Rituximab

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Rituximab (Rituxan®, MabThera®, Genentech/Roche) is a chimeric murine/human monoclonal IgG1k antibody directed against the CD20 antigen located at the surface of normal and malignant *B* lymphocytes.

In November 1997, FDA granted its approval and in June 1998 EMEA licensed the product for the treatment of relapsed refractory low-grade or follicular CD20 positive B cell non-Hodgkin's lymphoma (NHL). In February 2002, FDA authorized the use of rituximab as a component of the ibritumomab (Zevalin®) therapeutic regimen (see ibritumomab, Chap. 23). During 2006, the same Agency extended the use of rituximab in combination with methotrexate (MTX) as first therapy, to reduce signs and symptoms of moderately to severely active rheumatoid arthritis (RA) in adult patients who have had an inadequate response to one or more TNF antagonist therapies. The extension also included the use of this biomedicine in combination with CVP (cyclophosphamide, vincristine, and prednisolone) chemotherapy as first-line treatment of follicular CD20 positive B cell NHL. In 2008, the indications were further extended to include first-line treatment of B cell NHL in combination with CHOP (i.e., CVP+ adriamicine) or other anthracycline-based chemotherapy regimens. In February 2010, the extension to first-line therapy for CD20-positive chronic lymphocytic leukemia (CLL), in combination with fludarabine and cyclophosphamide (FC), was approved. During 2011 two new extensions were approved, concerning the use of rituximab as single-agent maintenance therapy in patients with previously untreated follicular, CD20-positive, B cell NHL who achieve a response to rituximab in combination with chemotherapy, and the use of rituximab in combination with glucocorticoids for the treatment of patients with Wegener's Granulomatosis (WG) and Microscopic Polyangiitis (MPA).

Between 2004 and 2010 EMEA granted similar extensions. In particular, the Agency approved the following rituximab uses: (i) combined with CVP

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chemotherapy, as first-line treatment, in patients with follicular lymphoma (2004); (ii) the use in RA patients resistant/intolerant to DMARDs and TNF therapy, in combination with MTX (2006); (iii) as first-line treatment of patients with CLL, in combination with chemotherapy (2008); (iv) for the treatment of advanced (stages III–IV) follicular lymphoma, in combination with any dedicated chemotherapy (2008); (v) for the treatment of patients with previously untreated and relapsed/refractory CLL (2009); and (vi) for the treatment of follicular lymphoma patients responding to induction therapy (2010).

However, the requests for extension to MTX-naive patients (as first-line treatment) and to MTX-IR patients (as second-line treatment) were not accepted. At present, rituximab has been approved in over 100 countries for most or all of the mentioned indications.

Pivotal trials for initial approval of NHL treatment were the Phase III controlled study 102-105 on 203 NHL patients, and supportive Phase I–II study 102–02, for a total of 240 enrolled patients. Six additional studies were presented: two completed Phase I–II studies, three Phase II ongoing studies, and one other study that was planned yet not implemented. Overall, 322 patients were evaluated for safety, and 306 for efficacy. Subsequent extension was supported by the several additional studies listed below:

NHL: (i) Pivotal Study EORTC20981 on 465 (334 exposed) patients, along with studies GLSG-FMC, SAKK35/98, and LYM-5 for rituximab maintenance therapy on about 500 patients; (ii) Study U0824 and ECOG1496, that enrolled 384 previously untreated patients; (iii) Main studies GLSH'00, OSHO-39, FL2000 and supportive studies from various Authors, for the extension in combination with any chemotherapy indicated for B cell follicular lymphoma on 987 (563 exposed) patients; and (iv) Study MO18264 (PRIMA) for maintenance therapy in 321 (162 exposed) patients responding to induction therapy.

DLBCL: Main studies SO15165, U071.5 s, and efficacy data from Study LNH98-5 for treatment in combination with CHOP on 486 patients.

CLL: Pivotal Study BO17072 on 546 patients, and supportive studies from various investigators for a total of 1564 (880 exposed) patients.

RA: (i) Pivotal WA17042 (REFLEX) on 520 (308 exposed) patients, and supportive studies WA16291 and WA17043 for a total of 585 patients; (ii) An extension of Study WA16291 continued in Study WA16855; (iii) IMAGE (WA17047) on 755 (513 exposed) patients, SERENE (WA17045) on 520 (209 exposed) patients, MIRROR (WA17044) on 377 exposed patients, and REFLEX extension study (WA17042D) on 468 patients. Studies, that supported the evaluation of rituximab when combined with various MTX regimen; and (iv) SUNRISE (U3384 g) and DANGER studies, that were included but not evaluated.

WG/MPA: The most recent approved indications are for WG, MPA, and ANCA-associated vasculopathy (AAV) group, and are based on data from the RAVE (NCT00104299) trial on 197 AAV patients.

Overall, a total of 3,587 NHL patients (739 treated, 1,427 for maintenance studies) were evaluated for NHL. CLL treatment was experienced on 1,564 patients (880 treated). RA studies were conducted in 2,385 patients (1,439 exposed), and

WG/MPA were analyzed on 197 patients [1–8]. At present, over 1,250 trials on various rituximab applications are completed, ongoing or recruiting.

35.1 Mechanism of Action

CD20 (Bp35) is a tetraspanning transmembrane human B lymphocyte-restricted differentiation antigen, located at the surface of normal and primate B lymphocytes and on human malignant B cells. During B cell maturation, this nonglycosylated hydrophobic phosphoprotein is first expressed on pre-B cells, but is lost during the final stage of maturation to plasma cells. In particular, CD20 is expressed in a subpopulation (about 30 %) of precursor B cells, in mature B lymphocytes, and in follicular dendritic reticulum cells. CD20 is also expressed by low-grade B cell NHL, precursor B cell neoplasms, precursor B-Lymphoblastic leukemia/lymphoma, (B-LBL), HCL, B-CLL (weak), and by B-PLL. It is also present in lymphoplasmacytic cells in Waldenström's macroglobulinemia, but is absent in myeloma and B cell ALL.

CD20 has a role in B cell activation/proliferation through the Src family tyrosine kinases, and enables optimal B cell immune response against T cell-independent antigens. Moreover, CD20 is also expressed in a minor population of T lymphocytes (2 %), pertaining to the memory cytotoxic compartment that tends to increase with age. It is also presumed that CD20 may act as a store operated calcium ion channel. However, no natural ligands are known, and no soluble CD20 forms have been detected.

Rituximab (formerly C2B8) is a glycosylated chimeric IgG1k murine/human monoclonal antibody directed against the human CD20 antigen. The antibody has murine light- and heavy-chain variable regions and human constant region sequences. Rituximab was developed from the fully murine parent ibritumomab, which recognizes the same epitope. The basic effector mechanisms of this antibody are related to the Fc portion, and include complement-dependent cytotoxicity (CDC) resulting from C1q binding, and antibody-dependent cellular cytotoxicity (ADCC), mediated by one or more of the Fcγ receptors at the surface of granulocytes, macrophages, and NK cells. However, rituximab is also able to induce cell death via apoptosis, yet it is not clear which of these potential aggressive mechanisms are prevalent/effective in vivo. Administration of rituximab induces a rapid and massive destruction of circulating and tissue-based normal and malignant B cells. In some studies, depletion was evaluated by assessing the presence of residual B cells by another marker (CD19) and resulted being over 95 %. However, rituximab has no effect on hemopoietic progenitor cells and T cells. In hematologic malignancies, B cell depletion in circulation and in tissue localizations occurs within 3 weeks, and recovery begins in 6 months, reaching normal values within one year after treatment. In RA patients, there is an almost total depletion of circulating B cells within 2 weeks of treatment, lasting about 6 months and reaching normal levels within one year in most patients. A delayed recovery occurs, over 3 years after treatment, in a subgroup of subjects (4 %).

Consequently, there is a reduction of both IgM and IgG serum levels, reaching concentrations below normal limits in 15–20 % of cases. A reduction of IgA was also observed in RA patients, being below normal levels in a minority of cases (<1 %). B cell depletion was also rapidly detectable in WG and MPA, and lasted up to one year [7, 8].

Other potential and less known depletion mechanisms are induced by rituximab. For example, rituximab is effective in restoring platelet levels in immune-mediated thrombocytopenia (ITCP), but CDC and ADCC depleting action on B cells seems not sufficient to explain its action, since levels of antiplatelet autoantibodies remain virtually unchanged in these patients, while the platelet count increases rapidly. Among the proposed additional involved mechanisms, there is a saturating binding effect of rituximab-coated B cells to macrophages via Fc receptors, which may compete with platelet phagocytosis actively occurring in ITCP [9].

In a recent overview on anti-CD20 mAbs, five mechanisms of action were recognized, and the therapeutic mAbs were grouped according to their capacity to induce the reorganization of CD20 molecules into lipid rafts upon binding [10]. In particular, Type I antibodies induce the formation of lipid rafts and efficiently activate the classical pathway of the complement system, while Type II antibodies do not translocate CD20 into lipid drafts, yet induce cell death upon direct binding, and poorly activate complement. Both types are capable of inducing ADCC in the presence of effector cells. Rituximab is a Type I mAb and expresses the cytolytic action mainly by ADCC via FcγR-expressing monocyte/macrophages, while CDC may play an additional role. In contrast, Type II mAbs, such as tositumomab, mostly act by direct apoptosis. These cytolytic functions may be differently expressed in various situations, being CDC more effective in circulation, where complement factors are highly represented, than on a solid tumor mass. Moreover, their efficacy varies in dependence of the tumor burden. In this case, multiple events are expected to be necessary in the presence of significant tumor masses. Further mechanisms are involved during therapy, such as the opsonization of mAb-covered targets mostly occurring as late effect. CD20 is not shed from the cell surface and is not endocytosed during the antibody binding [8]. However, Type I mAbs can be internalized when bound to some FcγR, more than Type II mAbs. As for rituximab, the internalization requires the cross-link between the Fc tail to FcγRIIb, an ITIM-containing inhibitory receptor, and the Fab portion to CD20 on the same target cell. This mechanism is crucial in reducing therapeutic efficiency; it lowers the available amount of mAb at the target cell surface, necessary for effector cells recruiting, and drags the rituximab-CD20 complex into lysosomes for degradation. Interestingly, the expression of FcγRIIb on different targets correlates with resistance to rituximab while the presence of FcγRIIIa, the ITAM-containing stimulatory receptor, is related to mAb efficacy [11].

However, a genetic polymorphism of this receptor produces differences in the binding of IgG1, and subsequent activation of ADCC. In particular, homozygous valine in the position 158 exerts higher ADCC capacity with respect to heterozygous or homozygous phenylalanine alternatives in the same position. About 49

different FcγRIIIa phenotypes have been identified in NHL patients showing differences in therapy response. Similarly, a correlation between B cell depletion induced by rituximab and receptor phenotype has been shown in SLE patients. Another mechanism potentially capable of influencing the therapeutic response is related to the rituximab-induced downregulation of IL-10 that enhances apoptosis and synergize with chemotherapeutic and steroid agents on the target [12]. Finally, rituximab seems to increase the number of Treg lymphocytes and prevents the activity of specific autoreactive T cells. These two additional mechanisms support in principle the administration of rituximab in autoimmune diseases. Overall, rituximab shows multiple and complex functions, which may explain the different target sensitivities and safety profiles under various clinical conditions. These mechanisms are shared with other anti-CD20 mAbs, but their efficiency is expressed with a different hierarchy among them. In fact, clinical experience indicates that cases of noncross-reactive resistance and synergistic effects between anti-CD20 mAbs and chemotherapy occur, and are possibly related to mechanistic diversities (see also Chap. 23, 29, 37).

35.2 Immunogenicity

As expected, anti-rituximab antibody response was detected after treatment, with different frequencies. In particular, HACA were present in 23 % of WG/MPA, followed by 11 % of RA and 1.1 % of NHL-treated patients. In the RA group, most HACA-positive cases had an objective clinical response, and about 1 % of HACA-positive RA patients had associated serious infusion reactions. Overall, among 3,095 RA patients treated with rituximab 13 % had HACA, (4 % of them had previous anti-rituximab antibodies detected at baseline), which may interfere in efficacy and safety profiles of this subgroup of patients. Interestingly, in some off-label trials enrolling RRMS patients HACA were observed in 25 % of rituximab recipients.

Because of the lowering of Ig levels, which is not profound but can be prolonged during rituximab treatments, live viral vaccines were contraindicated and nonlive vaccines may produce a reduced response. In one study on NHL patients, the primary response to a conventional antigen (KLH) was remarkably reduced (4 % vs. 69 % in healthy subjects), as well as the immune recall to tetanus toxoid (18 % vs. 81 % in healthy controls). Similar results were obtained in CLL patients, but not in RA patients where the primary response was reduced of about 50 %, and secondary response was comparable to controls [6–8].

35.3 Adverse Events

According to the 2012 update of the official label, safety data on rituximab are based on 2,783 (2,427 exposed) patients with malignancies, including 1180 NHL, 927 DLBCL, and 676 CLL. The safety profile for RA is based on 2,587 (540

exposed) patients, and for WG/MPA disorders on 197 subjects. The 2012 EMEA EPAR update reports evaluations on 3,189 exposed patients, including 1,193 untreated NHL, 166 in monotherapy, 881 in combination with CHOP, 322 with CVP, and 44 with FCM chemotherapy. Responders from these groups were put on maintenance therapy as 513 FL (505 controls). Relapsed/refractory FL consisted in 465 patients, of whom 234 in combination with CHOP (231 controls). Of 334 responders, 167 were put on maintenance therapy (167 controls). Of 399 previously untreated DLBCL, 202 were treated with rituximab in combination with CHOP chemotherapy (197 controls). The CLL cohort consisted in 817 previously untreated CLL and 552 relapsed/refractory CLL treated in association with FC chemotherapy. In particular, 403 of the untreated group (407 controls) and 276 of the relapsing/refractory patients (276 controls) received the combined therapy. The RA safety profile was based on 3,100 patients from clinical trials receiving at least one cycle of rituximab in combination with MTX, and followed from 6 months up to over 5 years. On this basis, and considering postmarketing data so far accumulated, a general safety profile of rituximab has been depicted, reporting most relevant and occurring AEs. Moreover, some peculiarities encountered in each treated disease have also been reported.

The updated official label includes BBW for *infusion reactions*, *tumor lysis syndrome* (TLS), severe *mucocutaneous reactions*, and *progressive multifocal leukoencephalopathy* (PML). They all can be fatal. The initial 1997 label contained only a warning for infusion reactions, while the 2004 update included a warning box for the first three SAEs. PML was included in 2007 on the basis of postmarketing reports.

Infusion reactions were predominantly seen as *cytokine release syndrome* (CRS) in >50 % of patients. Additional complications, such as hypotension and bronchospasm, associated in about 10 % of cases, can be serious. The reported incidence at first infusion was up to 77 % for patients with malignancies and 32 % for RA patients, and decreased to 9–11 % after the second infusion. In the smaller group of WG/MPA patients treated with rituximab (99), infusion reactions were reported as 12 % at first treatment, with a similar decreasing trend after the following administrations. Typical manifestations included urticaria, cardiovascular and respiratory hypersensitivity signs, ARDS, and anaphylactoid events. *TLS* is mainly expressed as renal failure and is observed in malignancies with a high number of circulating malignant cells or high tumor burden. *Mucocutaneous reactions* usually appear within the first 13 weeks of treatment as paraneoplastic pemphigus, Stevens-Johnson syndrome, lichenoid dermatitis, vesiculobullous dermatitis, and toxic epidermal necrolysis. *PML* after rituximab has been reported in 114 cases in one major database (WHO Drug Monitoring AE databank) and is fatal [13].

The most relevant reactions in the general profile of rituximab include *infections*, *cytopenias*, and *hypogammaglobulinemia*. However, due to the very different treatment indications for rituximab and the consequent encountered AEs, a general safety profile will be provided for malignant and nonmalignant diseases,

while additional relevant AEs peculiarities will be further described for each treated pathology.

The *safety profile in NHL and CLL* is based on patients treated with rituximab as monotherapy, or in combination with various chemotherapies. Under these circumstances, the most common drug-related AEs were infusion reactions occurring in the majority of patients, followed by infections (mostly bacterial and viral) and by cardiovascular events (mostly arrhythmias). Additional rare events include HBV hepatitis reactivation and PML. In particular, during monotherapy treatment of B cell malignancies, the overall incidence of infections was 13–17 % (serious 4 %) and occurred as bacterial (19 %), viral (10 %), and fungal (1 %), mainly as URTI and UTI. Localized Candida and Herpes zoster infections were also reported at higher incidence in the study groups. Cytopenias at induction and maintenance regimen were usually mild and transient. However, severe neutropenia (4–10 %), anemia (1 %), and thrombocytopenia (2 %) were observed as increased on control levels (<1–4 %). IgM and IgG levels decreased in about 14 % of patients. Ig levels were reduced also in control patients receiving chemotherapy alone, but they recovered up to normal levels. In long-term observations (2 year), IgG levels remained reduced in 60 % of patients in the study group, and in 36 % in controls [7].

When combined with chemotherapy, constitutional signs were present in 86 % of patients with malignancies (57 % severe/serious), hematological signs occurred in 67 % (48 %), followed by dermatological reactions as 44 % (2 %), respiratory signs as 38 % (4 %), metabolic/nutritional abnormalities as 38 % (3 %), gastrointestinal signs as 37 % (2 %), nervous system disorders in 32 % of cases (1 %), musculoskeletal signs as 26 % (3 %), and cardiovascular disorders as 25 % (3 %). In particular, severe leukopenia ranged 88–97 % in NHL when rituximab was associated with CHOP chemotherapy, and was present in 30 % of CLL patients treated in association with FC chemotherapy, with frequencies in controls of 79–88 % and of 19 %, respectively. Cardiovascular disorders during monotherapy were about 19 %, being arterial pressure unbalance the most frequent reported event. Serious cardiac events (ischemia/infarction, atrial fibrillation, LVF) were 3 % in the study group and 1 % in controls. Noteworthy, only functional cardiac disorders increased (7 %) in groups with associated CHOP chemotherapy. Cardiac disorders in CLL patients treated in combination with FC chemotherapy were low and equally distributed between study groups and controls. Cerebrovascular accidents, respiratory serious events (ILD), and gastrointestinal disorders were also observed at lower frequencies, mostly in patients treated with rituximab associated to chemotherapy.

Additional differences in the safety profiles, either in typology or frequency, were observed in patients with B cell malignancies. In *untreated NHL* patients, peripheral sensory neuropathy occurred in 30 % of treated patients (18 % in controls), rash/pruritus occurred in 17 % (5 %). Pulmonary and hepatotoxicity (17–18 % vs. 7–10 %), and neutropenia (8 % vs. 3 %) were also reported. Infections reached 37 % (22 % in controls) in some studies and were serious, together with neutropenia, in 4 % of treated patients (1 % in controls). In *DLBCL*,

rituximab was associated with CHOP chemotherapy, with 80–100 % of patients experiencing at least one AE. The events were severe/serious and drug-related in 20–40 % of cases. Infusion reactions were usually mild and observed in about 30 % of patients (severe in <10 %), mostly at first administration. Pyrexia (56 % vs. 46 % in controls), lung disorders (31 % vs. 24 %), cardiac disorders (29 % vs. 21 %), and chills (13 % vs. 4 %) were more frequently reported as severe/serious events. Other SAEs included thrombocytopenia (9 % vs. 7 %), cardiac toxicity (4.5 % vs. 1 %), and lung disorders (6 % vs. 3 %). Moreover, infections (about 45 %), neutropenia (>80 %), and anemia (15–20 %) were also among the registered events in both study groups and controls. Drug-related infections were about 19 %. Moderate hypogammaglobulinemia was present in 15–30 % of cases. In *CLL*, all AEs were 99 % versus 96 % in controls. Severe reactions (80 % vs. 74 %) and serious events (50 % vs. 48 %) were similarly distributed. Infusion reactions were frequent (59 and 7 % severe/serious). Neutropenia (49 % vs. 44 %), febrile neutropenia (15 % vs. 12 %), thrombocytopenia (11 % vs. 9 %), hypotension (2 % vs. 0 %), and hepatitis B reactivation (2 % vs. <1 %) characterize the safety profile. Prolonged neutropenia was observed in 25 % of cases. Overall, the general SOC safety profile in the study group was slightly higher than in controls, but no new/unexpected signals were detected. When the biomedicine was used in combination with FC chemotherapy, all AEs were observed in 83 % of cases in the study group and in 71 % in controls receiving only the FC chemotherapy. However, serious cardiac events (4 % vs. 3–4 %), cerebrovascular accidents, neurologic events (3–4 % vs. 3–4 %), leukopenia (30 % vs. 19 %), and pancytopenia (3 % vs. 1 %) were consistently lower in CLL than in NHL. Nonetheless, CRS, TLS, and some PML cases were observed in CLL treated patients and reported in the postmarketing experience.

Overall, in studies where rituximab was associated with various chemotherapies, the incidence of AEs in most SOC categories was higher than in patients receiving mAb monotherapy or chemotherapy alone. However, no synergistic drug effects on AEs induction could be detected. In contrast, the incidence of HACA in combined therapy was lower than in rituximab induction monotherapy. It was also low compared to treated patients with nonmalignant disorders.

During treatment of *RA* and *WG/MPA*, infections occurred in 39 and 62 % respectively, which also reflect the rates of infections in control groups (34 and 47 % respectively). They were mainly featured as URTI (7 %) and UTI (<5 %). Serious infections occurred in about 2 % (1 % in controls) and 11 % (10 %) in the two groups of patients, and involved LRTI including pneumonia, cellulitis, and UTI at ≤0.5 % rates. Overall, the most common AEs included constitutional signs (2–8 % in RA, 1–18 % in WG/MPA), dermatological reactions (2–5 and 10 % respectively), gastrointestinal signs (2–3 and 17 %), hypertension (8 and 12 %), and arthralgia (6 and 13 %). Moreover, in the WG/MPA group leukopenia/anemia (10–16 %), dyspnea (10 %), and epistaxis (11 %) were also reported.

Additional AEs peculiarities in RA-treated patients include vascular (7 % vs. 4 %), respiratory (5 % vs. 1 %), and dermatologic events (5 % vs. 2 %), while musculoskeletal complaints were prevalent in control groups. Infusion reactions

occurred in 36 % (serious <0.5 %) of patients under treatment, mostly (26 %) during the first administration. Most common SAEs after rituximab were infections, some of them fatal, with statistical significance when compared to controls (7–10 % vs. 3 %). Cardiac events were 10 % in the study group and 3 % in controls. However, serious cardiac events were more equally distributed (1.7 % vs. 1.3 % in controls), although they resulted fatal in one study as 0.4 % on 769 treated subjects (0 % in controls). Infections were low (1 % vs. <1 %) but serious. Hypophosphatemia (12 % vs. 10 %) and hyperuricemia (1.5 % vs. 0.4 %) were also observed as new cases, infusion-related and transient. In an enlarged cohort of RA-treated patients, hypophosphatemia was observed in 21 % of cases. AEs tended to be more frequent and severe in patients treated with higher doses of rituximab.

Taken together, the overall risk of serious AEs is more elevated in RA patients, and is more frequently associated to allergic reactions and to an increased incidence of HACA, as revealed by four extension studies (IMAGE, SERENE, SUNRISE, and MIRROR) where they were detected in 2–12 % of cases. PML cases occurred in rituximab-treated patients as well as in untreated RA, SLE, and vasculitis. HBV reactivation and PML cases were also reported. Moderate hypogammaglobulinemia was also detected, but was not associated with increase of infections or serious infections.

Finally, the safety profile in WG/MPA was further depicted in a relatively small cohort of treated patients (99) and was characterized by the presence of CRS during infusion, and by a consistent number of infections of any type (62 % vs. 47 % in controls), including URTI, UTI, and Herpes zoster infections. However, serious infection frequency was similar to controls (11 % vs. 10 %), being pneumonia the most common serious event. Overall, the general profile, except for CRS, TLS, and PML cases, may be considered acceptable. No opportunistic infections or cases of latent/overt tuberculosis were observed. No risk of increased malignancies has been raised so far. Subsequent courses of rituximab therapy do not seem to increase rates and types of AEs, up to four cycles [1–8].

Rituximab is also used in *Waldenstrom's disease*, which is classified as a lymphoplasmacytic lymphoma by WHO, and is mainly associated with chemotherapy. However, as a single agent rituximab is also used in patients with *IgM-related peripheral neuropathy*, with no concomitant evidence of symptomatic lymphoma. A peculiar drug-related AE is referred as *hyperviscosity syndrome*, caused by a transient increase of serum IgM levels, which usually almost returns to baseline level within 4 months. The syndrome is commonly expressed by local hemorrhage (epistaxis, gingival and retinal episodes), dizziness, and fatigue. The general safety profile in chemotherapy-associated treatment of Waldenstrom's disease is within the range of other lymphomas and includes hematologic (neutropenia, thrombocytopenia, and anemia) and nonhematologic signs (infusion reactions, constitutional signs, arrhythmias, etc.). In a recent study on 43 patients treated with multiple cycles of rituximab, fludrocortisone and cyclophosphamide, infusion reactions (49 %) were mostly mild and associated to first administration. One of them was severe and associated with rash. Major events included anemia

(30 %), neutropenia (12 %), thrombocytopenia (3 %), nausea/vomiting (21 %), arrhythmia (5 %), dyspnea (2 %), biliary/liver dysfunction (2 %), and polyuria (2 %). Hyperviscosity syndrome was absent. However, there was at least one episode of severe neutropenia in about 88 % of patients during the study, and it was long lasting in 44 % of them. Two cases of severe thrombocytopenia and one of serious hemolytic anemia were also observed. A total of nine infections (six severe) including pneumonia (5) and sepsis (1), UTI (2) and Herpes zoster infection (1) were observed during treatment. Three additional cases of pneumonia were observed as tardive and two of them were associated to late neutropenia. One patient required hospitalization for pemphigus vulgaris. None of the patients developed high-grade NHL [1–8, 14].

35.4 Off-Label Experience

Rituximab has been used in nephrotic syndrome, ITCP, immune-mediated glomerular diseases, refractory granulomatous ocular diseases, multiple sclerosis, peripheral nervous system autoimmune disorders, SLE, neuropsychiatric lupus, kidney transplant rejection, autoimmune hepatitis, primary biliary cirrhosis, mucous membrane pemphigoid systemic sclerosis, cryoglobulin vasculitis, pemphigus vulgaris, and chronic neutropenic leukemia. Rituximab has been also used for purging stem-cell transplants and for posttransplant treatment of residual disease. At present, over 1,250 trials are completed, ongoing or enrolling patients, and most of them investigate on-label indications (970). However, part of them also include or are expressly dedicated to off-label pathologies, such as Burkitt's Lymphoma (86), plasma cell neoplasms/Waldenström (84), ALL (64), HD (54), precancerous conditions (30), AML (18), autoimmune diseases (164), arthritis/joint disorders (77), blood protein disorders (70), urologic diseases and kidney transplant rejection (37), virus reactivations (36), thrombocytopenias and thrombotic microangiopathies (32), purpura (28), skin disorders (28), GVHD (22), HIV infections (22), nephritis/lupus nephritis/glomerulonephritis (18), SLE (14), multiple sclerosis (10), and vasculitis (14). Among all of these, nonmalignant pathologies offer the possibility of long-term evaluations of rituximab regimen for NHL or RA on different underlying diseases. Most of all, recent experiences on autoimmune and dermatologic disorders seem more promising and potentially expanding, such as in AIHA, ITCP, thrombotic thrombocytopenic purpura, SLE, refractory dermatomyositis, and cryoglobulinemias.

The removal of autoreactive B cell clones is the basic assumption of these off-label treatments, which should inhibit/eradicate autoimmune aggressions. A secondary effect has been attributed to the decrease of the B-APC function, which reduces potential T cell activation in autoimmune disorders.

Rituximab is increasingly used in refractory *pemphigus vulgaris*. In a wide overview of the past 12 years publications, 272 patients in 42 studies received rituximab either with the NHL protocol (180) or with RA protocol (92). Interestingly, the former was less effective and produced less serious infections, but

higher mortality, while the RA protocol was more effective, produced more infections, but with lower mortality rates. None produced sustained clinical remission. The overall major concern was the high infection rates, some of which were fatal. SAEs (16.6 %) associated to the NHL protocol in 48 patients included two cases of pneumonia (P. Carinii, one fatal), and single cases of septic shock (fatal), multi-bacterial sepsis, bacterial pneumonia, DVT/pulmonary embolism, hip arthritis (P. Aeruginosa), and gastritis/retinitis (CMV). Two late-onset neutropenias, one severe and one associated to bacterial pneumonia, were observed after 19–27 weeks from treatment. Seven case series on 88 subjects reported SAEs in 2.3 % of patients, including one septicemia (fatal) and one pyelonephritis.

SAEs associated to the RA protocol were reported in 20 % of patients and included three cases of sepsis, one fatal (S. Aureus), and one associated with spinal hemorrhage and paraplegia, three cases of pneumonia, six UTI, and two herpes infections (one ocular).

Additional SAEs (5.8 %) were observed in 51 patients enrolled in six case series including one gastric perforation (fatal), one cardiac complication, and late-onset neutropenic sepsis. Overall, the incidence of serious infections was about 4 % in the NHL protocol and 15 % in RA protocol, while mortality rates were 2.2 and 1.1 %, respectively [15]. The same Authors also reviewed the literature on *mucous membrane pemphigoid* (MMP). Serious adverse events included 10 % infections in 28 patients treated with one or two cycles of the NHL protocol associated with immunosuppressive and anti-inflammatory agents, and included one pyelonephritis, and one new-onset pulmonary TB (fatal), both associated with hypogammaglobulinemia. Although some benefits were reported in both reviews, serious infections and related mortality remained the major concerns, mainly because of their low occurrence in untreated patients [16].

Rituximab in ITCP treatment as second-line therapy has given more encouraging results, also due to a longer experience of more than 5 years.

In a systematic review on 313 ITCP adult patients treated with rituximab, AEs were observed in about 97 % of patients, and were mild/moderate in about 22 % of cases, mostly as infusion reactions (60 %). However, the rate of infusion reactions was variable across all studies. Serious and life-threatening events were reported in about 4 % of cases. Mortality was reported in 2.9 % of treated patients as caused by respiratory insufficiency, pneumonia, cerebral hemorrhage, hemorrhagic complications, infections, pulmonary embolism, and hepatic failure. This rate was in the range of similar studies. However, only two of nine reported deaths were referred to drug in study by the Authors [17]. In the first systematic review on *pediatric ITCP*, 352 patients were examined for efficacy in 18 studies. In 304 patients the diagnosis was primary ITCP, while 48 patients were diagnosed with secondary ITCP associated to other diseases, including Evans' syndrome, SLE, and autoimmune lymphoproliferative syndrome. Patients received a variety of IV doses (1–6) of rituximab. Safety data were considered in 23 studies reporting adverse events. In particular, 91/208 (44 %) patients reported 108 AEs as mild/moderate (84 %). The most common events included allergic reactions with

pruritus, urticaria, chills, and pyrexia. Serum sickness-like syndrome was observed in seven patients (6.5 %), and was severe in three cases. Infusion reactions were present in two cases, causing therapy discontinuation. Transient neutropenia was observed in three cases. Infections related to treatment in study were reported in 3.7 % of cases, and included pneumonia, one life-threatening enteroviral meningoencephalitis, and two cases of varicella. Finally, one patient developed cephalea with brain MRI abnormality, and one developed common variable immunodeficiency with prolonged hypogammaglobulinemia and increased susceptibility to infections.

Overall, a good response to therapy, both in primary and secondary ITCP, was accompanied by a limited number of mild/moderate AEs and a very limited number of serious infections. No death was reported [18].

Similar results have been reported in another recent review, which also provided some indications for reducing the impact of most common drug-related AEs. Among these are premedication and infusion slowing to minimize serum sickness signs, which occur more frequently in children, and prevaccinations against all encapsulated bacteria to reduce the risk of serious infections [19].

Recent *updates on SLE* have been provided by a number of open studies, along with secondary analysis of EXPLORER and LUNAR (lupus nephritis) trials and previous pivotal trials. All of these failed in terms of efficacy, except for a subgroup of African-American patients, who are known to develop more frequently lupus nephritis. This result stresses the opportunity to direct this therapy to specific and selected cohorts of patients, who could thus benefit from the reduction of AEs caused by alternative treatments such as corticosteroids or estrogens. Tolerability and patient dropout rates were similar to placebo.

Similar results were obtained in *Sjögren's syndrome* (SS), where some benefits were observed on peripheral neuropathy, vasculitis, and cryoglobulinemia, but worsening and serious effects were observed on CNS manifestations of SS, such as MS-like signs, transverse myelitis, anxiety, depression and cognitive dysfunction, all with MRI abnormalities. Interestingly, some SS patients treated with rituximab revealed a higher clonal expansion and mutational rate in IgA and IgG-expressing cells in the parotid tissue, despite the almost complete peripheral B cell depletion [20]. In contrast, a recent review and case report on *refractory neuropsychiatric SLE* (NPSLE), enrolling 36 patients treated with rituximab, showed a partial therapeutic response (in 85 % of cases, but with 45 % recurrence), yet along with a number of expected and moderate AEs. Infections were the most common registered event (29 %) and included pneumonia (four cases), Herpes zoster (two cases), UTI (two cases), infected decubitus ulceration (one case), chickenpox (one case), and enteritis infection (one case). No cases of severe infusion reactions or hematologic abnormalities were reported. Only one patient died due to SLE progression (fatal pancarditis) in the study group. The apparent discrepancy with results of EXPLORER and other studies were attributed to the exclusion of severe and refractory patients, including NPSLE [21, 22].

Other uncontrolled clinical studies on SLE have shown contrasting results. Nonetheless, no new signals were reported, and a consistent sparing effect on steroid-related AEs was confirmed. Once again discrepancies were attributed to patients selection, heterogeneity of SLE disease, and clinical assessment.

Experience on MS is expanding, on the assumption that B cell depletion reduces autoreactive B cell clones and the production of T cell mediated proinflammatory cytokine response. A number of open-label and controlled trials are completed or ongoing on relapsing remitting multiple sclerosis (RRMS) and on primary progressive disease (PPMS) with uncertain results. So far, the side effect profile in studies using rituximab in monotherapy is similar to that previously reported in RA and other autoimmune disorders. Infusion reactions were the most common ($\geq$10 %) drug-related AEs, and were mild/moderate in more than 90 % of cases (7 %, severe). Other severe events were similar in the study groups and in controls (about 14 %), as well as withdrawal rates (about 5 %). Similarly, overall infections were comparable in placebo and study groups (about 70 %), although nasopharyngitis, URTI, UTI, and sinusitis were prevalent in rituximab recipients (5–6 % over controls). No opportunistic infection was reported. However, long-term data are still lacking, and more prolonged observations in PPMS studies (about 2 years) indicate that serious infections are increased in rituximab older recipients (4.5 % vs. <1.0 %). Notably, in these patients HACA to rituximab were observed in about 25 % of RRMS recipients [23].

A number of *glomerular diseases*, including on-label AAV and various off-label disorders such as lupus nephritis, mixed cryoglobulinemia-associated glomerulonephritis, idiopathic membranous glomerulopathy, and focal glomerulosclerosis are treated with rituximab with variable success, on the assumption that autoantibodies play a crucial role in their development. Overall, the safety profile follows the mentioned AAV experience. However, the major advantage in the whole group of disorders is mAb low toxicity compared to conventional therapies, rather than its higher efficacy. In fact, conventional immunosuppressive regimens produce cumulative toxicity and heavily compromise the quality of life of these patients, also exposing to infertility and to a major risk of malignancies in the long term.

In a recent retrospective survey on 74 *pediatric* patients with various forms of *nephrotic syndrome* resistant to conventional therapy, who were treated with rituximab in Japan, the best achievement was the steroid sparing in terms of serious adverse effects. Among these, short stature, obesity, hypertension, cataract, glaucoma, spinal fracture, glucose intolerance, and psychological disturbance are steroid-related AEs, while hypertrichosis, nephropathy, gingival hypertrophy, and drug-resistance are related to CsA treatment. During treatment with rituximab it was possible to discontinue steroids in 77 % of cases, and CsA in 60 % of the recipients. About 50 % of patients recovered from these drug-related events, including CsA resistance. In contrast, rituximab-related AEs were mostly mild infusion reactions expressed as constitutional signs, pyrexia, rash, cardiac frequency disorders, and blood pressure unbalance. Severe events were limited to one infection and two cases of late granulocytopenia recovered without sequelae.

However, many patients relapsed, thus requiring repeated rituximab treatments and re-introduction of some immunosuppressive agents for maintenance [24].

When administered in eight patients with early diffuse *systemic sclerosis* (dSS) who were followed for 2 years, rituximab safety profile registered five SAEs, including one sepsis (fatal), a secondary infection after coronary bypass grafting (fatal), noninfectious pyrexia, secondary infection on a digital ulcer, and one episode of hyperventilation, which were all considered probably nondrug related. No neutropenia or hypogammaglobulinemia were observed. Minor AEs occurred in four patients (3.5 AEs/P) and included URTI (3), one skin infection, GI signs (4), cardiovascular manifestations (hypertension, thrombosis), one COPD exacerbation, depression (1), and one tendinitis. Interestingly, the treatment seemed to stabilize dermatological and internal organ progression of the disease [25].

In a recent review on *cryoglobulinemia vasculitis* (CryoVas) results seem more encouraging. However, about 50 % of treated patients developed AEs, and in a subset of the 23 patients (aged, Type II CryoVas), severe infections were observed in 26 % (about 3 fold rates of RA patients). Severe infections were also observed in a larger subsequent series of patients (242) treated with a different regimen, particularly when high doses of corticosteroids were associated, whereas death rates did not differ between the two therapeutic regimens [26].

A particular concern with some mAbs, as well as with chemotherapy and immunosuppressive regimens, is the *reactivation of viruses*, such as HBV and to a lesser extent HCV reactivation. In particular, HBV reactivation has been reported with considerable variation (1–23 %), and is a potentially fatal complication after rituximab-containing chemotherapy. HCV infection seems to be a risk factor mainly in DLBCL-treated patients, which can lead to therapy discontinuation and lymphoma progression [27].

Rituximab has been also used to treat *granulomatous ocular episcleritis* and *iritis associated to AAV or sarcoidosis*. In a retrospective case report analysis, nine patients (six after AAV, four after sarcoidosis) were treated with rituximab systemic therapy for 12–41 months. Two patients had recurrent herpetic mouth and skin infections. Two cases of neutropenia were also observed and the severe one caused discontinuation of rituximab for one year, with relapse of eye disease causing reintroduction of monotherapy and subsequent improvement [28].

Recently, rituximab has been experienced in *chronic fatigue syndrome* with some benefit, and four trials have been registered (one completed and three ongoing). Data form the completed study on 30 patients (15 treated) reported infusion reactions as mild (13 %), without serious AEs or major toxicity. Insomnia and psoriasis exacerbation were also observed (two cases each) [29]. As for the latter disease, a patent for the use of anti-CD20 mAbs has been registered in Norway (IPC8 Class: AA61K39395FI (USPC Class: 4241731).

Finally, serious and fatal viral infections and reactivations, including HBV, HCV, CMV, herpetic viruses, and JCV (responsible for PML fatalities) have been observed in off-label administrations. Moreover, progression of Kaposi's sarcoma has also been observed during off-label treatment with rituximab, mostly in HIV patients [7].

35.5 Postmarketing Surveillance

In the 2012 label, a number of postmarketing reports were considered as relevant on the basis of their severity, frequency of reporting, and causality with rituximab administration. They included hematologic (bone marrow hypoplasia, prolonged/late pancytopenia and neutropenia, and hypogammaglobulinemia), cardiac (fatal), infective (severe/serious, HIV-associated, PML), dermatologic (mucocutaneous reactions), intestinal (obstruction and perforation), pulmonary (fatal ILD and bronchiolitis obliterans), neurologic (RPLS), and malignant (Kaposi sarcoma progression) events. Moreover, a number of immune/autoimmune events were observed, which enlarge the spectrum of possible drug-related AEs at ocular (neuritis, uveitis) and systemic level (vasculitis, LLS, polyarticular arthritis, and serum sickness).

Initial observations on about 13,000 rituximab-treated patients with B cell malignancies reported 39 fatalities and 66 serious infusion reactions. Serious dermatological events included toxic necrolysis, paraneoplastic pemphigus, lichenoid dermatitis, and lichen planus.

In the FAERS database over 16,700 reports (3.5 AE/R) included infections (6.6 %), WBC disorders (5.7 %), respiratory (3.7 %), and GI signs (2.8 %) as the most signaled events.

In the EV database, 16,766 (16,633 serious) reports included 36,744 AEs (2.19 AEs/P). Most common AEs were infections (13 % of reports), respiratory signs (11 %), hematological events (10 %), nervous disorders (7 %), GI disorders (6 %), and infusion reactions (4 %). TLS (145 reports) and CRS (44 reports) were also registered. Infections included pneumonia (3 %), sepsis (2 %), neutropenic sepsis (0.2 %), HBV (3 %) and HCV (0.3 %) infections/reactivations, herpes zoster infections (1 %), CMV infections (0.8 %), UTI (0.6 %), and URTI (0.2 %). Most frequent cytopenias were neutropenia (6 %), febrile neutropenia (2 %), thrombocytopenia (3 %), and pancytopenia (2 %). Bone marrow failure was reported in 137 cases. Noteworthy, 423 cases of PML, and 37 cases of JCV infections, and 33 cases of leukoencephalopathy were also reported. Fatalities were about 6 %.

35.6 Remarks

Since initial observations in experimental animals (monkeys), the overall safety profile of rituximab appeared limited to the B cell line and reassuring. However, one apparent concern was about the long-term depleting effect on these cells, since B cell recovery was not complete after 90 days postdose [1]. After about 15 years of clinical experience, rituximab has confirmed the expectancies and remains a manageable biomedicine, mainly when compared to alternative therapies for the same indicated diseases. The lack of serious long-term toxicity and of myelosuppressive effects, although massively depleting the B cell compartment without

interfering with the stem cell compartment, remains the most valuable characteristic of this mAb. However, anemia and thrombocytopenia are among the encountered drug-related AEs.

Rituximab exerts its action through a number of effector mechanisms that still need to be fully elucidated, which support the selective and prolonged activity on the B cell compartment. However, recent experience is showing that genetic polymorphism of Fc receptors may explain differences in efficacy and safety, and possibly enlighten the progressive resistance to therapy observed with this drug, mainly when used as monotherapy [10]. The intervention of different mechanisms of action may also be involved in diversity of expressions at local level, both in efficacy and safety, as well as in early versus late interventions. For example, CDC is more likely to cause early events in circulation, while internalization of rituximab-CD20 complexes may led to resistance episodes and more persistent late events. On the other hand, they also explain some enhancing and synergic effects of rituximab, when associated with chemotherapy and/or other monoclonals, through mechanisms of sensitization of the targets.

The major concern of rituximab is about infections, often serious and sometimes fatal, which occur in most therapy regimen and in almost all treated diseases, while the risk for developing an immune or allergenic response is very low. The tumor burden has also an important impact on efficacy and expression of typical AEs, such as TLS and CRS, which are usually moderated by proper initial treatment regimens.

Particular concerns rise from off-label experiences, not only due to lack of controlled trials, extreme heterogeneity of uncontrolled studies, and observational data bias, but also because serious infections and related fatalities remain frequent also in cohorts of patients with low levels of diseases-related mortality. This approach exposes patients to an additional unjustified risk, when considering the overall low efficacy profiles so far experienced.

In some studies on ITCP, drug-related mortality rates were higher than in NHL cohorts, and the real efficacy of rituximab treatment was hard to estimate, due to the known spontaneous recovery of some ITCP in the absence of controlled trials [17]. In other clinical conditions overlapping multiple cytotoxic and immunosuppressive therapies do not allow to discriminate among AE causalities. In other published experiences, NHL or RA protocols were adopted for the treatment of other diseases without an apparent scientific rationale, and in the presence of a number of serious and typical rituximab-related AEs, such as severe and fatal infections [15, 16]. Therefore the off-label treatment with rituximab should be more carefully considered.

In both on- and off-label experience, the rate of Ig decrease is not severe and tends to recover, together with the number of B cells. This may appear in contrast with the rate and severity of encountered infections, thus suggesting the existence of other immunological impairments. In fact, postrituximab B cell late reconstitution appeared to be preferential and earlier for naive B cells, rather than for memory B cells, although transient decreases of CD4+ and CD8+T cells were also

observed, suggesting the possibility of a different remodulation of the immune lymphocyte compartment after rituximab therapy [23].

A different approach to reduce mAbs-related AEs incidence without hampering efficacy may come from ongoing studies on lower and fractionated doses. In fact, a higher dose of rituximab seems to reduce cell killing with respect to intermediate doses, due to hypothetical mechanisms of effector exhaustion, including complement consumption at high B cell burdens.

Both in NHL and SLE experience an underlying heterogeneity of the disease affects efficacy, but it is not yet clear if it influences also the AEs response. Studies in this direction are lacking and may help in selecting best curable subpopulations of individuals.

Amid the discouraging experience on SLE patients, rituximab seems to be more effective in refractory diseases than in nascent lupus nephritis, suggesting the possibility of selecting populations with a better risk/benefic outcome for this disease, such as Afro-Americans [20].

Overall, the risk of infections and of serious/fatal infectious complications seem elevated in many autoimmune diseases, including RA and pemphigus, which may be influenced by a variety of heavy immunosuppressive therapies. Whether rituximab confers a uniquely elevated risk remains unclear. Nonetheless, in most instances the toxic effects of chemotherapy and immunosuppressive therapy are more elevated than rituximab ones, and their association considerably contributes to lower the overall rates of AEs.

References

1. Rituximab (Rituxan®) BLA 97-0260 and BLA 97-0244 Clinical Review FDA, Nov 1997
2. Rituximab (MabThera®) WC500025817 Scientific Discussion EMEA, Jan 2005
3. Rituximab (MabThera®) WC500025822/3 EPAR Variations EMEA, Sept 2006
4. Rituximab (MabThera®) WC500025824 Assessment Report EMEA, June 2008
5. Rituximab (MabThera®) WC500025826 Assessment Report EMEA, Aug 2009
6. Rituximab (MabThera®) WC500099488/9 Assessment Reports EMEA, Oct 2010
7. Rituximab (MabThera®) WC500025821 EPAR Update Annex I EMEA, June 2012
8. Rituximab (Rituxan®) Prescribing Information, Genentech, Oct 2012
9. Braendstrup P, Bjerrum OW, Nielsen OJ et al (2005) Rituximab chimeric anti-CD20 monoclonal antibody treatment for adult refractory idiopathic thrombocytopenic purpura. Am J Hematol 78:275–280
10. Boross P, Leusen JHW (2012) Mechanisms of action of anti CD20 antibodies. Am J Cancer Res 2:676–690
11. Lim SH, Vaughan AT, Ashton-Key M et al (2011) Fc gamma receptor IIb on target B cells promotes rituximab internalization and reduces clinical efficacy. Blood 118:2530–2540
12. Rastetter W, Molina A, White CA (2004) Rituximab: expanding role in therapy for lymphomas and autoimmune diseases. Annu Rev Med 55:477–503
13. Keene DL, Legare A, Taylor E et al (2011) Monoclonal antibodies and progressive multifocal leukoencephalopathy. Can J Neurol Sci 38:565–571
14. Tedeschi A, Benevolo G, Varettoni M et al (2912) Fludarabine plus cyclophosphamide and rituximab in Waldenström macroglobulinemia. Cancer 118:434–443
15. Zakka LR, Shetty SS, Ahmed AR (2012) Rituximab in the treatment of pemphigus vulgaris. Dermatol Ther (Heidelberg) 2:17–29

16. Shetty S and Ahmed AR (2012) Critical analysis of the use of rituximab in mucous membrane pemphigoid: a review of the literature. J Am Acad Dermatol doi.org/10.1016/j.jaad.2012.10.018
17. Arnold DM, Dentali F, Crowther MA et al (2007) Systematic review: efficacy and safety of rituximab for adults with idiopathic thrombocytopenic purpura. Ann Intern Med 146:25–33
18. Liang Y, Zhang L, Gao J et al (2012) Rituximab in children with immune thrombocytopenia: a systematic review. PLoS One 7:e36698. doi:10.1371/journal.pone.0036698
19. Journeycake JM (2012) Childhood immune thrombocytopenia: role of rituximab, recombinant thrombopoietin, and other new therapeutics. Hematol Am Soc Hematol Educ Program 2012:444–449
20. Coca A, Sanz I (2012) Updates on B-cell immunotherapies for systemic lupus erythematosus and Sjögren's syndrome. Curr Opin Rheumatol 24:451–456
21. Wiesik-Szewczyk E, Olesinska M (2012) B-cell targeted therapy in systemic lupus erythematosus: potential of rituximab. Biologics 6:347–354
22. Narvaez J, Ríos-Rodriguez V, de la Fuente D et al (2012) Rituximab therapy in refractory neuropsychiatric lupus: current clinical evidence. Semin Arthritis Rheum 41:364–372
23. Lulu S and Waubant E (2012) Humoral-targeted immunotherapies in multiple sclerosis. Neurotherapeutics 10(1):34–43. doi: 10.1007/s13311-012-0164-3
24. Ito S, Kamei K, Ogura M et al (2012) Survey of rituximab treatment for childhood-onset refractory nephrotic syndrome. Pediatr Nephrol 27(2):331–332. doi:10.1007/s00467-012-2319-1
25. Smith V, Piette Y, van Praet JT et al (2012) Two-year results of an open pilot study of a 2-treatment course with rituximab in patients with early systemic sclerosis with diffuse skin involvement. J Rheumatol 167(5):1138–1144. doi:10.3899/jrheum.120778
26. Terrier B, Cacoub P (2013) Cryoglobulinemia vasculitis: an update. Curr Opin Rheumatol 25:10–18
27. Watanabe T, Tanaka Y (2012) Reactivation of hepatitis viruses following immunomodulating systemic chemotherapy. Hepatol Res. doi:10.1111/hepr.12014
28. Lower EE, Baughman RP, Kaufman AH (2012) Rituximab for refractory granulomatous eye disease. Clin Ophthalmol 6:1613–1618
29. Fluge Ø, Bruland O, Risa K et al (2011) Benefit from B lymphocyte depletion using the anti-CD20 antibody rituximab in chronic fatigue syndrome. A double-blind and placebo-controlled study. PLoS One 6:e26358