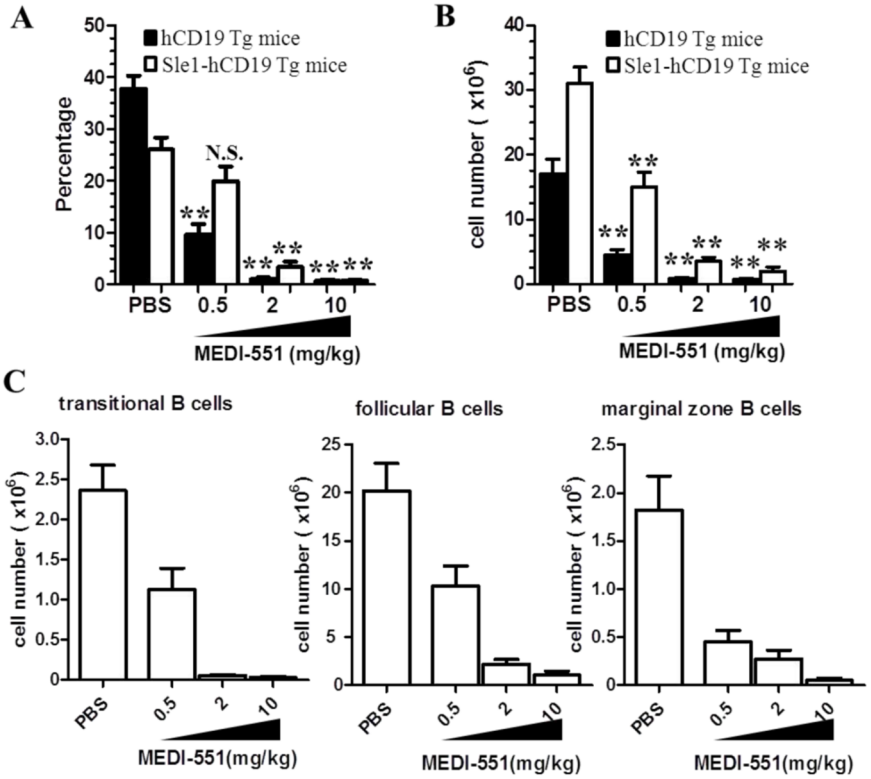

図 2.6.2.2－15　hCD19Tg マウス及び Sle1-hCD19Tg マウスにおける Inebilizumab の B 細胞減少作用

MEDI-551 = inebilizumab; PBS = phosphate-buffered saline; Tg = transgenic
Human CD19 transgenic (hCD19 Tg) mice and Sle1-hCD19Tg mice were treated with a single dose of MEDI-551 (0.5, 2, or 10 mg/kg) or PBS. B cells were detected by B220 staining in flow cytometry analysis of blood and spleen at 1 week following treatment.
A)　The percentage of B cells in the blood was significantly reduced following treatment with MEDI-551.
B)　The number of splenic B220+ B cells of Sle1-hCD19 Tg mice were almost twice that of hCD19 Tg mice: an average 30x106 per spleen and 17x106 per spleen, respectively. Following treatment with MEDI-551, the number of B cells in the spleen is significantly reduced in both mouse strains.
C)　Transitional B cells (B220+CD93+), follicular B cells (B220+ CD93-IgM+CD23+), and marginal zone B cell (B220+ CD93-IgM+CD23-) subsets of the spleen were all significantly reduced in the Sle1-hCD19 Tg mice treated with MEDI-551. All data are shown as mean +/- SEM. Data was compiled from 3 separate studies with n = 10-13 mice per group. (**p < 0.01 compared to PBS, t-test).

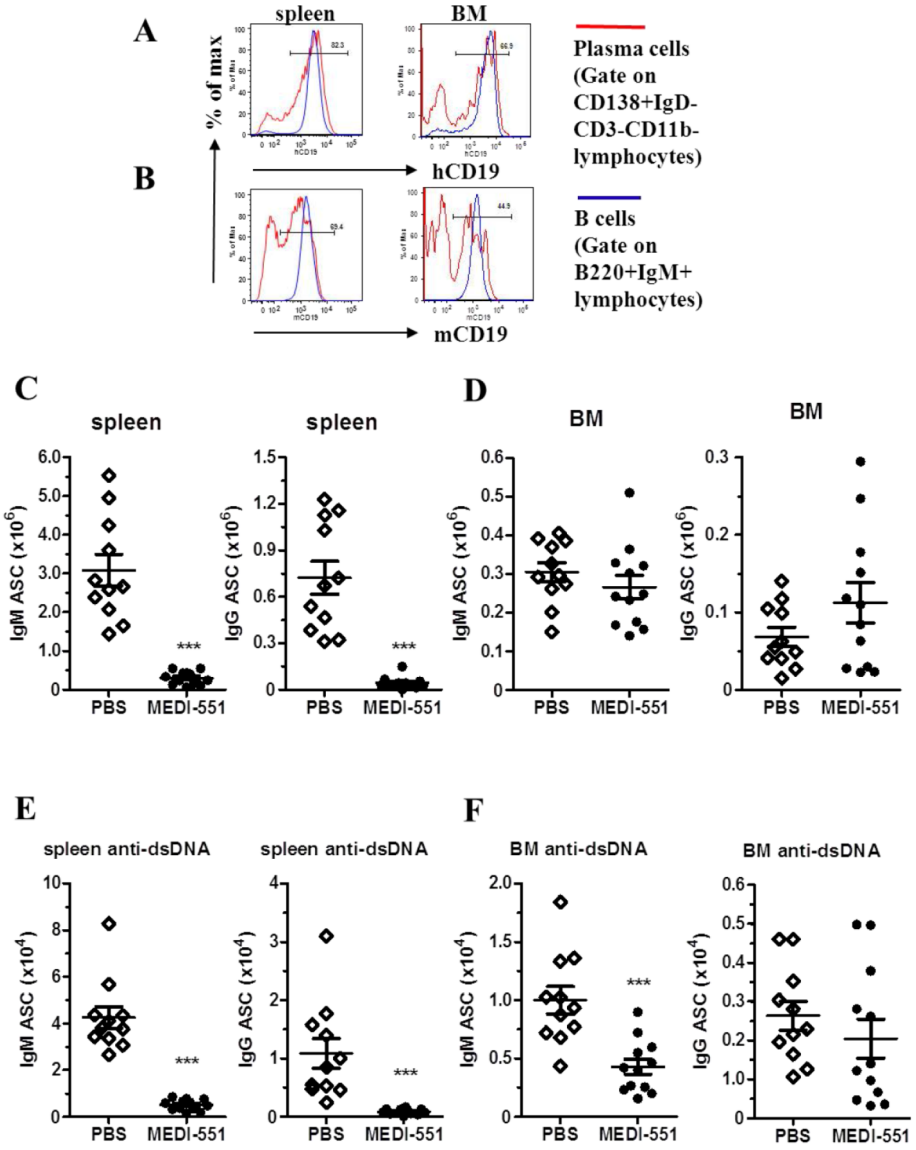

図 2.6.2.2－16　脾臓及び骨髄中の形質細胞における huCD19 発現並びに Inebilizumab による形質細胞減少作用

ASC = antibody secreting cells; BM = bone marrow; FACS = fluorescence activated cell sorting; hCD19 = human CD19; mCD19 = mouse CD19; MEDI-551 = inebilizumab; PBS = phosphate-buffered saline; SEM = standard error of the mean; Tg = transgenic

The expression of hCD19 on the plasma cells of Sle1-hCD19 Tg mice was determined by FACS analysis. Plasma cells were gated as CD138+IgD-CD3-CD11b- and the expression of hCD19 and mCD19 was determined in the spleen and bone marrow compared to total B cells (B220+).

A)　Data from one representative mouse indicates that the level of hCD19 on plasma cells in spleen and BM is 70- 80% and 45-67%, respectively.

B)　A similar level of mCD19 expression was detected on the same plasma cells.

C-F) At 12 weeks after treatment, Sle1-hCD19 Tg mice treated with 10 mg/kg MEDI-551 (followed by a maintenance dose of 400 µg/mouse intraperitoneal injection every 2 weeks) showed significant reduction in IgG and IgM ASC as detected by ELISpot in the spleen, but not in BM. Data are plotted for each animal in the PBS (open diamond) and MEDI-551 (black circle) treatment groups, mean and SEM are indicated (***p < 0.001 compared to PBS, t-test).

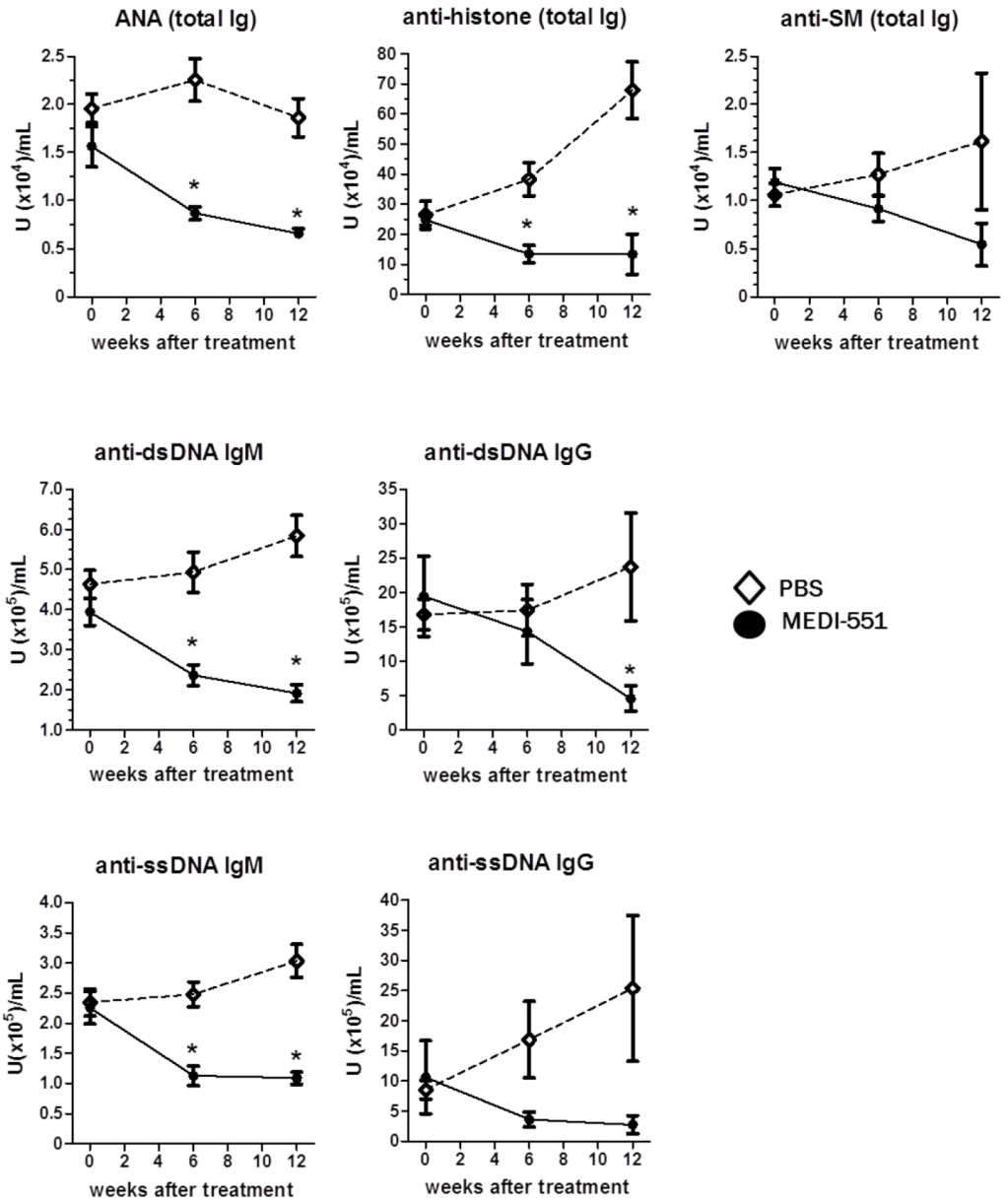

図 2.6.2.2－17　Sle1-hCD19Tg マウスにおける Inebilizumab による血清自己抗体減少作用

ANA = anti-nuclear antibodies; dsDNA = double-stranded DNA; ELISA = enzyme-linked immunosorbent assay; MEDI-551 = inebilizumab; PBS = phosphate-buffered saline; SEM = standard error of the mean; SM = Smith antigen; ssDNA = single-stranded DNA

ELISA data shown as mean +/-SEM, n = 12/group. (*$p < 0.05$, compared to PBS; t-test).

2.6.2.2.2.3　Inebilizumab による B 細胞及び形質細胞の減少と，実験的自己免疫性脳脊髄炎発症抑制

Inebilizumab による CD19 を標的とした B 細胞減少作用について，EAE モデルを用いて評価した（Chen et al., 2014）．このモデルでは，B 細胞減少作用並びに Ig 及び病態への影響を明らかにするため，組換え型ヒトミエリンオリゴデンドロサイト糖たん白質（rhMOG）で免疫前の，又は発症が明らかとなった後のいずれかにおいて，CD19 陽性 B 細胞を減少させる目的で huCD19 Tg マウスに Inebilizumab を単回投与した．rhMOG 免疫後に Inebilizumab を投与することにより，脾臓 B 細胞数が持続的に減少し，疾患の発症が抑制された（Chen et al., 2014）［図 2.6.2.2－18］．Inebilizumab 投与により，疾患の重症度が低下し，罹病期間が短縮した．疾患の発症率が低下し（対照抗体 100%に対し Inebilizumab 66.7%），重症度が低下した（対照抗体 2.94±0.24 に対し Inebilizumab 0.56±0.13，p<0.0001）ことからも明らかなように，Inebilizumab を単回投与したマウスでは EAE 重症度が一貫して低下し Day 23 までにほぼ完全に回復した．

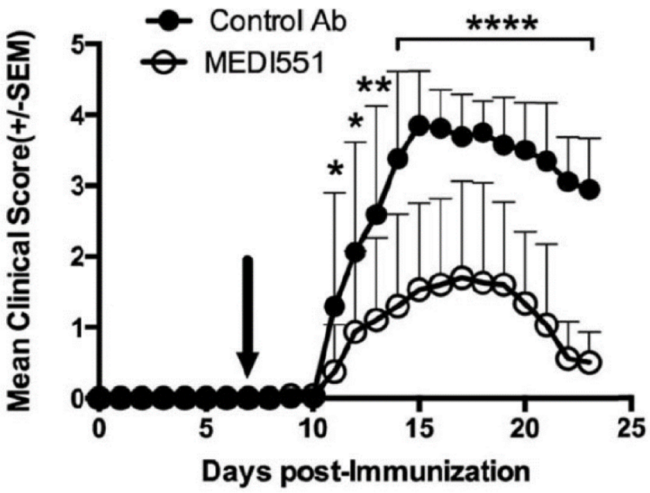

図 2.6.2.2－18　Inebilizumab による huCD19Tg マウス EAE 進行の抑制

Ab = antibody; EAE = experimental autoimmune encephalomyelitis; huCD19 Tg = human CD19 transgenic; MEDI- 551 = inebilizumab; SEM = standard error of the mean.
hCD19 Tg mice received a single dose of 250 µg MEDI-551or control Ab intraperitoneally at Day 7 after EAE induction. Filled circles indicate the control Ab-treated group (n = 17); open circles represent the MEDI-551 -treated group (n = 15). Black arrows indicate the day of Ab treatment. Three independent experiments were done and data from 2 experiments are shown. *p < 0.1; **p < 0.01; ****p ≤ 0.0001

重要な所見として，Inebilizumab 投与により，血清中及び CNS において自己反応性形質細胞及び MOG 特異的自己抗体の発現が抑制された［図 2.6.2.2－19］．このことは，CNS における MOG 特異的 T ヘルパー17（Th）細胞の有意な減少，及び MOG 特異的 CD4 陽性制御性 T 細胞の増加と一致している（Chen et al., 2014）．

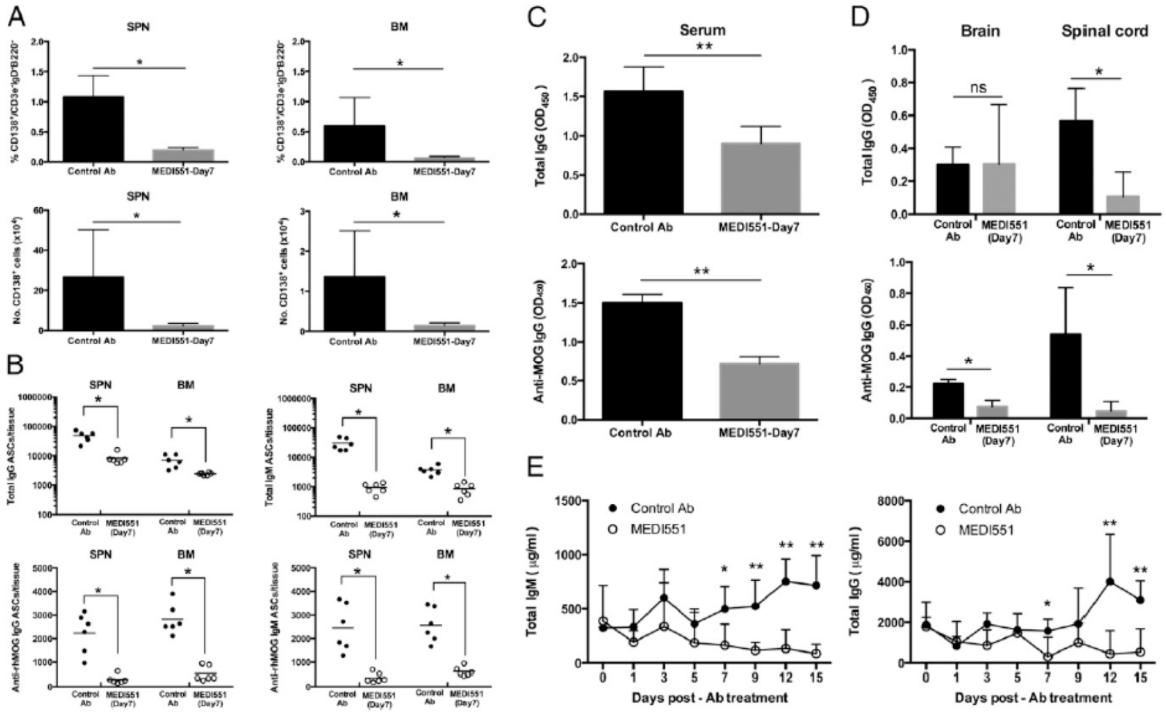

図 2.6.2.2－19　Inebilizumab による各種組織での形質細胞減少と総 IgG 及び MOG 特異的 IgG 濃度の低下

Ab = antibody; ASC = Ab-secreting cell; BM = bone marrow; CNS = central nervous system; ELISA = enzyme-linked immunosorbent assay; FACS = fluorescence-activated cell sorting; IgG = Immunoglobulin G; IgM = immunoglobulin M; LN = lymph node; MEDI-551 = inebilizumab; rhMOG = recombinant human myelin oligodendrocyte glycoprotein; ns = not significant; OD = optical density; PC plasma cell; SEM = standard error of the mean; SPN = spleen; Tg = transgenic

Human CD19 Tg mice were immunized with rhMOG on Day 0 and treated with 250 μg MEDI-551 or control antibody on Day 7. Tissues were harvested at peak of the disease and subjected to different assays. (A) FACS analysis was performed on isolated cells. Frequency and cell numbers of CD138+ PCs in the spleen and bone marrow from MEDI-551 Day 7 treated and control antibody treated mice were shown. After live and singlet gating, cells were gated on IgD and CD3e to identify IgD−CD3e− populations. IgD−CD3e− cells were then gated on B220 to exclude B220+ cells because some pre-B cells in the bone marrow are B220+CD138+. From the B220− population, CD138+ PCs were identified as CD138high CD19low. (B) Cells from the spleen and bone marrow were collected, and the numbers of total IgG/IgM and MOG-specific IgG/M antibody secreting cells were determined by ELIspot. Horizontal bar represents mean number of indicated ASC per tissue. Serum (C), brain, and spinal cord supernatants (D) were harvested at peak of the disease (Days 14‑16), and total IgG and MOG-specific IgG levels were determined by ELISA. Antibody titers were quantified as relative OD450 for total IgG or MOG-specific IgGs. Significant differences between the means of the 2 groups are indicated. Data from 4 to 6 mice per group were shown and were representative of 2 independent experiments. (E) MEDI-551 reduces existing serum Ig levels in hCD19 Tg mice. Naive hCD19 Tg mice were intraperitoneally injected with a single dose of 250 μg MEDI-551 on Day 0. Blood were collected every 2 days, and ELISAs detecting total IgM and total IgG were performed. Data from 5 mice per group were shown.

*$p < 0.05$; **$p < 0.01$.

これらのデータから，Inebilizumab 投与により，発症中の EAE モデルマウスの CNS における自己反応性形質細胞を含む，循環血中 CD19 陽性 B 細胞が完全に枯渇し，さらに組織浸潤 B 細胞が有意に減少することが示された．また，Inebilizumab 投与により，CNS において自己抗体が減少すると共に Ig 媒介性及び補体媒介性炎症が抑制された．このように Inebilizumab は前臨床の神経炎症性状態において病因性 B 細胞を標的とすることで高い有効性を示した．

2.6.2.3　副次的薬力学試験

Inebilizumab の副次的薬力学試験は実施していない．

2.6.2.4　安全性薬理試験

Inebilizumab の独立した安全性薬理試験は実施していないが，生命維持に重要な臓器（心血管系，呼吸器系，CNS など）への影響について huCD19 Tg マウスを用いた反復投与毒性試験（試験 [redacted]-2083，[redacted]-2084，[redacted]-2153，[redacted]-2237）の中で評価した（Module 2.6.6）．これらの毒性試験において，生命維持に重要な臓器に関連する有害作用は認められず，病理組織学的変化も認められなかった．また，一般状態に対する影響は認められなかった．更に，ヒト組織交差反応性試験では，心臓，肺，腎臓，脳，消化管を含む重要組織の細胞膜への Inebilizumab の結合は認められなかった．

2.6.2.5　薬力学的薬物相互作用試験

Inebilizumab の薬力学的薬物相互作用試験は実施していない．

2.6.2.6　考察及び結論

CD19 は CD19 遺伝子によってコードされる，免疫グロブリンスーパーファミリーに属する膜貫通型たん白質であり，B 細胞に限定して発現している（Cooper et al., 2004）．この細胞表面抗原は，重鎖再構成時から B 細胞成熟期間全体にわたり早期プロ B 細胞において発現し，そして大部分の形質細胞において発現している（Nadler et al., 1983）．

Inebilizumab は，親和性を最適化し糖鎖部分を改変したヒト化 IgG1 κ mAb であり，CD19 に結合する．Inebilizumab は，フコシル基転移酵素欠損チャイニーズハムスター卵巣細胞株において，ADCC を増強した均質に脱フコシル化された抗体として発現する．本稿に示した結果は，Inebilizumab の特徴である 1）ヒト CD19 に対する高い結合親和性，及び 2）非ヒト霊長類又はげっ歯類由来の CD19 に対する交差反応性がないことを強調するものである．更に，Inebilizumab は ADCC 機序を介して B 細胞を減少させ，CDC 活性を示さない．最後に，*in vitro* での ADCC アッセイにおいて，Inebilizumab は，健常ドナーB 細胞及び CD19 陽性形

質細胞に対してだけでなく，様々なB細胞株に対してもADCC活性を示した．

*In vivo*試験では，Inebilizumab投与によりEAEマウスのCD19陽性B細胞及び形質細胞が効果的に減少し，その結果，血液中及びCNS中の自己抗体が全体的に減少することが示された．これにより，Inebilizumabを投与したマウスの疾患スコアが有意に低下した．

Inebilizumabは，汎用される動物種（げっ歯類又はカニクイザル，アフリカミドリザル，ヒヒ等の非ヒト霊長類）において，これらの種のCD19と結合しない．huCD19 Tgマウスが薬理評価に適切なモデルであることを裏付けるデータが十分存在したことから，本モデルを用いてInebilizumabの薬物動態，薬力学及び安全性を評価した．

huCD19 Tgマウスの特性評価は過去に実施されており，本動物を用いることにより，血液中及び組織内のCD19陽性B細胞の減少及び回復の薬理学的効果を評価することが可能である．ヒトB細胞上のCD19へのInebilizumabの結合親和性と，本マウスB細胞上のヒトCD19への結合親和性とはほぼ同じであった．huCD19 Tgマウスにおいて，Inebilizumabは血液，脾臓，骨髄，及び腹腔から採取したB細胞と結合し，ヒトCD19は未熟・成熟B細胞，腹腔内のB細胞，及びB1a B細胞を含むすべてのB細胞集団に発現していた．この結果から，Inebilizumabは，ヒトB細胞への結合と同様に，huCD19 Tgマウス由来B細胞にも結合することが明らかとなった．Inebilizumabはヒト CD19に特異的であり，マウスのB細胞表面マーカー，またはB細胞以外の細胞とは反応しない．Inebilizumabは，huCD19 Tgマウスにおける強力なB細胞減少抗体である．B細胞の減少持続期間及び回復までの時間は用量依存性である．重要なことに，B細胞の減少後，アイソタイプ対照抗体投与動物と同様の細胞数及び成熟度に戻る．Inebilizumab投与の影響はB細胞に限定され，循環血中のその他の免疫細胞に対する明らかな影響は認められなかった．これは，このモデルにおけるhuCD19発現がB細胞に限定されることと一致する．Inebilizumabを投与したhuCD19 Tgマウスにおいて，サイトカイン及びケモカインの濃度は，BAFFを除き変化しなかった．BAFF濃度はB細胞減少中に上昇し，B細胞回復後にベースライン濃度に戻った．抗CD20 mAbであるリツキシマブを投与した被験者においても，BAFFの血清中濃度に同様の変化が認められている（Levesque and St Clair, 2008）．

上市品及び臨床試験において，複数のB細胞減少mAbが用いられており，これらのB細胞減少抗体の主要毒性は，オフターゲット毒性ではなく，その薬理作用に関連することを示すエビデンスが増えている．huCD19 Tgマウスモデルにより，薬理作用に関連したhuCD19陽性B細胞の減少と回復に関する情報と，PK/PDを適切にモデル化することで，臨床試験のための安全な開始用量を確立するために必要なトキシコキネティクスデータが得られる．

以上より，非臨床試験の結果は，自己抗体が病態発現に寄与するNMOSDのような全身性自己免疫疾患の治療のためにCD19陽性B細胞を減少させるInebilizumabの臨床試験を支持するものである．

2.6.2.7　参考文献

1. Ackerman ME, Dugast AS, McAndrew EG, Tsoukas S, Licht AF, Irvine DJ, et al. Enhanced phagocytic activity of HIV-specific antibodies correlates with natural production of immunoglobulins with skewed affinity for FcgammaR2a and FcgammaR2b. J Virol. 2013 May;87(10):5468-76.
2. Bradbury LE, Goldmacher VS, Tedder TF. The CD19 signal transduction complex of B lymphocytes. Deletion of the CD19 cytoplasmic domain alters signal transduction but not complex formation with TAPA-1 and Leu 13. J Immunol. 1993 Sep 15;151(6):2915-27.
3. Chen D, Blazek M, Ireland S, Ortega S, Kong X, Meeuwissen A, et al. Single dose of glycoengineered anti-CD19 antibody (MEDI551) disrupts experimental autoimmune encephalomyelitis by inhibiting pathogenic adaptive immune responses in the bone marrow and spinal cord while preserving peripheral regulatory mechanisms. J Immunol. 2014 Nov 15;193(10):4823-32.
4. Chen D, Gallagher S, Monson N, Herbst R, Wang Y. Inebilizumab, a B cell-depleting anti-CD19 Antibody for the Treatment of Autoimmune Neurological Diseases: insights from preclinical studies. J Clin Med, 2016; Nov 24; 5(12): 107
5. Chen D, Ireland SJ, Davis LS, Kong X, Stowe AM, Wang Y, et al. Autoreactive CD19+CD20- Plasma Cells contribute to disease severity of Experimental Autoimmune encephalomyelitis. J Immunol. 2016; Feb 15; 196:1541-1549.
6. Cooper LJ, Al-Kadhimi Z, DiGiusto D, Kalos M, Colcher D, Raubitschek A, et al. Development and application of CD19-specific T cells for adoptive immunotherapy of B cell malignancies. Blood Cells Mol Dis. 2004 Jul-Aug;33(1):83-9.
7. Dugast AS, Tonelli A, Berger CT, Ackerman ME, Sciaranghella G, Liu Q, et al. Decreased Fc receptor expression on innate immune cells is associated with impaired antibody-mediated cellular phagocytic activity in chronically HIV-1 infected individuals. Virology. 2011 Jul 5;415(2):160-7.
8. Groves CJ, Carrell J, Grady R, Rajan B, Morehouse CA, Halpin R, et al. CD19-positive antibody-secreting cells provide immune memory. Blood Adv. 2018;2(22):3163-3176.
9. Inaoki M, Sato S, Weintraub BC, Goodnow CC, Tedder TF. CD119-regulated signaling thresholds control peripheral tolerance and autoantibody production in B lymphocytes. J Exp Med. 1997 Dec 1; 186(11):1923-31.
10. Levesque MC, St Clair EW. B cell-directed therapies for autoimmune disease and correlates of disease response and relapse. J Allergy Clin Immunol. 2008 Jan;121(1):13-21.
11. Matlawska-Wasowska K, Ward E, Stevens S, Wang Y, Herbst R, Winter SS, et al. Macrophage and NK-mediated Killing of Precursor-B Acute Lymphoblastic Leukemia Cells Targeted with a- Fucosylated Anti-CD19 Humanized Antibodies. Leukemia. 2013 June; 27(6): 1263-74.
12. Nadler LM, Anderson KC, Marti G, Bates M, Park E, Daley JF, et al. B4, a human B

lymphocyte-associated antigen expressed on normal, mitogen-activated, and malignant B lymphocytes. J Immunol. 1983 Jul;131(1):244-50.

13. Richards JO, Karki S, Lazar GA, Chen H, Dang W, Desjarlais JR. Optimization of antibody binding to FcgammaRIIa enhances macrophage phagocytosis of tumor cells. Mol Cancer Ther. 2008 Aug;7(8):2517-27.
14. Sato S, Steeber DA, Tedder TF. The CD19 signal transduction molecule is a response regulator of B-lymphocyte differentiation. Proc Natl Acad Sci U S A. 1995 Dec 5; 92(25):11558-62.
15. Sato S, Steeber DA, Jansen PJ, Tedder TF. CD19 expression levels regulate B lymphocyte development: human CD19 restores normal function in mice lacking endogenous CD19. J Immunol. 1997 May 15; 158(10):4662-9.
16. Taylor DK, Ito E, Thorn M, Sundar K, Tedder T, Spatz LA. Loss of tolerance of anti-dsDNA B cells in mice overexpressing CD19. Mol Immunol. 2006 Apr; 43(11):1776-90.
17. Tebo AE, Kremsner PG, Luty AJ. Fcgamma receptor-mediated phagocytosis of Plasmodium falciparum-infected erythrocytes in vitro. Clin Exp Immunol. 2002 Nov;130(2):300-6.
18. Yazawa N, Hamaguchi Y, Poe JC, Tedder TF. Immunotherapy using unconjugated CD19 monoclonal antibodies in animal models for B lymphocyte malignancies and autoimmune disease. Proc Natl Acad Sci U S A. 2005 Oct 18;102(42):15178-83.
19. Zhou LJ, Smith HM, Waldschmidt TJ, Schwarting R, Daley J, Tedder TF. Tissue-specific expression of the human CD19 gene in transgenic mice inhibits antigen-independent B lymphocyte development. Mol Cell Biol. 1994 Jun; 14(6):3884-94.

ユプリズナ点滴静注 100 mg
製造販売承認申請書添付資料
添付資料

第 2 部（モジュール 2）

2.6　非臨床試験の概要文及び概要表
2.6.3　薬理試験概要表

田辺三菱製薬株式会社

目次

略語・略号一覧

略語・略号	略していない表現（英語）	略していない表現（日本語）
ADCC	antibody-dependent cellular cytotoxicity	抗体依存性細胞傷害
ADCP	antibody-dependent cellular phagocytosis	抗体依存性貪食
ALL	acute lymphoblastic leukemia	急性リンパ性白血病
BAFF	B cell activating factor	B 細胞活性化因子
CD	cluster of differentiation antigen	白血球分化抗原
EAE	experimental autoimmune encephalomyelitis	実験的自己免疫性脳脊髄炎
EC50	half-maximal effective concentration	50%有効濃度
ELISA	enzyme-linked immunosorbent assay	酵素免疫測定法
ELIspot	enzyme-linked immunosorbent spot assay	酵素免疫スポットアッセイ
FACS	fluorescence-activated cell sorting	蛍光活性化セルソーティング
Fab	antigen-binding fragment	抗原結合性フラグメント
Fc	fragment crystallizable	結晶化フラグメント
GLP	Good Laboratory Practice	医薬品の安全性に関する非臨床試験の実施の基準
huCD19 Tg	human CD19 transgenic	ヒト CD19 トランスジェニック
IV	intravenous	静脈内
Ig	immunoglobulin	免疫グロブリン
mAb	monoclonal antibody	モノクローナル抗体
MS	multiple sclerosis	多発性硬化症
NK	natural killer	ナチュラルキラー
PBMC	peripheral blood mononuclear cells	末梢血由来単核細胞
PCR	polymerase chain reaction	ポリメラーゼ連鎖反応

2.6.3　薬理試験概要表

2.6.3.1　薬理試験：一覧表

被験物質：Inebilizumab

表 2.6.3.1－1　Inebilizumab の効力を裏付ける試験の概要一覧表

Study Number	Type of Study	Study Title	Testing Facility	eCTD Location
[redacted]551-0001	In vitro (non-GLP)	Affinity Optimization of Monoclonal Antibody 3649	MedImmune	4.2.1.1
[redacted]551-0002		Binding Properties of anti-CD19 Antibodies	MedImmune	4.2.1.1
[redacted]551-0003		In Vitro Effector Function of MEDI-551 Against Multiple Lymphoblastic Cell Lines	MedImmune	4.2.1.1
[redacted]551-0004		In Vitro Effector Function of MEDI-551 with Healthy Donor- and Leukemia Patient-Derived Peripheral Blood B Cells	MedImmune	4.2.1.1
Matlawska-Wasowska et al., 2013		Macrophage and NK-mediated Killing of Precursor-B Acute Lymphoblastic Leukemia Cells Targeted with a-Fucosylated Anti-CD19 Humanized Antibodies	University of New Mexico Health Sciences Center	4.3
[redacted]551-0004		Differential Expression of CD19 and CD20 on Human Plasma Cells	MedImmune	4.2.1.1
[redacted]551-0002		MEDI-551 Mediated Depletion of Human Plasma Cells	MedImmune	4.2.1.1
[redacted]551-0008		Binding of Monoclonal Antibody 16C4 to Nonhuman Primate B Cells Measured by Flow	MedImmune	4.2.1.1
[redacted]551-0005		MEDI-551 Does Not Bind to Rabbit Peripheral B Cells	MedImmune	4.2.1.1
[redacted]551-0009		Binding of MEDI-551 to Human CD19 Expressed on Human B Cells and on Murine B Cells from Human CD19 Transgenic Mice	MedImmune	4.2.1.1

2.6.3.1　薬理試験：一覧表（続き）

表 2.6.3.1－1　Inebilizumab の効力を裏付ける試験の概要一覧表（続き）

Study Number	Type of Study	Study Title	Testing Facility	eCTD location
[redacted]551-0010	In vivo (non-GLP)	Longitudinal Study of B-cell Depletion by MEDI-551 in Human CD19 Transgenic Mice	MedImmune	4.2.1.1
[redacted]551-0003		MEDI-551 Treatment Depletes the Majority of Murine B Cells and Reduces Serum Titers of Autoantibodies in Sle1- human CD19 Transgenic Mice	MedImmune	4.2.1.1
Chen et al., 2014	In vivo (non-GLP)	Single dose of glycoengineered anti-CD19 antibody (MEDI551) disrupts experimental autoimmune encephalomyelitis by inhibiting pathogenic adaptive immune responses in the bone marrow and spinal cord while preserving peripheral regulatory mechanisms	University of Texas Southwestern Medical Center	4.3

CD19 = cluster of differentiation antigen 19; GLP = Good Laboratory Practice; MEDI-551 = Inebilizumab; NK = natural killer cell; 16C4 = affinity-optimized 3649 anti-human CD19.

2.6.3.2　効力を裏付ける試験

2.6.3.2.1　*in vitro* 薬理試験

被験物質：Inebilizumab

表 2.6.3.2－1　Inebilizumab の *in vitro* 薬理試験

Study Number (Type of Study)	**Test System**	**Test Methods**	**Noteworthy Findings**
[redacted]551-0001 (non-GLP)	*Escherichia coli*; human cell lines expressing varying amounts of CD19	PCR cloning; affinity-optimization of complementary-determining region; combinatorial screening of Fab constructs; conversion of Fab fragments to IgG	• Variant mAb 16C4 is derived from mAb 3649; the 2 mAbs differ by 3 amino acids. • MAb 16C4 binds approximately twice as strongly to human CD19 as does mAb 3649 (EC_{50} = 332.5 ng/mL for mAb 16C4; EC_{50} = 588.2 ng/mL for mAb 3649 [300.B4 cells, Figure 12-2])
[redacted]551-0002 (non-GLP)	Daudi human Burkitt lymphoma cells as model B cells	FACS; laser scanning confocal microscopy; internalization assays; measurement of surface binding; surface plasmon resonance	• The binding properties of mAb 16C4 (the fucosylated version of MEDI-551) are compatible with ADCC activity. • Loss of fucose from human IgG1-Fc results in enhanced binding to human Fc gamma RIIIA and mouse Fc gamma RIV (Table 12-1). • MAb 16C4 binds to B cells (Daudi cells) better than does its parental antibody (mAb 3649, Figure 12-1), and dissociates more slowly from the B cell surface than does mAb 3649 or rituximab (Figure 12-2). • MAb 16C4 has a low relative level of internalization when compared to the internalization of the parental antibody (Figure 12-4).

2.6.3.2.1 *in vitro* 薬理試験（続き）

表 2.6.3.2－1　Inebilizumab の *in vitro* 薬理試験（続き）

Study Number (Type of Study)	**Test System**	**Test Methods**	**Noteworthy Findings**
[redacted]551-0003 (non-GLP)	Natural killer cells; B lymphoma cell lines; human serum from healthy donors	Lactate dehydrogenase cytotoxicity assay; FACS; ADCC assays; antibody binding assays	• Inebilizumab induced ADCC when 2 different (human) natural killer effector cell sources were employed. • In ADCC assays, inebilizumab is effective against multiple lymphoblastic cell lines including those derived from Burkitt lymphoma, diffuse large cell lymphoma,and multiple myeloma, non-Hodgkin lymphoma, chronic lymphocytic leukemia, and ALL. • Inebilizumab does not mediate complement-dependent cytotoxicity. • Inebilizumab and rituximab cause similar maximum cytotoxicity, but inebilizumab achieves this result at a lower concentration than does rituximab (ADCC EC_{50} for inebilizumab = 8 ng/mL; ADCC EC_{50} for rituximab = 32 ng/mL [Toledo cells, Table 12-1]). • Inebilizumab selectively depleted CD19+ cells from multiple myeloma cell lines with mixed subpopulations of CD19+ and CD19- cells.

2.6.3.2.1　*in vitro* 薬理試験（続き）

表 2.6.3.2－1　Inebilizumab の *in vitro* 薬理試験（続き）

Study Number (Type of Study)	Test System	Test Methods	Noteworthy Findings
[redacted]551-0004 (non-GLP)	PBMC from 3 sources (healthy donors, donors with chronic lymphocytic leukemia, and donors with ALL)	FACS; ADCC assays; immunofluorescence assays	• Inebilizumab has strong ADCC properties. • Inebilizumab depleted B cells in PBMC samples from healthy donors, donors with chronic lymphocytic leukemia, and donors with ALL. • Inebilizumab depleted B cells at mAb concentrations lower than those needed for rituximab depletion of B cells (ADCC EC_{50} for MEDI-551 = 0.016 nM; ADCC EC_{50} for rituximab = 0.431 [Donor 343, Table 12-1]). • The ADCC induced by inebilizumab (in vitro) was superior to the ADCC induced by rituximab. • Inebilizumab is more effective than rituximab at depleting B cells when cognate surface antigen densities are low.
Matlawska-Wasowska et al., 2013	Primary human macrophages and pre-B ALL cell line (697 cells)	ADCP assay; immunofluorescence assay	• Inebilizumab induces ADCP activity in vitro. • Primary human macrophages rapidly form a synapse with inebilizumab coated target cells. This contact is followed up by the engulfment of target cells by the macrophage. • It's unclear that ADCP is the predominant mode of action to deplete B cells in vivo.

2.6.3.2.1　*in vitro* 薬理試験（続き）

表 2.6.3.2－1　Inebilizumab の in vitro 薬理試験（続き）

Study Number (Type of Study)	Test System	Test Methods	Noteworthy Findings
[redacted]551-0008 (non-GLP)	Whole blood from nonhuman primates; whole blood from healthy human donors	FACS; binding assays	• MAb 16C4 binds CD19 on human B cells but does not bind CD19 on B cells from cynomolgus monkeys. • MAb 16C4 binds poorly to B cells from baboon, rhesus monkey, and African green monkey. Binding mAb 16C4 to B cells of nonhuman primates was about 1/20 to 1/10 the binding observed for human B cells.
[redacted]551-0009 (non-GLP)	B cells from huCD19 transgenic mice; B cells from healthy human donors; Fab fragment of MEDI-551	FACS; B cell isolation; purification of recombinant MEDI-551 Fab fragment from mammalian cells; protein sequence alignments	• Human CD19 and mouse CD19 proteins are approximately 74% similar and 65% identical, as determined by BLAST (BLOSUM62 matrix). • In huCD19 transgenic mice, human CD19 is expressed in immature and mature B cells, B cells in the peritoneal cavity, and B1a B cells. • The expression pattern of human CD19 on B cells of huCD19 transgenic mice closely reflects the developmental expression pattern of CD19 on human B cells. • The lowest level of human CD19 expression was observed on early pro- and pre-B cells, and the level of mAb 16C4 binding was increased in mature B cells. • Inebilizumab is specific for human CD19 and does not react with murine surface markers on B cells, or on non-B cells. • The binding of inebilizumab to human blood B cells, and to huCD19 transgenic mouse blood and spleen B cells, is comparable.

2.6.3.2.1　*in vitro* 薬理試験（続き）

表 2.6.3.2－1　Inebilizumab の *in vitro* 薬理試験（続き）

Study Number (Type of Study)	Test System	Test Methods	Noteworthy Findings
[redacted]551-0002	Primary and in vitro differentiated human plasma cells; Natural killer cells	B cell and plasma cells isolation; FACS; In vitro differentiation assay	• In in vitro ADCC assays with NK cells as effectors, inebilizumab demonstrated significant depletion of plasma cells from fresh human bone marrow samples. • Inebilizumab also depleted plasma cells generated by in vitro differentiation from human PBMC.
[redacted]551-0004	Human peripheral blood; human tonsil; human spleen; human bone marrow	FACS; ELIspot assay	• CD20 expression is found on human B cells but is present on very few if any plasma cells in the blood, spleen, or bone marrow. In contrast, CD19 is expressed on plasmablasts and most plasma cells. • Using flow cytometry, a population of CD19- plasma cells was identified in the spleen and bone marrow as a subpopulation of plasma cells in these tissues. • The bone marrow CD19- and CD19+ plasma cells have nearly identical morphology, immunophenotype, mRNA expression, and function. Additionally, vaccine-specific IgG is produced by both CD19+ and CD19- antibody secreting subsets in the bone marrow and spleen.
[redacted]551-0005	Rabbit peripheral blood B cells	FACS	• MAb 16C4 binds CD19 on human B cells but does not bind CD19 on B cells from rabbit peripheral blood.

ADCC = antibody-dependent cellular cytotoxicity; ADCP = antibody-dependent cellular phagocytosis; ALL = acute lymphoblastic leukemia; BLAST = basic local alignment search tool; CD19 = cluster of differentiation antigen 19; EC_{50} = half-maximal effective concentration; ELISpot assay = enzyme-linked immunosorbent spot assay; FACS = fluorescence-activated cell sorting; GLP = Good Laboratory Practice; huCD19 = human CD19; IgG = immunoglobulin G; mAb = monoclonal antibody; MEDI-551 = inebilizumab; NK = natural killer; PBMC = peripheral blood mononuclear cells; PCR = polymerase chain reaction; 16C4 = affinity-optimized 3649 anti-human CD19.

2.6.3.2.2　*in vivo* 薬理試験

被験物質：Inebilizumab

表 2.6.3.2－2　Inebilizumab の in vivo 薬理試験

Study Number (Type of Study)	**Test System**	**Test Methods**	**Noteworthy Findings**
[redacted]551-0010 (in vivo, non-GLP)	huCD19 transgenic mice	FACS; ELISA assays; IV administration of MEDI-551	• Treatment with inebilizumab resulted in profound and prolonged depletion of peripheral blood B cells in huCD19 transgenic mice. • B cell depletion was rapid, and initial depletion of blood B cells was comparable for doses of 10, 50, and 250 µg/mouse. • The duration of B cell depletion is dose-dependent. At 10 µg/mouse, B cell levels began to return to normal by 1 week post injection and were fully recovered by 5 weeks. A single dose of 250 µg/mouse was sufficient to deplete B cells for more than 10 weeks. • The effect of treatment with inebilizumab was limited to B cells and did not have an obvious impact on other immune cells in circulation. • Serum immunoglobulins steadily increased with age in control mice, but this increase was blocked by treatment with inebilizumab. • Serum levels of the B cell-activating factor, BAFF, were elevated in all animals treated with inebilizumab, and these changes corresponded to B cell depletion and recovery. • Other cytokines and chemokines were not affected by inebilizumab treatment.

2.6.3.2.2　*in vivo* 薬理試験（続き）

表 2.6.3.2－2　Inebilizumab の in vivo 薬理試験（続き）

Study Number (Type of Study)	Test System	Test Methods	Noteworthy Findings
[redacted]551-0003	Sle1- human CD19 transgenic mice	FACS, ELISA assays	• Sle1-huCD19 transgenic mice spontaneously develop autoantibodies. • Inebilizumab treatment resulted in > 90% depletion of B cells in blood, spleen and bone marrow. • Germinal center B cells and plasma cells, especially in the spleen were also significantly reduced. • Mice treated with inebilizumab had lower serum immunoglobulin levels and reduced autoantibody titers, in comparison with control-treated mice.
Chen et al., 2014	human CD19 transgenic mice	FACS, ELISA assays	• Inebilizumab, given before or during ongoing experimental autoimmune encephalomyelitis, disrupts development of the disease. • Leukocyte infiltration into the spinal cord is significantly reduced, as well as short-lived and long- lived autoreactive CD138+ plasma cells in the spleen and bone marrow, respectively. • Potentially protective CD1d^{hi}CD5+ regulatory B cells show resistance to depletion, and myelin-specific Foxp3+ regulatory T cells are expanded. • Taken together, these results demonstrate that Inebilizumab disrupts experimental autoimmune encephalomyelitis by inhibiting multiple proinflammatory components, whereas preserving regulatory populations.

BAFF = B cell activating factor; CD19, CD20 = cluster of differentiation antigen 19, antigen 20; ELISA = enzyme-linked immunosorbent assay; EAE = experimental autoimmune encephalomyelitis; FACS = fluorescence-activated cell sorting; GLP = good laboratory practice; huCD19 = human CD19; IV = intravenous; mAb = monoclonal antibody; MS = multiple sclerosis; Sle1 = major susceptibility locus in murine model of systemic lupus erythematosus.

2.6.3.3　副次的薬理試験

Inebilizumab の副次的薬力学試験は実施していない.

2.6.3.4　安全性薬理試験

Inebilizumab の独立した安全性薬理試験は実施していないが，生命維持に重要な臓器（心血管系，呼吸器系，中枢神経系など）への影響ついて huCD19 Tg マウスを用いた反復投与毒性試験（試験[redacted]-2083，[redacted]-2084，[redacted]-2153，[redacted]-2237）の中で評価した．これらの毒性試験において，生命維持に重要な臓器に関連する有害作用は認められず，病理組織学的変化も認められなかった．また，一般状態に影響は認められなかった．更に，ヒト組織交差反応性試験では，心臓，肺，腎臓，脳，消化管を含む重要組織の細胞膜への Inebilizumab の結合は認められなかった．

2.6.3.5　薬力学的薬物相互作用試験

Inebilizumab の薬力学的薬物相互作用試験は実施していない.

2.6.3.6　参考文献

1. Chen D, Blazek M, Ireland S, Ortega S, Kong X, Meeuwissen A, et al. Single dose of glycoengineered anti-CD19 antibody (MEDI551) disrupts experimental autoimmune encephalomyelitis by inhibiting pathogenic adaptive immune responses in the bone marrow and spinal cord while preserving peripheral regulatory mechanisms. J Immunol. 2014 Nov 15;193(10):4823-32.
2. Matlawska-Wasowska K, Ward E, Stevens S, Wang Y, Herbst R, Winter SS, et al. Macrophage and NK-mediated Killing of Precursor-B Acute Lymphoblastic Leukemia Cells Targeted with a- Fucosylated Anti-CD19 Humanized Antibodies. Leukemia. 2013 June; 27(6): 1263-74.