Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3071618 A1 2019/02/14

(21) 3 071 618

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2018/08/08
(87) Date publication PCT/PCT Publication Date: 2019/02/14
(85) Entrée phase nationale/National Entry: 2020/01/14
(86) N° demande PCT/PCT Application No.: EP 2018/071462
(87) N° publication PCT/PCT Publication No.: 2019/030260
(30) Priorité/Priority: 2017/08/08 (US62/542,489)

(51) Cl.Int./Int.Cl. *C07K 16/28* (2006.01),
A61K 39/395 (2006.01)

(71) Demandeurs/Applicants:
F. HOFFMANN-LA ROCHE AG, CH;
NANOSTRING TECHNOLOGIES, INC., US

(72) Inventeur/Inventor:
OESTERGAARD, MIKKEL ZAHLE, CH

(74) Agent: GOWLING WLG (CANADA) LLP

(54) Titre : TRAITEMENT A L'OBINUTUZUMAB D'UN SOUS-GROUPE DE PATIENTS DLBCL
(54) Title: OBINUTUZUMAB TREATMENT OF A DLBCL PATIENT SUBGROUP

(57) Abrégé/Abstract:
The present invention relates to obinutuzumab (or its functional equivalents) for use in the treatment of a particular biomarker-defined DLBCL patient and a novel DLBCL patient subgroup, respectively. The present invention further relates to a method for treating DLBCL with obinutuzumab (or its functional equivalents) in a patient in need thereof, wherein said patient is a particular biomarker-defined DLBCL patient or belongs to a novel biomarker-defined DLBCL patient subgroup. The present invention further relates to the use of obinutuzumab (or its functional equivalents) for the preparation of a pharmaceutical composition for the treatment of DLBCL in the particular biomarker-defined DLBCL patient/novel DLBCL patient subgroup. The present invention further relates to a method for identifying a particular DLBCL patient/novel DLBCL patient subgroup and a method for diagnosing a novel form of DLBCL and a particular DLBCL patient/novel DLBCL patient subgroup, respectively.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 February 2019 (14.02.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/030260 A1

(51) International Patent Classification:
C07K 16/28 (2006.01) *A61K 39/395* (2006.01)

(21) International Application Number:
PCT/EP2018/071462

(22) International Filing Date:
08 August 2018 (08.08.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/542,489 08 August 2017 (08.08.2017) US

(71) Applicants: **F. HOFFMANN-LA ROCHE AG** [CH/CH]; Grenzacherstrasse 124, 4070 Basel (CH). **NANOSTRING TECHNOLOGIES, INC.** [US/US]; 530 Fairview Ave North, Seattle, Washington 98109 (US).

(72) Inventor: **OESTERGAARD, Mikkel Zahle**; Bergstasse 32, 8702 Zollikon (CH).

(74) Agent: **VOSSIUS & PARTNER (NO 31)**; Patentanwälte Rechtsanwälte mbB, Siebertstraße 3, 81675 München (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2019/030260 A1

(54) Title: OBINUTUZUMAB TREATMENT OF A DLBCL PATIENT SUBGROUP

(57) Abstract: The present invention relates to obinutuzumab (or its functional equivalents) for use in the treatment of a particular biomarker-defined DLBCL patient and a novel DLBCL patient subgroup, respectively. The present invention further relates to a method for treating DLBCL with obinutuzumab (or its functional equivalents) in a patient in need thereof, wherein said patient is a particular biomarker-defined DLBCL patient or belongs to a novel biomarker-defined DLBCL patient subgroup. The present invention further relates to the use of obinutuzumab (or its functional equivalents) for the preparation of a pharmaceutical composition for the treatment of DLBCL in the particular biomarker-defined DLBCL patient/novel DLBCL patient subgroup. The present invention further relates to a method for identifying a particular DLBCL patient/novel DLBCL patient subgroup and a method for diagnosing a novel form of DLBCL and a particular DLBCL patient/novel DLBCL patient subgroup, respectively.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 62

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 62

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

OBINUTUZUMAB TREATMENT OF A DLBCL PATIENT SUBGROUP

The present invention relates to obinutuzumab (or its functional equivalents) for use in the treatment of a particular biomarker-defined DLBCL patient and a novel DLBCL patient subgroup, respectively. The present invention further relates to a method for treating DLBCL with obinutuzumab (or its functional equivalents) in a patient in need thereof, wherein said patient is a particular biomarker-defined DLBCL patient or belongs to a novel biomarker-defined DLBCL patient subgroup. The present invention further relates to the use of obinutuzumab (or its functional equivalents) for the preparation of a pharmaceutical composition for the treatment of DLBCL in the particular biomarker-defined DLBCL patient/novel DLBCL patient subgroup. The present invention further relates to a method for identifying a particular DLBCL patient/novel DLBCL patient subgroup and a method for diagnosing a novel form of DLBCL and a particular DLBCL patient/novel DLBCL patient subgroup, respectively.

Diffuse large B-cell lymphoma (DLBCL) is the most common type of aggressive non-Hodgkin lymphoma (NHL). Immunochemotherapy with the anti-CD20 monoclonal antibody (mAb) rituximab (R), plus cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), is the standard-of-care treatment for previously untreated patients who present with advanced-stage disease (Coiffier, N. Engl. J. Med. 346, 2002, 235-242; Tilly, Ann. Oncol. 26, 2015, v116-v125 (suppl 5); NCCN Clincal Practice Guidelines in Oncology: Non-Hodgkin's Lymphomas, Version 3. 2016; see also www.NCCN.org). Studies have shown a complete and unconfirmed complete response (CR/CRu) rate of 76% (GELA trial) (Coiffier loc. cit.), and a 2-year failure-free survival rate of 77% (Habermann, J. Clin. Oncol. 24, 2006, 3121-3127). Although first-line (1L) treatment for DLBCL is curative for many patients (Maurer, J. Clin. Oncol. 32, 2014, 1066-1073), there is still a need to improve outcome for the 20–40% of patients who fail to achieve a remission or who relapse, and outcomes with salvage therapy remain poor (Sehn, Blood 125, 2015, 22-32).

Obinutuzumab (Gazyva™/Gazyvaro™ *GA101*; G) is a glycoengineered, type II anti-CD20 mAb with greater direct cell death induction, and antibody-dependent cellular cytotoxicity and phagocytosis than R (Herter, Mol. Cancer Ther. 12, 2013, 2031-2042; Mössner, Blood 115, 2010, 4393-4402; EP-B1 2380910; WO 2005/044859; see also Illidge, Expert Opin. Biol. Ther. 12(5), 2012, 543-5). In phase 3 studies of previously untreated patients with chronic lymphocytic leukemia (CLL) and coexisting conditions (CLL11), or follicular lymphoma (FL; GALLIUM), G proved more effective than R (Goede, N. Engl. J. Med. 370, 2014, 1101-1110; Marcus, N. Engl. J. Med. (Accepted May 2017: in press). In smaller studies, G monotherapy and G-CHOP have demonstrated promise in aggressive forms of non-Hodgkin lymphoma (NHL), including DLBCL (Morschhauser, J. Clin. Oncol. 31, 2013, 2912-2919; Zelenetz, Blood 122, 2013, 1820). Also Owen (Expert Opin. Biol. Ther. 12(3), 2012, 343-51) discusses the use of Obinutuzumab for the treatment of lymphoproliferative disorders. A multicenter, open-label, randomized, clinical phase 3 study (GOYA; see below for further details) compared the efficacy and safety of G-CHOP with R-CHOP in previously untreated patients with DLBCL. However, in GOYA, G-CHOP did not improve the clinical outcome (e.g. progression free survival (PFS)) relative to R-CHOP in previously untreated DLBCL (1L DLBCL) with respect to the entive 1L DLBCL patient group that was initially intended to be treated in the context of GOYA.

Scott (Blood 123(8), 2014, 1214-7; JCO 33(26), 2015, 2848 57; Am. Soc. Clin. Oncol. Educ. Book 2015, 35:e458–66) and others (Nowakowski, Am. Soc. Clin. Oncol. Educ. Book 2015, 35:e449–57) performed gene expression-based determination of cell-of-origin (COO) subtypes of DLBCL by using the NanoString Lymph2Cx assay (Scott 2014 and 2015 loc. cit.). In particular, Scott (2014 and 2015 loc. cit.) assigned the COO subtypes of DLBCL, germinal-center B-cell-like DLBCL (GCB DLBCL), activated B-cell-like DLBCL (ABC DLBCL) and unclassified DLBCL, on the basis of a 20-gene gene expression assay and Linear Predictor Scores (LPSs) of ~ <1900, ~ 1900 – ~ 2450 and ~ >2450, respectively, (cf. Scott 2014 loc. cit., Fig. 1). Scott (2014 and 2015 loc. cit.) also assessed the treatment effect of R-CHOP on these COO subtypes of DLBCL.

Punnoose (Blood 126, 2015, 3971; see also http://www.bloodjournal.org/content/126/23/3971) describes the prevalence and prognostic value of BCL2 and MYC protein expression within ABC and GCB COO

subtypes in patients with previously untreated DLBCL from MAIN, a phase III trial that evaluated bevacizumab plus R-CHOP (NCT 00486759).
Challa-Malladi (Cancer Cell 20, 2011, 728-40) discloses that combined genetic inactivation of β2-Microglobulin and CD58 reveals frequent escape from immune recognition in DLBCL.

Despite previous success in the treatment of DLBCL (for example due to the advances with R, in particular R-CHOP), however, there is still a high unmet medical need for some DLBCL patients (cf. NCCN clinical practice guidelines in oncology; non-Hodgkin's lymphoma, v 2.2015) for an improved treatment (cf. Sehn, loc. cit.).

Therefore, the technical problem underlying the present invention is to provide an improved treatment of DLBCL in certain patients.

The solution to said technical problem is provided herein below and characterized in the appended claims.

It was surprisingly found in the context of the invention that, of all DLBCL patients, some patients respond to a treatment with obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) by an improved clinical outcome, in particular as compared to a treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy). It was also surprisingly found in the context of the invention that subgroups of DLBCL patients can be identified/determined which respond to a treatment with obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) by an improved clinical outcome, in particular as compared to a treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy). It was also surprisingly found in the context of the invention that there are DLBCL patients which respond to a treatment with obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) by an improved clinical outcome, in particular as compared to a treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy). It is the main gist of the

invention that such particular DLBCL patients and subgroups of DLBCL patients, respectively, can indeed be defined; also referred to herein as "patient defined herein". It is a further gist of the invention that such a patient can be defined by biomarkers (also referred to herein as "predictive biomarkers" and "predictive biomarker-defined patient", respectively).

In particular, based on exploratory analyses of GOYA, it was demonstrated in the context of the invention, and is illustrated by the appended examples, that obinutuzumab is superior over rituximab (each in combination with a CHOP chemotherapy) in (a) subset(s) of GCB DLBCL patients (e.g. in a new molecular follicular lymphoma (FL)-like GCB DLBCL patients subgroup) and/or in DLBCL patients with mutations in CD58 and/or with low expression of CD58. This was the first time that an obinutuzumab benefit over R has been identified for certain patients, in particular for a patient defined herein.

For example, in the context of the present invention, the following biomarker-defined DLBCL patients that benefit from treatment with G (e.g. G-CHOP) over the treatment with R (e.g. R-CHOP) have been identified/determined:

- BCL2 translocated patients (see, for example, Fig. 4);
- BCL2 protein expression positive patients (see, for example, Fig. 5);
- BCL2 translocated patients that are BCL2 protein expression positive (see, for example, Fig. 6);
- (a) subset(s) of GCB DLBCL patients. These can, for example, be identified as:
    - subgroup (COO) classification of GCB patients by cutoffs of the Linear Predictor Score (LPS) into subgroup "strong-GCB" patients (patients with LPS < cutoff; e.g. as compared to the general GCB DLBCL cutoff of LPS ~ 1900) (see, for example, Fig. 7, 10 and 12);
    - GCB patients with high BCL2 gene expression;
    - GCB patients that are BCL2 protein expression positive;
    - GCB patients with BCL2 translocation;
    - GCB patients with BCL2 translocation that are BCL2 protein expression positive (see, for example, Fig. 8);

- CD58 mutated patients and/or patients with low expression of CD58 (see, for example, Fig. 9).

More particular, based on exploratory analyses of GOYA, it was demonstrated in the context of the invention, and is illustrated by the appended examples, that an assessment of the LPS as a continuous variable identified a subgroup of GCB patients that benefitted from G (in particular G-CHOP) over R (in particular R-CHOP). Even more particular, it was observed that weighted expression of a gene expression (GE) array profile (measured by LPS) was linked with benefit in outcome from G treatment (e.g. G-CHOP) over R treatment (e.g. R-CHOP) among GCB patients in GOYA.

On this basis, new LPS cutoffs defining a strong-GCB DLBCL subgroup of patients with benefit from G treatment over R treatment could be determined. These new cutoffs are substantially below the LPS cutoff usually allocated to GCB DLBCL (~ <1900)). For example, a new LPS cutoff of ≤749 was determined in multivariate simulation analyses (cf. Figure 10). In accordance with this example, 'strong-GCB' patients, defined as patients with an LPS of ≤749, represented 25% (233/933) of evaluable DLBCL patients and 43% (233/540) of evaluable GCB patients in GOYA. As an other example, a new LPS cutoff of ≤725 was determined in multivariate simulation analyses (cf. Figures 10, 12). In accordance with this example, 'strong-GCB' patients are defined more stringently as patients with an LPS of ≤725. These patients represent 25% (229/933) of evaluable DLBCL patients and 43% (229/540) of evaluable GCB patients in GOYA. An LPS cutoff around 725 was shown to reflect extraordinary robustness and high generalizability of results to independent cohorts ("patient defined herein"), i.e. strong-GCB DLBCL patients. This was shown by bootstrap simulations.

Strong-GCB patients treated with G (G-CHOP) achieved significantly better clinical outcomes, for example in terms of progression-free survival (PFS), event-free survival (EFS), and overall survival (OS), than those treated with R (R-CHOP) (cf. Table 4). High-level safety was similar with either treatment regimen.

In gene-set enrichment analyses on FoundationOne® Heme (FOH) data, strong-GCB patients were further characterized as significantly enriched for FL somatic mutation hallmarks, as compared to other GCB patients, referred to as "weak-GCB" patients (e.g. false-discovery rates, FDR, 3.54e-9). In particular, BCL2 translocations

and mutations/mutation rates in several m7-FLIPI genes (*BCL2*, *BCL6*, *CD70*, *CDKN2A*, *CREBBP*, *EP300*, *IGH* , *MEF2B*, *MYC*, *MYD88*, *PCLO*, *TNFAIP3*, *TNFRSF14*) were highly enriched in strong-GCB patients, and/or in DLBCL patients with BCL2 translocations and/or with high BCL2 expression, as compared to other DLBCL patients (at an FDR <5%; Figure 11). There was no evidence for transformed indolent NHL in the strong-GCB subset on central pathology review.

In sum, new clinically and molecularly distinct subtypes of DLBCL, in particular of GCB DLBCL, have been identified, inter alia a subtype referred to as 'strong-GCB'. The identified subtypes represent de novo DLBCLs. These exhibit molecular features of FL, such as FL-typical mutations (cf. Morin, Nature 476 (7360), 2011, 298-303), they clinically differ, however, from FL. Treatment with G (e.g. G-CHOP) confers a substantial clinical benefit over treatment with R (e.g. R-CHOP) in these new subsets of (GCB) DLBCL patients ("patient defined herein"), in particular of 1L (GCB) DLBCL.

Accordingly, the invention provides for means and methods for identifying/determining/diagnosing (a) subset(s) of (GCB) DLBCL patients that advantageously respond to obinutuzumab ("patient defined herein"), in particular more advantageously to obinutuzumab than to R. The identification/determination/diagnosis can be performed by several ways, e.g. by determining whether there is a BCL2 translocation and/or a BCL2 protein overexpression, wether there is (are) (a) genetic mutation(s) in CD58 and/or there is lowered CD58 expression, or by gene expression profiling/determining weighted gene expression (e.g. by employing the NanoString COO assay) and using novel cutoffs for the LPS (as described herein elsewhere).

More particular, the invention relates to a method for identifying a DLBCL patient (patent with/suffering from DLBCL) which responds to a treatment with obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) by reaching an improved clinical outcome as compared to a treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy). Said method comprising (the step of) determining (e.g. by using a (tumor) sample of a patient) whether a patient is a patient defined herein.

The invention further relates to a method for diagnosing in a patient a form of DLBCL which can be treated with obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) so that an improved clinical outcome as compared to a treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) is reached. Said method comprising (the step of) determining (e.g. by using a (tumor) sample of a patient) whether a patient is a patient defined herein. Said method (further) comprising (the step of) diagnosing said form of DLBCL if the patient is a patient defined herein.

The present invention also relates to the medical intervention/treatment of the patient defined herein by obinutuzumab, or by a functional equivalent thereof. In principle, the term "obinutuzumab" as used herein also embraces its functional equivalents (see below for further explanations/definitions).

In one aspect, the present invention relates to obinutuzumab for use in the treatment of DLBCL in a patient defined herein.

In the context of this use, it is, for example, envisaged that (i) it has been determined (e.g. by using a (tumor) sample of a patient) whether a patient to be treated is a patient defined herein, (ii) a patient to be treated has been identified according to the method for identifying of the invention, or (iii) a form of DLBCL has been diagnosed in a patient to be treated according to the method for diagnosing of the invention.

In another aspect, the present invention relates to obinutuzumab for use in the treatment of DLBCL in a patient defined herein, wherein it is, for example, envisaged that said treatment comprises the step of (i) determining (e.g. by using a (tumor) sample of a patient) whether a (DLBCL) patient to be treated is a patient as defined herein, (ii) identifying a DLBCL patient according to the method for identifying of the invention, or (iii) diagnosing in the patient a form of DLBCL according to the method for diagnosing of the invention.

In the context of the (step of) determining/identifying/diagnosing, a sample of a (DLBCL) patient, for example a tumor sample of a (DLBCL) patient, may be

employed. The determination may be in a sample of a (DLBCL) patient, for example in a tumor sample of a (DLBCL) patient. A non-limiting example of a particular sample to be employed in accordance with the invention is a sample of a tumor tissue/tumor biopsy, more particular a formalin-fixed, paraffin-embedded tumor tissue/tumor biopsy. Such a sample can, for example, be prepared as described in Scott (2014 and 2015 loc. cit.). Other suitable samples are described herein elsewhere.

In another aspect, the present invention relates to a method for treating DLBCL in a patient in need thereof, wherein said patient is a patient defined herein. The method may comprise the steps of obtaining a sample of a patient for whom DLBCL therapy is contemplated and/or testing the/a sample of a patient to determine whether said patient is a patient defined herein. It is envisaged that the method for treating of the invention comprises the step of administering a pharmaceutically effective amount of obinutuzumab to the patient to be treated. In the context of these steps, in particular the step of testing, a (tumor) sample of a (DLBCL) patient may be employed. The testing may be in a (tumor) sample of a (DLBCL) patient. What is said herein above and elsewhere with respect to a "sample" to be employed also applies here, mutatis mutandis.
In the context of these steps, in particular in the context of the step of testing (or instead of that step) also a method for identifying or diagnosing according to the invention may be employed.

In another aspect, the present invention relates to the use of obinutuzumab for the preparation of a pharmaceutical composition for the treatment of DLBCL in a patient defined herein. Said treatment may comprise the step of determining/identifying/diagnosing in accordance with the invention whether a (DLBCL) patient to be treated is a patient as defined herein. In the context of the step of determining/identifying/diagnosing, a sample of a (DLBCL) patient, for example a tumor sample of a (DLBCL) patient, may be employed. The determination may be in a sample of a (DLBCL) patient, for example in a tumor sample of a (DLBCL) patient. What is said herein elsewhere with respect to a "sample" to be employed applies here, mutatis mutandis. Likewise, what has been said above with respect to the use and treatment applies here, mutatis mutandis.

The patient to be treated in accordance with the invention ("patient defined herein") is a patient, in particular a patient with/suffering from DLBCL, that responses to a treatment with obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) by reaching an improved clinical outcome as compared to a treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy).

In one aspect/embodiment of the invention (aspect/embodiment A), the patient defined herein (which is to be treated with obinutuzumab) is a predictive biomarker-defined patient.

A biomarker is "predictive" in accordance with the invention if it can be used to identify a patient defined herein (optionally in combination with one or more other (predictive) biomarkers), i.e. a patient that responses to the treatment with obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) more advantageously than to the treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy). More particular, a biomarker is predictive if the treatment effect (i.e. G treatment as compared to R treatment) differs between the biomarker-defined (subgroups of) patients. It is preferred in this context, that the predictive biomarker(s) is (are) the biomarker(s) as defined herein elsewhere. Particular examples of predictive biomarkers to be assessed in the context of the invention are *CD58* (e.g. (a) genetic mutation(s) therein and/or low expression thereof), *BCL2* (e.g. translocations and/or high expression thereof) and one or more (preferably all) of the genes *TNFRSF13B*, *LIMD1*, *IRF4*, *CREB3L2*, *PIM2*, *CYB5R2*, *RAB7L1*, and *CCDC50*; and *MME, SERPINA9*, *ASB13*, *MAML3*, *ITPKB*, *MYBL1*, and *S1PR2* (e.g. the weighted expression thereof resulting in an LPS substantially below ~ 1900). In this context, reference is also made to what is disclosed herein below, in particular in the context of the aspects/embodiments B to G, infra.

In one aspect/embodiment of the invention (aspect/embodiment B), the patient defined herein (which is to be treated with obinutuzumab) is a patient with/suffering from molecular follicular lymphoma (FL)-like germinal-center B-cell (GCB) DLBCL.

"Molecular" in this context means that, on molecular level, the patients resemble FL patients (cf. Morin loc. cit.). It is, however, envisaged that, on clinical level/clinically, the patients do not resemble FL patients.

In accordance with the invention, the molecular FL-like GCB DLBCL according to this aspect/embodiment is preferably characterized as strong-GCB DLBCL in accordance with the invention and/or as DLBCL in patients with *BCL2* translocations and/or high BCL2 expression (see aspects/embodiments D and E, infra).

A patient suffering from molecular FL-like GCB DLBCL in accordance with the invention may also be characterized as a patient with one or (preferably) more mutation(s) in one or (preferably) more of the genes selected from the group consisting of *BCL2*, *BCL6*, *CD70*, *CDKN2A*, *CREBBP*, *EP300*, *IGH* , *MEF2B*, *MYC*, *MYD88*, *PCLO*, *TNFAIP3* and *TNFRSF14*. Although less preferred, a patient suffering from molecular FL-like GCB DLBCL in accordance with the invention may also be characterized as a patient with one or (preferably) more mutation(s) in one or (preferably) more of the genes selected from the group consisting of *BCL2*, *CREBBP*, *EP300*, *EZH2*, *MEF2B*, *PCLO*, and *TNFRSF14*, with one or (preferably) more mutation(s) in one or (preferably) more of the genes selected from the group consisting of *CREBBP*, *EP300*, *EZH2*, *MEF2B* and *TNFRSF14*, or with one or (preferably) more mutation(s) in one or (preferably) more of the genes selected from the group consisting of *EZH2*, *MEF2B* and *TNFRSF14*.
One particular, however non-limiting, example of an applicable mutation in this respect is a *BCL2*mutation, in particular a *BCL2* translocation (see below for details).

The mutation(s) can, for example, be identified by relying on the appended examples. The Foundation Medicine next-generation sequencing assay, FoundationOne® Heme, can, for example, be used in this respect (according to the manual of the distributor).

In one aspect/embodiment of the invention (aspect/embodiment C), the patient defined herein (which is to be treated with obinutuzumab) is a patient with one or more genetic mutation(s) in CD58 and/or with low expression of CD58.

CD58 (see also Challa-Malladi loc. cit.) is known to be involved in immune recognition of tumor cells and is expressed on tumor cells. CD58 binds to CD2 on effector CTLs and NK cells (thereby providing activating signals of immune effector cells). Presence of genetic aberrations in CD58 is associated with lost or aberrant CD58 surface expression (e.g. detectable by immunohistochemistry, IHC). 67% of DLBCL cases show aberrant CD58 protein expression; with same proportion in GCB and ABC COO subgroups.
Nucleotide sequences encoding CD58 and amino acid sequences of CD58, in particular *Homo sapiens* (human) CD58, are well known in the art. They can, for example, be downloaded by following the ***Uniform Resource Locator*** (***URL***) https://www.ncbi.nlm.nih.gov/search/?term=Homo+sapiens+CD58&utm_expid=.fAeHyO5JTBGxnObh2WlrCA.0&utm_referrer=https%3A%2F%2Fwww.ncbi.nlm.nih.gov%2Fsearch%2F%3Fterm%3DHomo%2Bsapiens%2BCD58. Nucleotide sequences encoding CD58, in particular *Homo sapiens* (human) CD58, are, for example, available via NCBI accession NOs: XM_017002869.2 (variant X1); NR_026665.1 (variant 3); NM_001779.2 (variant 1); NM_001144822.1 (variant 2). Amino acid sequences of CD58, in particular *Homo sapiens* (human) CD58, are, for example, available via NCBI accession NOs: XP_016858358.1 (isoform X1); NP_001138294.1 (isoform 2); NP_001770.1 (isoform 1). An example of a nucleotide sequence encoding *Homo sapiens* (human) CD58 is depicted in SEQ ID NO:11. An example of an amino acid sequence of *Homo sapiens* (human) CD58 is depicted in SEQ ID NO:12.

In principle, "expression" in the context of the invention is envisaged to mean both, gene expression, i.e. appearance of (primary) mRNA (transcription level), and protein expression, i.e. appearance of protein (translation level).

The appearance of (primary) mRNA can, for example, be measured/detected by *in situ* hybridization (ISH) techniques, for example by fluorescence ISH (FISH). Respective means and methods are known in the art and are, for example, described

in Zhang (Chin. J. Cancer. Res. 23(2), 2011, 160–4; see also https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3587538/).
Gene expression/appearance of (primary) mRNA, in particular CD58 gene expression/appearance of (primary) CD58 mRNA, can also be evaluated by using TruSeq® RNA sequencing (according to the manual of the distributor (Illumina®, Inc.)).

The appearance of protein can, for example, be measured/detected by IHC. Respective means and methods are known in the art and are, for example, described in Punnoose (loc cit.; see also http://www.bloodjournal.org/content/126/23/3971).
Protein expression/appearance of protein, in particular CD58 Protein expression, can also be measured/detected as described in Challa-Malladi loc. cit.

In general, means and methods for assessing CD58 expression and CD58 mutations are known in the art (see, for example, Challa-Malladi loc. cit.). Moreover, the skilled person can readily assess whether a given CD58 expression is "low" in accordance with the invention or whether there are (is) (a) CD58 mutation(s) in accordance with the invention. Furthermore, the skilled person can readily choose a suitable control in comparison to which a given CD58 expression is considered "lower" in accordance with the invention or in comparison to which it is considered that there are (is) (a) CD58 mutation(s) in accordance with the invention. In this context, the skilled person can, for example, also rely on Challa-Malladi (loc. cit.).

In the context of the invention, "low expression of CD58" means that CD58 is expressed at a substantially lower level, in particular as compared to a suitable control. In general, a "low expression of CD58" in accordance with the invention means that the CD58 expression is as low (e.g. ± 10% or less, ± 7.5% or less, ± 5% or less, ± 3% or less, ± 2% or less, ± 1% or less, or even ± 0%) as the CD58 expression in a responder in accordance with the invention (i.e. a patient which responds to a treatment with obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) by reaching an improved clinical outcome as compared to a treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy); "patient defined herein") and/or lower than the expression of

CD58 in a non-responder in accordance with the invention (i.e. a patient which responds to a treatment with obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) by not reaching an improved clinical outcome as compared to the treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy); non-"patient defined herein"). The skilled is readily able to assess when CD58 expression is "low" in this respect and to apply a suitable control. For example a control in this respect may be a common DLBCL population, more particular DLBCL patients which are not classified as pertaining to the subgroup of patients classified in accordance with the invention (non-"patient defined herein").

"Low expression of CD58" may be a CD58 expression lower than the median CD58 expression among a control group, e.g. among such above-mentioned control groups. For example, a "low expression of CD58" and a "lower level" of CD58 expression, respectively, in accordance with the invention may be a CD58 expression which is lower than the median CD58 expression among the observed GOYA patients.

As a unit of CD58 (gene) expression, the unit log2(nRPKM), which is the normalized Reads Per Kilobase Million, may be used. Median CD58 expression using this unit among the GOYA patients is around 5.3. Accordingly, a "low expression of CD58" and a "lower level" of CD58 expression, respectively, in accordance with the invention may be a CD58 expression which is lower than the CD58 expression in a control (group of) patient(s) (see above) corresponding to the unit log2(nRPKM) 5.3 (median CD58 expression in the control (group of) patient(s)). In other words, the CD58 expression in a patient to be treated in accordance with the invention may be substantially lower than the CD58 expression in a control (group of) patient(s) corresponding to the unit log2(nRPKM) of 5.3. That is, the CD58 expression in a patient to be treated in accordance with the invention may be a CD58 expression that corresponds to a unit log2(nRPKM) $\leq$ 5.2, $\leq$ 5.1, $\leq$ 5.0, $\leq$ 4.5, $\leq$ 4.0, $\leq$ 3.5, $\leq$ 3.0, $\leq$ 2.5 or $\leq$ 2.0.

As mentioned above, CD58 is expressed on tumor cells and on the surface of B-cells. Accordingly, in one particular aspect, "low expression of CD58" and a "lower level" of CD58 expression, respectively, in accordance with the invention means that CD58 is expressed on tumor cells and/or B-cells at a level which is substantially

lower than the CD58 expression on common DLBCL tumor cells and/or common DLBCL B-cells. "common DLBCL" in this context, for example, means that the tumor cells and B-cells, respectively, are derived from a non-responder in accordance with the invention, preferably from DLBCL tumor cells and DLBCL B-cells, respectively, derived from a DLBCL patient which is not classified as a patient defined herein.

For example, a "low expression of CD58" and a (substantially) "lower level" of CD58 expression, respectively, in accordance with the invention means that CD58 is expressed at a level which is at least 10% lower, at least 20% lower, at least 30% lower, at least 40% lower, at least 50% lower, at least 75% lower, or at least 100% lower, in particular as compared to the CD58 expression in a suitable control (e.g. common DLBCL patient/population; non-"patient defined herein"). This applies to both, gene expression and protein expression.

Mutations, in particular CD58 mutations can, for example, be identified by using the FoundationOne® Heme (FOH) panel (see, for example, He, Blood 127(24), 2017, 3004-14; see also the appended examples).

Examples of genetic mutations in CD58 which may be present (and detected) in a patient defined herein are short-variant mutations and/or copy-number variants.

The skilled person is readily able to choose an appropriate sample to be used when assessing/detecting CD58 expression or (a) CD58 mutation(s) in accordance with the invention (either as the test sample or as the control sample).

A particular example of a sample to be employed in the context of the invention (either as the test sample or as the control sample) for assessing/detecting whether there is a low CD58 expression is a sample (e.g. biopsy) of a (CD58-expressing) tumor and/or a sample (e.g. biopsy) which contains (CD58-expressing) B-cells.

A particular example of a sample to be employed in the context of the invention (either as the test sample or as the control sample) for assessing/detecting whether there are (is) (a) CD58 mutation(s) is a DNA sample.

In one aspect/embodiment of the invention (aspect/embodiment D), the patient defined herein (which is to be treated with obinutuzumab) is a patient with/suffering from strong-GCB DLBCL.

In accordance with the invention, a patient with/suffering from strong-GCB DLBCL can be identified by determining the (weighted) expression of (a set of genes comprising) one or more (preferably all) of the genes *TNFRSF13B*, *LIMD1*, *IRF4*, *CREB3L2*, *PIM2*, *CYB5R2*, *RAB7L1*, and *CCDC50* (genes overexpressed in ABC DLBCL); and *MME*, *SERPINA9*, *ASB13*, *MAML3*, *ITPKB*, *MYBL1*, and *S1PR2* (genes overexpressed in GCB DLBCL).

More particular, a patient with/suffering from strong-GCB DLBCL may be defined as a patient having a tumor with a certain (weighted) expression of (a set of genes comprising) one or more, preferably all, of the genes *TNFRSF13B*, *LIMD1*, *IRF4*, *CREB3L2*, *PIM2*, *CYB5R2*, *RAB7L1*, and *CCDC50*; and *MME*, *SERPINA9*, *ASB13*, *MAML3*, *ITPKB*, *MYBL1*, and *S1PR2*; and, optionally, one or more, preferably all, of the genes *R3HDM1*, *WDR55*, *ISY1*, *UBXN4*, and *TRIM56* (housekeeping genes).

It is particularly envisaged in the context of this aspect/embodiment of the invention that the weighted gene expression is assessed.

In particular, when the Linear Predictor Score (LPS) resulting from the weighted expression of a set of genes disclosed herein is below a certain cutoff, i.e. substantially below the LPS cutoff usually allocated to GCB DLBCL (~ <1900), the GCB DLBCL is regarded as "strong-GCB DLBCL". Likewise, when the weighted expression of a set of genes disclosed herein corresponds to the weighted expression of a set of genes disclosed herein from which an LPS below a certain cutoff results, i.e. an LPS substantially below the LPS cutoff usually allocated to GCB DLBCL (~ <1900), the GCB DLBCL is regarded as "strong-GCB DLBCL". In accordance with the invention, examples of particular cutoffs, i.e. resulting LPS, that can be applied, i.e. that define "strong-GCB DLBCL", are cutoffs (about) ≤ 1200, (about) ≤ 1141, (about) ≤ 1100, (about) ≤ 756, (about) ≤ 750, (about) ≤ 749, (about) ≤ 745, (about) ≤ 725 or (about) ≤ 699. Preferred cutoffs are (about) ≤ 750, (about) ≤

749 and (about) ≤ 725. Particularly preferred cutoffs are (about) ≤ 750 and (about) ≤ 725.

The set of genes to be employed in accordance with the invention, i.e. in accordance with the determination/identification/diagnosing of strong-GCB DLBCL, may further comprises one or more housekeeping genes, for example one or more (preferably all) of the housekeeping genes *R3HDM1*, *WDR55*, *ISY1*, *UBXN4*, and *TRIM56*.
The expression of the one or more of the other genes to be employed may be normalized to the expression of one or more housekeeping gene(s), e.g. housekeeping gene(s) as defined herein. The skilled person is readily able to normalize the expression of the one or more of these other genes (and of one or more other gene(s) that may be comprised in the set of genes to be employed in accordance with the invention) on the basis of one or more housekeeping genes, for example on the basis of the one or more housekeeping genes mentioned above. For example, at least 1, 2, 3, 4 or 5 (of these) housekeeping genes may be employed in this respect. For respective guidance, the skilled person can rely on, for example, Scott (2014 and 2015 loc. cit.).

In principle, also a set of genes comprising only a subset of the above-mentioned sets of genes may be employed in accordance with the invention. For example, such a subset may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19 of the above-mentioned entire set of genes. However, the more of these genes are assessed, the more it is preferred.
Examples of such subsets of genes that may be employed in accordance with the invention are subsets of genes that comprise at least 1, 2, 3, 4, 5, 6 or 7 of the genes *TNFRSF13B*, *LIMD1*, *IRF4*, *CREB3L2*, *PIM2*, *CYB5R2*, *RAB7L1*, and *CCDC50*), at least 1, 2, 3, 4, 5 or 6 of the genes *MME*, *SERPINA9*, *ASB13*, *MAML3*, *ITPKB*, *MYBL1*, and *S1PR2*, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 of the genes of these two subsets of genes. In principle, the higher numbers of genes are preferred.

It is also envisaged in the context of the invention that not only the particular sets of genes or subsets of genes as mentioned herein can be assessed in accordance with the invention; but also (sub)sets of genes which comprise one or more further genes

(e.g. 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 20 or more, 30 or more, 40 or more, 50 or more, 60 or more, 70 or more, 80 or more, 90 or more, 100 or more, 150 or more, 170 or more, or 180 or more further genes). This/these further gene(s) may, for example, be one or more of the (about 180) genes known to separate/distinguish GCB and ABC (and unclassified) DLBCL on the basis of their (wheighted) expression (see, in particular, Lenz (N. Engl. J. Med. 359 (2), 2008, 2313-23) and also Geiss (Nature Biotechnology 26 (3), 2008, 317-25)).

For example, a set of genes to be assessed in accordance with the invention, may comprise (at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19 of) the genes of any of the particular sets of genes or subsets of genes mentioned above and one or more further genes (e.g. 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 20 or more, 30 or more, 40 or more, 50 or more, 60 or more, 70 or more, 80 or more, 90 or more, 100 or more, 150 or more, 170 or more, or 180 or more further genes), for example, of the (about 180) genes known to separate/distinguish GCB and ABC (and unclassified) DLBCL on the basis of their (wheighted) expression (cf. Lenz and Geiss, loc. cit.).

For example, one (or more) other gene(s) may be added to one of the above-described sets or subsets of genes, e.g. to one of the panels of 20, 15, 8 or 7 genes mentioned above, or one (or more) of the genes of one of the above-described sets or subsets of genes, e.g. of the panels of 20, 15, 8 or 7 genes mentioned above, may be replaced by one (or more) other gene(s).

A non-limiting, but preferred, example of another gene to be employed in accordance with aspect/embodiment D is the *BLC2* gene.

An example of the means and methods which can be used to determine the expression, in particular weighted expression, of the above-mentioned genes in accordance with the invention is provided by NanoString (NanoString Technologies, Inc., Seattle, WA, USA; see also Lenz (loc. cit.) and Geiss (loc. cit.)). A non-limiting particular example by which the (weighted) expression of the above-mentioned genes can be determined is the NanoString Research Use Only LST assay. Another

example is the TruSeq® RNA tool (Illumina®, Inc.). Further examples are described in Wright (PNAS 100 (17), 2003, 9991-6). Also described in Wright (loc. cit.) is the general applicability of any suitable panel for (weighted) gene expression analyses.

In accordance with the mentioned examples of means and methods which can be used to determine the (weighted) expression, a patient with/suffering from strong-GCB DLBCL may also be defined as a patient having a tumor with a (wheighted) gene expression resulting in an LPS below the LPS usually allocated to GCB DLBCL (~ <1900; see above for examples of respective cutoffs), wherein the LPS is derived from a NanoString LST, e.g. from the NanoString Research Use Only LST (NanoString Technologies, Inc., Seattle, WA, USA), as the (weighted) gene expression of the genes in the Nanostring panel (or a tumor with a (weighted) gene expression which corresponds to such a (wheighted) gene expression). For example, the particular genes mentioned above may be in the Nanostring panel.

A patient with/suffering from strong-GCB DLBCL may also be defined as a patient having a tumor with a (wheighted) gene expression with/resulting in an LPS below the LPS usually allocated to GCB DLBCL (~ <1900; see above for examples of respective cutoffs), wherein the LPS results from a COO classification by (weighted) gene-expression profiling (for example by using a NanoString LST as described herein) (or a tumor with a (weighted) gene expression which corresponds to such a (weighted) gene expression).

In principle, COO classification (e.g. into strong-, unclassified- and weak-GCB DCBCL), can be based on gene-expression profiling, in particular weighted gene-expression profiling, (e.g. using a NanoString LST (like, for example, the NanoString Research Use Only LST) (NanoString Technologies, Inc., Seattle, WA, USA).

In principle, the meaning of "LPS" is known in the art and is accordingly understood by the skilled person in the context of the invention. In particular, the LPS in accordance with the invention is a continuous variable (weighted average for gene expression; e.g. of the genes mentioned herein, which may be in a Nanostring LST). In the sum of the patients assessed in GOYA, the LPS has a range from -1138 to 4504. Normally, as mentioned above, the LPS is used to classify patients into COO

subgroups GCB DLBCL, ABC DLBCL, and unclassified DLBCL (see above and Scott 2014 and 2015 loc. cit.). Default COO algorithm uses a bayesian approach with GCB/ABC classification based on ≥90% cutoff on likelihood of being GCB or ABC (unclassified works as a buffer).

More particular, the LPS in accordance with the invention is envisaged to be the weighted sum of the expression of the genes to be employed in the gene expression profiling (e.g. the above mentioned genes comprised in the mentioned (sub-)sets of genes). The weighted sum of gene expression can be calculated according to the following formula (Formula I):

$$LPS(X) = \sum_j a_j X_j,$$

wherein X stands for each sample, X_j is the gene expression for gene j and a_j is the coefficient for gene j. (see also Wright loc. cit.; in particular, the sections "*Gene Expression Data*" and "*Formulation of the DLBCL Subgroup Predictor*"; incorporated herein by reference)

In general, the person skilled in the art is able to determine the expression of genes, in particular the weighted expression of genes, in accordance with the teaching of the invention. Respective means and methods are known in the art and are, for example, described in Wright (loc. cit.), in particular, in the sections "*Gene Expression Data*" and "*Formulation of the DLBCL Subgroup Predictor*"; incorporated herein by reference. Wright (loc. cit.), for example, also outlines how the weighted gene expression algorithm can be used and how it can be transferred across gene expression platforms, like the NanoString LST and others. Comparable guidance is also provided in Lenz (loc. cit.).

In the context of the strong-GCB DLBCL classification in accordance with the invention and, in particular as described above, the following may further be considered:
Multivariate Cox regression and(/or) elastic net penalized regression (alpha=0.5) may be used to evaluate biomarker treatment effects. Simulations to identify the optimal cutoff, for example on the basis of the NanoString LST and the respective LPS, for

treatment effect may be performed using cross validation and(/or) bootstrapping. Multiple testing adjustment may be done by estimating false-discovery rates (FDRs), for example using the Benjamini-Hochberg procedure (e.g. significance <5% FDR). Pathway enrichment analysis may be performed by gene-set enrichment, e.g. by using gene sets defined by MSigDB hallmarks and(/or) a curated FL somatic mutation hallmark gene set.

In particular an LPS cutoff (e.g. as described herein elsewhere) may be determined in (a) simulation analyses (analysis), preferably in (a) multivariate simulation analyses (analysis).

The robustness of an LPS cutoff (e.g. as described herein elsewhere) may be shown by bootstrap simulations.

Moreover, instead of using the specific weighted algorithm of LPS, e.g. from the NanoString LST, the 1st principal component from a principal component analysis of the results, for example, of another expression analysis panel (e.g. the TruSeq® RNA tool (Illumina®, Inc.)) may be applied, evaluating, for example the above-mentioned genes (e.g. one or more of the ~180 genes known to separate GCB and ABC) by gene expression. It was also shown in accordance with the invention that there is a very high correlation between the (NanoString LST-derived) LPS and the 1st principal component.

In one aspect/embodiment of the invention (aspect/embodiment E), the patient defined herein (which is to be treated with obinutuzumab) is a patient with BCL2 translocations and/or high BCL2 expression. Preferably, a BCL2 translocated patient with high BCL2 expression is envisaged in the context of this aspect/embodiment.

BCL2 (see also Zhang loc. cit.; Punnoose loc. cit.; Iqbal, Clin Cancer Res 17(24), 2011, 7785 – 95; Iqbal, JCO 24(6), 2006, 961 - 8; Hu, Blood 121(20), 2013, 4021 – 31; Johnson, JCO 30(28), 2012, 3452 – 67; Green, JCO 30(28), 2012, 3460 - 67) is commonly known to be an anti-apoptotic protein whose overexpression opposes mitochondrial apoptotic pathways. BCL2 is known to be expressed in tumors of DLBCL patients.

Nucleotide sequences encoding BCL2 and amino acid sequences of BCL2, in particular *Homo sapiens* (human) BCL2, are well known in the art. They can, for example, be downloaded by following the ***Uniform Resource Locator*** (***URL***)

https://www.ncbi.nlm.nih.gov/search/?term=Homo+sapiens+BCL2&utm_expid=.fAeHyO5JTBGxnObh2WlrCA.0&utm_referrer=https%3A%2F%2Fwww.ncbi.nlm.nih.gov%2Fsearch%2F%3Fterm%3DHomo%2Bsapiens%2BBCL2. Nucleotide sequences encoding BCL2, in particular *Homo sapiens* (human) BCL2, are, for example, available via NCBI accession NOs: XM_017025917.2 (variant X3); XM_011526135.3 (variant X2); XR_935248.3 (variant X1); NM_000657.2 (variant beta); NM_000633.2 (variant alpha). Amino acid sequences of BCL2, in particular *Homo sapiens* (human) BCL2, are, for example, available via NCBI accession NOs: XP_016881406.1 (isoform X2); XP_011524437.1 (isoform X1); NP_000648.2 (isoform beta); NP_000624.2 (isoform alpha). An example of a nucleotide sequence encoding *Homo sapiens* (human) BCL2 is depicted in SEQ ID NO:13. An example of an amino acid sequence of *Homo sapiens* (human) BCL2 is depicted in SEQ ID NO:14.

Was has been generally said herein above with respect to "expression", the measurement/detection of (primary) mRNA and the measurement/detection of protein applies here, mutatis mutandis.

In general, means and methods for measuring/detecting BCL2 expression and BCL2 translocations are known in the art and are, for example, described in Zhang (loc. cit.) and Puunoose (loc. cit.). Moreover, the skilled person can readily assess whether a given BCL2 expression is „high" in accordance with the invention or whether there are (is) (a) BCL2 translocation(s) in accordance with the invention. Furthermore, the skilled person can readily choose a suitable control in comparison to which a given BCL2 expression is considered "higher" in accordance with the invention or incomparison to which it is considered that there are (is) (a) BCL2 translocation(s) in accordance with the invention. In this context, the skilled person can, for example, also rely on Zhang (loc. cit.) and Puunoose (loc. cit.).

BCL2 expression can, for example, be assessed by a Ventana immunohistochemistry (IHC) assay, for example by the Ventana investigational-use IHC assay (BCL2 antibody clone 124) (by attending to the manucal of the supplier). For example, high BCL2 expression can be defined in this context as moderate or strong staining in ≥50% tumor cells (see below for further details).

BCL2 protein expression/appearance of BCL2 protein can also be measured/detected as described in Punnoose (loc. cit.), Iqbal (2011 and 2006 loc. cit), Hu (loc. cit.), Johnson (loc. cit), Green (loc. cit.).
Gene expression/appearance of (primary) mRNA, in particular BCL2 gene expression/appearance of (primary) BCL2 mRNA can, for example, be evaluated as described in Zhang (loc. cit.) or by using TruSeq® RNA sequencing ((Illumina®, Inc.) according to the manual of the distributor).

In the context of the invention, "high expression of BCL2" means that BCL2 is expressed at a substantially higher level, in particular as compared to a suitable control. In general, a "high expression of BCL2" in accordance with the invention means that the BCL2 expression is as high (e.g. ± 10% or less, ± 7.5% or less, ± 5% or less, ± 3% or less, ± 2% or less, ± 1% or less or even ± 0%) as the BCL2 expression in a responder in accordance with the invention (i.e. a patient which responds to a treatment with obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) by reaching an improved clinical outcome as compared to a treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy); "patient defined herein") and/or higher than the expression of BCL2 in a non-responder in accordance with the invention (i.e. a patient which responds to a treatment with obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) by not reaching an improved clinical outcome as compared to the treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy); non-"patient defined herein"). The skilled is readily able to assess when BCL2 expression is "high" in this respect and to apply a suitable control. For example, a control in this respect may be a common DLBCL population, more particular DLBCL patients which are not classified as pertaining to the subgroup of patients classified in accordance with the invention (non-"patient defined herein"). "High expression of BCL2" may be a BCL2 expression higher than the median BCL2 expression among a control group, e.g. among such above-mentioned control groups. For example, a "high expression of BCL2" and a "higher level" of BCL2 expression, respectively, in accordance with the invention may be a BCL2

expression which is higher than the median BCL2 expression among the patients assessed in GOYA.

An example of a control, on the basis of which it can be considered in the context of the invention whether BCL2 expression is "high", is normal, i.e. non-tumor, tissue, more particular normal, i.e. non-tumor, lymphatic tissue. The tissue may be from a DLBCL patient. For example, it may be from the DLBCL patient to be treated. However, in principle, the tissue may also be of a normal/healthy subject.

A preferred example of a control, on the basis of which it can be considered in the context of the invention whether BCL2 expression is "high", is tumor tissue, more particular lymphatic tumor tissue form a non-responder in accordance with the invention (non-"patient defined herein"). It is preferred that the tissue is from a non-responding DLBCL patient (non-"patient defined herein" which is a DLBCL patient).

BCL2 expression is considered "high" if, for example, ≥30%, ≥40%, ≥50% or ≥60% of the tumor cells express BCL2 (for example show BCL2 staining in an IHC assay), in particular show moderate to strong BCL2 expression (for example show moderate to strong BCL2 staining in an IHC assay).

It is preferred in the context of the invention that BCL2 expression, in particular "high" BCL2 expression, incorporates both, the percentage of tumor cells which express BCL2 and the intensity of BCL2 expression in these cells.

When assessing whether a given BCL2 expression is "high" in accordance with the invention, the skilled person can also rely on Iqbal (2011 and 2006 loc. cit), Hu (loc. cit.), Johnson (loc. cit.) and Green (loc. cit).

More general, a "high expression of BCL2" and a (substantially) "higher level" of BCL2 expression, respectively, in accordance with the invention means that BCL2 is expressed at a level which is at least 10% higher, at least 20% higher, at least 30% higher, at least 40% higher, at least 50% higher, at least 75% higher, or at least 100% higher, in particular as compared to the BCL2 expression in a suitable control

(e.g. common DLBCL patient/population; non-"patient defined herein"). This applies to both, gene expression and protein expression.

The meaning of "(a) BCL2 translocation(s)" is well known in the art. Typically, a "BCL2 translocation" is a gene fusion between BCL2 and IgH (involving chromosoms 14 and 18). BCL2 translocation(s) are, for example, described in Zhang (loc. cit.)
BCL2 translocations can, for example, be assessed/detected by using the BCL2 Dual Color Break Apart technology (Vysis, Abbott Molecular), in particular by using Vysis LSI Dual Color Break Apart FISH Probes, (e.g. with a (FISH) cutoff at 5% (typically used) or 50%); by attending to the manual of the supplier.
BCL2 translocations can also be assessed/detcted with the Foundation Medicine next-generation sequencing assay, FoundationOne® Heme (by attending to the manucal of the supplier; see also He loc. cit.).
Means and methods for assessing/detecting BCL2 translocations are known in the art and are, for example, described in Zhang loc. cit.) and He (loc. cit.).

The skilled person is readily able to choose an appropriate sample to be used when assessing/detecting (a) BCL2 translocation (s) or BCL2 expression in accordance with the invention (either as the test sample or as the control sample).

A particular example of a sample to be employed in the context of the invention (either as the test sample or as the control sample) for assessing/detecting whether there is a high BCL2 expression is a sample (e.g. biopsy) of a (BCL2-expressing) tumor.

A particular example of a sample to be employed in the context of the invention (either as the test sampleor as the control sample) for assessing/detecting whether there are (is) BCL2 translocation(s) is a DNA sample.

In one aspect/embodiment of the invention (aspect/embodiment F), the patient defined herein (which is to be treated with obinutuzumab) is defined by a combination/an intersection of any 2, any 3, any 4 or any 5 of the patient definitions referred to in aspects/embodiments A, B, C, D and E supra. That is, the patient may be defined by a combination/an intersection of the patient definitions referred to in

aspects/embodiments A and B; A and C; A and D; A and E; B and C; B and D, B and E; C and D; C and E; A, B and C; A, C and D; A, D and E; A, B and D; A, B and E; A, C and E; B, C and D; B, C and E; B, D and E; C, D and E; A, B, C and D; A, C, D and E; B, C, D and E; and A, B, D and E. Preferred are combinations/intersections which comprise the definitions according to aspects/embodiments D and E.

In one aspect/embodiment of the invention (aspect/embodiment G), the patient defined herein (which is to be treated with obinutuzumab) is defined by a combination/an intersection of the patient definitions referred to in aspects/embodiments D and E (or A, D and E), supra. That is, the patient defined herein in accordance with this aspect/embodiment is a patient (i) with/suffering from strong-GCB DLBCL; and (ii) with BCL2 translocations and/or high BCL2 expression. This combination/intersection of patient definitions defines a preferred patient defined herein.

In general, as used in the context of the present invention, a non-limiting example of a "control" is preferably a "non-responder" control, for example a sample/cell/tissue obtained from one or more patients that do not suffer from the particular DLBCL as defined herein (non-"patient defined herein") and that are known to be not advantageously responsive to obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) as compared to rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) in accordance with the invention. Another example for a "non-responder" control is a cell line/sample/cell/tissue that shows no improved response to obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) as compared to rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) in an ex-vivo test. Another non-limiting example of a "control" is an "internal standard", for example purified or synthetically produced proteins, peptides, DNA and/or RNA, or a mixture thereof, where the amount of each protein/peptide/DNA/RNA is gauged by using the "non-responder" control described herein.

In principle, the patient to be treated in the context of the invention is envisaged to be a DLBCL patient. In other words, the patient is a patient with/suffering from DLBCL. Accordingly, it is particularly envisaged that also the patient defined with respect to any of the aspects/embodiments A, B, C, D, E, F, G, supra, is a DLBCL patient and a patient with/suffering from DLBCL, respectively. However, it is not necessarily required that a given patient is diagnosed as being a DLBCL patient, for example prior to (or after) the determination/identification/diagnosis of being a patient as defined herein, in particular as defined in one (or more) of the aspects/embodiments A to G, supra. It is, however, preferred that the patient to be treated in accordance with the invention is, in a first step, diagnosed as being a DLBCL patient, or at least as being a Lymphoma patient, and, in a second step, determined/identified/diagnosed as being a patient defined herein, in particular a patient as defined in one (or more) of the aspects/embodiments A to G, supra. In principle, in accordance with the invention, a given patient may, in a first step, also be determined/identified/diagnosed as being a patient defined herein, and, in a second step, diagnosed as being a DLBCL patient, or at least as being a Lymphoma patient. However, the latter option is less preferred and, as mentioned, the (foregoing or subsequent) step of diagnosing whether the patient to be treated is a (DLBC)L patient may also be omitted.

A non-limiting example of a way how the attending physician would choose whether a given patient is to be treated in accordance with the invention is provided in the following:

From a patient, for example with an abnormality raising the clinical suspicion of lymphoma (e.g. enlarged lymph nodes), a (tumor) sample (e.g. (tumor) biopsy) may be taken. The (tumor) sample may be diagnosed as (DLBC)L positive (e.g. by a pathologist). This may be one of the 2 two steps mentioned above.
As mentioned, this step may be omitted.
From a (remainder of the) (tumor) sample (e.g. (tumor) tissue/biopsy), or from another (tumor) sample of the same or another patient, or from another tumor of the same or another patient, protein, RNA (e.g. (primary) mRNA) and/or DNA may be extracted. The patient defined herein may then be determined/identified/diagnosed, i.e. the biomarker analysis/analyses in accordance with the invention may then be

performed, with the sampled protein, RNA (e.g. (primary) mRNA) and/or DNA. For example, the samples may be analyzed with the weighted gene expression assay (e.g. by using the NanoString LST) to obtain the LPS, tested for (a) genetic mutation(s) in *CD58* and/or for low expression of *CD58* and/or tested for BCL2 translocations and/or for high BCL2 expression. The results of the analysis/analyses then allow for classifying the patient into the DLBCL subgroups defined in accordance with the invention. In other words, results of the analysis/analyses then allow for classifying whether the patient is a "patient defined herein".
This may be the other one of the 2 two steps mentioned above (i.e. the obligatory step).

Non-limiting examples of the biomarker analysis/analyses may be employed in accordance with the invention according to the following:
A tumor sample, for example a diagnostic tumor sample, (e.g. tissue biopsy), for example formalin-fixed and(/or) paraffin-embedded, may be taken from a patient. RNA (or protein or DNA) may be extracted and gene expression may by analysed for strong-GCB classification, CD58 translocation/low expression and/or BCL2 mutation(s)/low expression. DNA may be extracted to evaluate (a) CD58 mutation(s). Tissue sections, in particular tumour tissue sections, may be cut and embedded, e.g. for IHC and/or (F)ISH analyses.

As mentioned, it is envisaged in the context of the invention to use obinutuzumab, or a fuctional equivalent of obinutuzumab, for treating the patient defined herein.

Obinutuzumab itself is well known in the art and is, for example, described in EP-B1 2380910 and WO 2005/044859. See below for further details as to obinutuzumab itself.

Also the meaning of "fuctional equivalent of obinutuzumab" is clear to the skilled person. In particular, the term "functional equivalent of obinutuzumab" refers to an antibody, in particular to a humanized Type II anti-CD20 antibody, which is more suitable for treating (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) the patient defined herein than rituximab (in particular in combination with a chemotherapy, more particular in combination with

a CHOP chemotherapy). In other words, this term refers to an antibody, in particular a human Type II anti-CD20 antibody, with features and mode of actions (MOAs) which render the antibody capable of treating a patient defined herein so that it responses by reaching an improved clinical outcome as compared to a treatment with rituximab. More particular, the term "functional equivalent of obinutuzumab" refers to an antibody, in particular an Type II anti-CD20 antibody, which has the same features and biological functions as obinutuzumab itself, in particular the same biological functions as obinutuzumab itself which render the antibody to be more suitable for treating the patient defined herein than rituximab.

Examples of the most relevant features and MOAs of an equivalent of obinutuzumab in accordance with the invention (and of obinutuzumab itself) are defined herein elsewhere. They can readily be determined by the skilled person.

It is, in principle, envisaged in the context of the invention that the term "functional equivalent of obinutuzumab" also covers biosimilars of obinutuzumab. In particular, it is envisaged that the meaning of that term also covers any biosimilar of obinutuzumab which is more suitable for treating the patient defined herein than rituximab. In other words, the "functional equivalent of obinutuzumab" may be a biosimilar of obinutuzumab which is capable of treating a patient defined herein so that it responses by reaching an improved clinical outcome as compared to a treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy).
In general, the meaning of "biosimilar" is well-known in the art. In this context, a "biosimilar" is known to be a biologic medical product which is almost an identical copy of an original biologic medical product and is also known as follow-on biologic or subsequent entry biologic. Biosimilars are officially approved versions of original "innovator" products. In this context, reference is, for example, made to the EMEA guidline on Similar Biological Medicine Products (CHMP/437/04 London, 2005).

In the context of the invention, obinutuzumab, in particular the functional equivalent of obinutuzumab, is envisaged to be an antibody, in particular a humanized Type II anti-CD20 antibody, comprising
(a) a heavy chain variable region as depicted in SEQ ID NO:1 and a light chain

variable region as depicted in SEQ ID NO:2 (this light chain variable region is also known as KV1 light chain variable region; "KV1" stands for the humanized light chain variable region of the murine B-Lyl monoclonal antibody; see EP-B1 2380910);

(b) a heavy chain variable region having the specificity determining residues of the heavy chain variable region of (a) and a light chain variable region having the specifity determining residues of the light chain variable region of (a); or

(c) a heavy chain variable region that is encoded by a nucleic acid sequence which is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to SEQ ID NO: 3 and a light chain variable region that is encoded by a nucleic acid sequence which is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to SEQ ID NO: 4 (the higher values are preferred).

It is preferred that obinutuzumab, in particular the functional equivalent of obinutuzumab to be employed in the context of the invention is a monoclonal antibody, in particular a monoclonal humanized Type II anit-CD20 antibody.

It is particularly preferred that the antibody to be employed in accordance with the invention is a Type II anti-CD20 antibody, in particular a humanized Type II anti-CD20 monoclonal antibody, or, preferably and, an antibody which comprises a glycoengineered Fc region, in particular a glycoengineered Fc region as defined herein below. It is further preferred that, in accordance with the invention, such an antibody, or any other antibody to be employed in accordance with the invention, shows substantially higher levels of ADCC activity, in particular as compared to a comparable Type I anti-CD20 antibody and/or as compared to a non-glycoengineered antibody (e.g. rituximab).

The meaning of "Type II" anti-CD20 antibody is well known in the art. In general, anti-CD20 monoclonal antibodies fall into two distinct categories based on their mechanism of action in eradicating lymphoma cells. Type I anti-CD20 antibodies primarily utilize complement to kill target cells, while Type II antibodies operate by different mechanisms, primarily apoptosis. Rituximab and 1F5 are examples of Type I anti-CD20 antibodies, whereas B 1 is an example of a Type II antibody. See, e.g.,

Cragg (Blood 103(7), 2004, 2738-2743); Teeling (Blood 104(6), 2004, 1793-1800), the entire contents of which are hereby incorporated by reference. Also obinutuzumab itself is a Type II antibody. See, e.g. EP-B1 2380910 and WO 2005/044859, the entire contents of which are hereby incorporated by reference.

The skilled person knows, but is at least readily able to determine, the relevant specificity determining residues of the heavy and light chain variable regions of obinutuzumab. As to respective guidance, the skilled person can, for example, rely on EP-B1 2380910 and WO 2005/044859.

In one aspect, obinutuzumab/the functional equivalent of obinutuzumab as employed in the context of the invention, in particular as defined in (b) and (c), supra, is envisaged to have, inter alia, one or more of the following features:

(i) capability of inducing higher levels of apoptosis when incubated with CD20-positive human cells relative to a control under identical conditions using rituximab;

(ii) capability of causing an increased $CD20^+$ tumor B-cell killing as compared to rituximab;

(iii) capability of causing an increased direct cell death as compared to rituximab (without being bound by theory, this is due to an alternative binding geometry (e.g. elbow hinge-modification));

(iv) capability of causing a decreased complement-dependent cytotoxicity (CDC) as compared to rituximab (without being bound by theory, this is due to an alternative binding geometry (e.g. elbow hinge-modification));

(v) capability of causing an increased antibody-dependent cellular cytotoxicity (ADCC) as compared to rituximab (without being bound by theory, this is due to glycoengineered Fc region);

(vi) capability of causing an increased antibody-dependent cellular phagocytosis (ADCP) as compared to rituximab (without being bound by theory, this is due to glycoengineered Fc region);

(vii) an increased affinity for FcγRIII receptors as compared to rituximab (without being bound by theory, this is due to glycoengineered Fc region);

(viii) capabilitiy to trigger, upon binding to CD20, less internalization of surface CD20 as compared to rituximab.

In another aspect, obinutuzumab/the functional equivalent of obinutuzumab as employed in the context of the invention, in particular as defined in (b) and (c), supra, is envisaged to show, inter alia, one ore more of the following MOAs:

(i) capability of inducing higher levels of apoptosis when incubated with CD20-positive human cells relative to a control under identical conditions using rituximab;

(iii) capability of causing an increased direct cell death as compared to rituximab (without being bound by theory, this is due to an alternative binding geometry (e.g. elbow hinge-modification));

(iv) capability of causing a decreased complement-dependent cytotoxicity (CDC) as compared to rituximab (without being bound by theory, this is due to an alternative binding geometry (e.g. elbow hinge-modification));

(v) capability of causing an increased antibody-dependent cellular cytotoxicity (ADCC) as compared to rituximab (without being bound by theory, this is due to glycoengineered Fc region);

(vi) capability of causing an increased antibody-dependent cellular phagocytosis (ADCP) as compared to rituximab (without being bound by theory, this is due to glycoengineered Fc region);

(vii) an increased affinity for FcγRIII receptors as compared to rituximab (without being bound by theory, this is due to glycoengineered Fc region);

(viii) capability to trigger, upon binding to CD20, less internalization of surface CD20 as compared to rituximab.

Means and methods which can be used to determine the relevant features of an antibody to be employed in accordance with the invention (e.g. biological functions, MOAs) are well-known in the art and can readily be applied by the skilled person.

Means and methods which can be used to determine the level of apoptosis, in particular whether a given antibody is capable of inducing higher levels of apoptosis when incubated with CD20-positive human cells relative to a control under identical conditions using rituximab, are known in the art and are, for example, described in EP-B1 2380910 and WO 2005/044859.

A "higher level of apoptosis" in accordance with the invention means, for example, at least 1.2-fold higher, at least 1.5-fold higher, at least 2-fold higher, at least 3-fold higher, at least 4-fold higher, at least 5-fold higher, or at least 10-fold higher as compared to the level of apoptosis resulting from a comparable application of rituximab.

Means and methods which can be used to determine $CD20^+$ tumor B-cell killing, in particular whether there is an increased $CD20^+$ tumor B-cell killing as compared to rituximab, are known in the art and are, for example, disclosed in EP-B1 2380910 and WO 2005/044859.
In accordance with the invention, $CD20^+$ tumor B-cell killing is "increased", if it is, for example, at least 1.2-fold higher, at least 1.5-fold higher, at least 2-fold higher, at least 3-fold higher, at least 4-fold higher, at least 5-fold higher, or at least 10-fold higher as compared to $CD20^+$ tumor B-cell killing resulting from a comparable application of rituximab.

Means and methods which can be used to determine direct cell death, in particular whether there is an increased direct cell death as compared to rituximab, are known in the art and are, for example, disclosed in EP-B1 2380910 and WO 2005/044859.
In accordance with the invention, direct cell death is "increased", if it is at least 1.2-fold higher, at least 1.5-fold higher, at least 2-fold higher, at least 3-fold higher, at least 4-fold higher, at least 5-fold higher or at least 10-fold higher as compared to direct cell death resulting from a comparable application of rituximab.

Means and methods which can be used to determine CDC, in particular whether there is a decreased CDC as compared to rituximab, are known in the art and are, for example, disclosed in Herter (loc. cit.), Mössner (loc. cit.), EP-B1 238090, WO 2005/044859, WO 2015/067586 and WO 2016/207312.
In accordance with the invention, CDC is "decreased", if it is, for example, at least 1.2-fold lower, at least 1.5-fold lower, at least 2-fold lower, at least 3-fold lower, at least 4-fold lower, at least 5-fold lower, or at least 10-fold lower as compared to the CDC resulting from a comparable application of rituximab.
The term "complement-dependent cytotoxicity (CDC)" refers to lysis of human tumor target cells by the antibody to be employed according to the invention in the

presence of complement. CDC is measured preferably by the treatment of a preparation of CD20 expressing cells with an anti-CD20 antibody to be employed according to the invention in the presence of complement. CDC is found if the antibody induces, for example at a concentration of 100 nM, the lysis (cell death) of, for example, 20%, or more of the tumor cells after, for example, 4 hours. The assay is performed preferably with ^{51}Cr or Eu labeled tumor cells and measurement of released ^{51}Cr or Eu. Controls include the incubation of the tumor target cells with complement but with rituximab and, optionally, without the antibody.
The skilled person is readily able to adapt this particular example of a CDC assay so as to be able to test whether the CDC activity is decreased upon the application of an antibody to be used in accordance with the invention as compared to the application of rituximab, as the case may be.

Means and methods which can be used to determine ADCC, in particular whether there is an increased ADCC as compared to rituximab, are known in the art and are, for example, disclosed in Herter (loc. cit.), Mössner (loc. cit.), Tobinai (Adv. Ther. 34, 2017, 324–56), EP-B1 2380910, WO 2005/044859, WO 2015/067596 and WO 2016/207312.
In accordance with the invention, ADCC, more generally, is "increased", if it is, for example, at least 1.2-fold higher, at least 1.5-fold higher, at least 2-fold higher, at least 3-fold higher, at least 4-fold higher, at least 5-fold higher, or at least 10-fold higher as compared to the ADCC resulting from a comparable application of rituximab.
One, non-limiting, accepted *in vitro* ADCC assay is as follows:

1) the assay uses target cells that are known to express the target antigen recognized by the antigen-binding region of the antibody (CD20);
2) the assay uses human peripheral blood mononuclear cells (PBMCs), isolated from blood of a randomly chosen healthy donor, as effector cells;
3) the assay is carried out according to the following protocol:
    i) the PBMCs are isolated using standard density centrifugation procedures and are suspended at 5×10^6 cells/ml in RPMI cell culture medium;
    ii) the target cells are grown by standard tissue culture methods, harvested from the exponential growth phase with a viability higher than 90%, washed in RPMI cell culture medium, labeled with 100 micro-Curies of

^{51}Cr, washed twice with cell culture medium, and resuspended in cell culture medium at a density of 10^5 cells/ml;

iii) 100 microliters of the final target cell suspension above are transferred to each well of a 96-well microtiter plate;

iv) the antibody is serially-diluted from 4000 ng/ml to 0.04 ng/ml in cell culture medium and 50 microliters of the resulting antibody solutions are added to the target cells in the 96-well microtiter plate, testing in triplicate various antibody concentrations covering the whole concentration range above;

v) for the maximum release (MR) controls, 3 additional wells in the plate containing the labeled target cells receive 50 microliters of a 2% (VN) aqueous solution of non-ionic detergent (Nonidet, Sigma, St. Louis), instead of the antibody solution (point iv above);

vi) for the spontaneous release (SR) controls, 3 additional wells in the plate containing the labeled target cells receive 50 microliters of RPMI cell culture medium instead of the antibody solution (point iv above);

vii) the 96-well microtiter plate is then centrifuged at 50 x g for 1 minute and incubated for 1 hour at 4°C;

viii) 50 microliters of the PBMC suspension (point i above) are added to each well to yield an effector:target cell ratio of 25:1 and the plates are placed in an incubator under 5% CO_2 atmosphere at 37 °C for 4 hours;

ix) the cell-free supernatant from each well is harvested and the experimentally released radioactivity (ER) is quantified using a gamma counter;

x) the percentage of specific lysis is calculated for each antibody concentration according to the formula (ER-MR)/(MR-SR) x 100, where ER is the average radioactivity quantified (see point ix above) for that antibody concentration, MR is the average radioactivity quantified (see point ix above) for the MR controls (see point v above), and SR is the average radioactivity quantified (see point ix above) for the SR controls (see point vi above);

4) "increased ADCC" is defined as either an increase in the maximum percentage of specific lysis observed within the antibody concentration range tested above, and/or a reduction in the concentration of antibody required to achieve one half of the maximum percentage of specific lysis observed within the antibody

concentration range tested above. The increase in ADCC is relative to the ADCC, measured with the above assay, mediated by the same antibody, produced by the same type of host cells, using the same standard production, purification, formulation and storage methods, which are known to those skilled in the art, but that has not been produced by host cells engineered to overexpress GnTIII.

The skilled person is readily able to adapt this particular example of an ADCC assay so as to be able to test whether the ADCC activity is increased upon the application of an antibody to be used in accordance with the invention as compared to the application of rituximab, as the case may be.

Means and methods which can be used to determine ADCP in particular whether there is an increased ADCP as compared to rituximab, are known in the art and are, for example, disclosed in Herter (loc. cit.) and Mössner (loc. cit.).
In accordance with the invention, ADCP is "increased", if it is, for example, at least 1.2-fold higher, at least 1.5-fold higher, at least 2-fold higher, at least 3-fold higher, at least 4-fold higher, at least 5-fold higher, or at least 10-fold higher as compared to the ADCP resulting from a comparable application of rituximab.

Means and methods which can be used to determine the affinity for FcγRIII receptors, in particular whether there is an increased affinity for FcγRIII receptors as compared to rituximab, are known in the art and are, for example, disclosed in Tobinai (loc. cit.)
In accordance with the invention, the affinity for FcγRIII receptors is "increased", if it is, for example, at least 1.2-fold higher, at least 1.5-fold higher, at least 2-fold higher, at least 3-fold higher, at least 4-fold higher, at least 5-fold higher, or at least 10-fold higher as compared to the the affinity for FcγRIII receptors resulting from a comparable application of rituximab.

Means and methods which can be used to determine the capability to trigger internalization of surface CD20 (upon binding to an anti-CD20 antibody), in particular whether there is the capability to trigger less internalization of surface CD20 (when binding to obinutuzumab) as compared to rituximab, are known in the art and are, for example, disclosed in Lim (Blood 118(9), 2011, 2530-40).

In accordance with the invention, internalization of surface CD20 is "less", if it is, for example, at least 1.2-fold lower, at least 1.5-fold lower, at least 2-fold lower, at least 3-fold lower, at least 4-fold lower, at least 5-fold lower, or at least 10-fold lower as compared to the capability to trigger surface CD20 internalization resulting from a comparable application of rituximab.

As mentioned, it is preferred in the context of the invention that obinutuzumab/the functional equivalent of obinutuzumab to be employed in the context of the invention comprises a glycoengineered Fc region. In this context, reference is also made to EP-B1 2380910 and WO 2005/044859; the entire content of which is incorporated herewith by reference.

It is particularly preferred in the context of the invention that the Fc-region of the antibody to be employed is glycoengineered so that the antibody has one or more of the features and MOAs, respectively, referred to above, more particular in sections (v), (vi) and (vii), supra. The feature/MOA of section (v) is most preferred in this respect (increase in ADCC).

Obinutuzumab/the functional equivalent of obinutuzumab to be employed in the context of the invention may have an increase in the fraction of non-fucosylated oligosaccharides attached to said glycoengineered Fc region.
Obinutuzumab/the functional equivalent of obinutuzumab to be employed in the context of the invention may have an increase in the fraction of bisected, non-fucosylated oligosaccharides attached to said glycoengineered Fc region.
Obinutuzumab/the functional equivalent of obinutuzumab to be employed in the context of the invention may have significantly higher levels of binding to human FcγRIII receptors relative to the non-glycoengineered antibody, and/or relative to rituximab.

As mentioned, it is preferred in the context of the invention that obinutuzumab/the functional equivalent of obinutuzumab to be employed in the context of the invention, in particular as defined in (b) and (c), supra, exhibits significantly higher levels of ADCC activity, in particular relative to the non-glycoengineered antibody, and/or relative to rituximab. Without being bound by theory, the significantly higher levels of

ADCC activity result from the glycoengineered Fc region (see above).

The person skilled in the art is readily able to glycoengineer the Fc-region of an antibody, so as to achieve an antibody to be employed in accordance with the invention, e.g., as mentioned above and in a manner to retrieve the relevant feature(s)/MOA(s). Moreover, the skilled person is readily able to deduce what an increase in the fraction of non-fucosylated oligosaccharides and an increase in the fraction of bisected non-fucosylated oligosaccharides in accordance with the present invention is. In this context, the skilled person can, inter alia, rely on the guidance provided by EP-B1 2380910 and WO 2005/044859; the contents of which are incorporated herewith by reference. Non-limiting examples of such increases are increases of at least 1.2-fold, at least 1.5-fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold or at least 10-fold (relative to the non-glycoengineered antibody).

In accordance with the present invention, the terms "identity" or "identical" or "percent identity" or “percentage identity” or “sequence identity” in the context of two (or more) nucleic acid sequences refer to two (or more) sequences or subsequences that are the same, or that have a specified percentage of nucleotides that are the same (preferably at least 80% identity, more preferably at least 85%, 90%, 95%, 96%, 97% or 98% identity, most preferably at least 99% identity), when compared and aligned for maximum correspondence over a window of comparison, or over a designated region as measured using a sequence comparison algorithm as known in the art, or by manual alignment and visual inspection. Sequences having, for example, 80% to 90% or greater sequence identity may be considered to be substantially identical. Such a definition also applies to the complement of a test sequence. The described identity may exist over a region that is at least about 15 to 25 nucleotides in length, over a region that is at least about 50 to 100 nucleotides in length or over a region that is at least about 800 to 1200 nucleotides in length (or over the entire length of the sequence). Those of skill in the art will know how to determine percent identity between/among sequences using, for example, algorithms such as those based on CLUSTALW computer program (Thompson Nucl. Acids Res. 2 (1994), 4673-4680) or FASTDB (Brutlag Comp. App. Biosci. 6 (1990), 237-245), as known in the art.

Although the FASTDB algorithm typically does not consider internal non-matching deletions or additions in sequences, i.e., gaps, in its calculation, this can be corrected manually to avoid an overestimation of the % identity. CLUSTALW, however, does take sequence gaps into account in its identity calculations. Also available to those having skill in this art are the BLAST and BLAST 2.0 algorithms (Altschul, (1997) Nucl. Acids Res. 25:3389-3402; Altschul (1993) J. Mol. Evol. 36:290-300; Altschul (1990) J. Mol. Biol. 215:403-410). The BLASTN program for nucleic acid sequences uses as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=4, and a comparison of both strands. The BLOSUM62 scoring matrix (Henikoff (1989) PNAS 89:10915) uses alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

In order to determine whether a nucleotide residue in a nucleic acid sequence corresponds to a certain position in a given nucleotide sequence, the skilled person can use means and methods well-known in the art, e.g., alignments, either manually or by using computer programs such as those mentioned herein. For example, BLAST 2.0, which stands for Basic Local Alignment Search Tool BLAST (Altschul (1997), loc. cit.; Altschul (1993), loc. cit.; Altschul (1990), loc. cit.), can be used to search for local sequence alignments. BLAST, as discussed above, produces alignments of nucleotide sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying similar sequences. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP). An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cut-off score set by the user. The BLAST approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper limit of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence whose match satisfies E is reported in the program output.

Analogous computer techniques using BLAST (Altschul (1997), loc. cit.; Altschul (1993), loc. cit.; Altschul (1990), loc. cit.) are used to search for identical or related

molecules in nucleotide databases such as GenBank or EMBL. This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

and it takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1-2% error; and at 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules. Another example for a program capable of generating sequence alignments is the CLUSTALW computer program (Thompson (1994) Nucl. Acids Res. 2:4673-4680) or FASTDB (Brutlag (1990) Comp. App. Biosci. 6:237-245), as known in the art.

In general, the terms "antibody", "antibodies" or "functional equivalents thereof" as used herein are art recognized terms and are understood to refer to molecules or active fragments of molecules that bind to known antigens, particularly to immunoglobulin molecules and to immunologically active portions of immunoglobulin molecules, i.e molecules that contain a binding site that immunospecifically binds an antigen. The immunoglobulin may, in principle, be of any type (IgG, IgM, IgD, IgE, IgA and IgY) or class (IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclasses of immunoglobulin molecule.
"Antibodies" are intended within the scope of the present invention to include monoclonal antibodies, polyclonal, chimeric, single chain, bispecific, simianized, human and humanized antibodies as well as active fragments thereof. Examples of active fragments of molecules that bind to known antigens include Fab and $F(ab')_2$ fragments, including the products of a Fab immunoglobulin expression library and epitope-binding fragments of any of the antibodies and fragments mentioned above.
These active fragments can be derived from a particular antibody (e.g. obinutuzumab) by a number of techniques. For example, purified monoclonal

antibodies can be cleaved with an enzyme, such as pepsin, and subjected to HPLC gel filtration. The appropriate fraction containing Fab fragments can then be collected and concentrated by membrane filtration and the like. For further description of general techniques for the isolation of active fragments of antibodies, see, for example, Khaw, B. A. et al. J. Nucl. Med. 23:1011-1019 (1982); Rousseaux et al. Methods Enzymology, 121:663-69, Academic Press, 1986.

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s).
A humanized antibody may further refer to an antibody having a variable region where one or more of its framework regions have human (or primate) amino acids. In addition, framework support residues may be altered to preserve binding affinity. Methods to obtain "humanized antibodies" are well known to those skilled in the art. (see, e.g., Queen et al., Proc. Natl. Acad. Sci. USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technoloy, 9:421 (1991)).
A "humanized antibody" may also be obtained by a novel genetic engineering approach that enables production of affinity-matured humanlike polyclonal antibodies in large animals such as, for example, rabbits (http://www.rctech.com/bioventures/therapeutic.php).

The term "monoclonal antibody" is also well recognized in the art and refers to an antibody that is mass produced in the laboratory from a single clone and that recognizes only one antigen. Monoclonal antibodies are typically made by fusing a normally short-lived, antibody-producing B cell to a fast-growing cell, such as a cancer cell (sometimes referred to as an "immortal" cell). The resulting hybrid cell, or hybridoma, multiplies rapidly, creating a clone that produces large quantities of the antibody.

The term "antigen" refers to an entity or fragment thereof which can induce an immune response in an organism, particularly an animal, more particularly a mammal including a human. The term includes immunogens and regions responsible for antigenicity or antigenic determinants.

As used herein, the term “soluble” means partially or completely dissolved in an aqueous solution.

Also as used herein, the term "immunogenic" refers to substances which elicit or enhance the production of antibodies, T-cells and other reactive immune cells directed against an immunogenic agent and contribute to an immune response in humans or animals.

The term "hybridoma" is art recognized and is understood by those of ordinary skill in the art to refer to a cell produced by the fusion of an antibody-producing cell and an immortal cell, e.g. a multiple myeloma cell. This hybrid cell is capable of producing a continuous supply of antibody. See the definition of "monoclonal antibody" above for a more detailed description of the method of fusion.

In one embodiment, the functional equivalent of obinutuzumab is envisaged to comprise the constant heavy chain region of obinutuzumab itself (e.g. amino acid positions 120 to 449 of SEQ ID NO. 5), or the constant light chain region of obinutuzumab itself (e.g. amino acid positions 116 to 219 of SEQ ID NO. 6), or both, the constant heavy and light chain regions of obinutuzumab itself. The amino acid sequence of the constant heavy and/or light chain region to be comprised in the functional equivalent of the obinutuzumab may be 100% identical to the amino acid sequence of the constant heavy and/or light chain region of obinutuzumab itself. However, it may also vary to some extent from this/these amino acid sequence(s). For example, it may be at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% identical to the respective amino acid sequence of obinutuzumab itself. It is, however, envisaged that such a variant constant heavy and/or light chain region still contributes to the relevant features of obinutuzumab and the functional equivalent of obinutuzumab, respectively (see above for details), in particular to the feature of being glycoengineered as defined herein and contributing to a significantly higher level of ADCC activity as defined herein, respectively.

Most preferably, the antibody to be used in accordance with the invention is

obinutuzumab itself (also know a Gazyva™/Gazyvaro™ and *GA101*; WHO Drug Information 27(1), 2013, 90, Recommended INN: List 69). As mentioned Obinutuzumab is well known in the art and is, for example, described in EP-B1 2380910 and WO 2005/044859. Obinutuzumab has the following structure:

Heavy chain
QVQLVQSGAE VKKPGSSVKV SCKASGYAFS YSWINWVRQA PGQGLEWMGR 50
IFPGDGDTDY NGKFKGRVTI TADKSTSTAY MELSSLRSED TAVYYCARNV 100
FDGYWLVYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD 150
YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY 200
ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP SVFLFPPKPK 250
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS 300
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV 350
YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL 400
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK 449

Light chain
DIVMTQTPLS LPVTPGEPAS ISCRSSKSLL HSNGITYLYW YLQKPGQSPQ 50'
LLIYQMSNLV SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP 100'
YTFGGGTKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK 150'
VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE 200'
VTHQGLSSPV TKSFNRGEC 219'

Disulfide bridges location
22-96 22"-96" 23'-93' 23"'-93"' 139'-199' 139"'-199"' 146-202 146"-202"
219'-222 219"'-222" 228-228" 231-231" 263-323 263"-323" 369-427 369"-427"

Glycosylation sites
H CH2 N84.4
299, 299" (enriched in bisected non-fucosylated oligosaccharides)

The antibody rituximab (medical product name: MabThera®; also known as Rituxan®) is also known in the art. It is, for example, described in EP-B1 1005870 (e.g. Figures 4 and 5). The amino acid sequence of the heavy chain of rituximab is the depicted in SEQ ID NO. 9. The amino acid sequence of the light chain of rituximab is depicted in SEQ ID NO. 10.

In accordance with the invention, the skilled person is readily able to assess whether a patient advantageously responds to the treatment with obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy), as compared to a treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy). In particular, the clinical outcome or clinical endpoints of the treatment can be assessed in this respect. Clinical outcomes/clinical endpoints which can be assessed in this respect are available to the skilled person and are, for

example, described in Goede (loc. cit.), Owen (Expert Opin. Biol. Ther. 12(3), 2012, 343-51) and Illidge (Expert Opin. Biol. Ther. 12(5), 2012, 543-5) and in the appended examples.

Preferred examples of a clinical outcome to be assessed in accordance with the invention are progression free survival (PFS), overall survival (OS) and/or event free survival (EFS). The superiority of Obinutuzumab over rituximab in accordance with the invention may also be determined on the basis of one ore more clinical endpoints. In principle, in accordance with the invention, the term clinical outcome is envisaged to refer to a time during the treatment and the term clinical endpoint is envisaged to refer to the time at (or after) the end of the treatment. In accordance with the invention, the clinical endpoint may be a primary clinical endpoint. Particular, however non-limiting, clinical outcomes and clinical endpoints are described in the appended examples.

The skilled person is readily able to decide whether a given clinical outcome is improved in accordance with the invention, i.e. improved as compared to a treatment with rituximab. For example, "improved" in this context means that the clinical outcome (resulting from the treatment with obinutuzumab/a functional equivalent of obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy)) is at least 3% higher, at least 5% higher, at least 7% higher, at least 10% higher, at least 15% higher, at least 20% higher, at least 25% higher, at least 30% higher, at least 40% higher, at least 50% higher, at least 75% higher, at least 100% higher, or at least 120% higher, as compared to the clinical outcome resulting from a comparable treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy).

The time at which the clinical outcome/clinical endpoint is assessed can readily be determined by the skilled person. In principle, it is determined at a timepoint when the difference in the clinical outcome/clinical endpoint between the two treatments (obinutuzumab treatment vs. rituximab treatment) becomes (significantly) evident. This time may, for example, be at least 1 month, at least 2 months, at least 3 months, at least 6 months, at least 12 months, at least 18 months, at least 24

months, at least 30 months, at least 36 months, at least 42 months, or at least 48 months, after the beginning of the treatment.

Preferably, the (DLBCL) patient to be treated in accordance with the invention is a human patient/human.
Most preferably, the (DLBCL) patient to be treated in accordance with the invention is a 1L DLBCL human patient. This means that the DLBCL patient is a previously untreated DLBCL patient.
However, in principle, also other patients may be treated in accordance with the invention, for example a non-human patient, for example, a pet (e.g. dog, cat, rabbit, rat or mouse), a cattle (e.g. cow, pig, sheep), a horse or a pony or a bird (e.g. chicken, turkey, parrot). Also other warm-blooded animals may be treated in accordance with the invention.
As mentioned, it is particularly envisaged in the context of the invention that the patient defined herein responds to a treatment with obinutuzumab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy) by reaching an improved clinical outcome as compared to a treatment with rituximab (in particular in combination with a chemotherapy, more particular in combination with a CHOP chemotherapy).

The antibody to be used in the context of the invention (i.e. obinutuzumab or a functional equivalent thereof) may be administered in combination with further agents. For example, one or more additional other cytotoxic or chemotherapeutic agent(s), or ionizing radiation enhancing the effects of such agent(s), may be co-administered; see, for example, EP-B1 2380910, WO 2005/044859, WO 2015/067586 and WO 2016/207312 for respective examples.

The terms "administered in combination with" or "co-administration", "co-administering", "combination therapy" or "combination treatment" refer to the administration of the antibody as described herein, and the other agent(s) as described herein, e.g. as separate formulations/applications (or as one single formulation/application). The co-administration can be simultaneous or sequential in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. Said antibody and said further agent(s)

are co-administered either simultaneously or sequentially (e.g. intravenous (i.v.)), for example through a continuous infusion. When both therapeutic agents are co-administered sequentially the dose administered either on the same day in two separate administrations, or one of the agents may be administered on day 1 and the second may be co-administered on day 2 to day 7, preferably on day 2 to 4. Thus in one embodiment the term "sequentially" means within (about) 7 days after the dose of the first component, preferably within (about) 4 days after the dose of the first component; and the term "simultaneously" means at the same time. The term "co-administration" with respect to the maintenance doses of the antibody and/or further agent(s) means that the maintenance doses can be either co-administered simultaneously, if the treatment cycle is appropriate for both drugs, e.g. every week or the further agent is, e.g., administered, e.g., every first to third day and said antibody is administered every week. Or the maintenance doses are co-administered sequentially, either within one or within several days.

In addition to the antibody, optionally in combination with the other agent(s), also (a) chemotherapeutic agent(s) or targeted therapies may be administered.
Such additional chemotherapeutic agents, which may be co-administered, include, but are not limited to, anti-neoplastic agents including alkylating agents including: nitrogen mustards, such as mechlorethamine, cyclophosphamide, ifosfamide, melphalan and chlorambucil; nitrosoureas, such as carmustine (BCNU), lomustine (CCNU), and semustine (methyl-CCNU); Temodal(TM) (temozolamide), ethylenimines/methylmelamine such as thriethylenemelamine (TEM), triethylene, thiophosphoramide (thiotepa), hexamethylmelamine (HMM, altretamine); alkyl sulfonates such as busulfan; triazines such as dacarbazine (DTIC); antimetabolites including folic acid analogs such as methotrexate and trimetrexate, pyrimidine analogs such as 5-fluorouracil (5FU), fluorodeoxyuridine, gemcitabine, cytosine arabinoside (AraC, cytarabine), 5-azacytidine, 2,2'-difluorodeoxycytidine, purine analogs such as 6-merca.rho.topurine, 6-thioguamne, azathioprine, T-deoxycoformycin (pentostatin), erythrohydroxynonyladenine (EHNA), fludarabine phosphate, and 2- chlorodeoxyadenosine (cladribine, 2-CdA); natural products including antimitotic drugs such as paclitaxel, vinca alkaloids including vinblastine (VLB), vincristine, and vinorelbine, taxotere, estramustine, and estramustine phosphate; pipodophylotoxins such as etoposide and teniposide; antibiotics such as

actinomycin D, daunomycin (rubidomycin), doxorubicin, mitoxantrone, idarubicin, bleomycins, plicamycin (mithramycin), mitomycin C, and actinomycin; enzymes such as L-asparaginase; biological response modifiers such as interferon-alpha, IL-2, G-CSF and GM-CSF; miscellaneous agents including platinum coordination complexes such as oxaliplatin, cisplatin and carboplatin, anthracenediones such as mitoxantrone, substituted urea such as hydroxyurea, methylhydrazine derivatives including N- methylhydrazine (MIH) and procarbazine, adrenocortical suppressants such as mitotane (o, p-DDD) and aminoglutethimide; hormones and antagonists including adrenocorticosteroid antagonists such as prednisone and equivalents, dexamethasone and aminoglutethimide; Gemzar(TM) (gemcitabine), progestin such as hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate; estrogen such as diethylstilbestrol and ethinyl estradiol equivalents; antiestrogen such as tamoxifen; androgens including testosterone propionate and fluoxymesterone/equivalents; antiandrogens such as flutamide, gonadotropin-releasing hormone analogs and leuprolide; and non-steroidal antiandrogens such as flutamide. Therapies targeting epigenetic mechanism including, but not limited to, histone deacetylase inhibitors, demethylating agents (e.g., Vidaza) and release of transcriptional repression (ATRA) therapies can also be combined with the antigen binding proteins. In one embodiment the chemotherapeutic agent is selected from the group consisting of taxanes (like e.g. paclitaxel (Taxol), docetaxel (Taxotere), modified paclitaxel (e.g., Abraxane and Opaxio), doxorubicin, sunitinib (Sutent), sorafenib (Nexavar), and other multikinase inhibitors, oxaliplatin, cisplatin and carboplatin, etoposide, gemcitabine, and vinblastine. In one embodiment the chemotherapeutic agent is selected from the group consisting of taxanes (like e.g. taxol (paclitaxel), docetaxel (Taxotere), modified paclitaxel (e.g. Abraxane and Opaxio). In one embodiment, the additional chemotherapeutic agent is selected from 5-fluorouracil (5-FU), leucovorin, irinotecan, or oxaliplatin. In one embodiment the chemotherapeutic agent is 5-fluorouracil, leucovorin and irinotecan (FOLFIRI). In one embodiment the chemotherapeutic agent is 5-fluorouracil, and oxaliplatin (FOLFOX).

In a preferred embodiment, the antibody defined herein (i.e. obinutuzumab and its functional equivalents) may be administered in combination with a chemotherapy, for example with a CHOP chemotherapy (more preferred) or with variants of a CHOP chemotherapy, like a CHOEP chemotherapy, a CHOP-14 chemotherapy or a ACVBP

chemotherapy (see, for example, the appended examples, infra, and also EP-B1 2380910, WO 2005/044859 and Scott, 2014 and 2015, loc. cit.). Therefore, in a preferred embodiment, the additional chemotherapeutic agents to be co-administered are selected from the group consisting of Cyclophosphamide, Hydroxydaunorubicin, Oncovein, Prednisone or Prednisolone and, optionally, Etoposide.

The antibody to be used in the context of the invention may be comprised in a composition, in particular in a pharmaceutical composition. The pharmaceutical composition may comprise a pharmaceutically acceptable carrier.

Suitable pharmaceutical compositions and pharmaceutically acceptable carriers are known in the art and are, for example, described in EP-B1 2380910, WO 2005/044859, WO 2015/067586 and WO 2016/207312.

Accordingly, in another aspect, a composition, e.g. a pharmaceutical composition, containing an antibody, or an antigen-binding portion thereof, as defined herein, optionally formulated together with a pharmaceutically acceptable carrier, is envisaged to be employed in accordance with the invention.

As used herein, "pharmaceutically acceptable carrier" includes any and all suitable solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption/resorption delaying agents, and the like, that are physiologically compatible. Preferably, the pharmaceutical composition and carrier, respectively is suitable for injection or infusion.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. In addition to water, the carrier can be, for example, an isotonic buffered saline solution.

Acceptable carriers, excipients, or stabilizers are envisaged to be nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and

methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™or polyethylene glycol (PEG).

A composition/antibody of the present invention can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Exemplary antibody formulations which, appropriately adapted, may be employed also in accordance with the invention are described in WO98/56418. This publication describes a liquid multidose formulation comprising 40 mg/mL antibody, 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol, 0.02% polysorbate 20 at pH 5.0 that has a minimum shelf life of two years storage at 2-8°C. Another antibody formulation comprises 10 mg/mL antibody in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injection, pH6.5.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a

particular patient, composition, and mode of administration, without being toxic to the patient (effective amount). The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like (other) factors well known in the medical arts.

The term "a method of treating" or its equivalent, when applied to, for example, DLBCL, and the patient defined herein, respectively, refers to a procedure or course of action that is, for example, designed to reduce or eliminate the number of DLBCL tumor cells in a patient, or to alleviate the symptoms of a DLBCL tumor. "A method of treating" DLBCL, however, may not necessarily mean that the DLBCL tumor cells will, in fact, be eliminated, that the number of cells will, in fact, be reduced, or that the symptoms of a DLBCL tumor will, in fact, be alleviated. Often, a method of treating DLBCL will be performed even with a low likelihood of success, but which, given the medical history and estimated survival expectancy of a patient, is nevertheless deemed to induce an overall beneficial course of action, in particular as compared to a rituximab treatment.

It is self-evident that the antibody is (to be) administered to the patient in a "therapeutically effective amount" (or simply "effective amount") which is the amount of the respective compound or combination that will elicit the biological or medical response, for example of a tissue, system, animal or human, that is being sought by the researcher, veterinarian, medical doctor or other clinician.

The amount of (co-)administration and the timing of (co-)administration will depend on the type (species, gender, age, weight, etc.) and condition of the patient being treated and the severity of the disease or condition being treated. The antibody, and optionally a further agent, are suitably (co-)administered to the patient at one time or over a series of treatments e.g. on the same day or on the day after.

Depending on the type and severity of the disease, about 0.1 mg/kg to 50 mg/kg (e.g. 0.1-20 mg/kg) of the antibody defined herein is an initial candidate dosage for co-administration to the patient.

A particular, however non-limiting, example of a scheme of administration (including administration routes and dosages) for obinutuzumab/a functional equivalent of obinutuzumab (more particular G-CHOP) is described in and provided by the appended examples (in particular example 1 and example 2 which describes the study design and treatments applied in the context of the GOYA study in detail). The skilled person is, if need be, readly able to adapt this example of a G administration scheme to any other G administration scheme which might be appropriate in accordance with the invention.

The antibody and pharmaceutical composition, respectively, to be employed in accordance with the invention may be provided together with an instruction manual or instruction leaflet. The instruction manual/leaflet may comprise guidance for the skilled person/attending physician on how to treat DLBCL and the patient defined herein in accordance with the invention. For example, the instruction manual/leaflet may comprise guidance as to the herein described mode of administration/administration regimen (for example route of administration, dosage regimen, time of administration, frequency of administration). In particular, the instruction manual/leaflet may comprise information as to the patient to be treated, i.e. the patient defined herein. In principle, what has been said herein elsewhere with respect to obinutuzumab, the patient to be treated, the mode of administration/administration regimen (including dosages etc.) etc. may be comprised in the instruction manual/leaflet.

A preferred sample to be employed in the context of the invention is derived from the patient's tumor tissue (e.g. as a biopsy). For example, formalin-fixed or, preferably and, paraffin-embedded tumor tissue may be employed (e.g. sections of tumor tissue on an object slide). However, also other samples are envisaged to be employed in the context of the invention, for example, sections/biopsies of other tissues, blood samples, serum samples, or other body fluid samples, and the like.

In this specification, a number of documents including patents/patent applications are cited. The disclosure of these documents, while not considered relevant for the patentability of this invention, is herewith incorporated by reference in its entirety. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

The invention will now be described by reference to the following figures and examples which are not to be construed as a limitation of the scope of the present invention.

The Figures show:

Figure 1

Disposition of patients in GOYA.

*Discontinued refers to patients who discontinued study (antibody) treatment.

†Median observation time was 29 months in each group; completed treatment refers to patients who completed study (antibody) treatment.

Patients were stratified at randomization by IPI score (low/low-intermediate, high-intermediate, and high-risk), planned number of CHOP cycles (8 vs. 6), and geographic region (Western Europe, Eastern Europe, South and Central America, North America, Asia, and others).

G-CHOP, obinutuzumab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone.

Figure 2

Kaplan-Meier estimates of PFS and OS in GOYA.

(A) investigator-assessed PFS (primary endpoint) by treatment, intent-to-treat population, (B) OS by treatment, intent-to-treat population (C) investigator-assessed PFS by cell-of-origin subtype (irrespective of study treatment) in patients with cell-of-origin data.

ABC, activated B-cell-like; CI, confidence interval; GCB, germinal-center B-cell-like; G-CHOP, obinutuzumab plus cyclophosphamide, doxorubicin, vincristine, and

prednisone; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone.

Figure 3

Unstratified hazard ratios for investigator-assessed PFS in GOYA patient subgroups.

(A) randomization stratification factors and (B) baseline characteristics.

ABC, activated B-cell-like; CI, confidence interval; DLBCL, diffuse large B-cell lymphoma;

ECOG PS, Eastern Cooperative Oncology Group performance status; GCB, germinal-center B-cell-like; G-CHOP, obinutuzumab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; IPI, International Prognostic Index; KM, Kaplan-Meier; PFS, progression-free survival; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone.

*Cases where 'yes' was ticked in the eCRF for extranodal involvement; 14 patients with extranodal sites 0 were ticked in error.

Figure 4

Gazyva benefit in BCL2 translocated patients in GOYA

Figure 5

Gazyva benefit in BCL2 protein expression positive patients in GOYA

Figure 6

Gazyva benefit in BCL2 translocated patients that are BCL2 protein expression positive in GOYA

Evaluation of treatment effect in quadrants defined by BCL2 IHC (pos/neg) and BCL2 FISH (pos/neg) in GOYA. Gazyva superiority shown to be specific to BCL2 IHC+/FISH+ pts.

Figure 7

Gazyva benefit in subgroups of GCB defined by various cutoffs of the Linear Predictor Score in GOYA

A: FOREST Plot 25%-36% cutoff (%)

B: FOREST Plot 25%-50% cutoff (%)

C: Benefit of Gazyva in strong-GCB subgroup for the primary endpoint (INV-PFS) at LPS cut-off of <749.

- HR* (95% CI) in strong-GCB GOYA pts
  - PFS: 0.33 [0.18 – 0.63], p-value=0.0007
- Strong-GCB pts represent the 25% (n=233/933) of all DLBCL pts in GOYA with LPS < 749.
- Strong-GCB pts make up 43% (n=233/540) of GCB pts in GOYA

Figure 8

Gazyva benefit in GCB patients that are BCL2 translocated and BCL2 protein expression positive in GOYA.

Figure 9

Gazyva benefit in CD58 mutated patients and/or patients with low CD58 gene expression in GOYA.

Figure 10

Multivariate simulation optimization of the LPS cutoff for G-CHOP benefit over R-CHOP on progression-free survival in biomarker-evaluable pts with COO analysis (N=xx).

LPS cutoff optimization for strong-GCB treatment effect in GOYA original data.

*Multivariate HR adjusted for treatment, International Prognostic Index, number of chemotherapy cycles (6 or 8), and geographical region. Blue line, point estimate of the HR, yellow line, 95% CI; CI, confidence interval; COO, cell of origin; HR, hazard ratio; LPS, Linear Predictor Score.

Figure 11

Molecular characterization of strong-GCB patients# in GOYA.

Strong-GCB patients have significantly higher prevalence of FL somatic mutation hallmarks.

(A) Prevalence of FL somatic mutation hallmarks* (any mutation type).

(B) Prevalence of BCL2 Translocations†

#Other biomarkers evaluated to characterize strong- from weak-GCB, where no significant difference in prevalence rate was identified, were: by gene expression, stromal-1/2 gene signatures, immune-response 1/2 gene signatures, CD20, and PTEN; by protein expression, BCL2, MYC, and BCL2/MYC double-expressors; and by gene translocations, MYC translocations and BCL2/MYC double-hit. ABC, activated B-cell; DLBCL, diffuse large B-cell lymphoma; FDR, false discovery rate; FISH, fluorescence in situ hybridization; FL, follicular lymphoma; GCB, germinal center B-cell; NGS, next-generation sequencing.

Figure 12

Distribution of optimal LPS cutoff across bootstrap samples

- Bootstrap multivariate simulations to further test robustness and generalizability of an optimal LPS identified using the "min.HR rule"
- Extreme peak across bootstrap samples is at LPS = 725
- LPS distribution with its unique peak, supports robustness of treatment effect signal
- Optimal LPS cutoff suggested for new potential confirmatory study is LPS ≤ 725
  - Historically all GOYA biomarker analyses has defined strong-GCB as < 749 (25% of pts in GOYA), including biomarker analyses presented in this OBRF
  - n = 4 pts with 725 < LPS < 749, all G-CHOP (1 event)

Figure 13

Kaplan-Meier Estimates of Time to Next Anti-Lymphoma Treatment (Secondary Endpoint) in the Intent-To-Treat Population.

CI, confidence interval; G-CHOP, obinutuzumab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone; HR, hazard ratio; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone.

Figure 14

Kaplan-Meier Estimates of Investigator-Assessed PFS by Treatment Arm in Patients With COO Data, Subgrouped by COO Subtype.

(A) GCB; (B) ABC; (C) Unclassified.

ABC, activated B-cell-like; CI, confidence interval; COO, cell of origin; GCB, germinal-center B-cell-like; G-CHOP, obinutuzumab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone; HR, hazard ratio; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone.

The present invention also refers to the following tables.

Table 1. Baseline Patient and Disease Characteristics (Intent-to-Treat Population)

Characteristic	G-CHOP (N = 706)*	R-CHOP (N = 712)*
Median age, years (range)	62.0 (18–86)	62.0 (18–83)
Male — no. (%)	369 (52.3)	383 (53.8)
Geographic region — no. (%)		
Asia	260 (36.8)	258 (36.2)
Western Europe	211 (29.9)	215 (30.2)
North America	109 (15.4)	107 (15.0)
Eastern Europe	97 (13.7)	99 (13.9)
Other	29 (4.1)	33 (4.6)
ECOG PS — no. (%)	n = 705	n = 712
0–1	618 (87.7)	613 (86.1)
2–3	87 (12.3)	99 (13.9)
Ann Arbor stage — no. (%)	n = 706	n = 711
I and II	170 (24.1)	171 (24.0)
III and IV	536 (75.9)	540 (75.8)
IPI risk group — no. (%)		
Low/low intermediate	376 (53.3)	409 (57.4)
High-intermediate	221 (31.3)	192 (27.0)
High	109 (15.4)	111 (15.6)
Planned chemotherapy cycles — no. (%)		
6	523 (74.1)	526 (73.9)
8	183 (25.9)	186 (26.1)
LDH elevated — no. (%)	n = 705	n = 708
	415 (58.9)	401 (56.6)
Extranodal involvement — no. (%)†	484 (68.6)	468 (65.7)
Bulky disease (≥7.5 cm) — no. (%)	261/703 (37.1)	262/710 (36.9)
Cell of origin	n = 471‡	n = 462‡
GCB	271 (57.5)	269 (58.2)
ABC	125 (26.5)	118 (25.5)
Unclassified	75 (15.9)	75 (16.2)

ABC, activated B cell-like (subgroup); ECOG PS, Eastern Cooperative Oncology Group performance status; G-CHOP, obinutuzumab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone; GCB, germinal-center B cell-like (subgroup); IPI, International Prognostic Index; LDH, lactate dehydrogenase; PET, positron emission tomography; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone.

*n = 706 for G-CHOP and n = 712 for R-CHOP for all parameters unless otherwise specified.

†Cases where 'yes' was ticked in the eCRF for extranodal involvement; 14 patients with extranodal sites 0 were ticked in error.

‡COO subtype classification was missing for 485 patients (G-CHOP, 235; R-CHOP, 250); includes samples from China which could not be analyzed due to lack of an export license – analysis of these samples is planned in the near future.

Table 2. Summary of Efficacy Endpoints (Intent-to-Treat Population)

	Investigator Assessment	
Endpoint	**G-CHOP N = 706)**	**R-CHOP N = 712)**
Median observation time, months (range)	29.0 (0.1–56.6)	28.9 (0.1–56.2)
Investigator-assessed PFS (primary endpoint)	N = 706	N = 712
Patients with event, no. (%)	201 (28.5)	215 (30.2)
3-year PFS, %	69.6	66.9
Stratified HR (95% CI) – P value (log-rank)*	0.92 (0.76–1.11), *P* = .3868	
IRC-assessed PFS	N = 706	N = 712
Patients with event, no. (%)	171 (24.2)	186 (26.1)
3-year PFS, %	72.5	70.6
Stratified HR (95% CI), *P* value (log-rank)*	0.89 (0.72 to 1.10), *P* = .2736	
OS	N = 706	N = 712
Patients with event, no. (%)	126 (17.8)	126 (17.7)
3-year OS, % (95% CI)	81.2 (77.9 to 84.1)	81.4 (78.1 to 84.3)
Stratified HR (95% CI)*	1.00 (0.78 to 1.28)	
DFS in patients with investigator-assessed CR	n = 397	n = 369
Patients with event, no. (%)	77 (19.4)	64 (17.3)
Stratified HR (95% CI)*	1.27 (0.91 to 1.77)	
Investigator-assessed EFS	N = 706	N = 712
Events, no. (%)	236 (33.4)	250 (35.1)
Stratified HR (95% CI)*	0.92 (0.77 to 1.11)	
Time to start of new anti-lymphoma treatment	N = 706	N = 712
Patients with event, no. (%)	213 (30.2)	230 (32.3)
Proportion event-free at 3 years, % (95% CI)	69.9 (66.2 to 73.2)	66.5 (62.7 to 70.1)
Stratified HR (95% CI)*	0.92 (0.76 to 1.11)	
Investigator-assessed response (with PET) at end of treatment†	n = 669	n = 665
ORR		
Proportion, no. (%)	518 (77.4)	518 (77.9)
Percentage difference (95% CI)	-0.47 (-5.01 to 4.08)	

CR		
Proportion, no. (%)	379 (56.7)	396 (59.5)
Difference (95% CI)	-2.90 (-8.27 to 2.48)	
Investigator-assessed response (without PET) at end of treatment†	N = 706	N = 712
ORR		
Proportion, no. (%)	577 (81.7)	572 (80.3)
Percentage difference (95% CI)	1.39 (-2.76 to 5.54)	
CR		
Proportion, no. (%)	248 (35.1)	241 (33.8)
Difference (95% CI)	1.28 (-3.74 to 6.30)	

CI, confidence interval; CR, complete response; DFS, disease-free survival; EFS, event-free survival; G-CHOP, obinutuzumab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone; HR, hazard ratio; IRC, Independent Review Committee; ORR, overall response rate; OS, overall survival; PET, positron emission tomography; PFS, progression-free survival; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone.

*Stratification factors were International Prognostic Index score and planned number of CHOP cycles (6 or 8).

†According to revised response criteria.[13]

Table 3. Summary of AEs Including Grade 3–5 and Serious AEs Reported by ≥ 5% of Patients in Either Group (At Preferred Term Level; Safety Population)

	G-CHOP (N = 704), No. (%)		R-CHOP (N = 703), No. (%)	
Number of deaths for any reason	126 (17.9)		122 (17.4)	
Number of patients withdrawn from study due to an AE	4 (0.6)		3 (0.4)	
Patients with at least one				
AE	683 (97.0%)		657 (93.5)	
Grade 3–5 AE	519 (73.7)		455 (64.7)	
AE with fatal outcome*	41 (5.8)		30 (4.3)	
Serious AE	300 (42.6)		264 (37.6)	
Treatment-related AEs	639 (90.8)		596 (84.8)	
AEs leading to withdrawal of any treatment	84 (11.9)		60 (8.5)	
AEs leading to dose reduction for any treatment	145 (20.6)		138 (19.6)	
	Grade 3–5 AEs	Serious AEs	Grade 3–5 AEs	Serious AEs
Blood and lymphatic system disorders				
Total number of patients with at least one AE	415 (58.9)	135 (19.2)	348 (49.5)	113 (16.1)
Neutropenia	325 (46.2)	52 (7.4)	268 (38.1)	40 (5.7)
Febrile neutropenia	123 (17.5)	81 (11.5)	107 (15.2)	72 (10.2)
Leukopenia	96 (13.6)	10 (1.4)	71 (10.1)	5 (0.7)
Anemia	51 (7.2)	9 (1.3)	53 (7.5)	6 (0.9)
Infections and infestations				
Total number of patients with at least one AE	135 (19.2)	121 (17.2)	109 (15.5)	94 (13.4)

Pneumonia	40 (5.7)	40 (5.7)	35 (5.0)	32 (4.6)

AE, adverse event; G-CHOP, obinutuzumab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone.

*Fatal AEs that were reported in more than one patient in either group, listed as preferred terms, were: septic shock (6 [0.9%] patients), pneumonia (5 [0.7%]), death (cause unknown; 3 [0.4%]), pulmonary embolism (2 [0.3%]) and cerebrovascular accident (2 [0.3%]) in the G-CHOP group and pneumonia (6 [0.9%]), sepsis (3 [0.4%]), cerebrovascular accident (2 [0.3%]) and death (cause unknown; 2 [0.3%]) in the R-CHOP group.

Table 4: Effect of G-CHOP and R-CHOP on clinical outcomes in strong-GCB and all other patients

Multivariate Analysis HR (G vs R)* [95% CI]; p-value 3-yr rate (%)	**Strong-GCB Pts (n=233)** **R-CHOP: n=121** **G-CHOP: n=112**	**All Other Pts (n=700)** **R-CHOP: n=341** **G-CHOP: n=359**
PFS	0.33 [0.18–0.63]; p=0.0007 R: 66%; G: 88%	0.99 [0.76–1.28]; p=0.9117 R: 66%; G: 66%
EFS	0.47 [0.28–0.78]; p=0.00344 R: 59%; G: 80%	1.01 [0.79–1.29]; p=0.9513 R: 63%; G: 62%
OS	0.41 [0.20–0.87]; p=0.019 R: 79%; G: 92%	1.10 [0.79–1.53]; p=0.582 R: 81%; G: 78%

*Adjusted for treatment arm, International Prognostic Index, number of chemotherapy cycles (6 or 8), and geographic region

CI, confidence interval; EFS, event-free survival; GCB, germinal center B-cell; HR, hazard ratio; OS, overall survival; PFS, progression-free survival (investigator-assessed); yr, year

Table 5. Study Drug Exposure

	G-CHOP (N = 704), No. (%)	R-CHOP (N = 703), No. (%)
Number of obinutuzumab or rituximab doses received, median (range)	10 (1–10)	8 (1–8)
Patients with modifications to any obinutuzumab or rituximab dose*	222 (31.5)	210 (29.9%)
Patients with modifications to obinutuzumab or rituximab doses in cycle 1*	192/702 (27.4)	155/703 (22.0)
Day 1	39/651 (6.0)	0
Day 8	41/624 (6.6)	0
Day 15		
Patients with delays to obinutuzumab or rituximab doses of > 7 days	92 (13.1)	64 (9.1)
Patients with ≥ 90% planned dose intensity of obinutuzumab or rituximab	671 (95.3)	697 (99.1)
Patients with ≥ 90% planned dose intensity of		
Cyclophosphamide	642 (91.3)	647 (92.0)
Doxorubicin	631 (89.8)	639 (90.9)
Prednisone	662 (94.0)	643 (91.5)
Vincristine	642 (91.3)	625 (88.9)

Duration of exposure to obinutuzumab or rituximab, weeks, median (range)	25.3 (1–32)	25.3 (0–32)
Cumulative dose of obinutuzumab or rituximab in mg, median (range)	10,000 (998–10,065)	5,133.5 (515–8,084)

G-CHOP, obinutuzumab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone.
*Including interruptions to infusions and slowing of infusion rates.

Table 6. Number (and %) of Patients Reporting AEs of Any Grade With an Incidence Rate of At Least 10% (At Preferred Term Level) in Either Treatment Arm, Listed by System Organ Class and Preferred Term (Safety Population)

	G-CHOP (N = 704), No. (%)	**R-CHOP (N = 703), No. (%)**
Blood and lymphatic system disorders		
Total number of patients with at least one AE	451 (64.1)	389 (55.3)
Neutropenia	340 (48.3)	286 (40.7)
Febrile neutropenia	127 (18.0)	108 (15.4)
Leukopenia	115 (16.3)	87 (12.4)
Anemia	95 (13.5)	99 (14.1)
Gastrointestinal disorders		
Total number of patients with at least one AE	428 (60.8)	410 (58.3)
Nausea	207 (29.4)	199 (28.3)
Constipation	165 (23.4)	172 (24.5)
Diarrhea	112 (15.9)	92 (13.1)
Vomiting	103 (14.6)	74 (10.5)
General disorders and administration site conditions		
Total number of patients with at least one AE	420 (59.7)	323 (45.9)
Fatigue	137 (19.5)	123 (17.5)
Pyrexia	142 (20.2)	83 (11.8)
Chills	133 (18.9)	37 (5.3)

Asthenia	71 (10.1)	76 (10.8)
Injury, poisoning and procedural complications		
Total number of patients with at least one AE	281 (39.9)	204 (29.0)
Infusion-related reaction	254 (36.1)	165 (23.5)
Metabolism and nutrition disorders		
Total number of patients with at least one AE	202 (28.7)	170 (24.2))
Decreased appetite	97 (13.8)	71 (10.1)
Nervous system disorders		
Total number of patients with at least one AE	336 (47.7)	299 (42.5)
Peripheral neuropathy	88 (12.5)	89 (12.7)
Headache	75 (10.7)	57 (8.1)
Psychiatric disorders		
Total number of patients with at least one AE	107 (15.2)	83 (11.8)
Insomnia	76 (10.8)	58 (8.3)
Respiratory, thoracic and mediastinal disorders		
Total number of patients with at least one AE	232 (33.0)	197 (28.0)
Cough	83 (11.8)	60 (8.5)
Skin and subcutaneous tissue disorders		
Total number of patients with at least one AE	226 (32.1)	226 (32.1)
Alopecia	145 (20.6)	142 (20.2)

AE, adverse event; G-CHOP, obinutuzumab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone.

Table 7. Summary of AEs of Particular Interest as Preferred Terms or Predefined Categories (Safety Population)

	All grades		Grades 3–5	
Category	G-CHOP (N = 704), No. (%)	R-CHOP (N = 703), No. (%)	G-CHOP (N = 704), No. (%)	R-CHOP (N = 703), No. (%)
Infections*	379 (53.8)	310 (44.1)	135 (19.2)	109 (15.5)
Opportunistic Infections†	13 (1.8)	15 (2.1)	8 (1.1)	9 (1.3)

Neutropenia[‡]	398 (56.5)	338 (48.1)	384 (54.5)	324 (46.1)
Infusion-related reactions[§]	319 (45.3)	223 (31.7)	69 (9.8)	24 (3.4)
Infusion-related reactions (antibody related)[§]	273 (38.8)	174 (24.8)	53 (7.5)	16 (2.3)
Tumor lysis syndrome	4 (0.6)	4 (0.6)	4 (0.6)	4 (0.6)
Cardiac events[¶]	75 (10.7)	53 (7.5)	33 (4.7)	20 (2.8)
Thrombocytopenia[‖]	55 (7.8)	18 (2.6)	31 (4.4)	10 (1.4)
Second malignancies**	15 (2.1)	15 (2.1)	12 (1.7)	13 (1.8)
Hepatitis B reactivation[††]	16 (2.3)	6 (0.9)	2 (0.3)	2 (0.3)
Progressive multifocal leukoencephalopathy	1 (0.1)	0	1 (0.1)	0
Gastrointestinal perforation[‡‡]	14 (2.0)	8 (1.1)	12 (1.7)	8 (1.1)
Perforation events	7 (1.0)	7 (1.0)	6 (0.9)	7 (1.0)
Abscesses/other	8 (1.1)	2 (0.3)	8 (1.1)	2 (0.3)
Hemorrhagic events[§§]	65 (9.2)	39 (5.5)	23 (3.3)	10 (1.4)

AE, adverse event; G-CHOP, obinutuzumab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone; HBV, hepatitis B infection; MedDRA, Medical Dictionary for Regulatory Activities; R-CHOP rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone/prednisolone.

*Any preferred term in the System Organ Class Infections and infestations.

[†]Standardized MedDRA query.

[‡]Neutropenia and associated complications reported as AEs, not including abnormal laboratory values.

[§]Related to any infused treatment and occurring during/within 24 hours of infusion.

[¶]Any preferred term in the System Organ Class Cardiac disorders.

**Any preferred term in the System Organ Class Neoplasms benign, malignant and unspecified (including cysts and polyps) that started 6 months after the first study drug intake.

[††]At least one of an increase in HBV DNA level of ≥100 IU/ml or an AE of hepatitis B reactivation.

[‡‡]Standardized MedDRA query, comprising perforation events (preferred terms in the System Organ Class Gastrointestinal disorders) and abscesses and other events (preferred terms in other System Organ Classes).

[§§]Standardized MedDRA query, comprising hemorrhagic cerebrovascular conditions, and hemorrhage (laboratory and non-laboratory terms).

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 62

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 62

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

PCT/EP2018/071462
F. Hoffmann-La Roche AG; NanoString Technologies, Inc.
Our Ref.: AA1470 PCT S3

AMENDED CLAIMS

1. A humanized Type II anti-CD20 antibody comprising
   (a) a heavy chain variable region as depicted in SEQ ID NO:1 or as comprised in SEQ ID NO:5 (amino acid residues 1 to 119) and a light chain variable region as depicted in SEQ ID NO:2 or as comprised in SEQ ID NO:6 (amino acid residues 1 to 115); or
   (b) a heavy chain variable region that is encoded by a nucleic acid sequence which is at least 80% identical to SEQ ID NO:3 or SEQ ID NO:7 and a light chain variable region that is encoded by a nucleic acid sequence which is at least 80% identical to SEQ ID NO:4 or SEQ ID NO:8,

   wherein said antibody is capable of causing an increased antibody-dependent cellular cytotoxicity (ADCC) as compared to rituximab, is capable of causing an increased antibody-dependent cellular phagocytosis (ADCP) as compared to rituximab and/or has an increased affinity for FcγRIII receptors as compared to rituximab,

   for use in the treatment of diffuse large B-cell lymphoma (DLBCL) in a patient which responds to a treatment with obinutuzumab by reaching an improved clinical outcome as compared to a treatment with rituximab, wherein said patient is
   (i) a patient with one or more mutation(s) in one or more of the gene(s) selected from the group consisting of *CREBBP*, *EP300*, *MEF2B*, *MYC*, *EZH2* and *TNFRSF14*;
   (ii) a patient with (a) genetic mutation(s) in *CD58* and/or with a low expression of *CD58* that corresponds to *log2(normalized Reads Per Kilobase Million (nRPKM))* ≤ 5.2;
   (iii) a patient with a cell-of-origin (COO) subtype of DLBCL being germinal-center B-cell-like (GCB) DLBCL as defined by a Linear Predictor Score (LPS) <1141 (strong-GCB DLBCL); and/or
   (iv) a *BCL2*-translocated DLBCL patient and/or a DLBCL patient with high *BCL2* expression wherein ≥50% of the tumor cells express *BCL2*.

2. The antibody for use according to claim 1, wherein said clinical outcome is progression free survival (PFS), overall survival (OS) and/or event free survival

(EFS).

3. The antibody for use according to claim 1 or 2, wherein said patient is:
   (i) a patient as defined in claim 1(i); and
   (ii) a patient as defined in claim 1(ii).

4. The antibody for use according to any one of claims 1 to 3, in particular according to claim 3(i), wherein said patient is
   (i) a patient as defined in claim 1(iii); and
   (ii) a patient as defined in claim 1(iv).

5. The antibody for use according to any one of claims 1 to 4, in particular according to claim 1(iii) or 4(i), wherein said patient is identified by determining the expression of a set of genes comprising one or more (preferably all) of the genes *TNFRSF13B*, *LIMD1*, *IRF4*, *CREB3L2*, *PIM2*, *CYB5R2*, *RAB7L1*, and *CCDC50*; and *MME*, *SERPINA9*, *ASB13*, *MAML3*, *ITPKB*, *MYBL1*, and *S1PR2*.

6. The antibody for use according to claim 4(i) or 5, wherein said patient with strong-GCB DLBCL is a patient having a tumor with a weighted expression of a set of genes comprising one or more (preferably all) of the genes *TNFRSF13B*, *LIMD1*, *IRF4*, *CREB3L2*, *PIM2*, *CYB5R2*, *RAB7L1*, and *CCDC50*; and *MME*, *SERPINA9*, *ASB13*, *MAML3*, *ITPKB*, *MYBL1*, and *S1PR2* resulting in a Linear Predictor Score (LPS) < 1141.

7. The antibody for use according to claim 5 or 6, wherein said set of genes further comprises one or more (preferably all) of the genes *R3HDM1*, *WDR55*, *ISY1*, *UBXN4*, and *TRIM56*.

8. The antibody for use according to any one of claims 5 to 7, wherein the expression of said one or more genes as defined in claims 5 or 6 is normalized to the expression of one or more (preferably all) genes as defined in claim 7.

9. The antibody for use according to any one of claims 1 to 8, wherein said LPS is the weighted sum of the expression of (said) genes calculated according to the following formula (formula I):

$$LPS(X) = \sum_j a_j X_j,$$

wherein X_j is the gene expression for gene j and a_j is the coefficient for gene j.

10. The antibody for use according to any one of claims 1 to 9, wherein the expression of (said) genes is determined by the NanoString Research Use Only Lymphoma Subtyping Test (LST) (NanoString Technologies, Inc., Seattle, WA, USA).

11. The antibody for use according to any one of claims 1 to 10, wherein said LPS is ≤ 1100.

12 The antibody for use according to any one of claims 1 to 11, wherein said LPS is ≤ 749.

13. The antibody for use according to any one of claim 1 to 12, wherein said LPS is ≤ 725.

14. The antibody for use according to any one of claims 1 to 13, wherein said antibody comprises a glycoengineered Fc region.

15. The antibody for use according to claim 14, wherein said antibody has an increase in the fraction of non-fucosylated oligosaccharides attached to said glycoengineered Fc region.

16. The antibody for use according to claim 14 or 15, wherein said antibody has an increase in the fraction of bisected, non-fucosylated oligosaccharides attached to said glycoengineered Fc region.

17. The antibody for use according to any one of claims 1 to 16, in particular according to claim 14, wherein said antibody has significantly higher levels of binding to human FcγRIII receptors relative to the non-glycoengineered antibody and/or relative to rituximab.

18. The antibody for use according to any one of claims 1 to 17, in particular according to claim 14, wherein said antibody has significantly higher levels of ADCC activity relative to the non-glycoengineered antibody and/or relative to rituximab.

19. The antibody for use according to any one of claims 1 to 18, wherein said

antibody is obinutuzumab (Gazyva™/Gazyvaro™; *GA101*).

20. The antibody for use according to any one of claims 1 to 19, wherein one or more additional other cytotoxic or chemotherapeutic agent(s) or ionizing radiation enhancing the effects of such agent(s), is (are) to be administered.

21. The antibody for use according to any one of claims 1 to 20, wherein said antibody is to be administered in combination with a CHOP chemotherapy.

22. The antibody for use according to any one of claims 1 to 21, wherein said antibody is comprised in a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

23. Method for identifying a DLBCL patient (patent with/suffering from DLBCL) which responds to a treatment with obinutuzumab by reaching an improved clinical outcome as compared to a treatment with rituximab,
said method comprising (the step of) determining (e.g. by using a (tumor) sample of a patient) whether a patient is a patient as defined in any one of claims 1 to 13.

24. Method for diagnosing in a patient a form of DLBCL which can be treated with obinutuzumab so that an improved clinical outcome as compared to a treatment with rituximab is reached,
said method comprising (the steps of) determining (e.g. by using a (tumor) sample of a patient) whether a patient is a patient as defined in any one of claims 1 to 13, and diagnosing said form of DLBCL if the patient is a patient as defined in any one of claims 1 to 13.

25. The antibody for use according to any one of claims 1 to 22, wherein (i) it has been determined (e.g. by using a (tumor) sample of a patient) whether said patient is a patient as defined in any one of claims 1 to 13, wherein (ii) said patient has been identified according to the method of claim 23, or wherein (iii) a form of DLBCL has been diagnosed in said patient according to the method of claim 24.

26. The antibody for use according to any one of claims 1 to 22 and 25, wherein said treatment comprises the step of (i) determining (e.g. by using a (tumor) sample of a patient) whether the patient to be treated is a patient as defined in any one of claims 1 to 13, (ii) identifying a DLBCL patient according to the

method of claim 23, or (iii) diagnosing in the patient a form of DLBCL according to the method of claim 24.

27. A method for treating DLBCL in a patient in need thereof, wherein said patient is a patient as defined in any one of claims 1 to 13, the method comprising the steps of
determining (e.g. by using a (tumor) sample of a patient) whether the patient is a patient as defined in any one of claims 1 to 13, and
administering a pharmaceutically effective amount of an antibody as defined in any one of claims 1 and 14 to 22 to said patient.

Figure 1

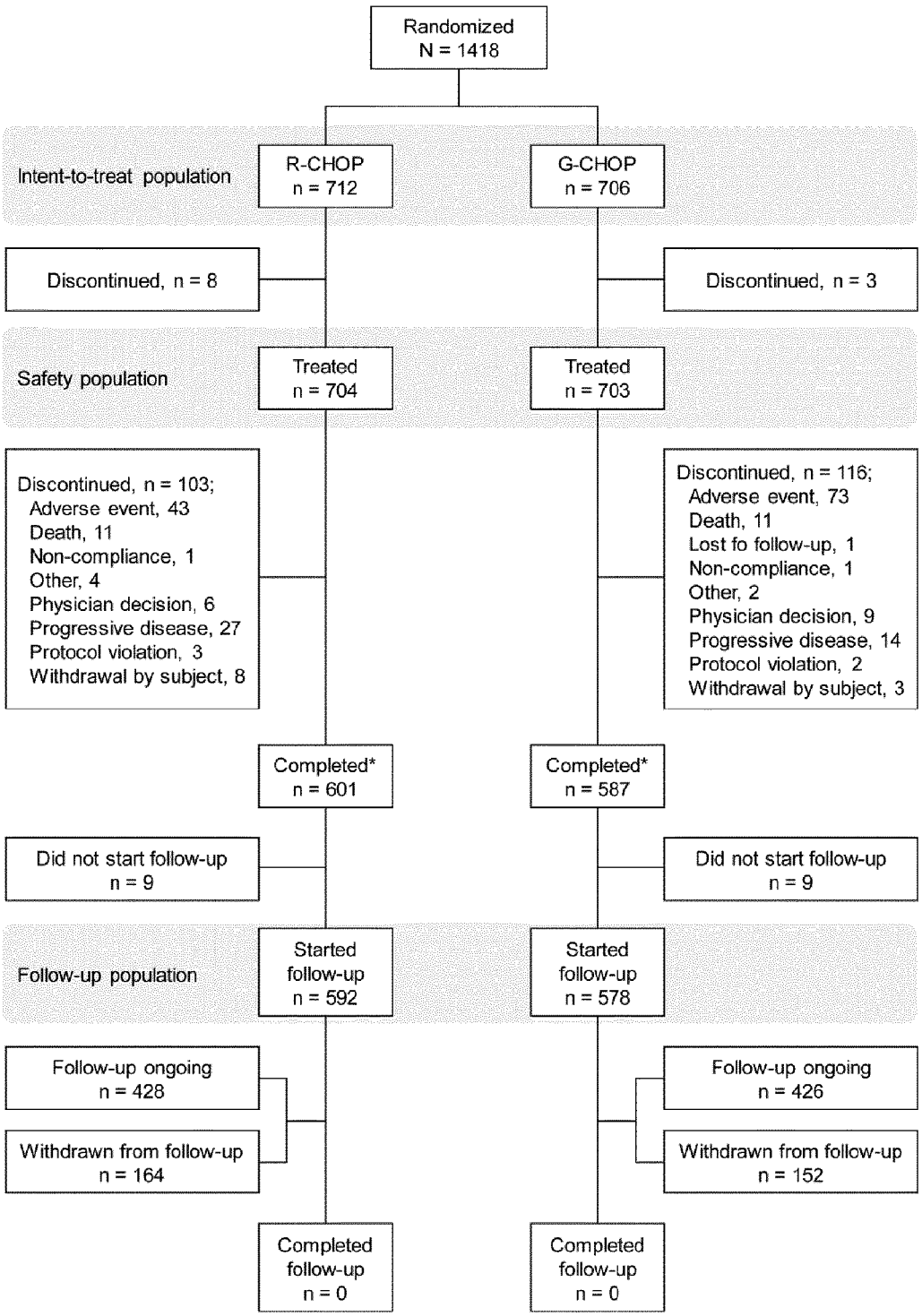
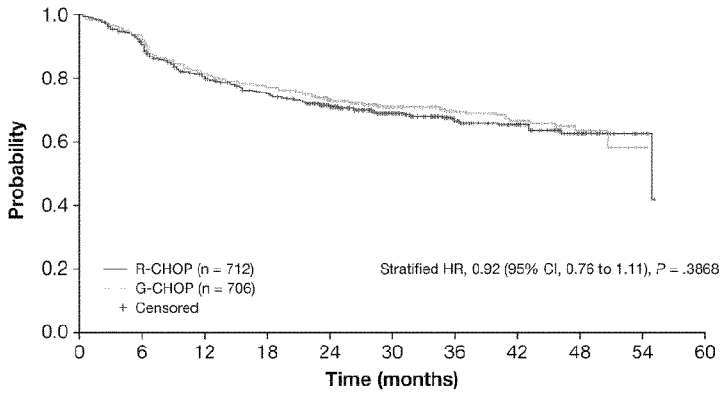

Randomized
N = 1418
Intent-to-treat population
R-CHOP
n = 712
G-CHOP
n = 706
Discontinued, n = 8
Discontinued, n = 3
Safety population
Treated
n = 704
Treated
n = 703
Discontinued, n = 103;
Adverse event, 43
Death, 11
Non-compliance, 1
Other, 4
Physician decision, 6
Progressive disease, 27
Protocol violation, 3
Withdrawal by subject, 8
Discontinued, n = 116;
Adverse event, 73
Death, 11
Lost fo follow-up, 1
Non-compliance, 1
Other, 2
Physician decision, 9
Progressive disease, 14
Protocol violation, 2
Withdrawal by subject, 3
Completed*
n = 601
Completed*
n = 587
Did not start follow-up
n = 9
Did not start follow-up
n = 9
Follow-up population
Started
follow-up
n = 592
Started
follow-up
n = 578
Follow-up ongoing
n = 428
Follow-up ongoing
n = 426
Withdrawn from follow-up
n = 164
Withdrawn from follow-up
n = 152
Completed
follow-up
n = 0
Completed
follow-up
n = 0

Figure 2

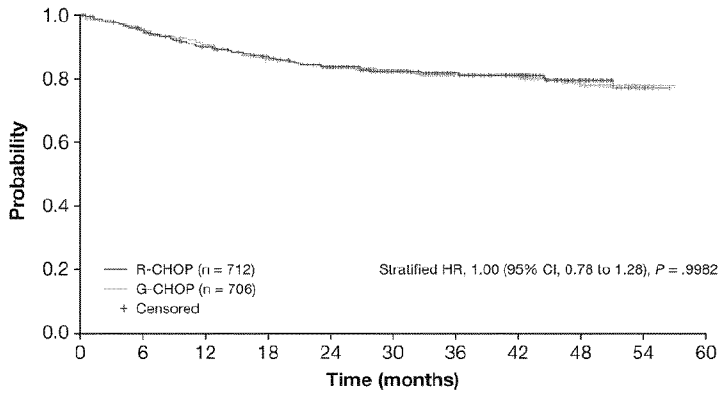
A



No. at risk:

R-CHOP	712	616	527	488	413	227	142	96	41	6
G-CHOP	706	622	540	502	425	240	158	102	39	2

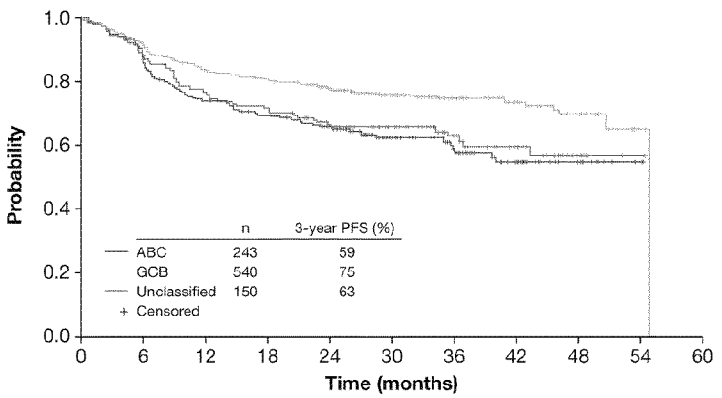
B



No. at risk:

R-CHOP	712	663	617	586	540	319	190	138	71	9
G-CHOP	706	659	616	582	552	316	201	138	67	8

C



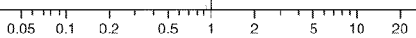
No. at risk

ABC	243	209	174	161	144	78	52	32	13	2
GCB	540	480	417	398	344	207	139	96	41	3
Unclassified	150	128	111	103	86	64	42	25	9	1

Figure 3

A

Stratification factors	Total N	R-CHOP (n = 712) n	Events	1-year KM rate	G-CHOP (n = 706) n	Events	1-year KM rate	HR	95% Wald CI
All patients	1,418	712	215	79.778	706	201	81.614	0.92	0.76 to 1.12
Number of planned CHOP cycles									
6	1,049	526	152	80.789	523	142	83.449	0.91	0.73 to 1.15
8	369	186	63	76.949	183	59	76.480	0.96	0.6 to 1.37
IPI									
Low-intermediate	785	409	99	84.168	376	86	87.510	0.93	0.70 to 1.24
High-intermediate	413	192	71	75.013	221	62	81.669	0.68	0.48 to 0.95
High	220	111	45	71.577	109	53	61.460	1.27	0.85 to 1.90
Geographic region									
Eastern Europe	196	99	28	77.354	97	32	80.726	1.10	0.66 to 1.82
Western Europe	426	215	61	79.653	211	51	85.429	0.79	0.54 to 1.14
North America	216	107	27	87.556	109	25	83.972	1.00	0.58 to 1.73
Asia	518	258	87	77.830	260	87	76.484	1.01	0.75 to 1.36
Other	62	33	12	77.047	29	6	93.103	0.46	0.17 to 1.22



B

Baseline factors	Total N	R-CHOP (n = 712) n	Events	1-year KM rate	G-CHOP (n = 706) n	Events	1-year KM rate	HR	95% Wald CI
All patients	1,418	712	215	79.778	706	201	81.614	0.92	0.76 to 1.12
Sex									
Female	666	329	100	78.126	337	83	85.406	0.75	0.56 to 1.00
Male	752	383	115	81.180	369	118	78.124	1.11	0.86 to 1.43
Age group									
<65	850	432	131	78.198	418	117	82.351	0.88	0.68 to 1.13
≥65	568	280	84	82.192	288	84	80.528	0.99	0.73 to 1.34
Race									
Asian	522	258	87	77.830	264	88	76.457	1.00	0.74 to 1.35
White	856	430	121	80.575	426	108	84.355	0.87	0.67 to 1.12
Other	40	24	7	86.542	16	5	93.333	1.10	0.34 to 3.49
ECOG									
0–1	1,231	613	180	81.540	618	161	83.774	0.86	0.69 to 1.06
2–3	186	99	35	68.590	87	40	65.750	1.41	0.89 to 2.23
Ann Arbor stage									
I	103	48	8	86.709	55	10	90.338	1.01	0.40 to 2.58
II	238	123	22	87.644	115	25	86.699	1.27	0.71 to 2.25
III	466	255	82	79.663	211	52	85.871	0.71	0.50 to 1.01
IV	610	285	103	75.197	325	114	75.572	0.96	0.73 to 1.25
Bulky disease									
Yes	523	262	94	71.548	261	79	78.333	0.78	0.58 to 1.05
No	890	448	120	84.771	442	122	83.481	1.06	0.82 to 1.36
Extranodal involvement*									
Yes	952	468	147	77.956	484	154	78.661	1.00	0.80 to 1.25
No	466	244	68	83.235	222	47	88.127	0.73	0.50 to 1.06
No of extranodal sites									
0	14	8	2	75.000	6	1	100.000	0.58	0.05 to 6.40
1	437	221	58	81.552	216	54	87.211	0.94	0.65 to 1.36
>1	503	240	87	74.793	263	99	71.520	1.02	0.77 to 1.36
Cell-of-origin									
GCB	540	269	74	81.022	271	55	86.062	0.72	0.5 to 1.01
ABC	243	118	47	73.714	125	45	74.634	0.86	0.57 to 1.29
Unclassified	150	75	27	73.973	75	27	80.572	1.02	0.60 to 1.75
Missing	485	250	627	83.214	235	74	80.634	1.18	0.85 to 1.64

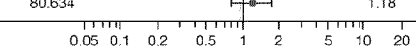


Figure 4

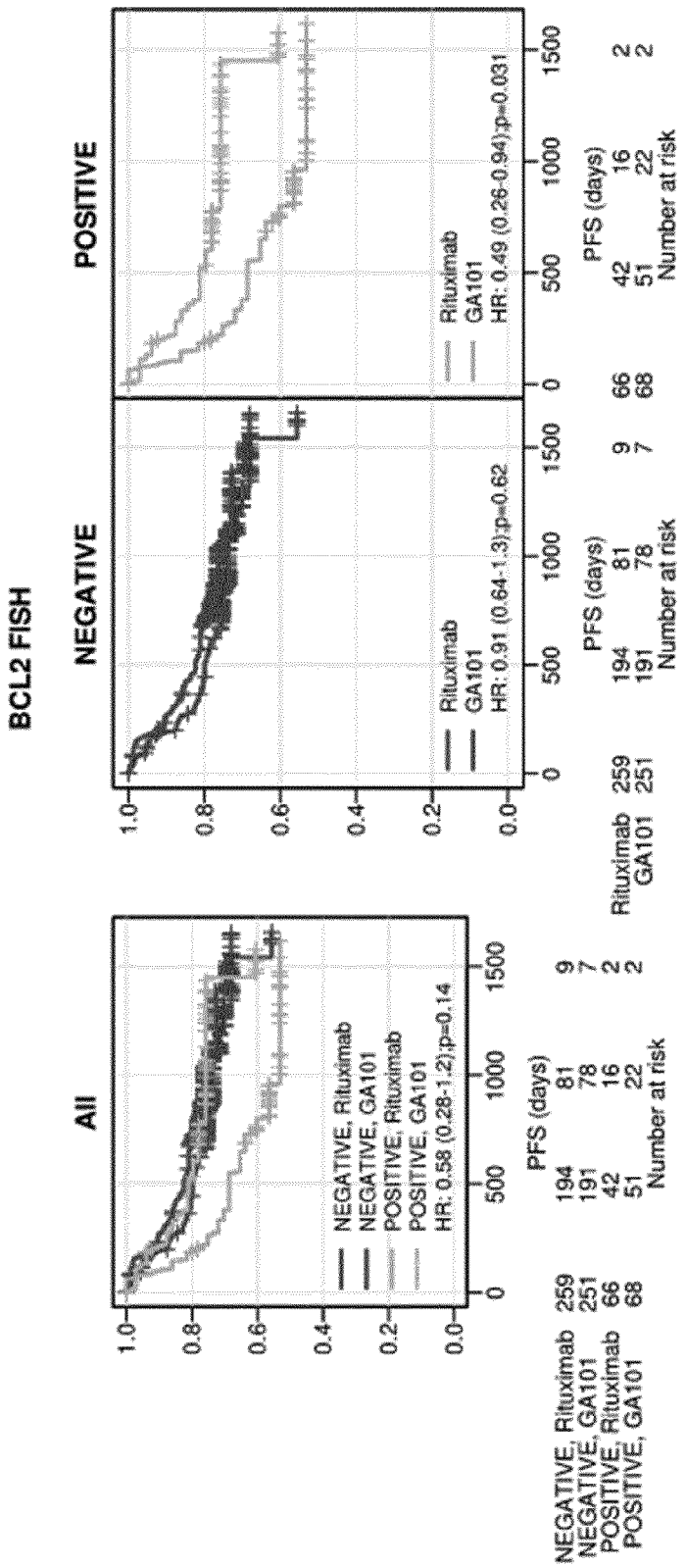

BCL2 FISH
All
NEGATIVE
POSITIVE
NEGATIVE, Rituximab
NEGATIVE, GA101
POSITIVE, Rituximab
POSITIVE, GA101
HR: 0.58 (0.28-1.2);p=0.14
Rituximab
GA101
HR: 0.91 (0.64-1.3);p=0.62
HR: 0.49 (0.26-0.94);p=0.031
PFS (days)
Number at risk
NEGATIVE, Rituximab 259 194 81 9
NEGATIVE, GA101 251 191 78 7
POSITIVE, Rituximab 66 42 16 2
POSITIVE, GA101 68 51 22 2

Figure 5

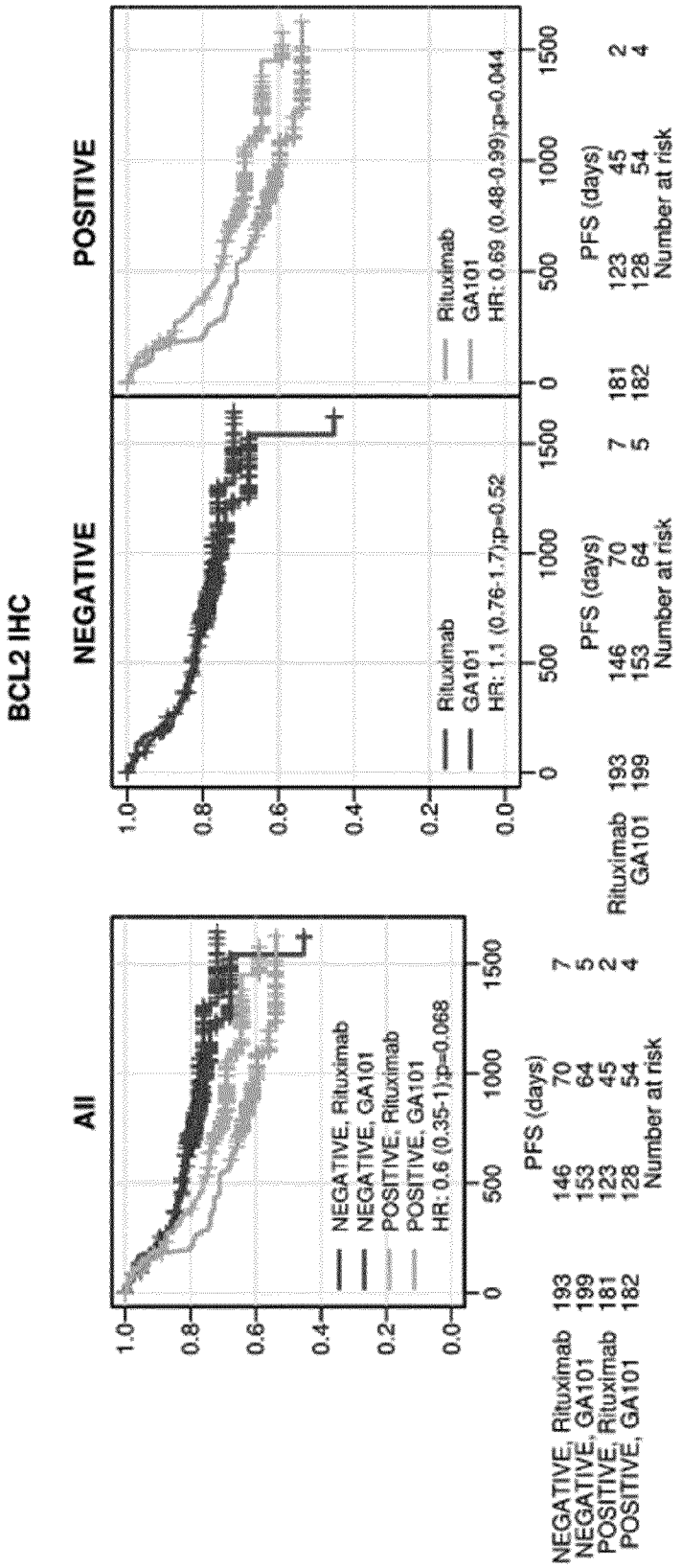

BCL2 IHC
All
NEGATIVE
POSITIVE
NEGATIVE, Rituximab
NEGATIVE, GA101
POSITIVE, Rituximab
POSITIVE, GA101
HR: 0.6 (0.35-1);p=0.068
HR: 1.1 (0.76-1.7);p=0.52
HR: 0.69 (0.48-0.99);p=0.044
Rituximab
GA101
PFS (days)
Number at risk
NEGATIVE, Rituximab 193 146 70 7
NEGATIVE, GA101 199 153 64 5
POSITIVE, Rituximab 181 123 45 2
POSITIVE, GA101 182 128 54 4

Figure 6

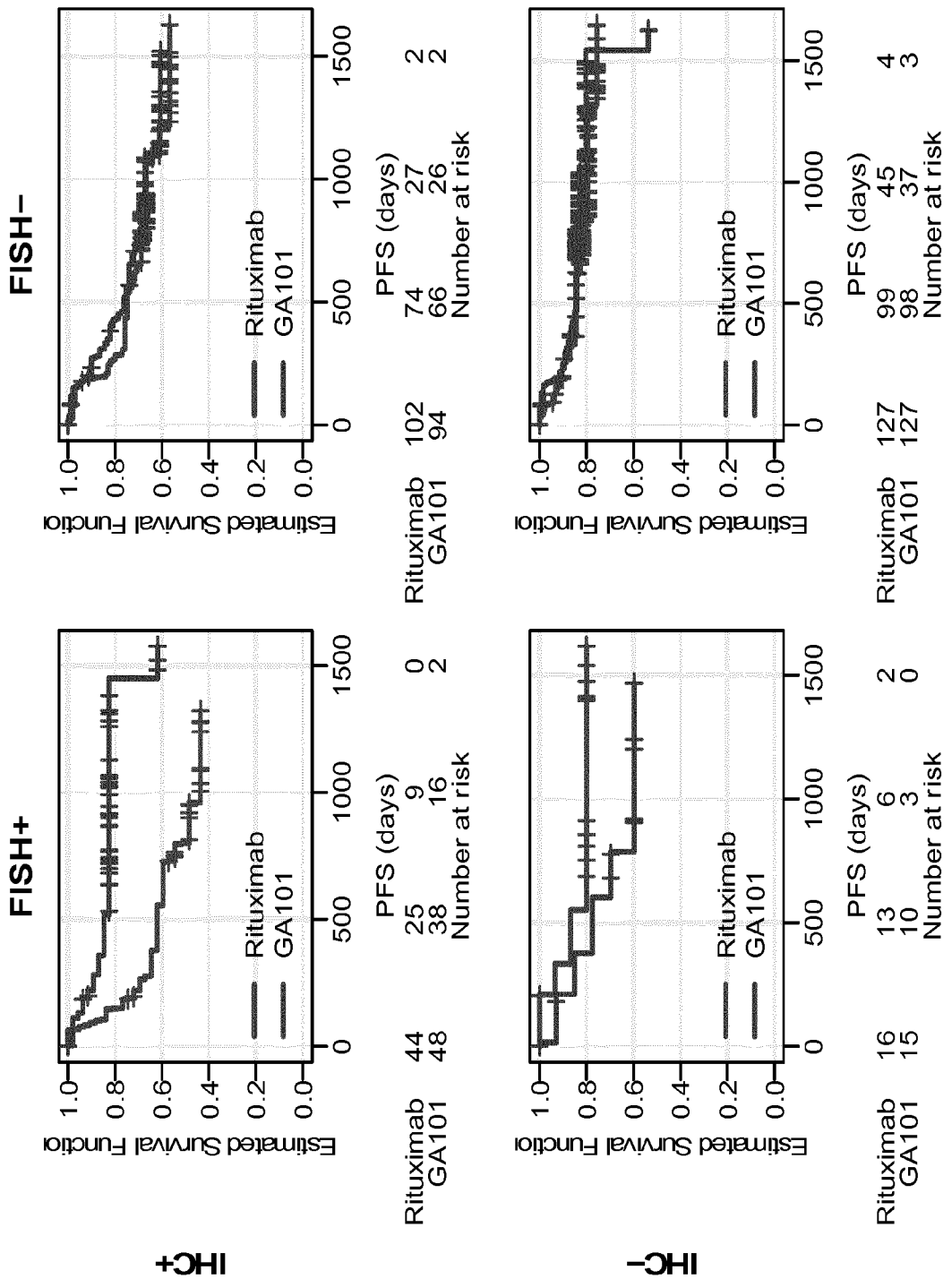

IHC+
IHC−
FISH+
FISH−
Estimated Survival Function
1.0
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
PFS (days)
Rituximab
GA101
Rituximab 44 25 9 0
GA101 48 38 16 2
Number at risk
Rituximab 16 13 6 2
GA101 15 10 3 0
Number at risk
Rituximab 102 74 27 2
GA101 94 66 26 2
Number at risk
Rituximab 127 99 45 4
GA101 127 98 37 3
Number at risk

Figure 7A

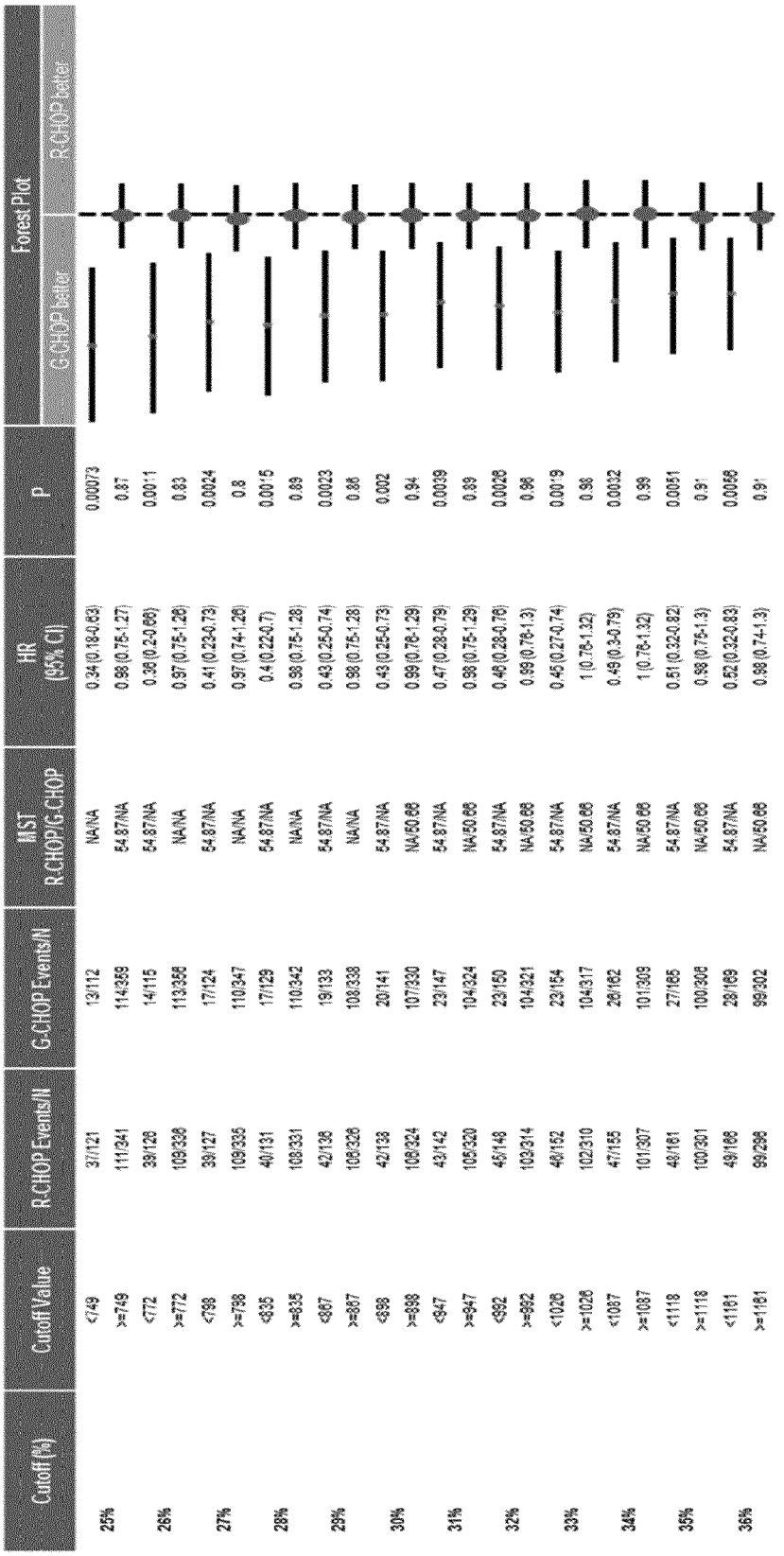

FOREST Plot: Association of treatment effect with survival outcome PFS within LPS (Linear Predictor Score) subgroups
Cutoff (%)
Cutoff Value
R-CHOP Events/N
G-CHOP Events/N
IHST R-CHOP/G-CHOP
HR (95% CI)
p
Forest Plot
G-CHOP better
R-CHOP better
25% <749 31/121 13/112 NA/NA 0.34 (0.18-0.63) 0.00073
>=749 111/341 114/359 54.87/NA 0.88 (0.75-1.27) 0.87
26% <772 39/128 14/115 54.87/NA 0.36 (0.2-0.66) 0.0011
>=772 109/338 113/356 NA/NA 0.97 (0.75-1.26) 0.83
27% <788 39/127 17/124 54.87/NA 0.41 (0.23-0.73) 0.0024
>=788 109/335 110/347 NA/NA 0.97 (0.74-1.26) 0.8
28% <835 40/131 17/129 54.87/NA 0.4 (0.22-0.7) 0.0015
>=835 108/331 110/342 NA/NA 0.98 (0.75-1.28) 0.89
29% <867 42/138 19/133 54.87/NA 0.43 (0.25-0.74) 0.0023
>=867 106/326 108/338 NA/NA 0.98 (0.75-1.28) 0.88
30% <898 42/138 20/141 54.87/NA 0.43 (0.25-0.73) 0.002
>=898 106/324 107/330 NA/50.66 0.99 (0.76-1.29) 0.94
31% <947 43/142 23/147 54.87/NA 0.47 (0.28-0.79) 0.0039
>=947 105/320 104/324 NA/50.66 0.98 (0.75-1.29) 0.89
32% <992 45/148 23/150 54.87/NA 0.45 (0.28-0.76) 0.0028
>=992 103/314 104/321 NA/50.66 0.99 (0.76-1.3) 0.96
33% <1026 46/152 23/154 54.87/NA 0.45 (0.27-0.74) 0.0019
>=1026 102/310 104/317 NA/50.66 1 (0.76-1.32) 0.96
34% <1067 47/155 26/162 54.87/NA 0.49 (0.3-0.79) 0.0032
>=1067 101/307 101/309 NA/50.66 1 (0.76-1.32) 0.99
35% <1118 48/161 27/165 54.87/NA 0.51 (0.32-0.82) 0.0051
>=1118 100/301 100/306 NA/50.66 0.98 (0.75-1.3) 0.91
36% <1161 49/166 28/169 54.87/NA 0.52 (0.32-0.83) 0.0068
>=1161 99/296 99/302 NA/50.66 0.98 (0.74-1.3) 0.91

Figure 7B

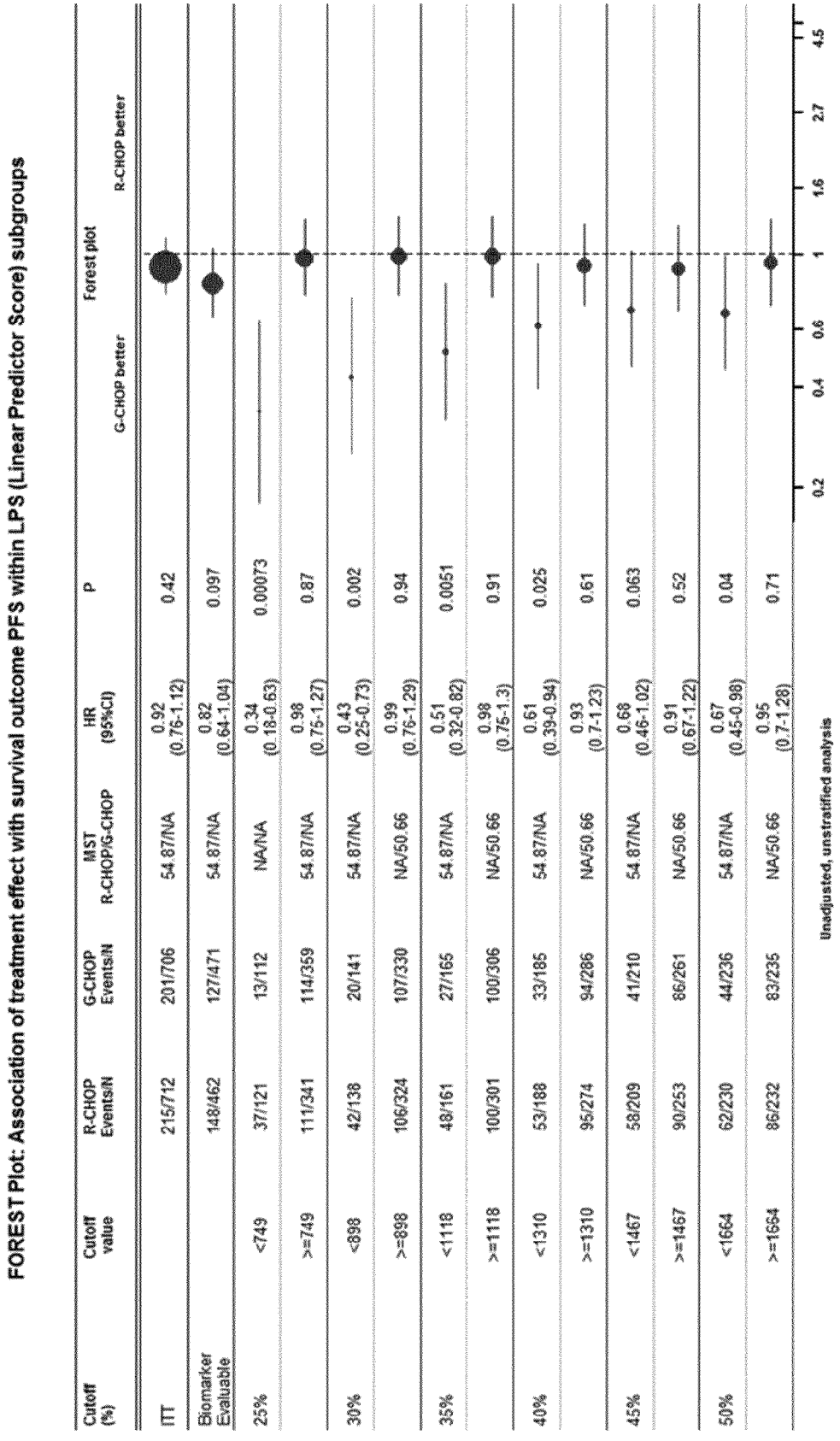

FOREST Plot: Association of treatment effect with survival outcome PFS within LPS (Linear Predictor Score) subgroups
Cutoff (%)
Cutoff value
R-CHOP Events/N
G-CHOP Events/N
MST R-CHOP/G-CHOP
HR (95%CI)
P
Forest plot
G-CHOP better
R-CHOP better
ITT
215/712
201/706
54.87/NA
0.92 (0.76-1.12)
0.42
Biomarker Evaluable
148/462
127/471
54.87/NA
0.82 (0.64-1.04)
0.097
25%
<749
37/121
13/112
NA/NA
0.34 (0.18-0.63)
0.00073
>=749
111/341
114/359
54.87/NA
0.98 (0.75-1.27)
0.87
30%
<898
42/138
20/141
54.87/NA
0.43 (0.25-0.73)
0.002
>=898
106/324
107/330
NA/50.66
0.99 (0.76-1.29)
0.94
35%
<1118
48/161
27/165
54.87/NA
0.51 (0.32-0.82)
0.0051
>=1118
100/301
100/306
NA/50.66
0.98 (0.75-1.3)
0.91
40%
<1310
53/188
33/185
54.87/NA
0.61 (0.39-0.94)
0.025
>=1310
95/274
94/286
NA/50.66
0.93 (0.7-1.23)
0.61
45%
<1467
58/209
41/210
54.87/NA
0.68 (0.46-1.02)
0.063
>=1467
90/253
86/261
NA/50.66
0.91 (0.67-1.22)
0.52
50%
<1664
62/230
44/236
54.87/NA
0.67 (0.45-0.98)
0.04
>=1664
86/232
83/235
NA/50.66
0.95 (0.7-1.28)
0.71
0.2
0.4
0.6
1
1.6
2.7
4.5
Unadjusted, unstratified analysis

Figure 7C

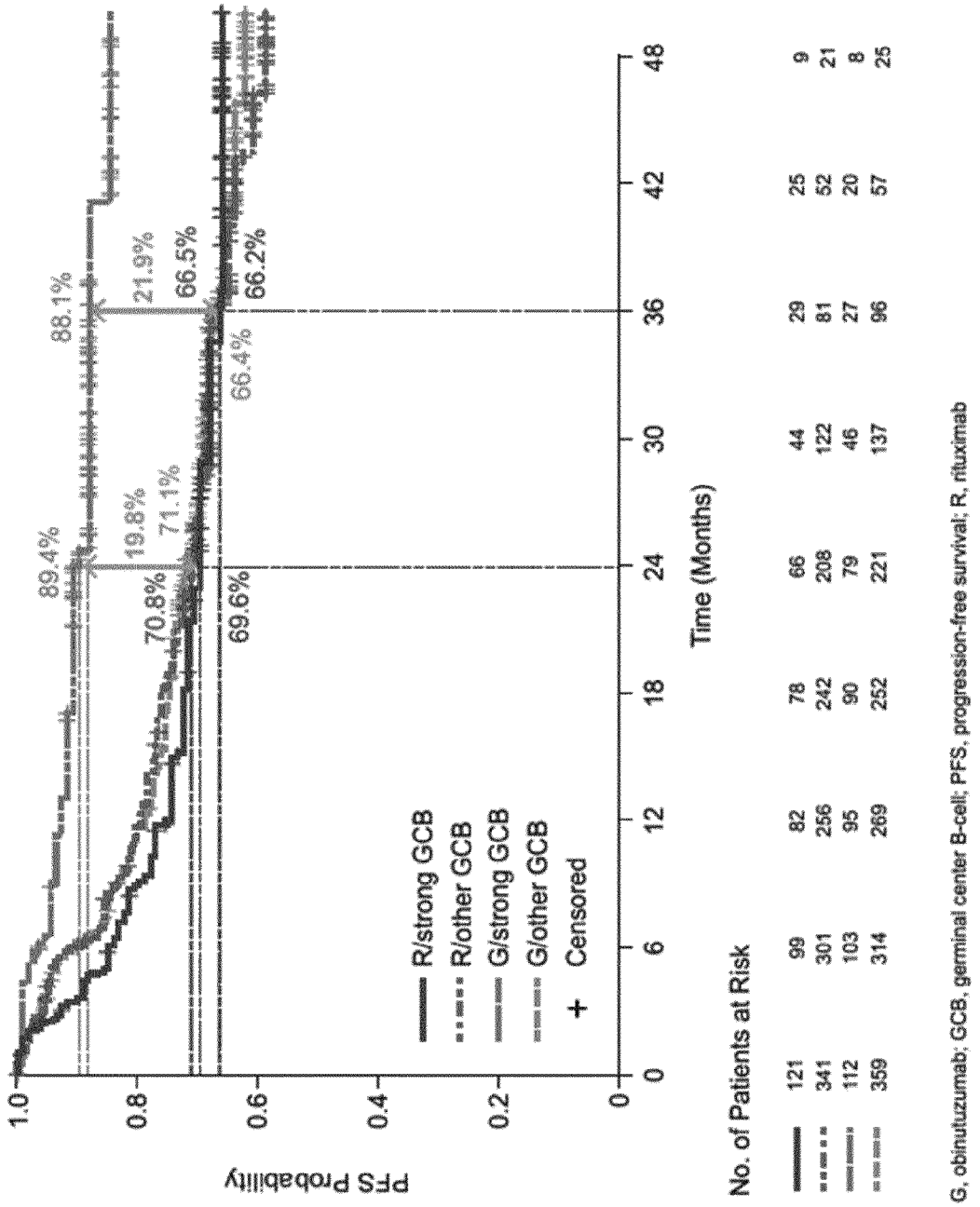

PFS Probability
1.0
0.8
0.6
0.4
0.2
0
0
6
12
18
24
30
36
42
48
Time (Months)
R/strong GCB
R/other GCB
G/strong GCB
G/other GCB
Censored
89.4%
88.1%
70.8%
71.1%
69.6%
66.4%
66.5%
66.2%
19.8%
21.9%
No. of Patients at Risk
121 99 82 78 66 44 29 25 9
341 301 256 242 208 122 81 52 21
112 103 95 90 79 46 27 20 8
359 314 269 252 221 137 96 57 25
G, obinutuzumab; GCB, germinal center B-cell; PFS, progression-free survival; R, rituximab

Figure 8

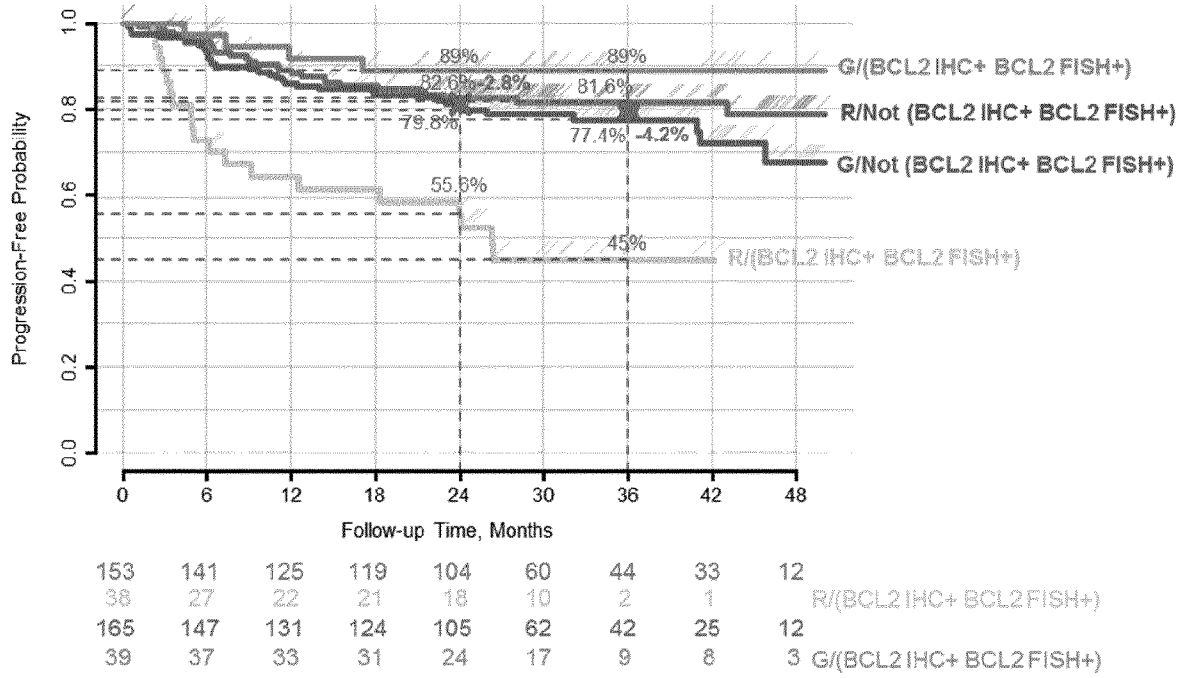
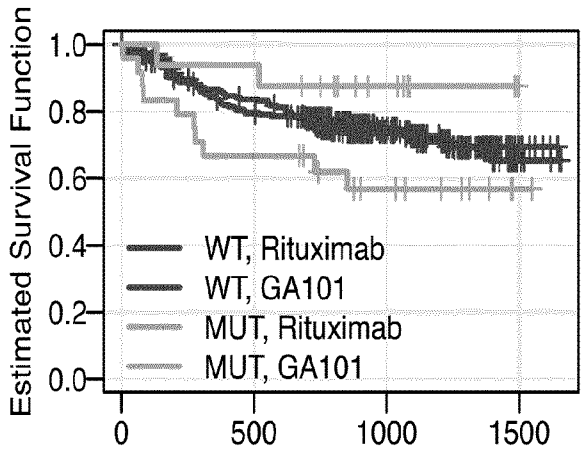


Figure 9

KM (PFS) in GOYA for CD58 mutations (any mutation type)

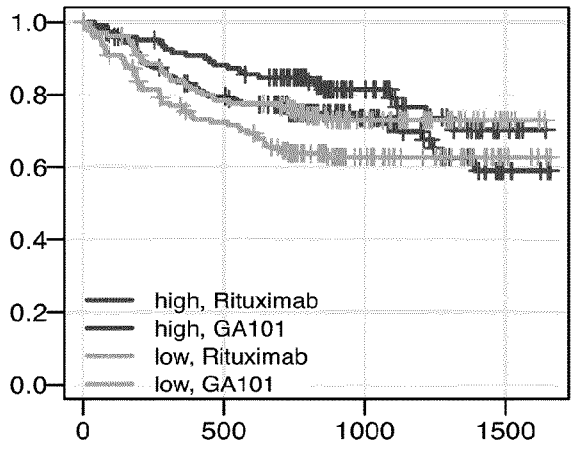


WT, Rituximab	228	172	73	8
WT, GA101	231	170	71	6
MUT, Rituximab	24	16	9	1
MUT, GA101	16	15	7	0

Number at risk

Figure 9 (continued)

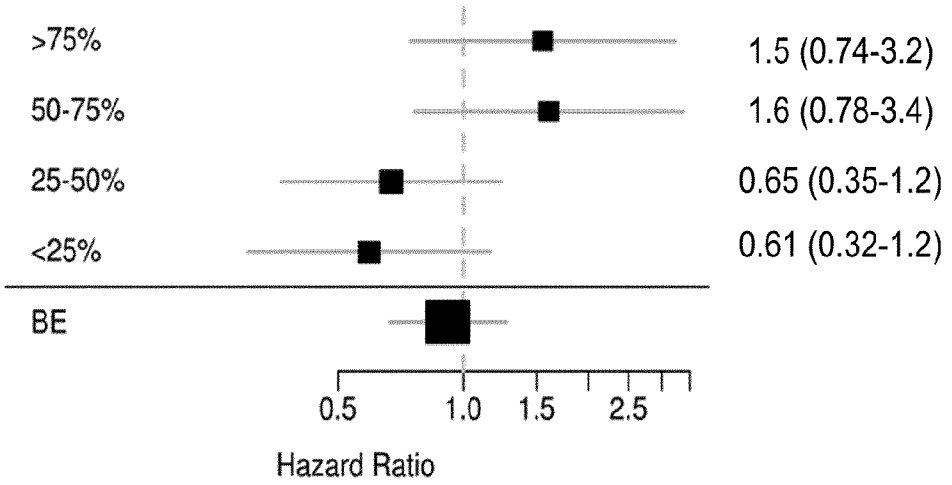
KM (PFS) for GOYA CD58 RNA Seq (median cut)



high, Rituximab	128	102	47	5
high, GA101	148	110	50	6
low, Rituximab	143	95	38	5
low, GA101	133	100	40	5

Number at risk

Forest plot evaluating treatment effect (G vs. R) in quartiles of CD58 gene expression



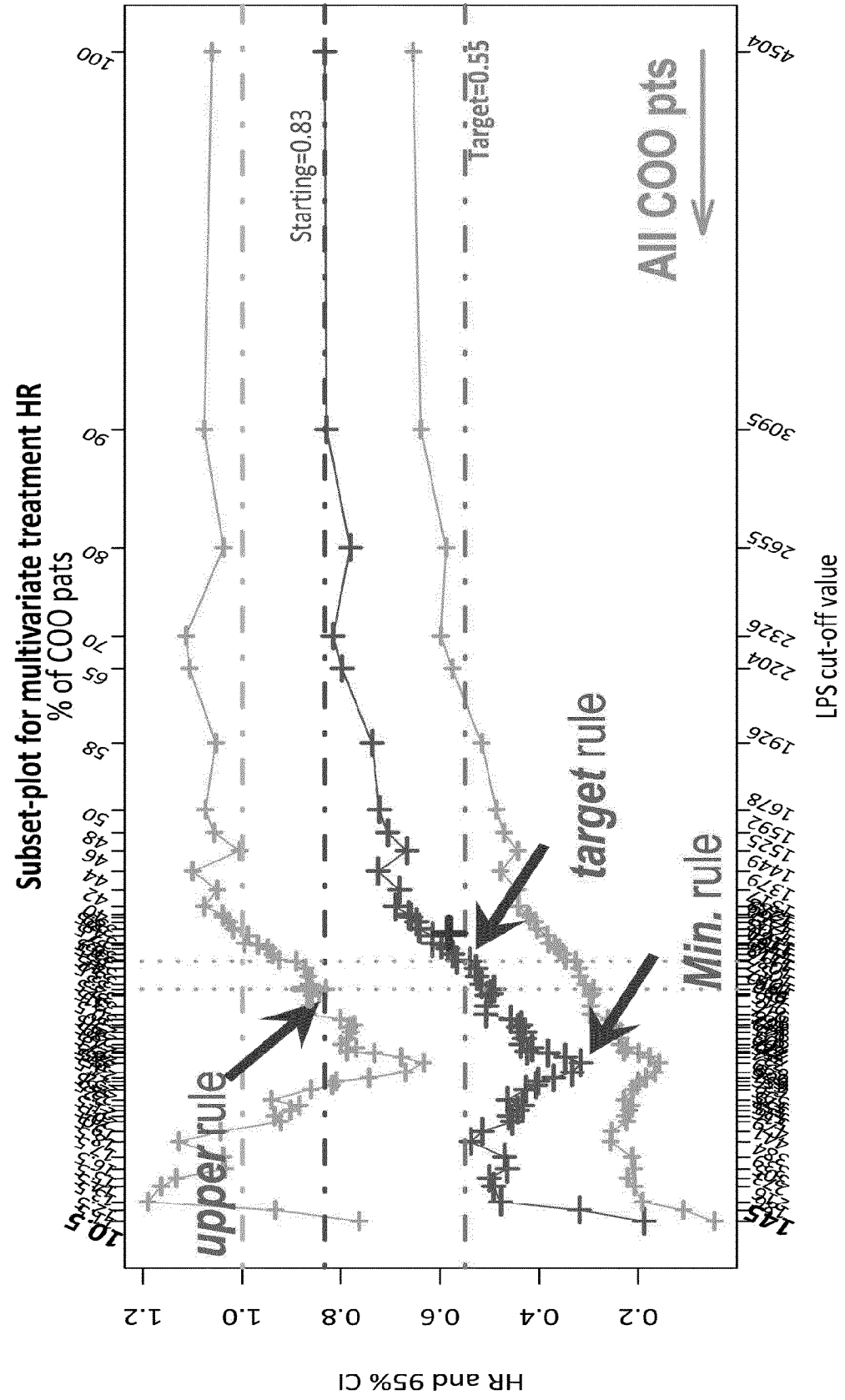
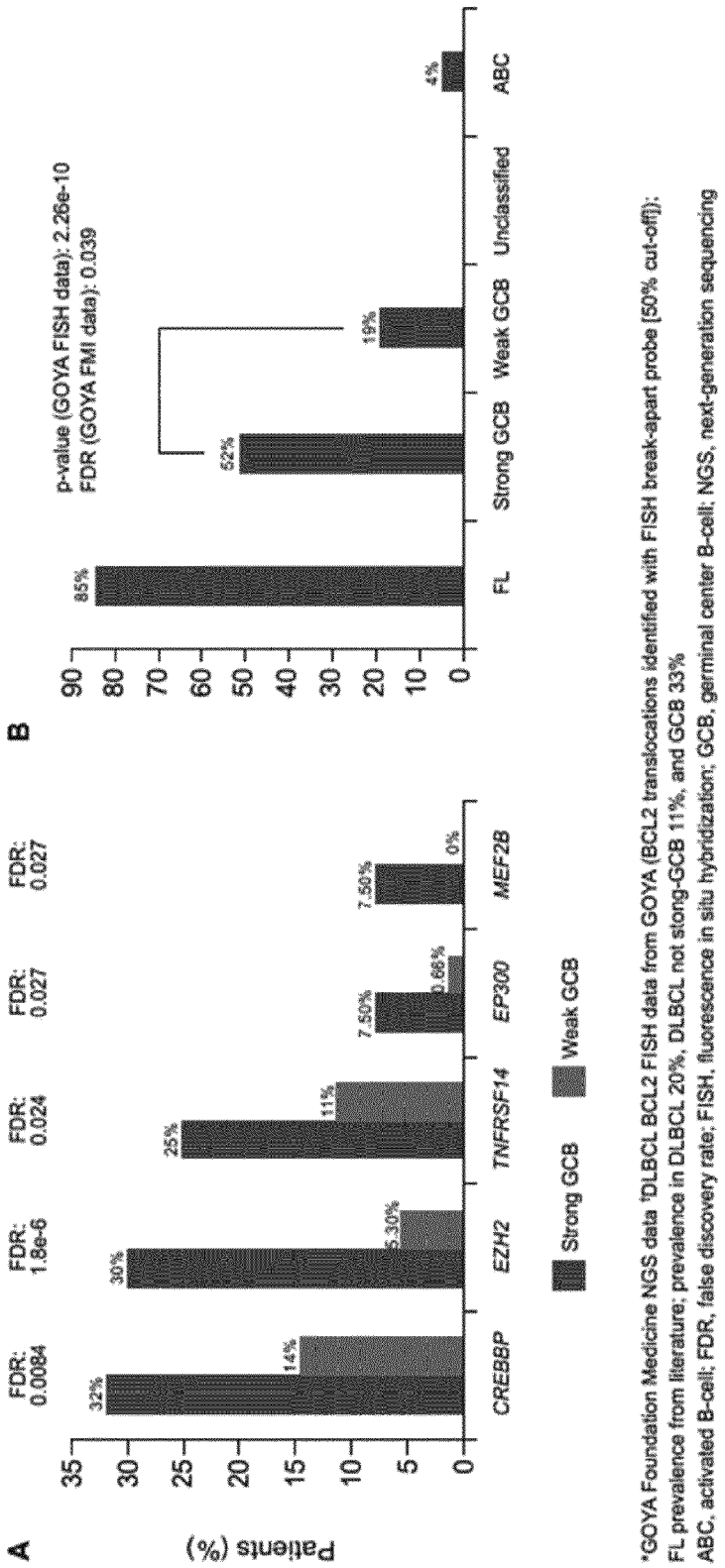

Subset-plot for multivariate treatment HR
% of COO pats
LPS cut-off value
HR and 95% CI
Starting=0.83
Target=0.55
All COO pts
upper rule
target rule
Min. rule

Figure 10

Figure 11



*GOYA Foundation Medicine NGS data †DLBCL BCL2 FISH data from GOYA (BCL2 translocations identified with FISH break-apart probe [50% cut-off]); FL prevalence from literature; prevalence in DLBCL 20%, DLBCL not stong-GCB 11%, and GCB 33%
ABC, activated B-cell; FDR, false discovery rate; FISH, fluorescence in situ hybridization; GCB, germinal center B-cell; NGS, next-generation sequencing

Figure 12

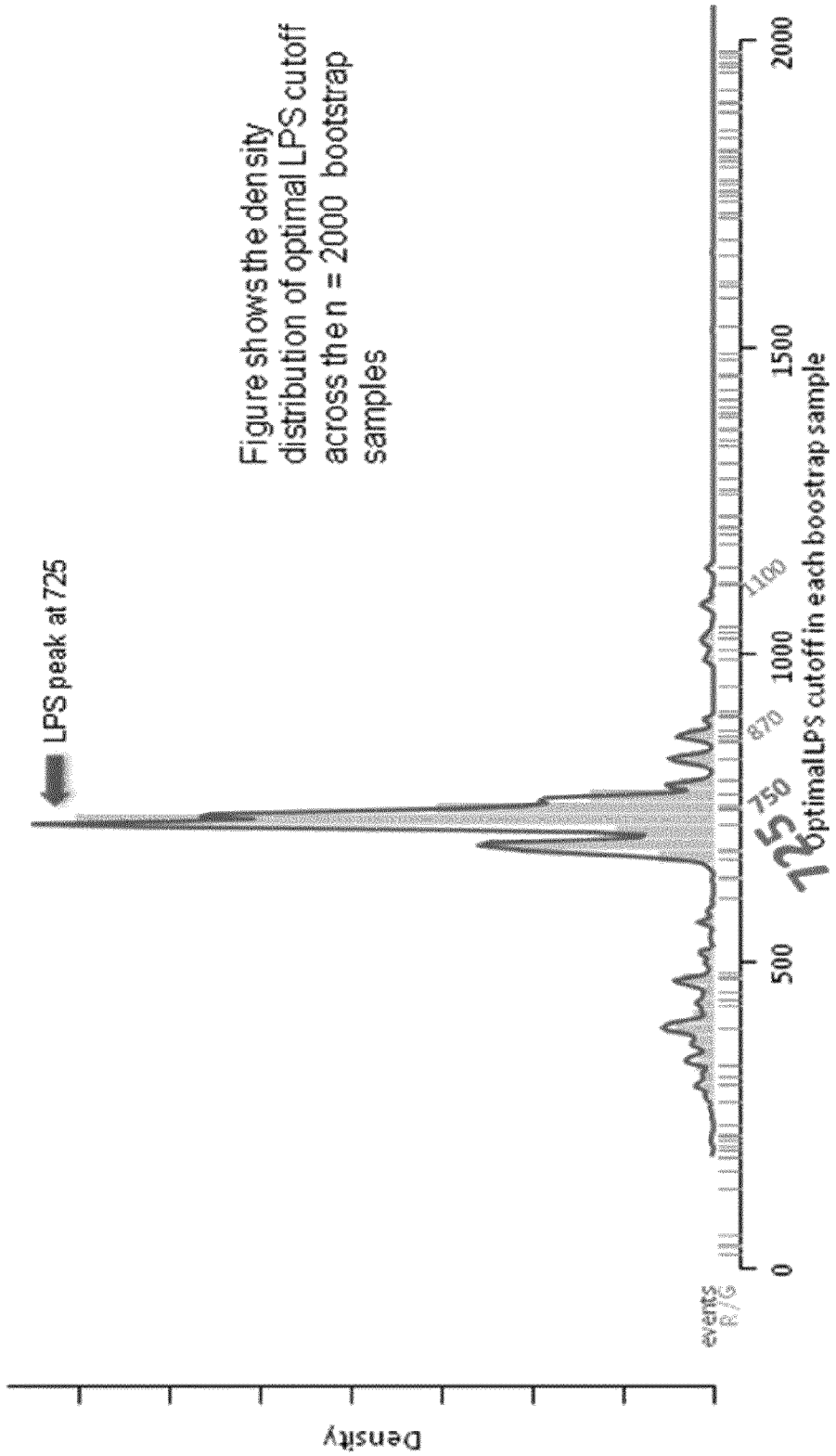
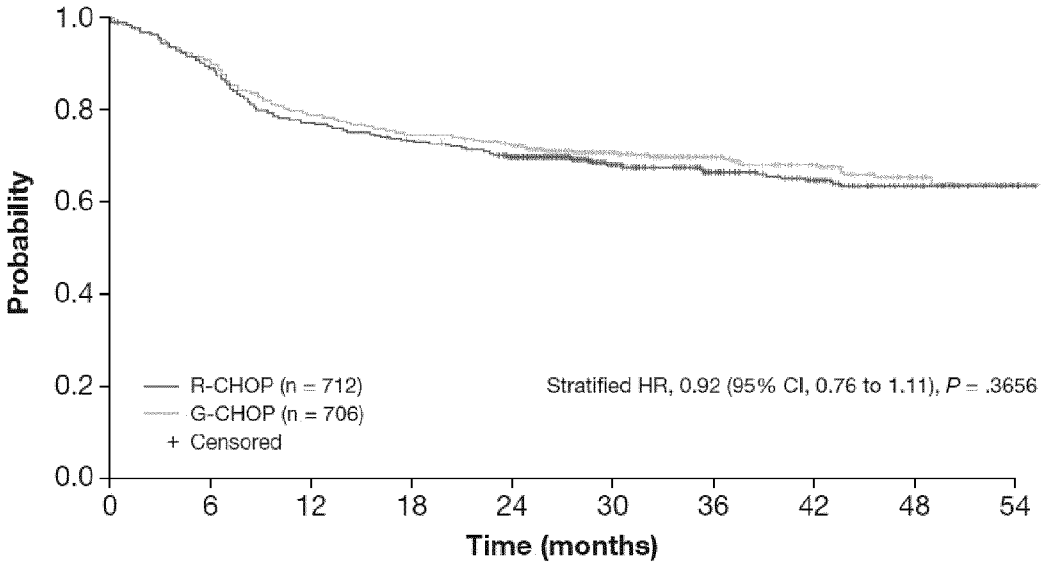


Figure shows the density distribution of optimal LPS cutoff across the n = 2000 bootstrap samples
LPS peak at 725
Density
events
0
500
750
870
1000
1100
1500
2000
Optimal LPS cutoff in each boostrap sample

Figure 13



No. at risk:

R-CHOP	712	623	538	505	460	270	166	118	57	8
G-CHOP	706	627	540	509	482	275	178	120	57	6

Figure 14 (to be continued)

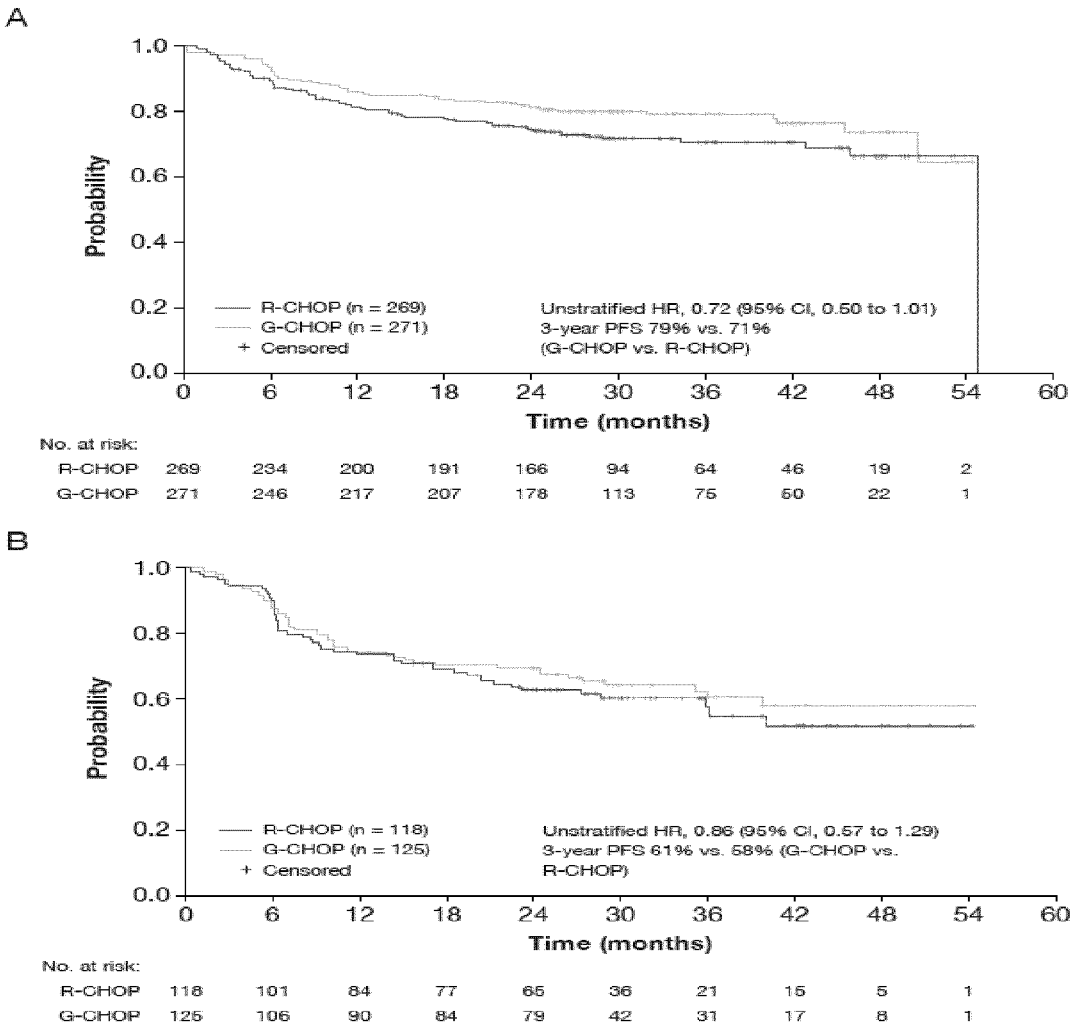
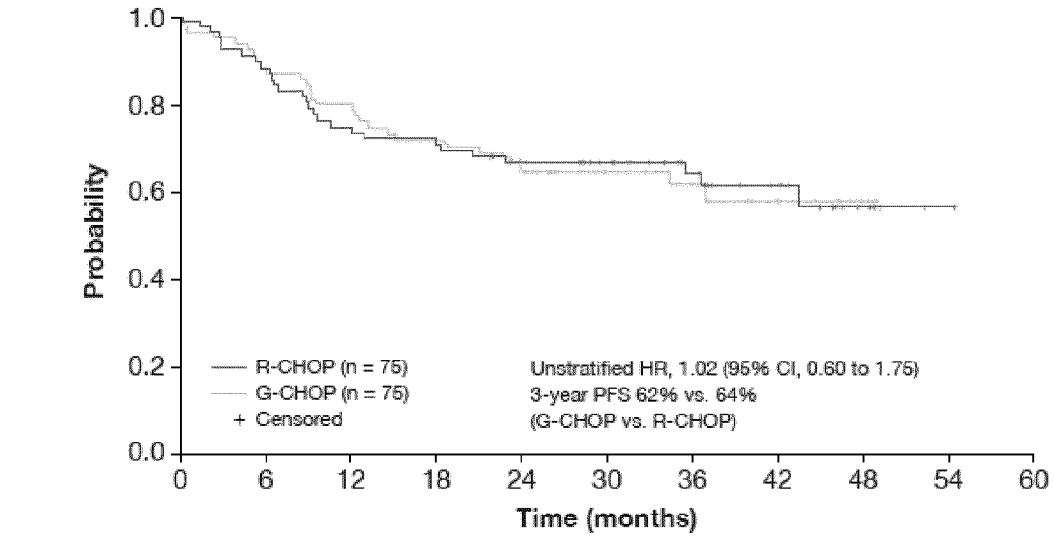


Figure 14 (continued)

C



No. at risk:

R-CHOP	75	65	54	52	43	36	25	15	6	1
G-CHOP	75	63	57	51	43	28	17	10	3	