

Optimizing the aggregation propensity of therapeutic monoclonal antibodies against cancer and autoimmune diseases: a computational study

ABBREVIATIONS: mAbs, monoclonal antibodies; CDRs, Complementarity Determining Regions; FDA, U.S. Food and Drug Administration; L1, light loop 1; L2, light loop 2; L3, light loop 3; H1, heavy loop 1; H2, heavy loop 2; H3, heavy loop 3.

Abstract

Monoclonal antibodies (mAbs) represent the most promising and rapidly growing class of therapeutic compounds for treating a wide variety of human chronic and acute diseases. Despite their benefits, mAbs obtained from human or other sources have deficiencies such as short *in vivo* life and low stability. A major drawback in the exploitation of antibodies is their tendency to form aggregates. As a result, severe immunological reactions in patients have also been recorded.

In this study, we investigated the susceptibility of a set of therapeutic monoclonal antibodies to form aggregates. We selected antibodies that have all been approved by the U. S. Food and Drug Administration and are indicated for the treatment of various types of cancer and autoimmune diseases. The AMYLPRED 2 consensus method was used to predict ‘aggregation-prone’ regions on the surface of these proteins.

These regions are conserved and observed in almost all monoclonal antibodies commercially available. Considering the amino acid sequences of these antibodies, common groups of ‘aggregation-prone’ regions were identified. We successfully reduced or even fully eliminated ‘aggregation-prone’ groups by specific ‘mutations’ of amino acids with exposed side chains. This information may be useful in future studies of monoclonal antibodies by improving existing therapeutic products or by designing novel ones.

KEYWORDS: Aggregation-prone regions; Monoclonal antibodies; Aggregation propensity; Cancer; Autoimmune diseases

Introduction

Monoclonal antibodies (mAbs) are tetramers of two identical pairs of polypeptide chains, the heavy and the light chains [1]. The light chain consists of one variable and one constant domain and the heavy chain consists of one variable and three or more constant domains [1]. All the domains have a similar tertiary structure called immunoglobulin fold, which is characterized by two anti-parallel beta sheets [2]. The antigen-binding site consists of six loops, three from the light chain and three from the heavy chain [3]. These loops are also known as complementarity determining regions (CDRs) and they are responsible for the recognition and the binding between antibody and antigen [4].

Given almost any substance, it is possible to produce monoclonal antibodies that specifically bind to it [5]. MAbs are made by identical immune cells that are clones of a unique parent B-cell, have monovalent affinity, bind to the same epitope and can be reproduced endlessly *in vitro* [6].

They are used in treatment schemes for autoimmune [7], cardiovascular [8] and infectious diseases [9], cancers [10,11] and others. They can also be used as diagnostic [12] and biotechnological tools [13].

Monoclonal antibodies in high concentrations, in their storage and usage, form aggregations [14], which are an increasing concern in therapeutic development. Protein aggregation causes side effects and immunogenic responses in clinical trials [15]. Aggregates may also result in reduction of antibody therapeutic activity, while severe immunological reactions in patients have also been recorded [16]. Thus, it is important to understand the aggregation mechanisms of mAbs under different circumstances [17] and their potential biological implications in the pharmaceutical industry in order to improve their efficacy and make them successful biopharmaceutical products [18].

Several computational studies have been carried out and a variety of monoclonal antibodies have been extensively studied and engineered [19]. A number of computational tools are also available [20] to predict aggregation-prone regions in therapeutic proteins. These tools focus on predicting the aggregation-prone regions (small peptides) and finding the potential sequence and structure factors that contribute toward aggregation formation in proteins [21]. As a result, a number of monoclonal antibodies have been improved, used again and for other cases too.

Taking into account all these insights, our purpose was to propose novel models of therapeutic monoclonal antibodies with improved solubility based on an amino acids substitution approach. Our findings can be applied on rational design of novel therapeutic candidates or improvement of existing bio-therapeutics.

Materials and methods

Dataset collection

In order to study the monoclonal antibodies, we carried out an extensive research in the literature and available databases. We analyzed antibodies that have been approved by the U.S. Food and Drug Administration (FDA) and have been indicated for the treatment of various acute and chronic diseases like cancer and autoimmune diseases. They are all IgG1 kappa immunoglobulins and they have several and different mechanisms of action.

We collected the amino acid sequences of antibodies from the Protein Data Bank [22]. We obtained also the three-dimensional structures of antibodies from the Protein Data Bank (PDB ID for Alemtuzumab: **1CE1**, PDB ID for Bevacizumab: **1BJ1**, PDB ID for Cetuximab: **1YY8**, PDB ID for Ofatumumab: **3GIZ**, PDB ID for Pertuzumab: **1L71**, PDB ID for Rituximab: **2OSL**, PDB ID for Trastuzumab: **1N8Z**, PDB ID for Adalimumab: **3WD5**, PDB ID for Infliximab: **4G3Y**, PDB ID for Natalizumab: **4IRZ**, PDB ID for Ustekinumab: **3HMX**).

Additional information about the monoclonal antibodies was obtained from the Drug Bank [23] and the Monoclonal Antibodies Database, part of the IMGT®, the international ImMunoGeneTics information system® [24] in order to perform sequence analysis.

Monoclonal antibodies against cancer

Detailed information about monoclonal antibodies against cancer is presented in Supplementary Material, in section S1. Table S1 also contains specific information about the monoclonal antibodies used.

Monoclonal antibodies against autoimmune diseases

Detailed information about monoclonal antibodies against autoimmune diseases is presented in Supplementary Material, in section S2. Table S2 also contains specific information about the monoclonal antibodies used.

Aggregation-prone regions

In order to predict ‘aggregation-prone’ regions on the surface of antibodies, we used AMYLPRED2 [25], a consensus method, which predicts ‘aggregation-prone’ peptides on an amino acid sequence, from sequence alone, developed in our lab. For each monoclonal antibody, the algorithm was applied to the amino acid sequence of each chain separately.

Furthermore, sequence alignments of heavy chains and light chains were performed utilizing ClustalW [26], to identify similarities and differences between the antibodies used.

Criteria of the amino-acid mutation

Three criteria were followed in order to select the amino acids that may be substituted by others in the amino acid sequences of the monoclonal antibodies of the dataset.

The first criterion was the accessibility of these amino acids to the solvent in the 3D-structure. It is undesirable to change amino acids that are not on the surface of the protein, because these

changes will probably affect the 3D-structure and the stability of the molecule. For this reason, we located the amino acid residues of the antibodies exposed to the solvent with the help of the DSSP algorithm [27]. Residues that are in contact with at least two molecules of water and they are predicted by AMYLPRED2 [25] to be in ‘aggregation-prone’ regions, were selected as possible candidates to be substituted by others.

The second criterion was that the residues to be substituted should not be found in complementarity determining regions (CDRs). Residues in these regions are responsible for the recognition and the interaction with the antigen-target. For these reason, any change in these regions may cause unexpected effects to the functionality of the antibody.

The third criterion was to replace amino acid residues with exposed side chains, in the predicted ‘aggregation-prone’ regions, considering the experimental aggregation propensities of the 20 natural amino acids [28]. According to the propensities, residues that are ‘aggregation-prone’ have positive values and those that are not have negative values. The higher the tension to form aggregations, the greater the positive value is and the same applies for the negative values respectively.

We also took into account the 3D-structure (Fab fragment) of the antibodies in order to be certain that our ‘mutations’ do not affect overall the 3D-structure.

Clusters

After performing sequence alignments of all the monoclonal antibodies in the dataset, for the light (Fig. 1) and the heavy (Fig. 2) chain separately, we discovered some common groups of ‘aggregation-prone’ regions in the amino acid sequences of the antibodies that are present in almost all the monoclonal antibodies that are now on the market [29].

These ‘aggregation-prone’ regions were grouped into clusters according to their positions in the 3D-structure (Figs. 3A, B). With specific changes of amino acid residues, only in the peptides

that are present in ‘aggregation-prone’ regions, we successfully diminished ‘aggregation-prone’ clusters or even made them to disappear completely (Figs. 3A, B).

The procedure of selecting the amino acids that will substitute the initial ones was really tedious, because of the fact that for each residue, in each chain, in each antibody of the dataset all possibilities were tested manually. Consequently, residues with exposed side chains, in ‘aggregation-prone’ regions, were substituted by others with lower tendency to form aggregates, but with similar physicochemical properties and the potential to form aggregates was reduced or even eliminated in some cases.

Novel models - Evaluation and improvements

After the substitutions, novel amino acid sequences were created. The AMYLPRED2 [25] consensus method was used again to predict ‘aggregation-prone’ regions in the novel amino acid sequences.

The Modeller 9.13 [30] software was used for homology modeling and novel models of the ‘mutated’ proteins were calculated, utilizing as templates the native structures. The side chains of the ‘mutated’ residues were examined and optimized, in order to ensure that the side chains are still exposed and to avoid steric hindrance. This was done utilizing Chimera 1.8 [31] and its capability to minimize the energy of the structure. The PROCHECK [32] and WHAT_CHECK [33], from software WHAT IF [34], algorithms were used to check the validity and the quality of the novel models.

Results

The sequence alignments of all monoclonal antibodies in the dataset, for the light and the heavy chain separately, are presented in Figs. 1 and 2, respectively. The CDRs for light and heavy chain are named L1, L2, L3 and H1, H2, H3 accordingly. The ‘aggregation-prone’ regions, that

AMYPRED2 [25] consensus method predicted, are also illustrated and grouped into clusters according to their position in the 3D-structure (Figs. 3A and 3B, BEFORE and Figs. 1 and 2). These clusters were called Cluster1 – Cluster5. Only two specific examples for the monoclonal antibodies RITUXIMAB and ADALIMUMAB are shown in Figs. 3A and 3B.

Performing specific changes of amino acid residues, only in the clusters, ‘aggregation-prone’ clusters were successfully diminished or even eliminated completely (shown explicitly in Figs. 3A and 3B, AFTER).

The clusters that we grouped the common ‘aggregation-prone’ regions, according to the 3D-structure of the proteins, are also presented in Table 1 in the column ‘Clusters’. The column ‘Regions’ contains the common ‘aggregation-prone’ regions, the column ‘Chain’ the chain of the regions and the column ‘Peptides’ presents the different peptides that were found in the common ‘aggregation-prone’ regions. The amino acids that were substituted are illustrated in bold and they are underlined. The column ‘After Substitutions’ contains the peptides after the substitutions. In some cases, some ‘aggregation-prone’ peptides were eliminated completely and this is depicted with the symbol (√). In other cases the ‘aggregation-prone’ peptides were diminished sufficiently and the peptides that remain are presented in the table.

Taking a closer look to the amino acids that were substituted, the amino acids Threonine (THR, T) and the amino acid Serine (SER, S) were mostly substituted. Other amino acids that were considerably substituted by others, were the amino acids Tyrosine (TYR, Y), Valine (VAL, V), Isoleucine (ISO, I) and Leucine (LEU, L). Thus, polar and hydrophobic amino acids which seem to favor aggregation should be substituted by other polar or charged amino acids, considering the experimental aggregation propensities of the 20 natural amino acids [28].

Every ‘aggregation-prone’ peptide, which belongs to a cluster, with a combination of one or two substitutions, will no longer be ‘aggregation-prone’. There are several potential combinations of

substitutions that may bring the desirable result. The novel models that are proposed, after the substitutions, will be less ‘aggregation-prone’ and should not provoke immunological reactions to patients. This remains to be proven by more refined experimental work.

We conclude that, comparing the space-filling models before and after the substitutions, with specific replacements of exposed amino acid residues, only in the clusters that contain common ‘aggregation-prone’ regions, the potential of antibodies to form aggregates will be reduced or even eliminated. Accordingly, their solubility should also be improved without affecting the potency of the bio-therapeutics.

Discussion

This computational study can be generalized in other monoclonal antibodies that are used therapeutically for other diseases and should experimentally be verified in order to confirm the validity of the observed results and that there will be no side effects. A general computational method for improvement of the solubility of all monoclonal antibodies will also be proposed soon (work in preparation).

We hope and anticipate that the combination of predictions, experiments and molecular models of the candidate targets can be applied on novel and existing bio-therapeutics with promising results. Finally, the continuous development and application of rational protein design technology will enable improvements in the efficacy and safety of protein therapeutics.

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FIGURE LEGENDS

Fig. 1. Sequence alignment of light chains of all the monoclonal antibodies of the dataset. ClustalW was used for the alignment [26]. The molecule name and the PDB ID are mentioned. The three CDRs are highlighted in yellow and named L1, L2 and L3. The predicted aggregation-prone regions are shown in red. The clusters of 'aggregation-prone' regions are also indicated (Cluster1, Cluster2, Cluster3, Cluster4).

Fig. 2. Sequence alignment of heavy chains of all the monoclonal antibodies of the dataset. ClustalW was used for the alignment [26]. The molecule name and the PDB ID are mentioned. The three CDRs are highlighted in yellow and named H1, H2 and H3. The predicted aggregation-prone regions are in red. The clusters of ‘aggregation-prone’ regions are also indicated (Cluster3, Cluster5).

Fig. 3.

A. Space-filling models of the monoclonal antibody Rituximab (Rituxan) (PDB ID: 2OSL) produced utilizing the software Chimera 1.8 [31]. The molecule name and the PDB ID are mentioned. Light chain in grey, heavy chain in blue, ‘aggregation-prone’ regions in red, CDRs in yellow, ‘aggregation-prone’ regions in CDRs in cyan and the residues that will be substituted are depicted with green color. The ‘BEFORE’ images illustrate the stage before the substitutions and the names of the clusters, for front and back view. The ‘AFTER’ images represent the structures after the substitutions and the substitutions made (residue that was substituted and position), for front and back view.

B. Space-filling models of the monoclonal antibody Adalimumab (Humira) (PDB ID: 3WD5) produced utilizing the software Chimera 1.8 [31]. The molecule name and the PDB ID are mentioned. Light chain in grey, heavy chain in blue, ‘aggregation-prone’ regions in red, CDRs in yellow, ‘aggregation-prone’ regions in CDRs in cyan and the residues that will be substituted are depicted with green color. The ‘BEFORE’ images illustrate the stage before the substitutions and the names of the clusters, for front and back view. The ‘AFTER’ images represent the structures after the substitutions and the substitutions made (residue that was substituted and position), for front and back view.

Table 1

Summary of the clusters of the common ‘aggregation-prone’ regions in the antibodies used for this study. The clusters of the ‘aggregation-prone’ regions, the ‘aggregation-prone’ regions, the chain, the specific peptides for each region (the amino acids that were substituted are in bold letters and underlined) and the final results after the substitutions (the ‘aggregation-prone’ peptides that were eliminated completely are depicted with (√) and the ones that remain are shown).

Clusters	Regions	Chain	Peptides	After Substitutions
Cluster1	RVTITC	LIGHT	RV <u>T</u> ITC	√
		LIGHT	RV <u>T</u> ITC	√

Cluster1	[FY]T[FL]TISS	LIGHT	FTFTI <u>SS</u>	FTFTI <u>S</u>
		LIGHT	F <u>T</u> LT <u>I</u> SS	√
Cluster2	I[VL]LTQ	LIGHT	I <u>L</u> L <u>T</u> Q	√
		LIGHT	I[VL]L <u>T</u> Q	√
Cluster2	[AV]ILS[AV]	LIGHT	[AV] <u>I</u> LS[AV]	√
Cluster3	PSVFIF	LIGHT	PSVF <u>I</u> F	√
		LIGHT	PSV <u>F</u> IF	√
		LIGHT	PS <u>V</u> FI	FI
Cluster3	TASVVCLLNNFY	LIGHT	<u>T</u> ASVVCLLNN <u>F</u> <u>Y</u>	SVVCLLNN
		LIGHT	<u>T</u> ASVVCLLNNFY	SVVCLLNNF
Cluster3	YSLSSVVTV	HEAVY	<u>Y</u> SLSSV <u>V</u> <u>T</u> <u>V</u>	√
Cluster3	GTQTYICNVN	HEAVY	GTQT <u>Y</u> ICNVN	√
		HEAVY	GTQ <u>T</u> <u>Y</u> ICNVN	√
		HEAVY	GTQ <u>T</u> Y <u>I</u> CNV	√
		HEAVY	<u>T</u> <u>Y</u> <u>T</u> <u>C</u>	√
Cluster4	A[TV]YYC	LIGHT	A <u>T</u> YYC	√
		LIGHT	A <u>T</u> <u>Y</u> YC	√
		LIGHT	A <u>Y</u> YYC	√
Cluster5	[TS]A[VIL]YYC	HEAVY	TA <u>V</u> YYC	YYC
		HEAVY	<u>S</u> A <u>V</u> YYC	√
		HEAVY	A <u>V</u> YYC	√
		HEAVY	<u>T</u> A <u>V</u> YYC	√
		HEAVY	TA <u>I</u> YYC	√
		HEAVY	<u>T</u> A <u>L</u> YYC	A <u>L</u> YY
Cluster5	G[TS][TL]VTVS[SA]	HEAVY	GT <u>L</u> V <u>T</u> VS[SA]	√
		HEAVY	GT <u>T</u> V <u>T</u> VS[SA]	√
		HEAVY	<u>T</u> LT <u>V</u> SS	√
		HEAVY	GS <u>L</u> V <u>T</u> VS[SA]	√
		HEAVY	GT <u>L</u> V <u>T</u> V <u>S</u>	√
BURIED	GCLVK	HEAVY	GCLVK	GCLVK
BURIED	W[IVL][GSA]	HEAVY	W[IVL][GSA]	W[IVL][GSA]

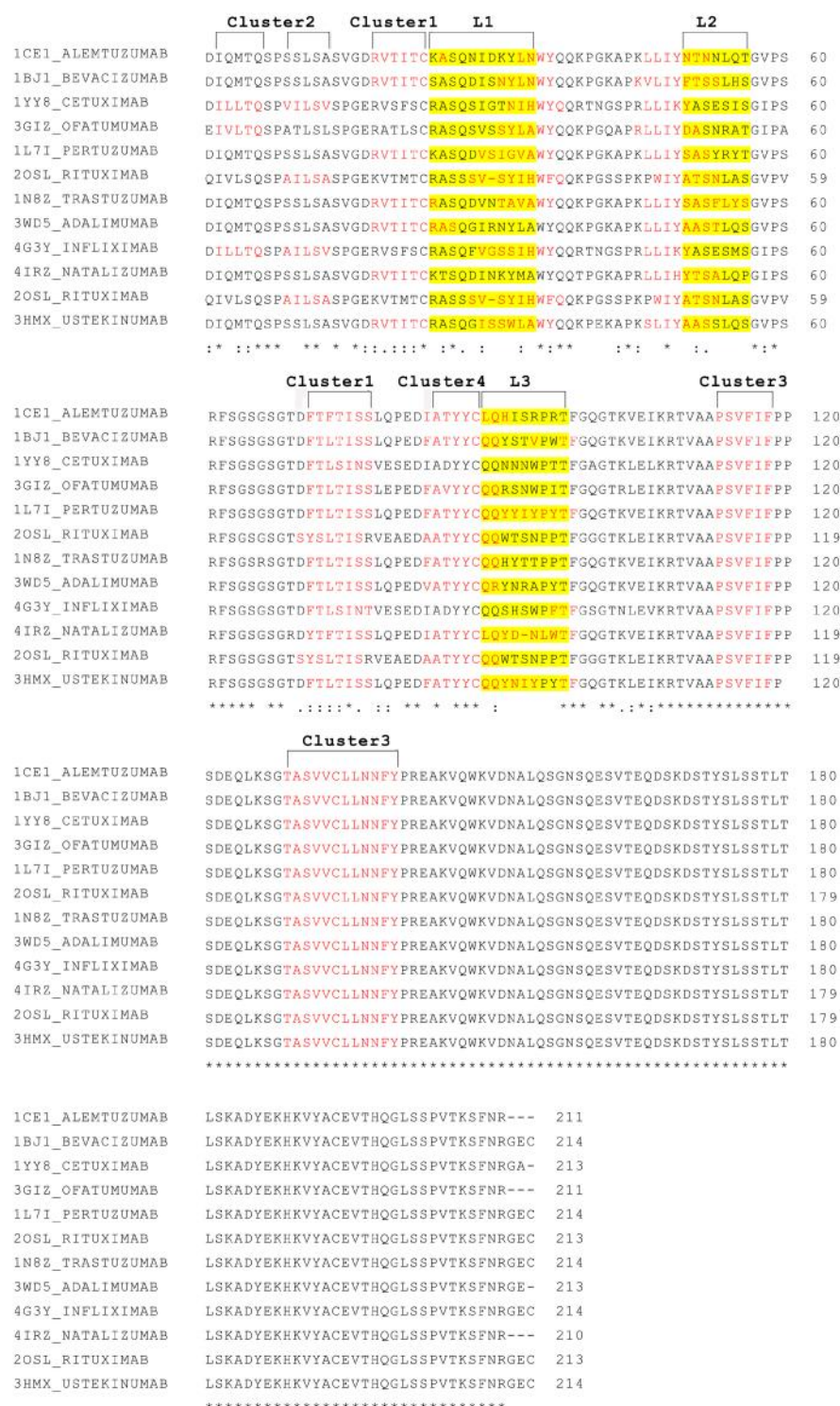


Fig. 1

Fig. 2

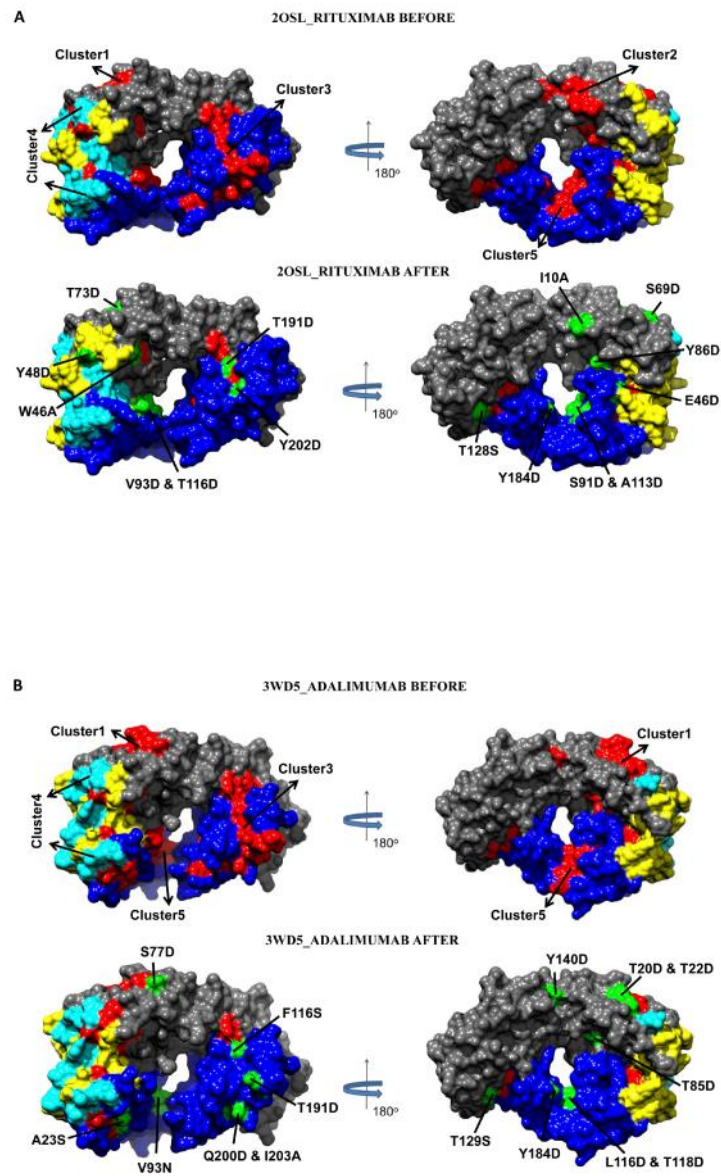


Fig. 3

Supplementary Material

S1. Monoclonal antibodies against cancer

Alemtuzumab [1] (CAMPATH®, developed by Genzyme Corp. under the name Campath-1H or LDP-03) is a recombinant DNA-derived humanized monoclonal IgG1-kappa antibody that is directed against the cell surface glycoprotein CD52 (21-28 kD) [2]. It is used against lymphocyte antigens and it consists of human variable framework, human constant regions and complementarity determining regions from a murine (rat) monoclonal antibody (Campath-1G). Alemtuzumab is used for the treatment of B-cell chronic lymphocytic leukemia (CLL) [3] [4] and multiple sclerosis [5].

Campath is used to treat leukemia by exploiting antibody mediated lysis of CD52 presenting cells. The CD52 antigen is a cell surface protein found on essentially all B and T lymphocytes, a majority of monocytes [6], macrophages and most granulocytes. In leukemia there is an excess of B and T cells, so Campath binds to the CD52 antigen and permits selective reduction of lymphocyte populations.

Bevacizumab [7] (AVASTIN®, developed by Genentech Inc. under the name 12-IgG1 or rhuMab-VEGF) is a recombinant humanized monoclonal IgG1 antibody that binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF). Bevacizumab consists of human framework regions and the complementarity determining regions of a murine antibody that binds to VEGF. It is used as part of combination therapy for metastatic colorectal cancer [8], HER2-negative metastatic breast cancer, glioblastoma [9] [10], non-small cell lung cancers [11], cervical carcinoma and renal cell carcinoma.

Bevacizumab is an antineoplastic agent that prevents or reduces the formation of blood vessels (angiogenesis) thereby preventing or reducing metastatic disease progressing [12]. Bevacizumab binds to VEGF and prevents the interaction of VEGF to its receptors (Flt-1 and KDR) on the surface of endothelial cells. This prevents blood vessel proliferation, endothelial cell proliferation and in response retardation of metastatic tumor growth occurs.

Cetuximab [13] (ERBITUX®, developed by Bristol-Myers Squibb, Eli Lilly, Merck & Co. Inc. under the name Fab C225 or IMC-225) is a chimeric monoclonal IgG1-kappa antibody that binds to Epidermal growth factor receptor (EGFR). Cetuximab is composed of the Fv regions of the 225 murine EGFR monoclonal antibody, specific for the N-terminal portion of human EGFR. It has human heavy and light chain constant regions. It is a widely used drug for the treatment of metastatic colorectal cancer [14], of head and neck cancer [15].

Used in the treatment of EGFR-expressing metastatic colorectal cancer, cetuximab binds specifically to the epidermal growth factor receptor (EGFR, HER1, c-ErbB-1) [16] on both normal and tumor cells. EGFR is over-expressed in many colorectal cancers. Cetuximab competitively inhibits the binding of epidermal growth factor (EGF) and other ligands, such as transforming growth factor–alpha (TGF alpha). Binding of cetuximab to the EGFR blocks phosphorylation and activation of receptor-associated kinases, resulting in inhibition of cell growth and thus in the elimination of metastatic spread so as to the induction of apoptosis, decreased matrix metalloproteinase secretion and reduced vascular endothelial growth factor production [17] [18].

Ofatumumab [19] (ARZERRA®, developed Genmab A/S under the name HuMax-CD20®) is a human monoclonal IgG1-kappa antibody, which is directed against MS4A1 (membrane-

spanning 4-domains subfamily A member 1, CD20). It is a widely used drug for the treatment of chronic lymphocytic leukemia (CLL) [20] and other types of cancer [21].

Ofatumumab inhibits early-stage B lymphocyte activation and it directs the body's immune system to fight normal and cancerous B-cells [22]. It binds to the CD20 molecule, which is found on the surface of B-cells [23], the type of cell which becomes cancerous in chronic lymphocytic leukemia (CLL). The CD20 molecule is also found on over 90% of B-cell lymphomas, as well as other lymphoid tumors of B-cell origin [24]. In these types of cancer where B-cells can over-proliferate, treatment is crucial to reduce their number.

Pertuzumab [25] (PERJETA®, developed by Genentech Inc. under the name OMNITARG™ or rhuMAB 2C4) is a recombinant humanized monoclonal IgG1-kappa antibody that targets the extracellular dimerization domain (Subdomain II) of the human epidermal growth factor receptor 2 protein (HER2). Pertuzumab is indicated for use in combination with trastuzumab and docetaxel for the treatment of patients with HER2-positive metastatic breast cancer who have not received prior anti-HER2 therapy or chemotherapy for metastatic disease [26].

Pertuzumab binds to the HER2 receptor and inhibits the ability of HER2 to interact with other HER family members (HER1, HER2, HER3, and HER4) on the surface of cancer cells [27]. The HER signaling pathway plays a role in the formation and growth of numerous cancers, and previous clinical trials of pertuzumab in a single agent setting had suggested clinical activity - including stable disease - in heavily pretreated patients with advanced ovarian and breast cancers.

Rituximab [28] (MABTHERA®, developed by Roche and RITUXAN®, developed by Biogen Idec and Genentech under the name IDEC-C2B8) is a genetically engineered chimeric

monoclonal IgG1-kappa antibody which is directed against the CD20 antigen, found on the surface of normal and malignant B lymphocytes. It contains murine light and heavy chain variable region sequences and human constant region sequences. Rituximab is a widely used drug for the treatment of rheumatoid arthritis (RA) [29], non-Hodgkin's lymphoma (NHL) [30] [31] and chronic lymphocytic leukemia (CLL) [32].

Rituximab binds to the CD20 antigen, which is predominantly expressed on mature B cells and on >90% of B-cell non-Hodgkin's lymphomas. The antibody leads to selective killing of B-cells. The Fab regions of Rituximab bind to the CD20 antigen on B lymphocytes, while the Fc domain recruits antibodies and complements to mediate cell lysis [33].

Trastuzumab [34] (HERCEPTIN®, developed by Genentech Inc. under the name 4D5V8 or Herceptin) is a recombinant humanized monoclonal IgG1-kappa antibody that selectively binds with high affinity in a cell-based assay to the extracellular domain of the human epidermal growth factor receptor (EGFR) protein. Trastuzumab is a widely used drug for the treatment of early stage HER2-positive breast cancer [35], metastatic breast cancer that substantially overexpresses HER2 and gastric cancers [36].

HER2 protein's overexpression is observed in 25%-30% of primary breast cancers [37]. Trastuzumab has been shown, both in vitro assays and in animals, to inhibit the proliferation of human tumor cells that overexpress HER2. It is a mediator of antibody dependent cellular cytotoxicity (ADCC), in that the binding of the antibody to HER2 overexpressing cells leads to preferential cell death [38]

S2. Monoclonal antibodies against autoimmune diseases

Adalimumab [39] (HUMIRA®, developed by AbbVie Inc. under the name D2E7) is a human monoclonal IgG1-kappa antibody against the human tumor necrosis factor-alpha (TNF-α). It is produced by recombinant DNA technology using a mammalian cell expression system. Adalimumab is a widely used drug for the treatment of rheumatoid arthritis (RA) [40], psoriatic arthritis [41], ankylosing spondylitis [42], psoriasis [43], ulcerative colitis [44], juvenile idiopathic arthritis [45] and Crohn's disease [46].

Adalimumab binds specifically to TNF-α and blocks its general cytokine effects, thereby reducing TNF-induced inflammation and halting tissue destruction. More precisely, it binds to TNF-α and blocks its interaction with the p55 and p75 cell surface TNF receptors. Adalimumab also lyses surface TNF expressing cells *in vitro* in the presence of complement.

Infliximab [47] (REMICADE®, developed by Janssen Biotech Inc. under the name cA2, TA-650) is a chimeric monoclonal IgG1-kappa antibody which is directed against the human tumor necrosis factor-alpha (TNF-α). It is composed of human constant and murine variable regions. Infliximab is a widely used drug for the treatment of Crohn's disease [48] and ulcerative colitis [49]. It is also used to manage signs and symptoms of rheumatoid arthritis [50], ankylosing spondylitis [51], psoriatic arthritis [52], psoriasis and juvenile idiopathic arthritis [53].

Infliximab binds with high affinity to the soluble and transmembrane forms of TNF-α and inhibits binding of TNF-α with its receptors, which leads to an overall reduction in inflammation [54]. This reduces production of pro-inflammatory cytokines such as interleukins (IL1 & IL6). This also limits leukocyte migration and expression of adhesion molecules by

endothelial cells and leukocytes. Infliximab does not neutralize TNF-beta (lymphotoxin a), a related cytokine that utilizes the same receptors as TNF-alpha.

Natalizumab [55] (TYSABRI®, developed by Biogen Idec under name AN100226) is a humanized monoclonal IgG4-kappa antibody, which is directed against the integrin alpha4 subunit (ITGA4). It is produced in murine myeloma cells, it contains human framework regions and the complementarity determining regions of a murine antibody that binds to α 4-integrin. Natalizumab was voluntarily withdrawn from the U.S. market because of risk of progressive multifocal leukoencephalopathy (PML). It was returned to the market in July, 2006. It is a widely used drug for the treatment of multiple sclerosis [56] and Crohn's disease [57].

A4-integrin is required for white blood cells to move into organs, therefore, natalizumab prevents these immune cells from crossing blood vessel walls to reach affected organs thereby decreasing inflammation. Natalizumab binds to the α 4-subunit of α 4b 1 and α 4b 7 integrins expressed on the surface of all leukocytes except neutrophils, and inhibits the α 4-mediated adhesion of leukocytes to their counter-receptor(s).

Rituximab (see section S1)

Ustekinumab [58] (STELARA®, developed by Janssen Biotech Inc. under the name CNTO 1275, TT-20) is a laboratory-manufactured, human monoclonal IgG1-kappa antibody which is directed against the interleukin 12 beta (IL12B) and the interleukin 23 (IL-23). Ustekinumab is a widely used drug for the treatment of psoriasis [59], Crohn's disease [60], multiple sclerosis [61] and psoriatic arthritis [62].

Ustekinumab is designed to interfere with the triggering of the body's inflammatory response through the suppression of certain cytokines. Specifically, it blocks interleukin IL-12 and IL-23 (via the p40 subunit of IL-12 and IL-23), which help activate certain T-cells.

Table S1. Monoclonal antibodies against cancer used in this study.

	MAbs	Trade Name	Source	Type	Target	PDB Id	Clinical Indications	FDA Approval	Clinical Trials
1	Alemtuzumab	CAMPATH®	Humanized	IgG1-kappa	CD52	1CE1, 1BEY	Chronic lymphocytic leukemia	2001	Phase M
							Multiple sclerosis	2013	Phase M
							Kidney transplant rejection		Phase I/II
2	Bevacizumab	AVASTIN®	Humanized	IgG1-kappa	VEGF-A	1BJ1	Colorectal cancer	2004	Phase M
							Lung cancer	2006	Phase M
							Breast cancer	2008	Phase M
							Renal cell carcinoma	2009	Phase M
							Glioblastoma	2011	Phase M
							Cervical carcinoma	2014	Phase M
3	Cetuximab	ERBITUX®	Chimeric	IgG1-kappa	EGFR	1YY8, 1YY9	Colorectal cancer	2004	Phase M
4	Ofatumumab	ARZERRA®	Human	IgG1-kappa	CD20	3GIZ	Chronic lymphocytic leukemia	2009	Phase M

							Non-Hodgkin's lymphoma		Phase III
5	Pertuzumab	PERJETA®	Humanized	IgG1-kappa	ERBB2	1L7I, 1S78	Breast cancer	2012	Phase M
							Prostate cancer		Phase II
6	Rituximab	RITUXAN®	Chimeric	IgG1-kappa	CD20	2OSL	Non-Hodgkin's lymphoma	1997	Phase M
							Chronic lymphocytic leukemia	2010	Phase M
7	Trastuzumab	HERCEPTIN®	Humanized	IgG1-kappa	ERBB2	1N8Z	Breast cancer	1998	Phase M
							Gastric cancer	2010	Phase M

4	Rituximab	RITUXAN® MABTHERA®	Chimeric	IgG1-kappa	CD20	2OSL	Rheumatoid arthritis	2006	Phase M
5	Ustekinumab	STELARA®	Human	IgG1-kappa	IL12A IL12B	3HMX 3HMW	Psoriasis	2009	Phase M
							Psoriatic arthritis	2013	Phase M
							Multiple sclerosis		Phase II
							Crohn's disease		Phase II

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