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Patent Evaluation on Monoclonal Antibody (MAB) Therapy with Binding Specificity to CD20 B-Cell Surface Antigen Bp35 and the Manufacturing Process of Biological &Antibodies and Derivatives

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Abstract

The present article relates to the treatment of hematologic malignancies associated with high numbers of circulating tumour cells by the administration of a therapeutically effective amount of an antibody that binds to the B-cell surface antigen Bp35 (CD20). The process involves the discovery that hematologic malignancies and, in particular, those characterized by high numbers of tumour cells in the blood may be effectively treated by the administration of a therapeutic anti-CD20 antibody. These malignancies include, in particular, CLL, B-PLL, and transformed non-Hodgkin's lymphoma and also explains the commercial process of the manufacturing process of biological & antibodies and derivatives.

Discussion

Rituximab is a chimeric mouse/human monoclonal antibody (MAB) therapy with binding specificity to CD20 and produced by mammalian cell (Chinese Hamster Ovary) suspension culture in a nutrient medium containing gentamicin. Gentamicin is not detectable in the final product it was the first therapeutic antibody approved for oncology patients and was the top-selling oncology drug for nearly a .decade with sales reaching $8.58 billion in 2016. Rituxan (Schwaber & Cohen, 1973) is a sterile, clear, colourless, preservative-free liquid concentration typically for intravenous administration.

Rituxan is supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single-use vials. The product is formulated in 9 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dehydrate, 0.7 mg/mL polysorbate 80, and water for Injection. The pH of the product is 6.5. (See Rituxan® Product Label dated February, 2010, Biogen Idec Inc., and Genentech Inc.) Rituximab (marketed under the trade names Rituxan® in the United States and MabThera® in Europe) is a genetically engineered chimeric murine/human monoclonal IgG1 kappa antibody directed against the CD20 antigen. Rituximab has an approximate molecular weight of 145 kD. Rituximab has a binding affinity for the CD20 (Köhler& Milstein, 1975) antigen of approximately 8.0 NM. Rituximab is produced by mammalian cell (Chinese Hamster Ovary) suspension culture in a nutrient medium containing gentamicin. Gentamicin is not detectable in the final product.

Biogen Idec Inc., and Genentech Inc, since its initial approval in 1997, it has improved outcomes in all B-cell malignancies, including diffuse large B-cell lymphoma, follicular lymphoma, and chronic lymphocytic leukemia (Koprowski et al., 1978). Despite widespread use, most mechanistic data have been gathered from in vitro studies while the roles of the various response mechanisms in humans are still largely undetermined. Polymorphisms in Fc gamma receptor and complement protein genes have been implicated as potential predictors of differential response to rituximab, but have not yet shown sufficient influence to impact clinical decisions.

Unlike most targeted therapies developed today, no known biomarkers to indicate target engagement/tumor response have been identified, aside from reduced tumor burden. The lack of companion biomarkers beyond CD20 (Nadler et al., 1980) itself has made it difficult to predict which patients will respond to any given anti-CD20 antibody. In the past decade, two new anti-CD20 antibodies have been approved: ofatumumab, which binds a distinct epitope of CD20, and obinutuzumab, a MAB (Shin& Morrison, 1989) derived from rituximab with modifications to the Fc portion and to its glycosylation. Both are fully humanized and have biological activity that is

distinct from that of rituximab. In addition to these new anti-CD20 antibodies (Riechmann et al., 1988), another imminent change in targeted lymphoma treatment is the multitude of biosimilars that are becoming available as rituximab's patent expires. While the widespread use of rituximab itself will likely continue, its biosimilars will increase global access to the therapy. This review discusses current research into mechanisms and potential biomarkers of rituximab response, as well as its biosimilars and the newer CD20 binding MAB therapies. Increased ability to assess the effectiveness of rituximab in an individual patient, along with the availability of alternative anti-CD20 (Armitage et al., 2017) antibodies will likely lead to dramatic changes in how we use CD20 antibodies going forward.

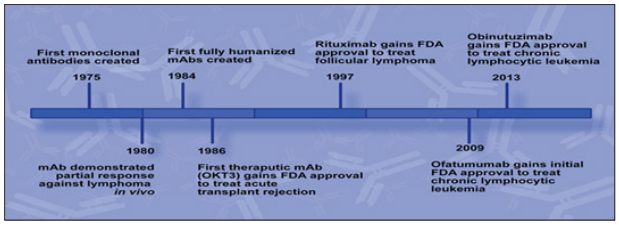


Figure 1: The History of Ritixumab and patents on Methods for producing chimeric, primate, primatized®, humanized and human antibodies

While the general concept of immunotherapies has been around for over a century, effective antibody therapies were not feasible before the ability to generate MABs using continuously growing cell lines (Figure 1). In 1975, Köhler and Milstein (Shankland et al., 2012) generated the first hybridoma cell lines capable of producing MABs by immunizing mice against sheep red blood cells followed by isolation of B-lymphocytes(Beers et al., 2010) from the murine spleens and subsequent fusion of those cells with a myeloma cell line (1). The medical and industrial potential of their achievement was quickly realized and has rapidly become a booming biotechnology industry.

The present patent evaluation relates to the treatment of hematologic malignancies associated with high numbers of circulating tumor cells by the administration of a therapeutically effective amount of an antibody that binds to the B-cell surface (Feugier, 2015) antigen Bp35 (CD20). The paper explains the discovery that hematologic malignancies and, in particular, those characterized by high numbers of tumor cells in the blood may be effectively treated by the administration of a therapeutic anti-CD20 antibody. These malignancies include, in particular, CLL, B-PLL (Lanini et al., 2011) and transformed non-Hodgkin's lymphoma. This discovery is surprising notwithstanding the reported great success of RITUXAN® for the treatment of relapsed and previously treated low-grade non-Hodgkin's lymphoma (Cang et al., 2012). In particular, this discovery is surprising given the very high numbers of tumor cells observed in such patients and also given the fact that such malignant cells, e.g., CLL cells, typically do not express the CD20 antigen at the high densities which is characteristic of some B-cell lymphomas, such as relapsed and previously-treated low-grade non-Hodgkin's lymphomas (Cang et al., 2012). Consequently, it could not have been reasonably predicted that the CD20 antigen would constitute an appropriate target for therapeutic antibody therapy of such malignancies. Treatment of according to the invention will comprise the administration of a therapeutically effective amount of anti-CD20 antibody, rituximab which administration may be effected alone or in conjunction with other treatment(s), e.g., chemotherapy, radiotherapy (e.g., whole-body irradiation, or treatment with radiolabelled antibodies). In addition, combination therapy with cytokines may be useful to upregulate CD20 on the surface of the lymphoma cells. Methods for producing chimeric, primate, primatized®, humanized and human antibodies are well known in the art. See, e.g., (Kasi et al., 2012; U.S. Patent 5,530,101, issued to Queen et al) U.S. Patent 5,530,101, issued to Queen et al, U.S. Patent 5,225,539, issued to Winter et al, U.S. Patents 4,816,397 and 4,816,567, issued to Boss et al, and Cabilly et al, respectively. EP 1616 572 B As noted, a particularly preferred chimeric anti-CD20 antibody is RITUXAN®, which is a chimeric gamma 1 anti-human CD20 antibody.

The complete amino acid and corresponding nucleic acid sequence for this antibody may be found in U.S. Patent 5,736,137. This antibody, which is produced in a proprietary CHO cell expression system commercialized by IDEC Pharmaceuticals Corporation, is made by a CHO (Cang et al., 2012; Kasi et al., 2012; U.S. Patent 5,530,101, issued to Queen et al., (n.d); Keating et al., 2010) cell transfectoma which was deposited on November 4, 1992, under the provisions of the Budapest Treaty at the American Type Culture Collection, located at 12301 Parklawn Drive, Rockville, MD 20852. This cell line was determined to be viable and will be replaced should it become non-viable during the term of the deposit. This cell line was made irrevocably available upon issuance of the 5,736,137 patent and is available without restriction from the ATCC (Cartron & Watier, 2017; Casan et al., 2018; Tobinai et al., 2017; Illidge, 2010; Rizzieri, 2016). This cell line will also be available without restriction during the lifetime of any patent that may issue based on this application. The subject anti-CD20 antibody will be administered by various routes of administration, typically parenteral. This is intended to include intravenous, intramuscular, subcutaneous, rectal, vaginal, and administration with intravenous infusion being preferred. The anti-CD20 antibody will be formulated for therapeutic usage by standard methods, e.g., by addition of pharmaceutically acceptable buffers, e.g., sterile saline, sterile buffered water, propylene glycol, and combinations thereof. Effective dosages will depend on the condition of the patient, age, weight, or any other treatments, among other factors. Such administration may be affected by various protocols, e.g., weekly, bi-weekly, or monthly, dependent on the dosage administered and patient response. Also, it may be desirable to combine such administration with other treatments, e.g., radioactive therapy, both targeted and non-targeted, chemotherapies, and lymphokine (Tobinai et al., 2017; Illidge, 2010; Rizzieri, 2016; Tierney et al., 2007; Zacher et al., 2014) or cytokine administration, e.g., interleukins, interferons, TNF's, colony-stimulating factors, etc. Typically, treatment will be affected

weekly, for about 2 to 10 weeks, more typically about 4 weeks. Also, stepped-up dosing schedules may be even more preferable. A particularly preferred chemotherapeutic regimen that may be used in conjunction with the subject antibody immunotherapy comprises CHOP immunotherapy, which comprises the administration of a combination of cyclophosphamide, doxorubicin, vincristine and prednisone. Other known chemotherapeutics (Casasnovas et al., 2017; Marcus et al., 2017; Hiddemann et al., 2018; Townsend et al., 2018; Sehn et al., 2015) include methotrexate, cisplatin, toremifene and tamoxifen. The following Examples are not intended, nor are they to be construed, as limiting the invention. The Examples are intended to provide clinical evidence in support of the efficacy of the invention was explained below examples.

EXAMPLE 1: Shadman et al. (2018); Khouri et al. (2015); Witzig et al. (2002); Gordon et al. (2004); Guzauskas et al. (2017); Sun and Carlson (2017): 2 patients in whom we noted a rapid reduction of blood tumor cells, which was associated with severe pulmonary infusion-related toxicity and thrombocytopenia, were studied. Also, 2 additional patients were collected from physician-submitted reports of adverse events related to RITUXAN® treatment. Pre-treatment characterization of these patients included a median age of 60 years (range 26-73) with the diagnosis of B-Prolymphocytic Leukemia (B-PLL), Chronic Lymphocytic Leukemia (CLL), or transformed non-Hodgkin's lymphoma. All of these patients had elevated leukocyte counts as a consequence of blood tumor involvement, bulky adenopathy and organomegaly. All four patients developed a unique syndrome of severe infusion-related reactions characterized by fever, rigors, bronchospasm with associated hypoxemia, requiring temporary cessation of RITUXAN® therapy. Concurrent with these symptoms, a rapid decrement in circulating tumor cell load (mean pre-treatment 98 x 103L; range 73-132 vs. mean post-treatment 11 x 103L; range 37-24.6) with mild electrolyte evidence of rapid tumor lysis. Thrombocytopenia, a finding not commonly associated with RITUXAN® therapy was noted in all four patients (mean pre-treatment 145 x 103L; range 57-277 vs. mean post-treatment Sn x 109/L; range 2-120), requiring transfusion in one case. Symptoms of this syndrome required hospitalization but resolved with supportive care. Subsequent RITUXAN® treatment were well tolerated in all patients. Two subsequent patients with CLL have been treated with high blood tumor counts utilizing stepped-up dosing (100 mg day 1 followed by rest of therapy on day 1) with demonstrated efficacy, thrombocytopenia but minimal infusion-related toxicity RITUXAN® administration in patients with hematologic malignancies who have blood tumor cell involvement may be associated with a higher frequency of severe initial infusion-related reactions and thrombocytopenia (Marcus et al., 2017) mandating careful clinical monitoring. Given the preliminary activity of RITUXAN® in these patients, future studies in CLL and PLL, utilizing a stepped-up dosing schedule, is to be conducted.

EXAMPLE II: Unlabelled immunoglobulins (MAB) are attractive for the treatment of NHL as they may: mediate cytotoxicity with complement (CDC) or effector cells (ADCC); effect apoptosis; be less toxic, less immunogenic and possibly more effective than (Marcus et al., 2017; Hiddemann et al., 2018; Townsend et al., 2018; Sehn et al., 2015; Vitolo et al., 2017; Maloney et al., 2016) toxin- or drug-conjugated Mabs; not require the complex procedures needed for radiolabeled Mab therapy (RIT), and not produce the myelosuppression typical of high-dose RIT. Until recently, use of Mabs in the treatment of EP 1 616 572 B1 4 5 10 15 20 25 30 35 40 45 50 55 hematologic malignancies has been limited. However, the chimeric anti-CD20 Mab, RITUXAN®, has a low toxicity profile and significant clinical efficacy and is now approved by the Food and Drug Administration (US FDA 11/97; EU 6/98) for the treatment of relapsed or refractory, low-grade or follicular (R=LG/F) NHL. In a single-agent clinical trial (PIII), of 166 patients with R-LG/F NHL treated with RITUXAN® at 375 mg/m2 weekly for four infusions (study 102-05), the overall response rate (ORR) was 48% (6% CR and 42% PR). Median time to progression for responders was 13.1 months and duration of response 11.2 months. Median circulating B-lymphocyte counts dropped to zero following the first dose. Fisher et al. (2005); Prasad (2014) CD3, CD4, CD8 and NK cell counts remained unchanged. B-cell recovery in peripheral blood began at 6-9 months and was complete by 9-12 months. No significant changes in serum complement levels were noted. The mechanism for action remains unresolved with CDC, ADCC, apoptosis and/or others being considered. In spite of the absence of a clinical/laboratory correlation, the contribution of CDC cannot be discounted. We have seen a correlation between higher absolute NK cell count at baseline and response to the Mab.

Cell Type	# Patients CR+PR	Abs. Count	# Patients NR	Abs. Count	P-value
NK	98	180	15	98	0.02
MK+ANC	98	185	15	102	0.02
ANC	101	3.7	15	3.4	0.40
CD3+	98	761	15	576	0.37
Platelets	101	187	15	206	0.32
Note: N = 166 patients from study 102-05, and 37 from 102-06. Abs. Count: NK, CD3 = cells/mm3; ANC, Pts. = cells x 10e3/mm3. P value for the difference between Abs. Counts.					

DCC may be an important mechanism for the clinical activity seen in patients treated with RITUXAN®. Agents which enhance effector cell number and activity may synergize with the mab. Studies of RITUXAN® in combination with cytokines, e.g., I1-2, G-CSF, GM-CSF, INF, are also ongoing.

EXAMPLE III: Phase I/II Study of RITUXAN® in CLL RITUXAN® is a monoclonal antibody targeting CD20 that has significant activity in the treatment of low-grade lymphoma (LGL). When given at a dosage of 375 mg/m3 weekly/ four response rate in relapsed patients (PTS) was 43% (Mc Claughlin et al., KOO, Vol. 14, 1998). Patients with small lymphocytic lymphoma had lower response rates (13%) than patients with other subtypes of LGL and lower serum levels of RITUXAN®. Reduced response seen in SLL could be related to lower density of CD20 antigen and/or higher circulating B-cell counts. Both factors would be expected to impact (negatively) on response seen in CLL. In an attempt to maximize activities in CLL are conducting a Phase I/II study. All patients receive a first dose of 375 mg/m3 to minimize infusion-relapsed side effects. Subsequent weekly dosages (3) remain the same but are given at an increased dose level. Sixteen patients have been treated at dosages of 500-1500 mg/m3. Medium age was 66 years (range, 25-78). Eighty-one percent had end-stage III-IV disease. Medium white blood cell count was 40 x 109/L (range, 4-200), Hgb 11.6 g/dl (range, 7.7-14.7), platelets 75 x 109/L (range, 16-160), median β2 immunoglobulin was 4.5 mg/L (range, 3.1-9.2). Median numbers of prior therapies was 2.5 (range 1-9). Sixty percent of patients were refractory to treatment. Two patients developed severe hypertension with the first dose (375 mgm3); another one received further therapy. Smith (2003); Cheson (2010); Pawluczkowycz et al. (2009); Fisher et al. (2005); Prasad (2014) Toxicity at subsequent escalated dosages has been mild although no patient at the 1500 mg/m3 dose level has been fully evaluated. Eight patients have completed therapy (4 at 500 mg/m3, 3 at 650 mg/m3, 1 at 825 mg/m3). One patient treated at 560 mg/m3 achieved full remission. One patient has progressive lymphocytosis on treatment and all other patients had reduction in peripheral blood lymphocytosis but less effect on lymph nodes. Dose escalation studies are ongoing.

EXAMPLE IV: Use of cytokines to upregulate the expression of CD20 [0026] another approach to improving response in CLL patients is to upregulate the CD20 antigen using cytokines. In an in vitro study, mononuclear cells from CLL patients were incubated for 24 hours with various cytokines. Flow cytometry results showed significant up-regulation by IL-4, GM-CSF, and TNF-alpha. Venugopal P, Sivararnan S, Huang Cell Type # Patients CR+PR Abs. Count # Patients NR Abs. Count P-value NK 98 180 15 98 0.02 MK+ANC 98 185 15 102 0.02 ANC 101 3.7 15 3.4 0.40 CD3+ 98 761 15 576 0.37 Platelets 101 187 15 206 0.32 Note: N = 166 patients from study 102-05, and 37 from 102-06. Abs. Count: NK, CD3 = cells/mm3; ANC, Pts. = cells x 10e3/mm3. P value for the difference between Abs. Counts. EP 1 616 572 B1 5 5 10 15 20 25 30 35 40 45 50 55 X, Chopra H, O'Brein T, Jajeh A, Preisler H. Upregulation of CD20 expression in Chronic Lymphocytic Leukemia (CLL) cells by in vitro exposure to cytokines. Blood 1998; 10:247a. In fact, recent data suggests that the upregulation of CD20 observed on CLL cells may be limited to tumor cells (Venogopal et al. Poster – Pan Pacific Lymphoma meeting, June 1999. Cytokine-induced upregulation of CD20 antigen expression in Chronic Lymphocytic Leukemia (CLL) (Vose et al., 2013; Quackenbush et al., 2015; Press et al., 2013; Shadman et al., 2018) cells may be limited to tumor cells). Preliminary data also suggest that interferon alpha also upregulates CD20 on CLL cells after only 24 hours when applied at a concentration of 500 to 1000 U/ml Thus, by administering certain cytokines to CLL patients prior to or concurrently with administration of Rituximab®, the expression of CD20 on the surface of malignant B-cells may be upregulated, thereby rendering CD20, as well as other cell surface markers such as CD19, a more attractive target for immunotherapy. Vitolo et al. (2017); Maloney et al. (2016); van Imhoff et al. (2017); Vose et al. (2013) A collaborative study has been initiated to test for optimal cytokine doses for CD20 upregulation in vivo. The study protocol involves treating ten patients initially with GM-CSF at 250 mcg/m2 SQ QD X 3, ten patients with IL-4 mcg/kg SQ QD X 3, and ten patients with G-CSF at 5 mcg/kg SQ QD X 3. Mononuclear cells will be separated by Ficon Hypaque centrifugation for apoptotic studies to determine if upregulation of CD20 translates to enhanced killing of tumor cells by Rituximab®.

EXAMPLE V: Combination Antibody and Chemotherapy Protocol .Antibody treatment of CLL can be combined with other conventional chemotherapeutic treatments known to be useful for the treatment of CLL. The most frequently used single agent for CLL is chlorambucil (leukeran), given either as 0.1 mg/kg daily or 0.4 to 1.0 mg/kg every 4 weeks. Chlorambucil is often combined with oral prednisone (30 to 100 mg/m2/d), which is useful in the management of autoimmune cytopenias. Cyclophosphamide is an alternative to chlorambucil, the usual dose being 1-2 g/m2 every 3-4 weeks together with vincristine and steroids (Sun & Carlson, 2017; Salles et al., 2017; Smith, 2003; Cheson, 2010; Pawluczkowycz et al., 2009; Fisher et al., 2005) (e.g., COP regimen). Various drug combinations have been used for CLL, including COP (cyclophosphamide, Oncovin, and prednisone), and CHOP (these three drugs plus doxorubicin). Fludarabine has shown an effect in the treatment of CLL, and gave an ORR of 50% in a group of patients treated with 25-30 mg/m2/d every 3-4 weeks. http://www.cancernetwork.com.

Although some patients have been shown to be refractory for fludarabine. Such patients may also be resistant to 2-CdA because often, patients who are refractory to fludarabine are also refractory to 2-CDA (O'Brien et al. N. Engl. J. Med. 330: 319-322 (1994)). Hence, anti-CD20 antibody therapy will be particularly useful for patients who are refractory or who have relapsed after treatment with chemotherapeutic drugs. Rituximab® therapy may also be combined with radiotherapy in these patients. TBI with a low fraction size of 15 cGy to total doses of 75 to 150 cGy has been shown to be effective in about one-third of patientsA Phase II trial is currently being conducted by CALGB in CLL patients. Rituximab®

and fludarabine are administered concurrently, followed by Rituximab® consolidation versus fludarabine induction followed by Rituximab®.

CONCLUSION

The goals of the study are

1. to determine in fludarabine treated CLL patients the complete response (CR) rate and toxicity profile of concurrent and consolidative Rituximab® therapy (Arm I) and of consolidative Rituximab® therapy (Arm II);
2. to assess the CR rate in patients receiving concurrent therapy with Rituximab® and fludarabine (the inductive phase of Arm I);
3. to assess the frequency of conversion of a partial response (PR) to a CR or stable disease to either PR or CR in CLL patients receiving consolidative therapy with Rituximab®;
4. to follow the effects of therapy with Rituximab® and fludarabine on the immunologic markers CD4, CD8, IgG, IgA and IgM; and
5. to examine progression-free survival and overall survival in Arms I and II. Although the present updated patent evaluation patents on the invention have been described in some detail by way of illustration and example, for purposes of clarity and understanding it will be apparent that certain changes and modifications may be practical within the scope of the above.

Further to this how the Manufacturing Process of Biological, Antibodies and derivatives was carried out industrially was explained stepwise.

1. Upstream Process

Stage 1: Fermentation and Clarification

Process starts with thawing of vial from cell bank to generate the inoculum. The cells from the vials are propagated using flasks and seed fermenters to get required quantity of inoculum for production bioreactor. The cells are allowed to grow in the production fermenter for defined number of days in the batch mode. Feeds of nutrients like glucose and Micronutrients are fed in the fermenter on different periods. N the Fermenter is harvested at the end of the fermentation and taken for further processing like Centrifugation, Homozinisation and clarification. The clarified processed bulk is called Inclusion bodies (IBs) and will be stored at -20 degree centigrade. The cells are removed from the centrifuge is then processed and killed in kill tank through steam and alkali.

2. Downstream Processing

Stage 2: Refolding And Chromatography Refolding (Microbial products only)

Purified Inclusion bodies from will be dissolved and subjected to alkaline conditions to unfold the protein to linear structure under defined conditions. Then the refolding buffer will be prepared and dissolved IBs will be transferred to the refolding buffer. Then the mixture will be incubated at 2-8 degree centigrade for 24 hrs and the reaction will quenched by adding acids. The refolded protein solution will be filtered through 1 Micron filter and subjected for chromatography-1.

Chromatography I:

During Chromatography I, column packed with defined matrix is conditioned by using buffers. The filtered refolded protein solution is then passed through the column and the product present in the solution binds with the Matrix (Stationary phase). The protein will bind to the column. The absorbed product is removed from the column by elution buffers. The column is then regenerated to remove impurities or unbound proteins by flushing with alkaline and acidic buffers like sodium hydroxide and acetic acid respectively. The column and system is stored in storage solution like mild alkali solution or solvents like ethanol.

Chromatography II:

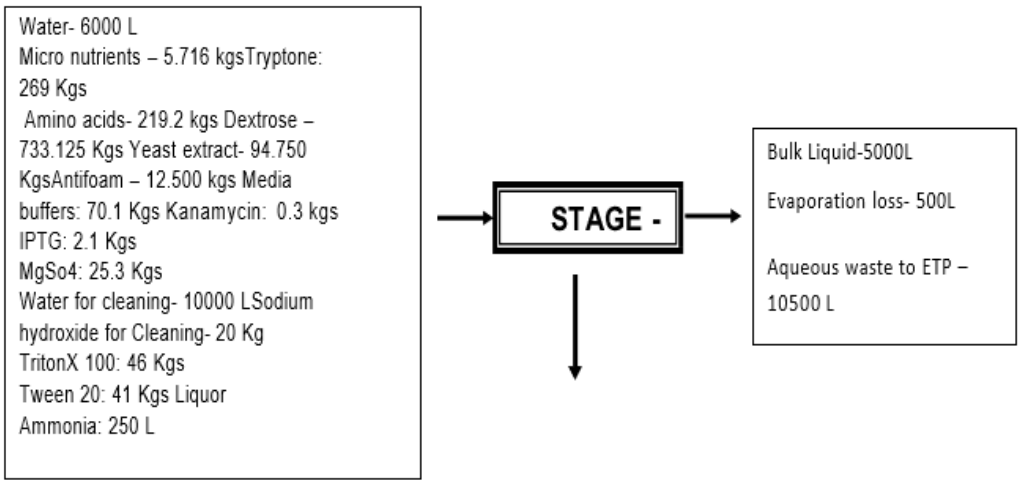
In this stage the product obtained from chromatography-1 will be passed through the Chromatography II column conditioned with binding buffers. The absorbed product is removed from the column by elution buffers which are in combination of solvents like n-propanol and sodium sulphate. The column is then regenerated to remove absorbed impurities by flushing with solvents. The column and system is stored in storage solution like mild alkali solution or solvents like ethanol.

Chromatography III:

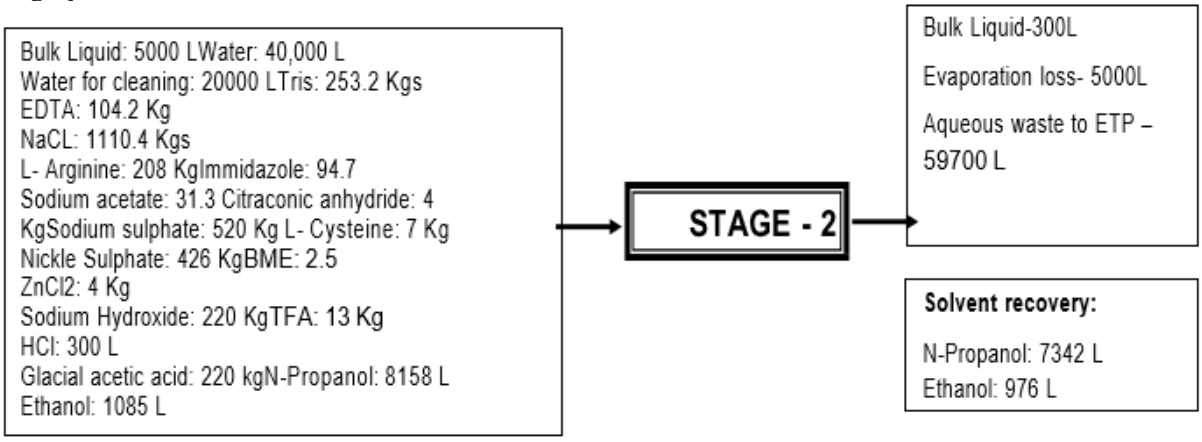
Product from Chromatography II will be passed through the Chromatography III column which is preconditioned under high pressure. The absorbed product is removed from the column by elution buffers which are in combination of solvents like n-propanol and sodium sulphate in gradient mode. The protein is collected in fractions as per the defined criteria. The column is then regenerated to remove absorbed impurities by flushing with solvents. The column and system is stored in storage solution like mild alkali solution or solvents like ethanol. The eluted protein is finally pooled and precipitated by adding zinc sulphate. The final precipitant (slurry) is separated by filtration and collected in vessel or container.

Stage 03: Lyophilisation/ Filtration

In this step the final slurry obtained from chromatography III is poured in trays and subjected to freezing conditions in Lyophiliser. The lyophilized product will be collected in containers and stored at 2-8 degree centigrade. The lyophiliser will be cleaned by using alkali and saturated steam.



Filtration: In this step final product will be filtrated by using single use 0.2 micron pore size filters aseptically. Filtrated product will be stored in the storage container under +2 to + 8 degree centigrade temperature. Single use 0.2 micron pore size filter post usage integrity will be tested.



- No pyrophoric & highly reactive materials are used in manufacturing process.
- No restricted/banned chemicals is used in the manufacturing process.

References

1. Schwaber, J. & Cohen, E. P. (1973 Aug 17). Human × Mouse Somatic Cell Hybrid Clone Secreting Immunoglobulins of Both Parental Types. *Nature, 244*, 444–447. DOI: 10.1038/244444a0
2. Köhler, G. & Milstein, C. (1975 Aug 7). Continuous Cultures of Fused Cells Secreting Antibody of Predefined Specificity. *Nature, 256*(5517), 495–497. DOI: 10.1038/256495a0
3. Koprowski, H., Steplewski, Z., Herlyn, D. & Herlyn, M. (1978 July 1). Study of Antibodies against Human Melanoma Produced by Somatic Cell Hybrids. *Proc. Natl. Acad. Sci. USA, 75*(7), 3405–3409. https://doi.org/10.1073/pnas.75.7.3405
4. Nadler, L. M., Stashenko, P., Hardy, R., Kaplan, W. D., Button, L. N., Kufe, D. W., Antman, K. H. & Schlossman, S. F. (1980). Serotherapy of a Patient with a Monoclonal Antibody Directed against a Human Lymphoma-Associated Antigen. *Cancer Res, 40*(9), 3147–3154. Retrieved from https://pubmed.ncbi.nlm.nih.gov/7427932/
5. Shin, S.U. & Morrison, S. L. (1989). Production and Properties of Chimeric Antibody Molecules. *Methods Enzymol, 178*, 459–476. DOI: 10.1016/0076-6879(89)78034-4
6. Riechmann, L., Clark, M., Waldmann, H. & Winter G. (1988). Reshaping Human Antibodies for Therapy. *Nature, 332*(6162), 323–327. DOI: 10.1038/332323a0
7. Armitage, J. O., Gascoyne, R. D., Lunning, M. A. & Cavalli, F. (2017 Jul 15). Non-Hodgkin lymphoma. *Lancet, 390* (10091), 298–310. DOI: 10.1016/S0140-6736(16)32407-2
8. Shankland, K. R., Armitage, J. O. & Hancock, B. W. (2012). Non-Hodgkin lymphoma. *Lancet, 380*(9844), 848–857. DOI: 10.1016/S0140-6736(12)60605-9
9. Beers, S. A., Chan, C. H., French, R. R., Cragg, M. S. & Glennie, M. J. (2010). CD20 as a target for therapeutic type I and II monoclonal antibodies. *Semin Hematol, 47*(2), 107-14. DOI: 10.1053/j.seminhematol.2010.01.001
10. Feugier, P. (2015). A review of rituximab, the first anti-CD20 monoclonal antibody used in the treatment of B non-Hodgkin's lymphomas. *Future Oncol, 11*(9), 1327–

1342. DOI: 10.2217/fon.15.57
11. Lanini, S., Molloy, A. C., Fine, P. E., Prentice, A. G., Ippolito, G. & Kibbler, C. C. (2011). Risk of infection in patients with lymphoma receiving rituximab: Systematic review and meta-analysis. *BMC Med, 9*, 36. DOI: 10.1186/1741-7015-9-36
12. Cang, S., Mukhi, N., Wang, K. & Liu, D. (2012). Novel CD20 monoclonal antibodies for lymphoma therapy. *J Hematol Oncol, 5,* 64. DOI: 10.1186/1756-8722-5-64
13. Kasi, P. M., Tawbi, H. A., Oddis, C. V. & Kulkarni, H. S. (2012). Clinical review: Serious adverse events associated with the use of rituximab—a critical care perspective. *Crit Care, 16*(4), 231. DOI: 10.1186/cc11304
14. U.S. Patent 5,530,101, issued to Queen et al, U.S. Patent 5,225,539, issued to Winter et al, U.S. Patents 4,816,397 and 4,816,567, issued to Boss et al, and Cabilly et al, respectively. EP 1616 572 B
15. Keating, M. J., Dritselis, A., Yasothan, U. & Kirkpatrick, P. (2010). Ofatumumab. *Nat. Rev. Drug Discov, 9*(2), 101–102. Retrieved from https://www.unboundmedicine.com/medline/citation/20118960/Ofatumumab_
16. Freeman, C. L. & Sehn, L. (2018). Anti-CD20 directed therapy of B cell lymphomas: Are new agents really better?. *Curr Oncol Rep, 20*(12), 103. DOI: 10.1007/s11912-018-0748-0
17. Soe, Z. N. & Allsup, D. (2017). The use of ofatumumab in the treatment of B-cell malignancies. *Future Oncol, 13*(29), 2611–2628. DOI: 10.2217/fon-2017-0275
18. Cartron, G. & Watier, H. (2017). Obinutuzumab: What is there to learn from clinical trials?. *Blood, 130*(5), 581–589. DOI: 10.1182/blood-2017-03-771832
19. Casan, J. M. L., Wong, J., Northcott, M. J. & Opat, S. (2018). Anti-CD20 monoclonal antibodies: Reviewing a revolution. *Hum Vaccines Immunother, 14*(12), 2820–2841. DOI: 10.1080/21645515.2018.1508624
20. Tobinai, K., Klein, C., Oya, N. & Fingerle-Rowson, G. (2017). A review of obinutuzumab (GA101), a novel type II anti-CD20 monoclonal antibody, for the treatment of patients with B-cell malignancies. *Adv Ther, 34*(2), 324–356. DOI: 10.1007/s12325-016-0451-1
21. Illidge, T. M. (2010). Radio immunotherapy of lymphoma: A treatment approach ahead of its time or past its sell-by date?. *J Clin Oncol, 28*(1), 2944–2946. https://doi.org/10.1200/JCO.2009.26.8748
22. Rizzieri, D. (2016). Zevalin(®) (ibritumomab tiuxetan): After more than a decade of treatment experience, what have we learned?. *Crit Rev Oncol Hematol, 105*, 5–17. DOI: 10.1016/j.critrevonc.2016.07.008
23. Tierney, J. F., Stewart, L. A., Ghersi, D., Burdett, S. & Sydes, M. R. (2007). Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials 8*, 1-16. Retrieved from https://trialsjournal.biomedcentral.com/articles/10.1186/1745-6215-8-16
24. Zacher, J., Kasenda, B., Engert, A. & Skoetz, N. (2014). The role of additional radiotherapy for primary central nervous system lymphoma. *Cochrane Database Syst Rev, 6.* Doi: 10.1002/14651858.CD009211.pub2
25. Casasnovas, R. O., Salles, G. A., Oberic, L., Bodet-Milin, C., Briere, J., Morschhauser, F., Tilly, H., Ribrag, V., Lamy, T. Pr., Thieblemont, C., Maisonneuve, H., Gressin, R., Bouabdallah, K., Haioun, C., Damaj, G., Fornecker, L. M., Bouabdallah, R., Feugier, P., Delarue, R., …… Gouill, S. L. (2017). Obinutuzumab versus rituximab in combination with ACVBP-14 or CHOP-14 following a PET Driven strategy in Aa-IPI 1–3 DLBCL patients (< 60 years): Third planned interim and final analyses of the gained trial. *Blood, 130*(1), 190. DOI: https://doi.org/10.1182/blood.V130.Suppl_1.190.190
26. Marcus, R., Davies, A., Ando, K., Klapper, W., Opat, S., Owen, C., Phillips, E., Sangha, R., Schlag, R., Seymour, J. F., Townsend, W., Trněný, M., Wenger, M., Fingerle-Rowson, G., Rufibach, K., Moore, T., Herold, M. & Hiddemann, W. (2017). Obinutuzumab for the first-line treatment of follicular lymphoma. *N. Engl. J. Med. 377*(14), 1331–1344. DOI: 10.1056/NEJMoa1614598
27. Hiddemann, W., Barbui, A. M., Canales, M. A., Cannell, P. K., Collins, G. P., Dürig, J., Forstpointner, R., Herold, M., Hertzberg, Mark., Klanova, M., Radford, J., Seymour, J. F., Tobinai, K., Trotman, J., Burciu, A., Fingerle-Rowson, G., Wolbers, M., Nielsen, T. & Marcus, R. E. (2018). Immunochemotherapy with obinutuzumab or rituximab for previously untreated follicular lymphoma in the GALLIUM study: Influence of chemotherapy on efficacy and safety. *J Clin Oncol, 36*(23), 2395–2404. Retrieved from https://ascopubs.org/doi/pdf/10.1200/JCO.2017.76.8960
28. Townsend, W., Buske, C., Cartron, G., Cunningham, D., Dyer, M. J. S., Gribben, J. G., Hess, G., Ishikawa, T., Keller, U., Kneba, M., Malladi, R., Neidhart, J. D., Rusconi, C., Zhu, J., Catalani, O., Knapp, A., Zeuner, H., Herold, M., Hiddemann, W., .. Marcus, R. (2018). Obinutuzumab-based immunochemotherapy prolongs progression-free survival and time to next anti-lymphoma treatment in patients with previously untreated follicular lymphoma: Four-year results from the phase III GALLIUM study. *Blood, 132*(1), 1597. Retrieved from https://ashpublications.org/blood/article/132/Supplement%201/1597/272983/Obinutuzumab-Based-Immunochemotherapy-Prolongs
29. Sehn, L. H., Goy, A., Offner, F. C., Martinelli, G., Caballero, M. D., Gadeberg, O., Baetz, T., Zelenetz, A. D., Gaidano, G., Fayad, L. E., Buckstein, R., Friedberg, J. W., Crump, M., Jaksic, B., Zinzani, P. L., Padmanabhan, Iyer. S., Sahinm, D., Chai, A., Fingerle-Rowson, G., … Press, O. W. (2015). Randomized phase II trial comparing obinutuzumab (GA101) with rituximab in patients with relapsed CD20+ indolent B-Cell non-Hodgkin lymphoma: Final analysis of the GAUSS study. *J Clin Oncol, 33*(30), 3467–3474. DOI: 10.1200/JCO.2014.59.2139
30. 30. Shadman, M., Li, H., Rimsza, L., Leonard, J. P., Kaminski, M. S., Braziel, R. M., Spier, C. M., Gopal, A. K., Maloney, D. G., Cheson, B. D., Dakhil, S., LeBlanc, M., Smith, S. M., Fisher, R. I., Friedberg, J. W. & Press, O. W. (2018). Continued excellent outcomes in previously untreated patients with follicular lymphoma after treatment with CHOP plus rituximab or CHOP plus (131)I-tositumomab: Long-term follow-up of phase III

randomized study SWOG-S0016. *J Clin Oncol, 36*(7), 697–703. DOI: 10.1200/JCO.2017.74.5083

31. Khouri, I. F., Sui, D., Turturro, F., Erwin, W. D., Bassett, R. L., Korbling, M., Valverde, R., Ahmed, S., Alousi, M. A., Anderlini, P., Bashir, Q., Ciurea, S. O., Oran, B., Olson, A., Popat, U. R., Patel, K. K., Qazilbash, M. H., Fanale, M. A., Fayad, L. E., … Jessop, A. (2015). In-Vivo Purging with Rituximab (R) Followed By Z/BEAM Vs BEAM/R Autologous Stem Cell Conditioning for Relapsed Diffuse Large B-Cell Lymphoma (DLBCL) Patients (pts): Mature Results from a Combined Analysis of 3 Trials. *Blood, 126*(23), 3192. Retrieved from https://ashpublications.org/blood/article/126/23/3192/90883/In-Vivo-Purging-with-Rituximab-R-Followed-By-Z
32. Witzig, T. E., Gordon, L. I., Cabanillas, F., Czuczman, M. S., Emmanouilides, C., Joyce, R., Pohlman, B. L., Bartlett, N. L., Wiseman, G. A., Padre, N., Grillo-López, A. J., Multani, P. & White, C. A. (2002). Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. *J Clin Oncol, 20*(10), 2453–2463. DOI: 10.1200/JCO.2002.11.076
33. Gordon, L. I., Witzig, T., Molina, A., Czuczman, M., Emmanouilides, C., Joyce, R., Vo, K., Theuer, C., Pohlman, B., Bartlett, N., Wiseman, G., Darif, M. & White, C. (2004). Yttrium 90-labeled ibritumomab tiuxetan radioimmunotherapy produces high response rates and durable remissions in patients with previously treated B-cell lymphoma. *Clin. Lymphoma, 5*(2), 98–101. DOI: 10.3816/clm.2004.n.015
34. Guzauskas, G. F., Masaquel, A., Thuresson, Per-Olof., Li, J., Reyes, C. M. & Pharm, D. V. (2017). Is obinutuzumab plus chemotherapy followed by obinutuzumab monotherapy for previously untreated follicular lymphoma patients cost-effective in the US? *Blood 130*, 5603
35. Sun, L. A. & Carlson, J. J. (2017). Cost-utility of obinutuzumab in previously untreated follicular lymphoma. Value Health 20, A110-a111
36. Vitolo, U., Trněný, M., Belada, D., Burke, J. M., Carella, A. M., Chua, N., Abrisqueta, P., Demeter, J., Flinn, I., Hong, X., Kim, W. S., Pinto, A., Shi, Y. K., Tatsumi, Y., Oestergaard, M. Z., Wenger, M., Fingerle-Rowson, G., Catalani, O., Nielsen, T., Martelli, M., …. Sehn, L. H. (2017). Obinutuzumab or rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone in previously untreated diffuse large B-cell lymphoma. *J Clin Oncol, 35*(31), 3529-3537. DOI: 10.1200/JCO.2017.73.3402
37. Maloney, D. G. Fukuhara, N., Ogura, M., Larouche, J. F., Tournilhac, O., Coleman, M., Hong, X., Fennessy, M., Cota, M., Woessner, M. & Tobinai, k. (2016). A phase III study of ofatumumab vs rituximab in indolent B-cell non-Hodgkin lymphoma relapsed after rituximab containing therapy (HOMER): Results of the interim analysis. *Haematologica, 101*, 102. Retrieved from https://www.google.com/search?q=A+phase+III+study+of+ofatumumab+vs+rituximab+in+indolent+B-cell+non-Hodgkin+lymphoma+relapsed+after+rituximab+containing+therapy+%28HOME https://www.google.com/search?q=A+phase+III+study+of+ofatumumab+vs+rituximab+in+indolent+B-cell+non-Hodgkin+lymphoma+relapsed+after+rituximab+containing+therapy+%28HOMsults+of+the+interim+analysis&rlz=1C1UEAD_enIN972IN972&sxsrf=APq-WBtsE9st9kI3w0FtlPwjFQ9aLTfy0w%3A1650861795956&ei=4yZmYtD-OfWI4-EPxcaU8Ao&ved=0ahUKEwiQ_K63s673AhV1xDgGHUUjBa4Q4dUDCA4&uact=5&oq=A+phase+III+study+of+ofatumumab+vs+rituximab+in+indolent+B-cell+non-Hodgkin+lymphoma+relapsed+after+ritghYughgoQxoAnAAeACAAQCIAQCSAQCYAQCgAQGgAQKwAQrAAQE&sclient=gws-wiz
38. Fisher, R. I., Kaminski, M. S., Wahl, R. L., Knox, S. J., Zelenetz, A. D., Vose, J. M., Leonard, J. P., Kroll, S., Goldsmith, S. J. & Coleman, M. (2005). Tositumomab and iodine-131 tositumomab produces durable complete remissions in a subset of heavily pretreated patients with low-grade and transformed non-Hodgkin's lymphomas. *J Clin Oncol, 23*(30), 7565–7573. DOI: 10.1200/JCO.2004.00.9217
39. Prasad, V. (2014). The withdrawal of drugs for commercial reasons: The incomplete story of tositumomab. *JAMA Intern Med, 174*(12), 1887–1888. DOI: 10.1001/jamainternmed.2014.5756
40. Smith, M. R. (2003). Rituximab (monoclonal anti-CD20 antibody): mechanisms of action and resistance. *Oncogene, 22*(47), 7359–7368. DOI: 10.1038/sj.onc.1206939
41. Cheson, B. D. (2010). Ofatumumab, a novel anti-CD20 monoclonal antibody for the treatment of B-cell malignancies. *J Clin Oncol, 28*(21), 3525–3530. DOI: 10.1200/JCO.2010.27.9836
42. Pawluczkowycz, A. W., Beurskens, F. J., Beum, P. V., Lindorfer, M. A., van de Winkel, J. G., Parren, P. W. & Taylor, R. P. (2009). Binding of submaximal C1q promotes complement-dependent cytotoxicity (CDC) of B cells opsonized with anti-CD20 mAbs ofatumumab (OFA) or rituximab (RTX): Considerably higher levels of CDC are induced by OFA than by RTX. *J Immunol, 183*(1), 749–758. DOI: 10.4049/jimmunol.0900632
43. Vose, J. M., Carter, S., Burns, L. J., Ayala, E., Press, O. W., Moskowitz, C. H., Stadtmauer, E. A., Mineshi, S., Ambinder, R., Fenske, T., Horowitz, M., Fisher, R. & Tomblyn, M. (2013). Phase III randomized study of rituximab/carmustine, etoposide, cytarabine, and melphalan (BEAM) compared with iodine-131 tositumomab/BEAM with autologous hematopoietic cell transplantation for relapsed diffuse large B-cell lymphoma: Results from the BMT CTN 0401. *J Clin Oncol, 31*(13), 1662–1668. DOI: 10.1200/JCO.2012.45.9453
44. Quackenbush, R. C., Horner, T. J., Williams, V. C., Giampietro, P. & Lin, T. S. (2015). Patients with relapsed follicular lymphoma treated with rituximab versus tositumomab and iodine I-131 tositumomab. *Leuk. Lymphoma, 56*(3), 779–781. DOI: 10.3109/10428194.2014.927461

45. Press, O. W., Unger J. M., Rimsza L. M., Friedberg, J. W., LeBlanc, M., Czuczman, M. S., Kaminski, M., Braziel, R. M., Spier, C., Gopal, A. K., Maloney, D. G., Cheson, B. D., Dakhil, S. R., Miller, T. P. & Fisher, R. I. (2013). Phase III randomized intergroup trial of CHOP plus rituximab compared with CHOP chemotherapy plus (131)iodine-tositumomab for previously untreated follicular non-Hodgkin lymphoma: SWOG S0016. *J Clin Oncol, 31*(3), 314–320. DOI: 10.1200/JCO.2012.42.4101
46. van Imhoff, G. W., McMillan, A., Matasar, M. J., Radford, J., Ardeshna, K. M., Kuliczkowski, K., Kim, W., Hong, X., Goerloev, J. S., Davies, A., Barrigón, MDC., Ogura, M., Leppä, S., Fennessy, M., Liao, Q., van der Holt, B., Lisby, S. & Hagenbeek, A. (2017). Ofatumumab versus rituximab salvage chemoimmunotherapy in relapsed or refractory diffuse large B-cell lymphoma: The ORCHARRD study. *J Clin Oncol, 35*(5), 544–551. DOI: https://doi.org/10.1200/jco.2016.69.0198
47. Salles, G., Barrett, M., Foà, R., Maurer, J., O'Brien, S., Valente, N., Wenger, M. & Maloney, D. G. (2017). Rituximab in B-cell hematologic malignancies: A review of 20 years of clinical experience. *Adv Ther, 34*(10), 2232–2273. DOI: 10.1007/s12325-017-0612-x
48. Lopez-Guillermo, A., Canales, M. A., Dlouhy, I., Briones, J., Caballero, D., Sancho, J. M., Vilchez, S. M., Moraleda, J. M., Terol, M. J., Salar, A., Palomera, L., Gardella, S., Jarque, I., Ferrer, S., Bargay, J., Lopez, A., Panizo, C., Muntanola, A., Montalban, C., …. Tomas, J. F. (2013). A randomized phase II study comparing consolidation with a single dose of 90y ibritumomab tiuxetan (Zevalin®) (Z) Vs. maintenance with rituximab (R) for two years in patients with newly diagnosed Follicular Lymphoma responding to R-CHOP. Preliminary results at 36 months from randomization. *Blood, 122*(21), 369. DOI: https://doi.org/10.1182/blood.V122.21.369.369
49. Hansel, T. T., Kropshofer, H., Singer, T., Mitchell, J. A. & George, A. J. (2010). The safety and side effects of monoclonal antibodies. *Nat Rev Drug Discov, 9*(4), 325–338. https://doi.org/10.1038/nrd3003
50. Higgins, Julian, P. T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M. J. & Welch, V. A. (Eds.) (2019). Cochrane Handbook for Systematic Reviews of Interventions. (2nd Eds.) Wiley-Blackwell. Retrieved from chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://dariososafoula.files.wordpress.com/2017/01/cochrane-handbook-for-systematic-reviews-of-interventions-2019-1.pdf