTACAGAATGGCACAGG) and one 3′locked nucleic acid modified primer: GAAGACACATTTTTACTCCCAA+C for FcγRIIIa-158V versus CTCTGAAGACACATTTTTACTCCCAA+A for FcγRIIIa-158F. Amplifications were performed in a 25µL reaction with 100 nM dNTPs, 1.5 mM MgCl2, 2.5 units of Taq polymerase (Thermo Fisher Scientific, Waltham, MA), 20 ng genomic DNA, and 200 nM of each primer. The first cycle consisted of 5 min at 94°C followed by a first set of 5 cycles (30 s at 94°C, 40 s at 61°C, and 60 s at 72°C) and a second set of 30 cycles (30 s at 94°C, 40 s at 56°C and 60 s at 72°C) with a final 8 min elongation phase at 72°C. The specificity of these PCRs was verified by direct sequencing of the DNA products.

Fisher's test and t test were used to compare the two groups. OS and EFS were evaluated, using Kaplan-Meier analysis.

Results

Table 1. Participants demographic and clinical characteristics

	Total (n=39)	VV/VF (n=14)	FF (n=25)
Median age	76.35 (66-89)	79 (71-89)	76 (66-87)
Sex Male/Female	11/28	3/11	8/17
Stage Ann Arbor			
I-II	10 (26%)	5 (36%)	5 (20%)
III-IV	29 (74%)	9 (64%)	20 (80%)
IPI			
Low/Low intermediate	23 (70%)	14 (67%)	9 (75%)
High/High intermediate	10 (30%)	7 (33%)	3 (25%)

There were no significant differences regarding age, gender, stage and IPI between the two groups. Although no significant influence of FcγRIIIa polymorphisms on response to treatment was observed, the FF group was associated with a trend toward an inferior CR rate at the three months and six months PET examinations. At the end of immunochemotherapy, all the patients of the VV/VF group were in CR, but 4 (16%) of the patients from the FF group were in PR. Survival estimates showed no statistically significant differences in EFS and OS according to the genotype. EFS in the FF group was 71% (18 patients), vs. 64% (9 patients) in the VF/VV group (p=0.12). OS was 67% (17 patients) vs 71% (10 patients) in the FF and VV/VF groups, respectively (p=0.16).

Discussion

Therapeutic response and survival data of patients with DLBCL have improved significantly over recent years, but there are some patients who do not respond well or progress despite therapy. IPI is widely used to determine patients' prognosis but this score needs to be perfected (Sehn et al 2007). The level of CD20 expression on tumoral cells (McLaughlin et al 1998), low rituximab serum concentrations (Berinstein et al 1998), or the presence of high tumor burden (Coiffier et al 1998) may explain the lack of efficacy of rituximab in DLBCL patients. The causes of treatment failure are unknown, but it has been previously suggested that polymorphisms of the FcγRIIIa gene are associated with the response to rituximab. This polymorphism was shown to be predictive for rituximab monotherapy in NHL and Waldenström macroglobulinemia (Cartron et al 2002; Treon et al 2005; Weng & Levy 2003), but not in chronic lymphocytic leukemia (Farag et al 2004).

The focus of our study was to search for associations between FcγRIIIa polymorphisms and outcome of DLBCL patients, aged over 65 years, treated with R-PMitCEBO. To our knowledge there are five publications on the role of FcγRIIIa polymorphisms in DLBCL treated with R-CHOP, from which only one study on

Korean patients shows a significant association with the response to immunochemotherapy (Kim et al 2006). However, there are no studies on the elderly patients treated with R-PMitCEBO. Our data support the majority of the findings published on DLBCL R-CHOP treated patients by showing no interaction between FcγRIIIa polymorphisms and treatment outcome. Although not significant, these analyses indicate a trend toward an unfavorable outcome, regarding response to therapy and OS, for the FF group, under R-PMitCEBO therapy. This similar trend was reported for patients treated with R-CHOP in the largest study on the role of FcγRIIIa polymorphisms, but even though the sample size was important, the differences in outcome did not reach significance (Ahlgrimm et al 2011). One possible explanation is that polymorphisms in FcγRs affect response only when patients are treated with rituximab monotherapy and not immunochemotherapy (Fabisiewicz et al 2011). This might be due to a main tumor cell killing mechanism other than ADCC, when rituximab is associated to cytotoxic agents.

Conclusion

Our study demonstrates no association between FcγRIIIa polymorphisms and R-PMitCEBO treatment outcome suggesting that ADCC may not be the major mechanism of lymphoma cell killing on this group of patients. The new monoclonal antibodies engineered to overcome the differences in binding of their Fc parts, due to polymorphisms, seem not to offer an important benefit for DLBCL patients treated with immunochemotherapy.

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