ELSEVIER

Critical Reviews in Oncology/Hematology 64 (2007) 210–225

CRITICAL REVIEWS IN Oncology Hematology Incorporating Geriatric Oncology

www.elsevier.com/locate/critrevonc

Structure–function relationships of the variable domains of monoclonal antibodies approved for cancer treatment

Charlotte Magdelaine-Beuzelin[a,b], Quentin Kaas[c], Vanessa Wehbi[a], Marc Ohresser[a], Roy Jefferis[d], Marie-Paule Lefranc[c], Hervé Watier[a,b,*]

[a] *Université François Rabelais de Tours, EA 3853 "Immuno-Pharmaco-Génétique des Anticorps thérapeutiques", France*
[b] *CHRU de Tours, Laboratoire d'Immunologie, France*
[c] *IMGT, Laboratoire d'ImmunoGénétique Moléculaire, LIGM, Institut de Génétique Humaine, UPR CNRS 1142, Montpellier, France*
[d] *Immunity and Infection, University of Birmingham, United Kingdom*

Accepted 20 April 2007

Contents

Abstract

Due to their exquisite specificity for a given epitope on the target antigen, recombinant monoclonal antibodies (rmAb) can deliver "targeted therapy" in oncology. This review focuses on the structural bases of "antigen specificity" to aid clinical researchers and pharmacologists in managing these new drugs. The fine structure of the Fv (Fragment variable) module (combination of VH and VL domains) from the five unconjugated antibodies currently approved for cancer treatment, namely rituximab, cetuximab, alemtuzumab, trastuzumab and bevacizumab, is presented and analysed. Co-crystal and functional studies are reviewed to define rmAb residues contributing to antigen binding site (paratope)–epitope interfaces. The genetic origin of these recombinant monoclonal antibodies, determined through the IMGT/3Dstructure-DB database and IMGT/V-QUEST (http://imgt.cines.fr), is presented, allowing the evaluation of homologies between antibodies and their closest germline human counterparts and hence their possible immunogenicity. Overall, the IMGT standards appear as a first and crucial step in the evaluation of recombinant antibodies.

Keywords: Therapeutic antibodies; Variable domain; Paratope; Epitope; Oncology; Cancer; Rituximab; Cetuximab; Alemtuzumab; Trastuzumab; Bevacizumab; Monoclonal antibody; IMGT

There is an increase pressure in oncology to develop targeted therapies that avoid the numerous side effects associated with conventional chemotherapy. Recombinant monoclonal antibodies (rmAbs) and tyrosine kinase inhibitors (TKI) are the two main classes of drug with potential to provide such therapeutics. TKI are small molecule

* Corresponding author at: Laboratoire d'Immunologie, Faculté de Médecine, 10 boulevard Tonnellé, 37032 Tours Cedex, France.
Tel.: +33 247 47 38 74; fax: +33 234 38 94 12.
E-mail address: watier@med.univ-tours.fr (H. Watier).

1040-8428/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.critrevonc.2007.04.011

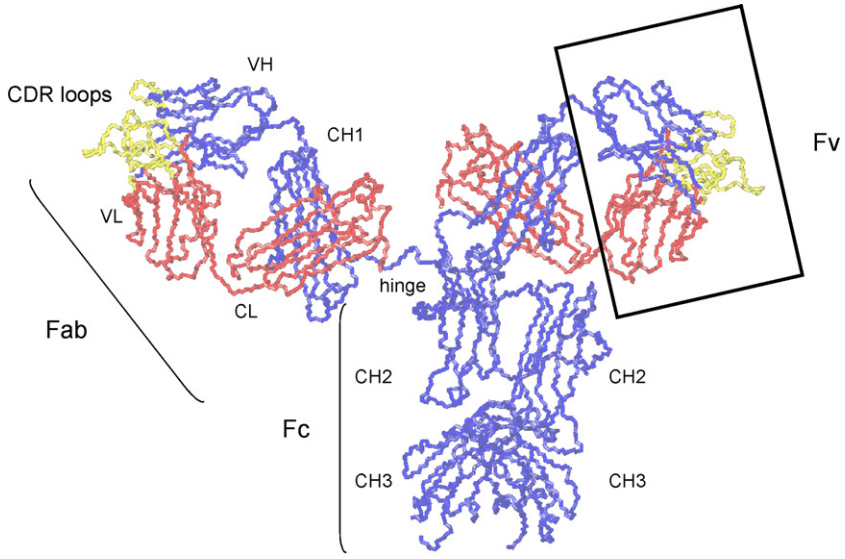


Fig. 1. Three-dimensional structure of a human IgG1κ. Alpha carbon backbone representation of the monoclonal antibody b12 directed against the HIV envelope glycoprotein gp210, the first and up-to-now the only structure of an entire human IgG1κ resolved by crystallography [8] (PDB and IMGT/3Dstructure-DB, http://imgt.cines.fr: 1hzh). Figure generated with VMD software [9]. The two identical γ1 heavy chains are indicated in blue and the two identical κ light chains in red, which are linked together with disulfide bonds. The four polypeptide chains are folded into domains: one variable domain (VH) and three constant domains (CH1, CH2, CH3) for the γ heavy chain with the hinge region between CH1 and CH2, and one variable domain (VL) and one constant domain (CL) for the light chain (for κ chain: V-KAPPA and C-KAPPA) (for review [7]). The three arms of the antibody correspond to two Fab (Fragment antigen binding) and one Fc (Fragment crystallized). Each Fab carries one antigen binding site or paratope (CDR loops in yellow) at the top of the Fv (Fragment variable) module (association of the VH and VL, here V-KAPPA, domains). In constrast to schematic, symmetric and fixed Y-shaped representations usually presented in textbooks, this crystal structure illustrates the asymmetry of the antibody, which results from the high degree of freedom of its three arms around the inter-heavy chain disulfide bridges in the hinge region. It allows the molecule to be very flexible: each Fab, and therefore each Fv, may rotate and bend.

drugs that act intracellularly whereas rmAbs are large biomolecules (biopharmaceuticals, *i.e.* recombinant proteins) acting extracellularly, either on membrane bound antigens or antigens of the tumour microenvironment. TKI are not entirely selective for their molecular target as they display some degree of cross-inhibition towards other tyrosine kinase receptors [1,2]; rmAbs are specific for the targeted antigen, which may be expressed on normal tissue in addition to tumour cells. Specificity is the defining characteristic of antibodies due to the various selection processes operative in the development of humoral immune responses. Similarly, rmAbs developed from combinatorial libraries are subject to selection for specificity and increased affinity by *in vitro* techniques that mirror molecular mechanisms operating *in vivo*.

There are currently five unconjugated rmAbs approved for cancer treatment, namely rituximab, cetuximab, alemtuzumab, trastuzumab and bevacizumab, and there is increasing understanding of their mechanism(s) of action, *in vitro* and *in vivo* [3–6]. These rmAbs were generated by molecular engineering with selection for specificity and affinity; however, three-dimensional (3D) structural data have now been published for four of them that allow a more detailed analysis of the structural features that account for specificity. The present review focuses on the structure of the variable domains of these therapeutic antibodies, in relation with their high specificity and affinity for a given epitope on the target antigen. Comparison of the V-REGION sequences of chimeric and humanized antibodies with those of human sequences allows insights into possible correlations with observed immunogenicity. These insights will inform for the future development of new antibody specificities.

1. Chimeric and humanized antibodies

Technologies for the generation of full human rmAbs have been developed relatively recently and no fully human rmAb has been approved for cancer treatment to-date, however, several are currently in phase III trials.[1] The five unconjugated therapeutic antibodies currently in use in oncology, are rmAbs constructed from genes encoding murine or rat mAb selected for their specificity and based on the format of a human IgG1κ, *i.e.* the *IGHG1* and *IGKC* genes encoding the constant region of the γ1 heavy chains and of the κ light chains, respectively, are from human, to decrease their potential immunogenicity. Each has unique VH and VL (V-KAPPA) sequences that "pair" to generate unique Fv (Fragment variable) modules (for review [7]) (Fig. 1).

[1] A fully human antibody, panitumumab (Vectibix®), a human IgG2 kappa rmAb (ABX-EGF) that binds to the human EGFR received FDA approval on 27 September 2006 (after submission of this article). In the absence of 3D structure, the conclusions on the antibody/antigen interactions remains unchanged. Information on the panitumumab sequences and IMGT Collier de Perles will be provided, when available, in The IMGT Biotechnology page, Monoclonal antibodies with clinical indications, http://imgt.cines.fr.

Table 1
Presentation of the six rmAbs used in oncology

rmAbs	Main indication	Date of FDA approval[a], EMEA approval[b]	Patent number (available on http://ep.espacenet.com)
Chimeric rmAbs			
cetuximab (Erbitux®)	Treatment of patients with advanced colorectal cancer that has spread to other parts of the body	12 February 2004, 29 June 2004	[49]
rituximab (MabThera®, Rituxan®)[c]	Treatment of patients with relapsed or refractory low-grade or follicular, B cell non-Hodgkin's lymphoma	26 November 1997, 2 June 1998	[50]
Humanized rmAbs			
alemtuzumab (MabCampath®)	Treatment of patients with B cell chronic lymphocytic leukemia who have been treated with alkylating agents and who have falled fludarabine therapy	5 July 2001, 6 July 2001	[51]
bevacizumab (Avastin®)	First-line treatment for patients with metastatic colorectal cancer	26 February 2004, 12 January 2005	[52]
trastuzumab (Herceptin®)	Treatment of patients with metastatic breast cancer whose tumours overexpress the HER-2 protein and who have received one or more chemotherapy regimens for their metastatic disease	25 September 1998, 28 August 2000	[53]
Fully human rmAb			
panitumumab (Vectibix®)	Treatment of EGFR-expressing, metastatic colorectal carcinoma with disease progression on or following fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens	27 September 2006	[54]

Indications, date of approval and patent number.
[a] FDA: U.S. Food and Drugs Administration (http://www.fda.gov).
[b] EMEA: European Medicines Agency (http://www.emea.eu.int/).
[c] Rituxan® in USA, Canada and Japan, MabThera® in the remaining world.

Depending on the homology of the Fv sequences with human sequences the rmAbs are described as "chimeric" or "humanized". In "chimeric" rmAbs, the Fv is entirely non-human in origin, *e.g.* mouse for cetuximab and rituximab. In "humanized" rmAbs the complementarity determining regions (CDR), which confer specificity, are of non-human origin (rat or mouse) and replace the CDRs of a human Fv, *i.e.* the framework regions (FR) are of human origin, *e.g.* alemtuzumab, bevacizumab and trastuzumab (Table 1). Besides CDR grafting, more recent humanization technologies have been also developed, such as resurfacing or human engineering [10].

A schematic representation of a Fv is shown in Fig. 2. During the *in vivo* synthesis of an immunoglobulin (IG) in a B cell, the VL domain (here, V-KAPPA) or V–J-REGION results from the rearrangement of a V gene (IGKV) to a J gene (IGKJ), the VH domain or V–D–J-REGION results from the rearrangement of a IGHV gene, a IGHD gene and a IGHJ gene. In addition to the combinatorial diversity created by these gene rearrangements, the VH V–D–J junction diversity is considerably increased by the N-diversity which results from the trimming of the V-, D- and J-REGION ends to be joined and the random addition of nucleotides by the terminal deoxynucleotidyltransferase (TdT) [7]. Further diversity is introduced within a secondary immune response by somatic hypermutation of VH and VL sequences. Sequence diversity is particularly focused at the V–J and V–D–J junctions [11] that generate the CDR3 hypervariable regions that have a major role in determining antibody specificity forming contacts with the epitope [12] (Fig. 2). Whatever the species origin, there is no global structural differences in the V domain structure. Therefore, the primary amino acid

Fig. 2. Structure of the Fv of bevacizumab. (A) Linear representation of the two domains V-KAPPA and VH which form the Fv of bevacizumab. The V-KAPPA (V–J-REGION) results from the rearrangement of a IGKV gene and a IGKJ gene. The VH (V–D–J-REGION) results from the rearrangement of a IGHV gene, a IGHD gene and a IGHJ gene. N1 and N2 indicate N-REGIONs not encoded in the germline genes and which result from the N-diversity mechanism at the V–D–J junction (for review [7]). Framework regions (FR) and complementarity determining regions (CDR) alternate. FR and CDR are delimited according to the IMGT unique numbering for V-DOMAIN [13]. The number of amino acid residues is indicated below each FR and CDR. (B) Lateral and (C) top view of the alpha carbon backbone representation of the Fv of bevacizumab [14] (PDB and IMGT/3Dstructure-DB: 1cz8). CDR-IMGT are coloured according to the IMGT colour menu. VH CDR1-IMGT (red), CDR2-IMGT (orange) and CDR3-IMGT (purple); V-KAPPA CDR1-IMGT (blue), CDR2-IMGT (green) and CDR3-IMGT (green blue). The N (cyan) and C (magenta) ends of each region are also coloured. The conserved C23, W41 and L89, and the six FR-IMGT positions limiting the CDR-IMGT regions, also designated as anchor positions (positions 26 and 39 for the CDR1-IMGT, 55 and 66 for the CDR2-IMGT, 104 and 118 for the CDR3-IMGT) are shown in white. Nine antiparallel beta strands (in grey) constitute the FR-IMGT. The folding drives the formation of the CDR-IMGT loops which are localised at the top of the Fv and form the antigen binding site or paratope The lengths of the CDR-IMGT are indicated between brackets separated by dots, figures are generated using VMD software [9].

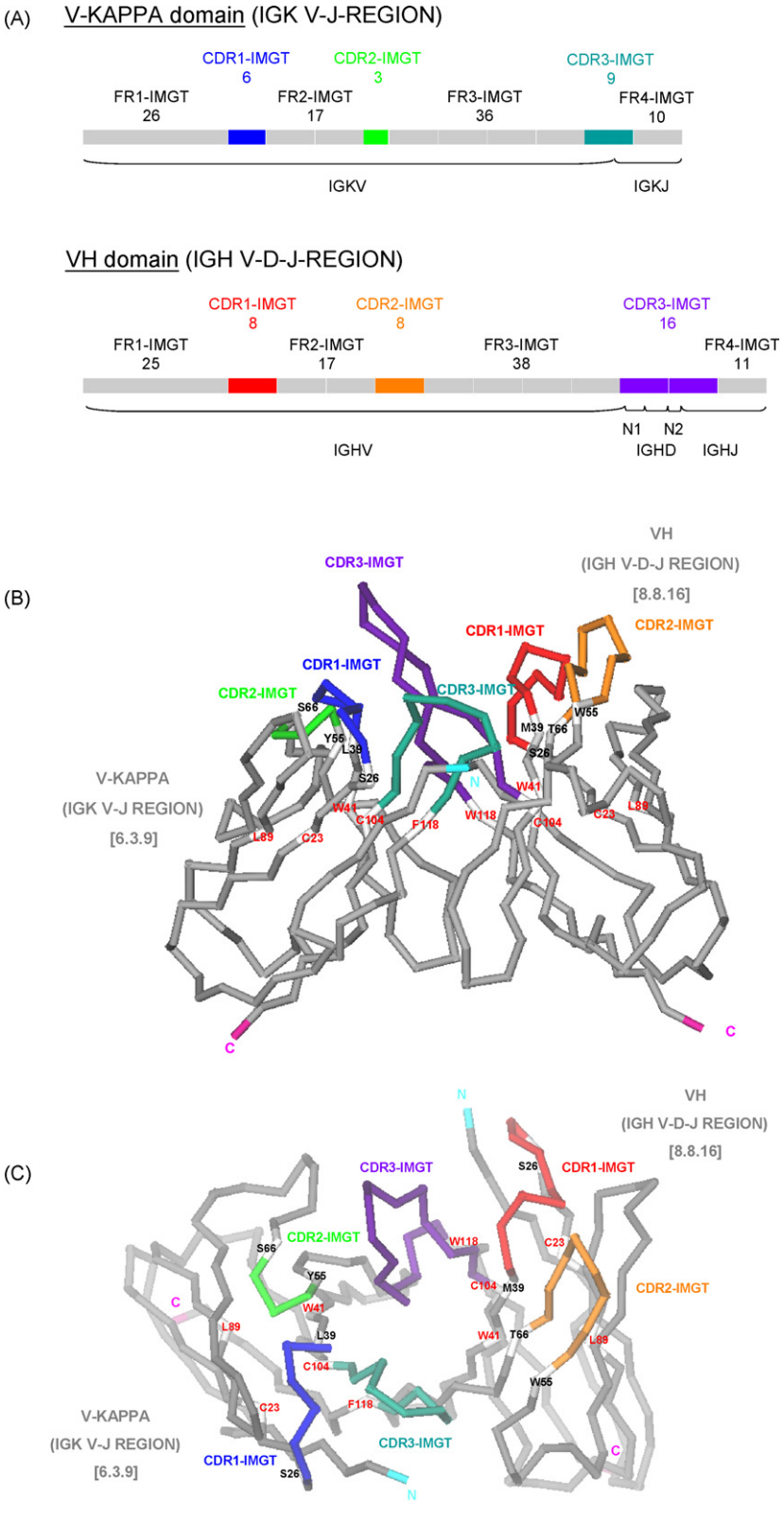
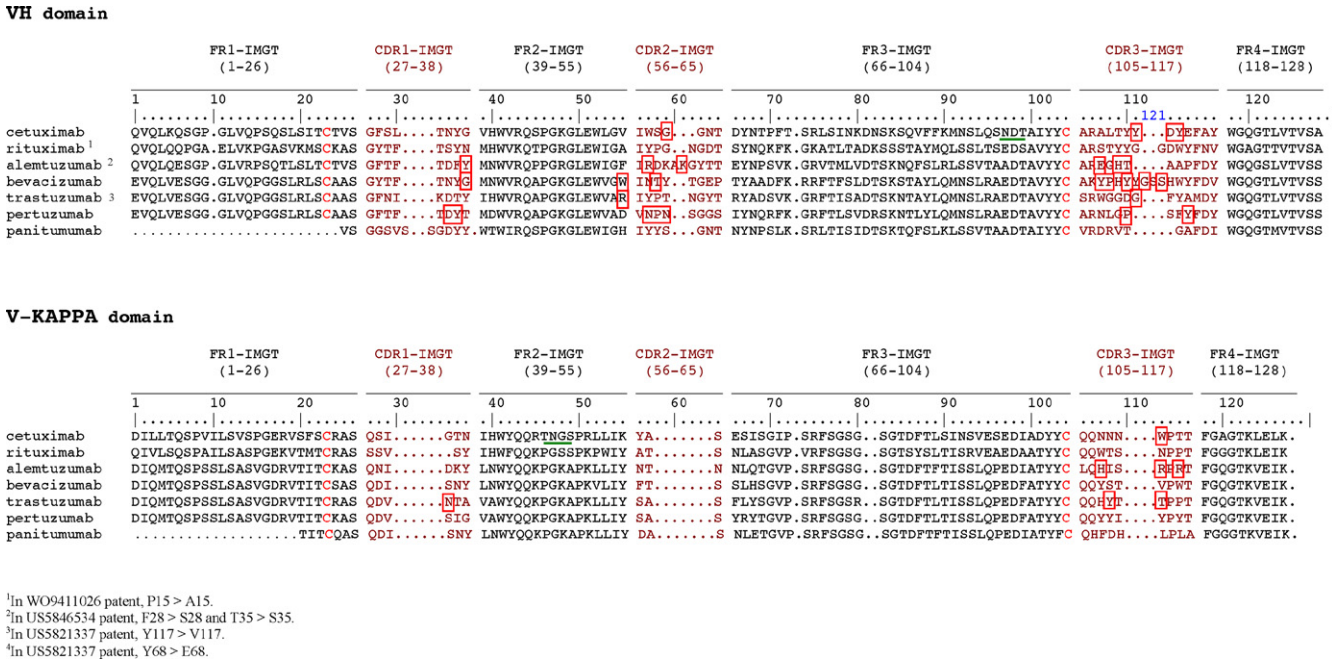

(A)
V-KAPPA domain (IGK V-J-REGION)
CDR1-IMGT 6
CDR2-IMGT 3
CDR3-IMGT 9
FR1-IMGT 26
FR2-IMGT 17
FR3-IMGT 36
FR4-IMGT 10
IGKV
IGKJ
VH domain (IGH V-D-J-REGION)
CDR1-IMGT 8
CDR2-IMGT 8
CDR3-IMGT 16
FR1-IMGT 25
FR2-IMGT 17
FR3-IMGT 38
FR4-IMGT 11
N1
N2
IGHV
IGHD
IGHJ
(B)
VH
(IGH V-D-J REGION)
[8.8.16]
CDR3-IMGT
CDR1-IMGT
CDR2-IMGT
V-KAPPA
(IGK V-J REGION)
[6.3.9]
(C)
VH
(IGH V-D-J REGION)
[8.8.16]
V-KAPPA
(IGK V-J REGION)
[6.3.9]

Fig. 2.



[1]In WO9411026 patent, P15 > A15.
[2]In US5846534 patent, F28 > S28 and T35 > S35.
[3]In US5821337 patent, Y117 > V117.
[4]In US5821337 patent, Y68 > E68.

Fig. 3. IMGT protein displays of the VH and V-KAPPA domains of the cetuximab, rituximab, alemtuzumab, bevacizumab, trastuzumab, pertuzumab and panitumumab antibodies. Amino acid sequences are from 1yy9 (cetuximab), AX556949 and AX556951 (rituximab VH and V-KAPPA), 1ce1 (alemtuzumab), 1cz8 (bevacizumab), 1n8z (trastuzumab), 1s78 (pertuzumab), and DD214915 and DD214916 (panitumumab). Amino acid differences with patents from Table 1 are indicated. FR-IMGT and CDR-IMGT delimitations and gaps, represented by dots, are defined according to the IMGT unique numbering for V-DOMAIN [13]. The *N*-glycosylation site motifs in the cetuximab VH and V-KAPPA are underlined but only the VH motif is glycosylated (see text). Amino acids identified in IMGT/3Dstructure-DB [22] as having hydrogen bonds with the antigens (EGFR for cetuximab, CD52 mimotope for alemtuzumab, VEGF for bevacizumab, ERBB2 for trastuzumab and pertuzumab) (Table 2) are shown in boxes. The numbers 1 2 1 in the VH CDR3-IMGT numbering correspond to the additional positions 111.1, 112.2 and 112.1, respectively [13].

sequences of the VH and V-KAPPA of the five unconjugated anti-cancer rmAbs can be aligned and are displayed in Fig. 3 using the IMGT unique numbering and definition of the FR-IMGT and CDR-IMGT [13].

In a "chimeric" antibody (*e.g.* rituximab and cetuximab), the original specificity of the murine antibody is conserved since the murine variable domains are unmodified. In a "humanized" antibody (*e.g.* alemtuzumab, bevacizumab and trastuzumab), only the CDRs of the original rat or murine antibody are retained, being grafted into human framework gene sequences, thus conferring the specificity of the original rat or murine antibody to a human antibody. Humanization was introduced in order to reduce the potential immunogenicity of the rat or murine variable domains [15]. When designing a humanized antibody it is important to select human VH and VL framework regions with highest homology to the original rat or murine ones in order to maintain the 3D orientation of the CDR loops [16,17]. This is usually achieved by comparing the sequences of the variable domains of the selected rat or murine monoclonal antibody to human sequences and 3D structures available in databases. It should be emphasized that the three humanized antibodies were designed using the Kabat system for defining the CDRs [18] before the CDR-IMGT standardization was available [13,19,20]. As a consequence the initial humanized form of the Campath antibody lacked specificity and affinity, therefore alemtuzumab was "designed" with mutations at two positions [21] which were thought to be FR but which clearly

Fig. 4. IMGT Colliers de Perles of the VH and V-KAPPA domains of the alemtuzumab, bevacizumab, cetuximab, trastuzumab, rituximab and pertuzumab antibodies. IMGT Colliers de Perles are shown on one layer and two layers. Amino acids are shown in the one-letter abbreviation. Positions at which hydrophobic amino acids (hydropathy index with positive value: I, V, L, F, C, M, A) and tryptophan (W) are found in more than 50% of analysed sequences are shown in blue. All proline (P) are shown in yellow. The CDR1-IMGT (BC loop), CDR2-IMGT (C′C″ loop) and CDR3-IMGT (FG loop) are limited by amino acids shown in squares (anchor positions), which belong to the neighbouring strands (FR-IMGT in V-DOMAINs). Arrows indicate the direction of the beta strands and their designations in 3D structures (from IMGT Repertoire, http://imgt.cines.fr) [25]. For the VH domains, CDR1-IMGT is in red, CDR2-IMGT in orange and CDR3-IMGT is in purple. For the VL domains (here V-KAPPA), CDR1-IMGT is in blue, CDR2-IMGT in green and CDR3-IMGT is in green blue [25]. The IMGT Colliers de Perles on two layers show, on the forefront, the GFCC′C″ strands and, on the back, the ABED strands. Hatched circles correspond to missing positions according to the IMGT unique numbering for V-DOMAIN [13]. IMGT Colliers de Perles are from IMGT/3Dstructure-DB entries (chain entry identifiers are shown between parentheses), or obtained, for rituximab whose 3D structure is not available, using the IMGT/Collier de Perles tool at the IMGT/3Dstructure-DB Query page [22]. Amino acid one-letter abbreviation: A (Ala), alanine; C (Cys), cysteine; D (Asp), aspartic acid; E (Glu), glutamic acid; F (Phe), phenylalanine; G (Gly), glycine; H (His), histidine; I (Ileu), isoleucine; K (Lys), lysine; L (Leu), leucine; M (Met), methionine; N (Asn), asparagine; P (Pro), proline; Q (Gln), glutamine; R (Arg), arginine; S (Ser), serine; T (Thr), threonine; V (Val), valine; W (Trp), tryptophan; Y (Tyr), tyrosine.

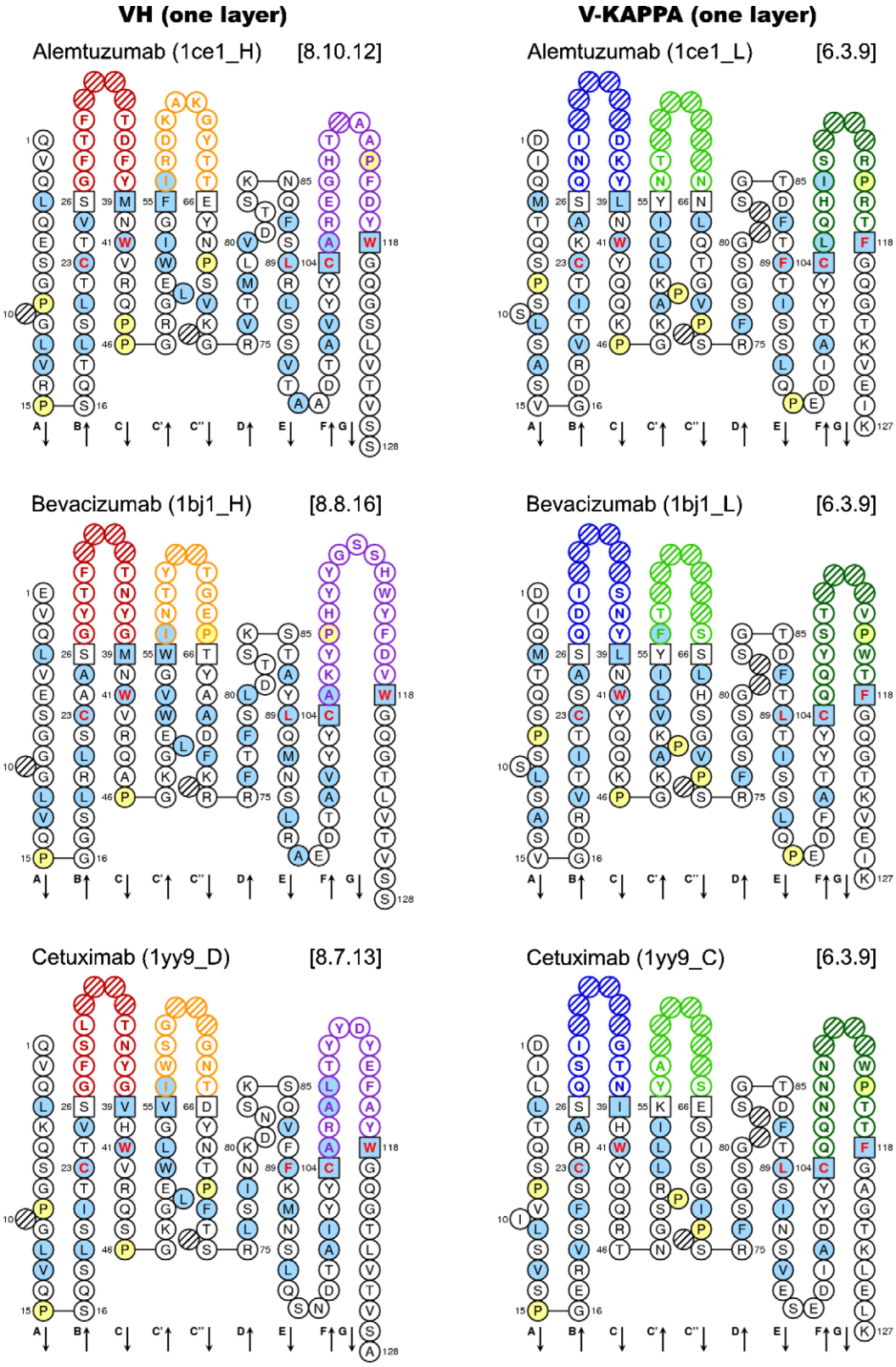


Fig. 4. (*Continued*)

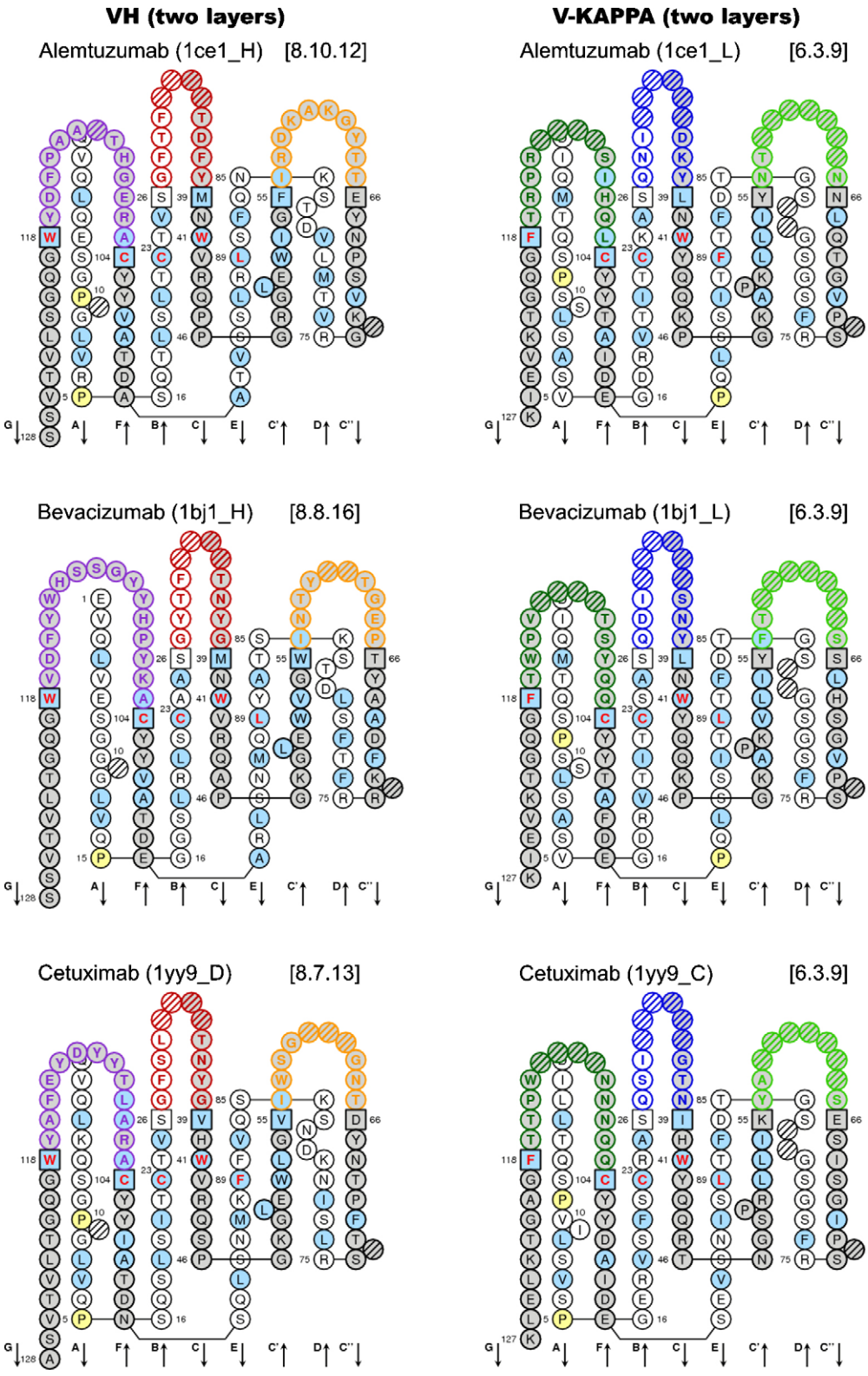


Fig. 4. (*Continued*)

belong to the CDR1-IMGT (positions 28 and 35 according to the IMGT unique numbering for V-DOMAIN [13]) (Fig. 3). Moreover, positions 66–74 of VH and positions 66–69 of V-KAPPA were grafted from rat onto human genes [15] as they were thought to be CDR but they clearly belong to the FR-IMGT, and keeping these positions as "human" in rmAbs would probably diminish their potential immunogenicity (for sequences of the original rat and humanized Campath vari-

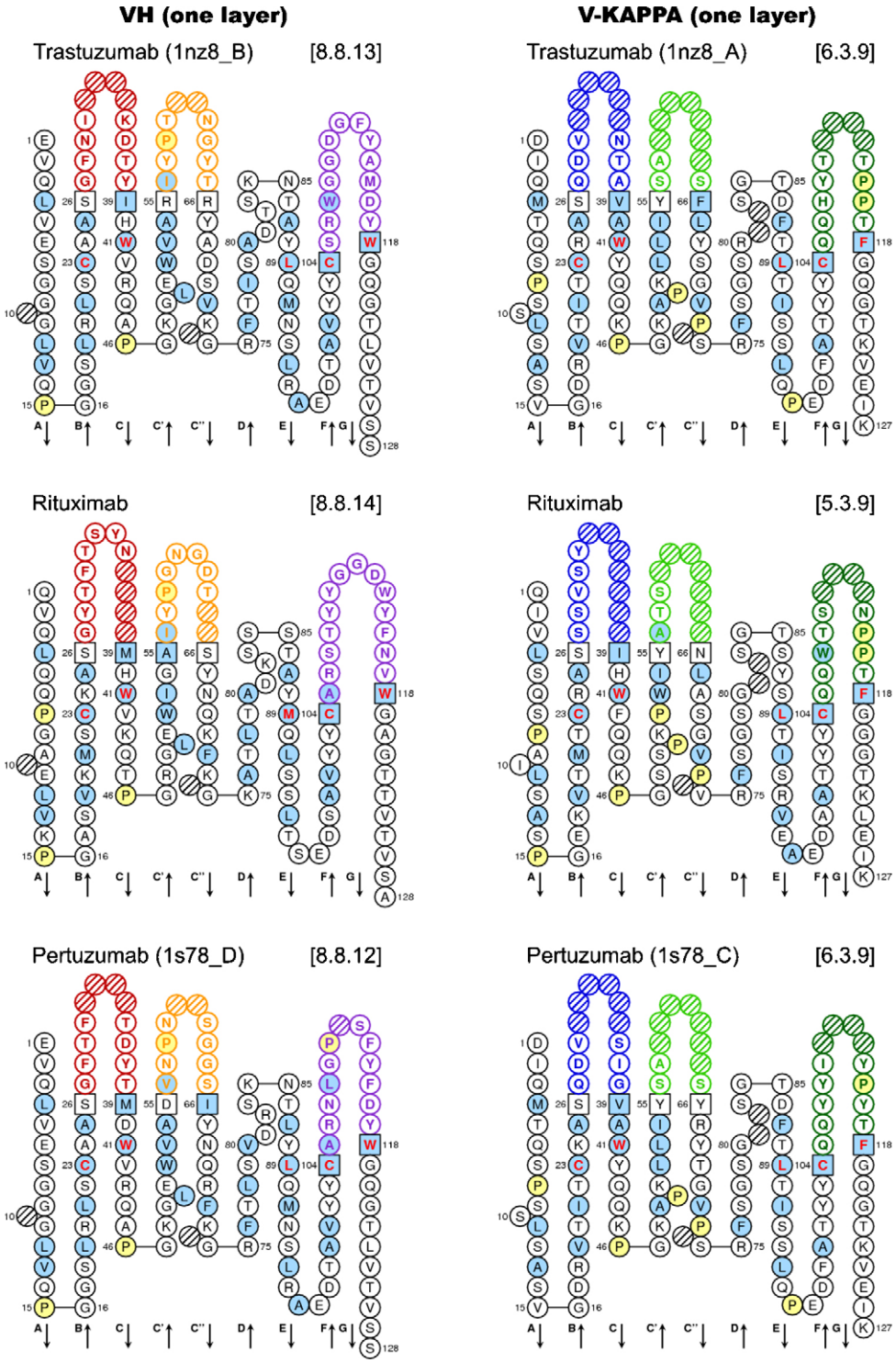


Fig. 4. (*Continued*)

able domains, see "Antibody humanization" in The IMGT Biotechnology page, http://imgt.cines.fr). This observation is confirmed by the fact that alemtuzumab can be captured by an anti-rat IgG specific antibody in an ELISA, not supposed to recognise the alemtuzumab idiotype beared by the CDRs [23].

2. IMGT Collier de Perles

The IMGT Colliers de Perles are two-dimensional (2D) standardized graphical representations of amino acid sequences of domains [13,24], and more particularly of V-DOMAINs [13,25]. The IMGT Colliers de Perles of the

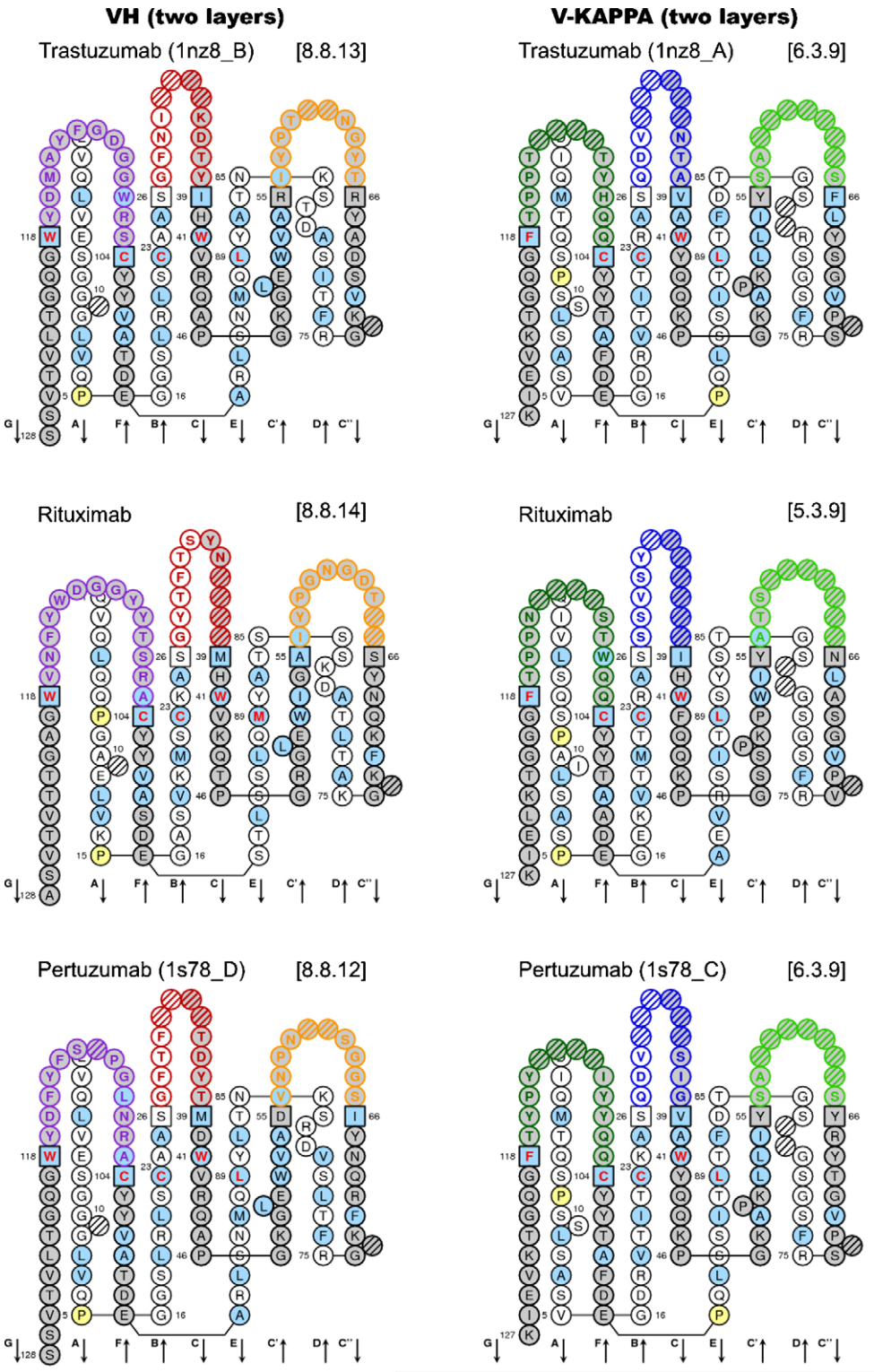


Fig. 4. (*Continued*).

VH and V-KAPPA of the five rmAbs are shown in Fig. 4. The IMGT Collier de Perles on one layer allows to readily define the FR-IMGT beta strands and the CDR-IMGT loops in the amino acid sequence of the variable domains. This emphasizes the usefulness of the IMGT Collier de Perles in designing humanized antibodies since the CDR-IMGT loops are the regions which need to be considered for the humanization. The CDR1-IMGT and CDR2-IMGT anchor positions need also to be checked as they determine the orientation of the CDR loops. The IMGT Collier de Perles on

two layers shows the relative localisation of the strands and loops in space even if 3D structures are not yet available (for example, rituximab). For known 3D structures, they allow the display of hydrogen bonds in IMGT/3Dstructure-DB, http://imgt.cines.fr, and they give access to the contacts of each amino acid (IMGT Residue@Position card) by clicking on a given position in the IMGT Collier de Perles [22].

Glycosylation sites are observed in ∼15% of normal human V regions, therefore, it is of interest to note that both the VH and V-KAPPA sequences of the cetuximab include a glycosylation site motif [N-X-T/S] (where X is any amino acid except proline) whereas none is observed in the other rmAbs (Fig. 3). The two motifs are opposite to the CDR loops. The motif localised at position 97 (at the tip of the EF loop) in the cetuximab VH is glycosylated and bears a complex diantennary oligosaccharide structure containing a very diverse mixture of glycans as usually observed for other rmAbs at the Fab domain [26], however, the motif at position 47 (at the CC′ beta turn) in the V-KAPPA is unoccupied (Jefferis R, et al., unpublished data). Indeed, glycosylation is only effective in two thirds of potential *N*-glycosylation sites. When present in Fv regions, *N*-glycosylation has been reported to have a positive, negative or neutral impact on specificity, affinity, stability, solubility, etc. [27]. In the particular case of cetuximab, VH *N*-glycosylation probably does not interfere with antigen binding site since it is localised at the opposite side of the paratope, but may contribute to the immunogenicity of framework regions of this antibody since cetuximab is produced in rodent cells and could bear terminal residues (*e.g.* Galα(1 → 3) or Neu5Gc) not present in the human species.

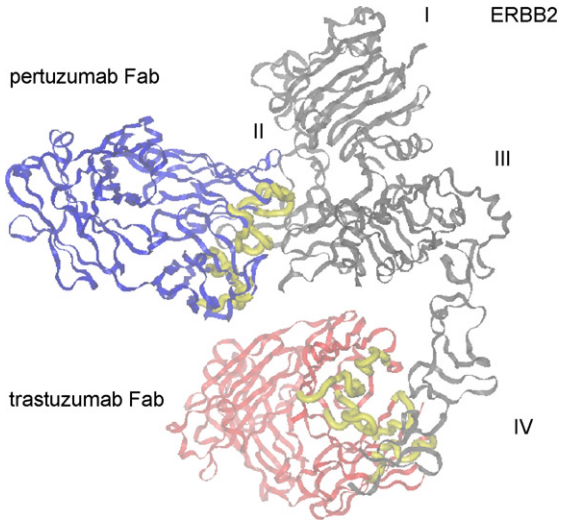


Fig. 5. Superimposition of trastuzumab Fab-ERBB2 and pertuzumab Fab-ERBB2 co-crystal structures. The ribbon structure of the extracellular domain of ERBB2 (also called NEU/HER-2) in grey shows that it is divided into four domains: I is the most distal and IV the most proximal from the cell membrane. Trastuzumab Fab [28] (PDB and IMGT/3Dstructure-DB: 1v8z) and pertuzumab Fab [29] (PDB and IMGT/3Dstructure-DB: 1s78), 3D structures are indicated in red and blue, respectively. The CDR loops of each antibody are represented as yellow tubes, connected to the framework regions of the variable domains represented as ribbons. The two antibodies are directed against two different epitopes on the receptor, on domain II for pertuzumab and on domain IV for trastuzumab. Figure generated with VMD [9].

3. Antibody/antigen interactions

To date, the 3D structure of bevacizumab in complex with the vascular endothelial growth factor, VEGF [14], of cetuximab in complex with the extracellular domain of the epidermal growth factor receptor, EGFR (ERBB1) [6] and of trastuzumab in complex with the extracellular domain of ERBB2 (also called NEU/HER-2) (Fig. 5) [28] have been reported. Most of the contact residues of bevacizumab with VEGF are located in the three CDR-IMGT loops of the VH domain, particularly the VH CDR3-IMGT [30] (Fig. 2B and Table 2). The epitope targeted by bevacizumab partly overlaps VEGF residues that interact with the binding to VEGFR1 (Flt-1, FLT1) and VEGFR2 (FLK1, KDR), the two VEGF receptors [14].

The 3D structures of the anti-EGFR cetuximab [6] and of the anti-ERBB2 trastuzumab [28], together with that

Table 2
Amino acids of rmAbs involved in hydrogen bonds with the antigens, from IMGT/3Dstructure-DB, http://imgt.cines.fr

rmAbs[a]	Number of H bonds[b]	VH			V-KAPPA	
		CDR1-IMGT	CDR2-IMGT	CDR3-IMGT	CDR1-IMGT	CDR3-IMGT
cetuximab (1yy9)	6 (5 + 1)		59G	111Y 112D 113Y		114W
alemtuzumab (1ce1)	14 (9 + 5)	38Y	57R 61K	107E 109H 110T		107H 114R 116R
bevacizumab (1bj1)	14 (14 + 0)	38G	55W 58T	107Y 108P 109Y 110Y 111.1G 112.1S		
trastuzumab (1n8z)	6 (2 + 4)		55R	111G	36N	108Y 114T
pertuzumab (1s78)	9 (9 + 0)	36D 37Y	57N 58P 59N	110P 114Y		

Amino acid positions and CDR-IMGT delimitations are according to the IMGT unique numbering for V-DOMAIN [13]. Amino acids involved in one, two or three H bonds are not underlined, single underlined or double underlined, respectively. Amino acids involved in H bonds are shown in boxes in Fig. 3.

[a] IMGT/3Dstructure-DB entries (same code as PDB) are shown between parentheses.

[b] Hydrogen (H) bond identification is from IMGT/3Dstructure-DB contact analysis [22], http://imgt.cines.fr. Total number of H bonds is shown with, between parentheses, number of H bonds for VH and V-KAPPA.

of another anti-ERBB2 currently under development (pertuzumab) [29] allow a comprehensive description of the functional domains of this family of receptors. Cetuximab binds the domain III of the EGFR, mainly through its VH CDR2-IMGT and CDR3-IMGT. More particularly, tyrosine 111 in VH CDR3-IMGT (Fig. 3 and Table 2) protrudes into a pocket located in domain III of EGFR. By binding to this particular epitope, cetuximab blocks the interaction of EGF with its receptor, hence the antagonist activity of cetuximab [6].

Pertuzumab binds the domain II of ERBB2, a domain involved in the pairing of ERBB2 with other members of the ERBB family [29], through the three VH CDR-IMGT (Table 2). Since heterodimerization of ERBB2 is required for signalling, pertuzumab acts as an "indirect" antagonist of ERBB2 partners [6].

Trastuzumab recognises three loops on domain IV of ERBB2 [28], a domain which lies very close to the cell membrane (Fig. 5). CDR3-IMGT of both VH and V-KAPPA seem to be involved in this interaction by the formation of a pocket [28], but no more details are available about the residues required for the recognition of the epitope (Table 2). The domain IV of ERBB2 is probably subjected to the action of one or several metalloproteases as ADAM17 (TACE) [30] or MMP-15 (Slikowski M, pers. commun.) whose functions are to cleave ERBB2 and to release its ectodomain in the microenvironment. This soluble ectodomain may serve as decoy for trastuzumab, and decreases its clinical efficacy [31]. Interestingly, it has been shown that trastuzumab, but not pertuzumab, inhibits ERBB2 shedding by steric hindrance. One of the cleavage sites on ERBB2 has been identified and localised to a membrane proximal region very close to the epitope bound by trastuzumab [32]. However, there is no clear relation between the fact that trastuzumab blocks ERBB2 shedding and the precise mechanism of action of trastuzumab which is only active on tumours overexpressing ERBB2. The most common hypotheses are that trastuzumab prevents ERBB2 homodimerization and/or induces receptor internalisation, which ultimately lead to an arrest in ERBB2 signalling [28].

The 3D structure of alemtuzumab in complex with a synthetic peptide mimicking its natural antigen CD52 is also available; the natural CD52 ligand is a highly glycosylated and glycosyl-phosphatidyl-inositol (GPI) anchored short polypeptide [33]. The ability of alemtuzumab to bind a peptidic mimic of CD52 [33] depends on the three VH CDR-IMGT and a noticeable contribution of V-KAPPA CDR3-IMGT (Table 2). No distinctive biological function has yet been assigned to the CD52 molecule and alemtuzumab does not seem to have antagonistic or agonistic activities on CD52-expressing cells; therefore the activity of alemtuzumab is assumed to be the recruitment of immune effector mechanisms [34].

The 3D structure of rituximab, one of the most widely used anti-cancer rmAb, has not been determined. This is probably explained by the nature of its target antigen, the membrane-spanning 4-domains, subfamily A, member 1, MS4A1 (CD20), which has four hydrophobic transmembrane regions. Binding of rituximab to CD20 is highly dependent on the presence of alanine at position 170 and proline at position 172 in the extracellular loop of CD20 [35,36]. Studies of the interaction of rituximab with soluble recombinant CD20 [37] also demonstrate that the binding of rituximab is dependent upon the oxidation state of cysteines in the extracellular domain of the CD20 protein, *i.e.* binding is ablated for reduced and alkylated CD20, but further structural studies are strongly awaited.

Overall, these structural data show that VH and particularly its CDR3-IMGT rather than V-KAPPA contribute to antibody/antigen interaction, particularly because more hydrogen bonds are created with this VH domain. Based on these observations, therapeutic antibodies follow the general rule of antibodies.

4. Variable domain genetic analysis

The variable domains of the antibodies were analysed using IMGT/3Dstructure-DB [22] for the amino acid sequences and 3D structures and IMGT/V-QUEST [38] for the nucleotide sequences (IMGT®, the international ImMunoGeneTics Information System®; http://imgt.cines.fr (Founder and director: Marie-Paule Lefranc, Montpellier, France)) [39]. IMGT/3Dstructure-DB and IMGT/V-QUEST identify the closest V, (D) and J genes and delimit the FR-IMGT and CDR-IMGT delimitations (Fig. 2A and Table 3). IMGT/V-QUEST compares the nucleotide sequences with the IMGT mouse or human germline reference directory, which comprises one reference sequence for each gene and allele of the mouse (IMGT Repertoire) or human [7] repertoires from IMGT/GENE-DB; http://imgt.cines.fr [40].

The variable domains of the chimeric antibodies, cetuximab and rituximab, were compared to the IMGT murine reference directory (Table 2). The cetuximab VH derives from the mouse *IGHV2-2**02, *IGHD2-4**01 and *IGHJ3**01 genes and the rituximab VH derives from the mouse *IGHV1-12**01, *IGHD1-2**01 and *IGHJ1**01 genes (Table 3). Concerning the light chains, the cetuximab V-KAPPA derives from the mouse *IGKV5-48**01 and *IGKJ5**01 genes, and the rituximab V-KAPPA derives from the mouse *IGKV4-72**01 and *IGKJ2**01 genes (Table 3).

The comparison of the V-REGION of the VH and V-KAPPA nucleotide sequences with the IMGT/V-QUEST germline sequences allows the identification of residues that result from somatic hypermutations that occurred in the donor mouse. Cetuximab is highly mutated since there are seven somatic hypermutations in the IGH V-REGION, all of them leading to amino acid (aa) changes (1 in CDR1-IMGT, 1 in CDR2-IMGT and 5 in FR3-IMGT) and five mutations in the *IGK* V-REGION, four of them leading to aa changes (1 in FR1-IMGT, 1 in CDR1-IMGT and 2 in CDR3-IMGT). Interestingly, six of the aa replacements (3 in VH and 3

Table 3
IMGT V, (D) and J genes involved in the building of rmAbs used in oncology

Antibodies	IMGT/3Dstructure-DB			VH		V-KAPPA	
	IMGT protein name	Fab	Fab in complex	IGHV, IGHD and IGHJ genes	CDR-IMGT lengths	IGKV and IGHJ genes	CDR-IMGT lengths
Chimeric antibodies: comparison with mouse (*Mus musculus*) germline V, (D) and J genes							
cetuximab (Erbitux®)	Fab C225, IMC-225	1yy8	1yy9 (with EGFR)	*IGHV2-2**02 (92.78%) (55.10% human *IGHV3-33*), *IGHD2-4**01 *IGHJ3**01 (93.33%) (80% human *IGHJ4*)	[8.7.13]	*IGKV5-48**01 (95.79%) (66.32% human *IGKV6-21*), *IGKJ5**01 (91.66%) (75% human *IGKJ2*)	[6.3.9]
rituximab (MabThera®, Rituxan®)	IDEC-C2B8	[a]	[a]	*IGHV1-12**01 (92.70%) (72.45% human *IGHV1-46*), *IGHD1-2**01 *IGHJ1**01 (82.35%) (64.70% human *IGHJ2*)	[8.8.14]	*IGKV4-72**01 (95.79%) (61.46% human *IGKV3-20*), *IGKJ2**01 (91.66%)(83.33% human *IGKJ2*)	[5.3.9]
Humanized antibodies: comparison with human (*Homo sapiens*) germline V and J genes. The IGHD genes are derived from mouse or rat and are not shown							
alemtuzumab (MabCampath®)	CAMPATH-1H	1bey	1ce1 (with CD52 mimotope)	*IGHV4-59**01 (73%), *IGHJ4**01 (92.86%)	[8.10.12]	*IGKV1-33**01 (86.32%), *IGKJ1**01 (91.66%)	[6.3.9]
bevacizumab (Avastin®)	Fab-12		1bj1 (with VEGF)	*IGHV7-4-1**02 (72.40%), *IGHJ2**01 (82.35%)	[8.8.16]	*IGKV1-33**01 (87.40%), *IGKJ1**01 (100%)	[6.3.9]
trastuzumab (Herceptin®)	Herceptin, 4D5v8		1n8z (with ERBB2)	*IGHV3-66**01 (81.63%), *IGHJ6**01 (76.47%)	[8.8.13]	*IGKV1-39**01 (86.32%), *IGKJ1**01 (91.66%)	[6.3.9]
Fully human antibody: comparison with human (*Homo sapiens*) germline V, D and J genes							
panitumumab (Vectibix®)	ABX-EGF E7.6.3	[b]	[b]	*IGHV4-61**01 (86.84%)[b], *IGHD3-3**01 or *02 *IGHJ3**02 (93.75%)	[10.7.11]	*IGKV1-33**01 (94.73%)[b], *IGKJ4**01 (91.66%)	[6.3.9]

Percentage of identity was calculated by comparison of the amino acid sequences with the closest mouse or human V-REGION and J-REGION. V-REGIONs were compared from position 1 to 106 for VH and from position 1 to 111 for V-KAPPA, using IMGT/DomainGapAlign at the IMGT Home page, http://imgt.cines.fr. Given the short length of the J-REGION (12–17 amino acids), the percentages are only given as an indication.

[a] There is no 3D structure available for rituximab Fab. Sequences are from IMGT/LIGM-DB: AX556949 (heavy chain) and AX556951 (κ chain).

[b] There is no 3D structure available for panitumumab. Sequences are from IMGT/LIGM-DB: DD214915 (VH) and DD214916 (V-KAPPA). Note that the N-terminal 23 and 19 amino acids (and codons) are missing in the VH and V-KAPPA sequences, respectively and in the patents and could not be analysed.

in V-KAPPA) correspond to serine to asparagine (S > N) change.

For rituximab, there are 20 mutations in the IGH V-REGION, affecting 16 codons, and leading to 10 aa changes (5 in FR1-IMGT, 3 in FR2-IMGT and 2 in FR3-IMGT) and 5 mutations in the IGK V-REGION, 4 of them leading to aa changes (2 in FR2-IMGT, 1 in FR3-IMGT and 1 in CDR3-IMGT).

This kind of somatic mutation analysis is not meaningful with humanized antibodies because of their construction. However it is interesting to compare the humanized genes with the closest human genes, using IMGT/DomainGapAlign. In their present form, the humanized antibodies alemtuzumab, bevacizumab and trastuzumab IGH V-REGIONs have a percentage of identity at the amino acid level of 73%, 72% and 81% with the closest germline human *IGHV4-59**01, *IGHV7-4-1**02 and *IGHV3-66**01, respectively (Table 3). The three humanized antibodies IGK V-REGIONs have a similar percentage of identity of 86–87% at the amino acid level with the closest germline human *IGKV1-33**01 for alemtuzumab and bevacizumab, and with *IGKV1-39**01 for trastuzumab (Table 3).

Deciphering the genetic origin of VH and V-KAPPA domains were successful for each rmAb studied, nicely illustrating the fundamental processes of repertoire maturation. The potential divergence of these newly generated sequences from their human germline counterparts also raise the question of their potential immunogenicity.

5. Immunogenicity

The comparison of the murine V-REGION and J-REGION sequences of VH and V-KAPPA from chimeric antibodies with the IMGT human reference directory is shown in Table 3. The percentages of identity are significantly lower than those obtained when compared with the IMGT mouse reference directory, and illustrate the inter-species divergence at the level of the variable domains. For a more precise evaluation of the inter-species differences between the frameworks, we compare the amino acid sequences of the four FR-IMGT (FR1-IMGT: positions 1–26, FR2-IMGT: 39–55, FR3-IMGT: 66–104, and FR4-IMGT: 118–128 for VH or 127 for V-KAPPA) of each murine V domain with the closest human sequence found (Table 3). We used IMGT/DomainGapAlign (http://imgt.cines.fr) at the IMGT Home page for comparison of the FR1-, FR2- and FR3-IMGT, and the Alignment of alleles IGHJ and IGKJ (Overview) in IMGT Repertoire for comparison of the FR4-IMGT. We observed 59.34% (54/91 aa) and 70.78% (63/89 aa) identity for the four FR-IMGT of the cetuximab VH and V-KAPPA domains, respectively, and 74.72% (68/91 aa) and 67.41% (60/89 aa) identity for the four FR-IMGT of the rituximab VH and V-KAPPA domains, respectively. Despite this sequence divergence in the FR that could reinforce immunogenicity of the idiotype itself, these two antibodies are not really immunogenic in cancer patients [41]. This could be due to the fact that their immune system is compromised and/or that they may be receiving cytotoxic drugs. In fact, human anti-chimeric antibodies (HACA) are observed in a higher percentage of patients receiving rituximab for an autoimmune disease [42]. Nevertheless, it should be mentioned that only some patients develop HACA. Since many factors external to the drug itself have been described to influence immunogenicity [43], it is therefore difficult to assign to any threshold of dissimilarity between the murine and human rmAbs proteins a role in the induction of a neutralizing response. When information will be available from large cohorts of patients, particularly from clinical studies comparing different rmAbs administered to similar patients, with identical regimens, it could become possible to find some correlation between the immunogenicity of a given rmAb and the global percentage of identity and physico-chemical characteristics of amino acids exposed at the surface of VH and VL domains in comparison to those truly expressed in humans, taking into account mutations observed in expressed repertoires [44]. The variability of anti-drug responses in patients depends on both accessibility of B cell epitopes and the processing of T cell epitopes by the highly polymorphic HLA class II molecules. Potentiel T cell epitopes within rmAbs primary sequences and their interaction with HLA class II molecules can now been mapped *in silico*, and this approach could be in an attempt to predict rmAbs immunogenicity [45].

Very logically, when VH and V-KAPPA sequences from humanized antibodies are compared with the IMGT human reference directory the percentage of identity appears higher than when chimeric rmAbs are analysed with the same directory (Table 3). When comparing the four FR-IMGT of the humanized sequence with the closest human sequences found, as described above, we observed, for the VH and V-KAPPA, respectively, 84.61% (77/91 aa) and 97.75% (87/89 aa) for alemtuzumab, 74.72% (68/91 aa) and 92.13% (82/89 aa) for bevacizumab, and 90.10% (82/91 aa) and 93.25% (83/89 aa) for trastuzumab. It is worthwhile to note that, whereas the FR percentage of identity is superior to 90% for four of six domains, this percentage is 84.61% for the alemtuzumab VH and only 74.72% for the bevacizumab VH, a percentage similar to that observed in the chimeric rituximab.

The humanized rmAbs are also weekly immunogenic in cancer patients [46], as are chimeric antibodies. Several studies have shown that humanization decreases but does not eliminate immunogenicity problems [47,48]. However, a rigorous comparison between a chimeric antibody and its humanized counterpart in humans in terms of immunogenicity remains crucially lacking.

In a first step, IMGT standardized criteria for statistical analysis, based on the definition of 11 IMGT amino acid chemical characteristics classes, have provided IMGT Colliers de Perles statistical profiles for the human expressed IGHV and IGKV repertoires [44]. Amino acid assignment of the chimeric and humanized rmAbs IMGT Colliers de Perles (Fig. 4) to IMGT aa chemical classes was compared

to the FR-IMGT positions which define the human IGHV and IGKV chemical profiles (41 and 59 positions at >80% threshold, respectively, see Plate 3 in [44]). Despite marked differences in the percentage of similarity as discussed earlier, there are no marked differences in IMGT chemical classes for the VH of humanized (0: trastuzumab; 1: bevacizumab; 2: alemtuzumab) and chimeric (1: cetuximab; 2: rituximab) antibodies. Similarly, the V-KAPPA of the three humanized rmAbs show only one (trastuzumab, alemtuzumab) or two (bevacizumab) changes in IMGT chemical classes. In contrast, the V-KAPPA of the chimeric cetuximab and rituximab have 6 and 8 changes in IMGT aa chemical classes, respectively. The comparison of domain sequences with the IMGT Colliers de Perles statistical profiles will be useful to identify potential immunogenic residues at given positions.

In conclusion, analysing the variable domains of rmAbs currently approved for cancer treatment by using the IMGT/3Dstructure-DB database and the IMGT/V-QUEST tool has proven to be particularly useful to interpret further their antigen binding properties and their genetic origin. Jumping from this analysis to the prediction of immunogenicity of chimeric or humanized rmAbs is not currently possible. Nevertheless, the IMGT standards provide the means to analyse and compare the chemical and genetic properties of different rmAbs and thus represent a first and crucial step in the evaluation of immunogenicity. Future approaches will have to take into account the genetic polymorphisms in humans, particularly in MHC molecules and factors modulating immune response in patients.

Reviewers

Jin Lu, Ph.D., Principal Research Scientist in Bioinformatics, Centocor Inc. (A Johnson and Johnson Company), 145 King of Prussia Road, Radnor, PA 19087, USA.

Thierry Wurch, Ph.D., Head, Cellular and Molecular Biology Unit, Institut de Recherche Pierre Fabre, 5, Avenue Napoleon III, BP 497, F-74164 St Julien-en-Genevois, France.

Acknowledgements

We are grateful to the IMGT team for helpful discussion, Prof. P. Bardos and Mrs. D. Degenne for their critical reading of the manuscript and La Fondation Langlois and l'Association pour la Recherche sur le Cancer (ARC) for their support. IMGT is a registered Centre National de la Recherche Scientifique (CNRS) mark. IMGT is a National RIO Bioinformatics Platform since 2001 (CNRS, INSERM, CEA, INRA). IMGT was funded in part by the BIOMED1 (BIOCT930038), Biotechnology BIOTECH2 (BIO4CT960037) and 5th PCRDT Quality of Life and Management of Living Resources (QLG2-2000-01287) programmes of the European Union and received subventions from ARC. IMGT is currently supported by the CNRS, the MENESR (Université Montpellier II Plan Pluri-Formation), BIOSTIC-LR2004, Région Languedoc-Roussillon, ACI-IMPBIO IMP82-2004, the Réseau National des Génopoles RNG, GIS-AGENAE, Agence Nationale de la Recherche ANR (BIOSYS06_135457) and the European Union ImmunoGrid project (IST-2004-0280069).

References

[1] Mol CD, Fabbro D, Hosfield DJ. Structural insights into the conformational selectivity of STI-571 and related kinase inhibitors. Curr Opin Drug Discov Dev 2004;7(5):639–48.
[2] Brehmer D, Greff Z, Godl K, et al. Cellular targets of gefitinib. Cancer Res 2005;65:379–82.
[3] Goldstein NI, Prewett M, Zuklys K, Rockwell P, Mendelsohn J. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. Clin Cancer Res 1995;1:1311–8.
[4] Albanell J, Codony J, Rovira A, Mellado B, Gascon P. Mechanism of action of anti-HER2 monoclonal antibodies: scientific update on trastuzumab and 2C4. Adv Exp Med Biol 2003;532:253–68.
[5] Cartron G, Watier H, Golay J, Solal-Celigny P. From the bench to the bedside: ways to improve rituximab efficacy. Blood 2004;104:2635–42.
[6] Li S, Schmitz KR, Jeffrey PD, Wiltzius JJ, Kussie P, Ferguson KM. Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. Cancer Cell 2005;7:301–11.
[7] Lefranc M-P, Lefranc G. The immunoglobulin FactsBook. London, UK: Academic Press; 2001. p. 1–458.
[8] Saphire EO, Parren PW, Pantophlet R, et al. Crystal structure of a neutralizing human IGG against HIV-1: a template for vaccine design. Science 2001;293(5532):1155–9.
[9] Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. J Mol Graph 1996;14, 33–8, 27–8.
[10] Li J, Sai T, Berger M, et al. Human antibodies for immunotherapy development generated via a human B cell hybridoma technology. Proc Natl Acad Sci USA 2006;103:3557–62.
[11] Yousfi Monod M, Giudicelli V, Chaume D, Lefranc M-P. IMGT/JunctionAnalysis: the first tool for the analysis of the immunoglobulin and T cell receptor complex V–J and V–D–J JUNCTIONs. Bioinformatics 2004;20:I379–85.
[12] Chothia C, Lesk AM. Canonical structures for the hypervariable regions of immunoglobulins. J Mol Biol 1987;196:901–17.
[13] Lefranc M-P, Pommié C, Ruiz M, et al. IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. Dev Comp Immunol 2003;27:55–77.
[14] Muller YA, Chen Y, Christinger HW, et al. VEGF and the Fab fragment of a humanized neutralizing antibody: crystal structure of the complex at 2.4 A resolution and mutational analysis of the interface. Structure 1998;6:1153–67.
[15] Riechmann L, Clark M, Waldmann H, Winter G. Reshaping human antibodies for therapy. Nature 1988;332:323–7.
[16] Winter G, Harris WJ. Humanized antibodies. Immunol Today 1993;14:243–6.
[17] Poul M-A, Lefranc M-P. Structural correspondences between mouse and human immunoglobulin VH genes: application to the humanization of mouse monoclonal antibodies. Ann NY Acad Sci 1995;764:47–9.
[18] Kabat EA, et al. Sequences of proteins of immunological interest. Washington, DC: NIH Publication; 1991. p. 91–3242.
[19] Lefranc M-P. Unique database numbering system for immunogenetic analysis. Immunol Today 1997;18:509.
[20] Lefranc M-P. The IMGT unique numbering for Immunoglobulins, T cell receptors and Ig-like domains. Immunologist 1999;7:132–6.

[21] Cheetham GM, Hale G, Waldmann H, Bloomer AC. Crystal structures of a rat anti-CD52 (CAMPATH-1) therapeutic antibody Fab fragment and its humanized counterpart. J Mol Biol 1998;284:85–99.
[22] Kaas Q, Ruiz M, Lefranc M-P. IMGT/3Dstructure-DB and IMGT/StructuralQuery, a database and a tool for immunoglobulin, T cell receptor and MHC structural data. Nucl Acids Res 2004;32:D208–10.
[23] Jilani I, Keating M, Giles FJ, O'Brien S, Kantarjian HM, Albitar M. Alemtuzumab: validation of a sensitive and simple enzyme-linked immunosorbent assay. Leuk Res 2004;28:1255–62.
[24] Lefranc M-P, Pommié C, Kaas Q, et al. IMGT unique numbering for immunoglobulin and T cell receptor constant domains and Ig superfamily C-like domains. Dev Comp Immunol 2005;29:185–203.
[25] Ruiz M, Lefranc M-P. IMGT gene identification and Colliers de Perles of human immunoglobulin with known 3D structures. Immunogenetics 2002;53:857–83.
[26] Qian J, Liu T, Yang L, Daus A, Crowley R, Zhou Q. Structural characterization of N-linked oligosaccharides on monoclonal antibody cetuximab by the combination of orthogonal matrix-assisted laser desorption/ionization hybrid quadrupole-quadrupole time-of-flight tandem mass spectrometry and sequential enzymatic digestion. Anal Biochem 2007;364:8–18.
[27] Jefferis R. Glycosylation of recombinant antibody therapeutics. Biotechnol Progr 2005;21:11–6.
[28] Cho HS, Mason K, Ramyar KX, et al. Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. Nature 2003;421:756–60.
[29] Franklin MC, Carey KD, Vajdos FF, Leahy DJ, de Vos AM, Sliwkowski MX. Insights into ERBB signaling from the structure of the ERBb2-pertuzumab complex. Cancer Cell 2004;5:317–28.
[30] Codony-Servat J, Albanell J, Lopez-Talavera JC, Arribas J, Baselga J. Cleavage of the HER2 ectodomain is a pervanadateactivable process that is inhibited by the tissue inhibitor of metalloproteases-1 in breast cancer cells. Cancer Res 1999;59:1196–201.
[31] Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J. Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. Cancer Res 2001;61:4744–9.
[32] Yuan CX, Lasut AL, Wynn R, et al. Purification of Her-2 extracellular domain and identification of its cleavage site. Protein Exp Purif 2003;29:217–22.
[33] James LC, Hale G, Waldmann H, Bloomer AC. 1.9 Å structure of the therapeutic antibody CAMPATH-1H Fab in complex with a synthetic peptide antigen. J Mol Biol 1999;289:293–301.
[34] Schulz H, Winkler U, Staak JO, Engert A. The monoclonal antibodies Campath-1H and rituximab in the therapy of chronic lymphocytic leukemia. Onkologie 2000;23:526–32.
[35] Polyak MJ, Deans JP. Alanine-170 and proline-172 are critical determinants for extracellular CD20 epitopes; heterogeneity in the fine specificity of CD20 monoclonal antibodies is defined by additional requirements imposed by both amino acid sequence and quaternary structure. Blood 2002;99:3256–62.
[36] Teeling JL, Mackus WJ, Wiegman LJ, et al. The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J Immunol 2006;177:362–71.
[37] Ernst JA, Li H, Kim HS, Nakamura GR, Yansura DG, Vandlen RL. Isolation and characterization of the B-cell marker CD20. Biochemistry 2005;44:15150–8.
[38] Giudicelli V, Chaume D, Lefranc M-P. IMGT/V-QUEST, an integrated software program for immunoglobulin and T cell receptor V–J and V–D–J rearrangement analysis. Nucl Acids Res 2004;32:W435–40.
[39] Lefranc M-P, Giudicelli V, Kaas Q, et al. IMGT, the international ImMunoGeneTics Information System®. Nucl Acids Res 2005;33:D593–7.
[40] Giudicelli V, Chaume D, Lefranc M-P. IMGT/GENE-DB: a comprehensive database for human and mouse immunoglobulin and T cell receptor genes. Nucl Acids Res 2005;33:D256–61.
[41] Maeda T, Yamada Y, Tawara M, et al. Successful treatment with a chimeric anti-CD20 monoclonal antibody (IDEC-C2B8, rituximab) for a patient with relapsed mantle cell lymphoma who developed a human anti-chimeric antibody. Int J Hematol 2001;74:70–5.
[42] Looney RJ, Anolik JH, Campbell D, et al. B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. Arthritis Rheum 2004;50:2580–9.
[43] Pendley C, Schantz A, Wagner C. Immunogenicity of therapeutic monoclonal antibodies. Curr Opin Mol Ther 2003;5:172–9.
[44] Pommié C, Levadoux S, Sabatier R, Lefranc G, Lefranc M-P. IMGT standardized criteria for statistical analysis of immunoglobulin V-REGION amino acid properties. J Mol Recogn 2004;17:17–32.
[45] Van Walle I, Gansemans Y, Parren PW, Stas P, Lasters I. Immunogenicity screening in protein drug development. Expert Opin Biol Ther 2007;7:405–18.
[46] Hale G, Rebello P, Brettman LR, et al. Blood concentrations of alemtuzumab and antiglobulin responses in patients with chronic lymphocytic leukemia following intravenous or subcutaneous routes of administration. Blood 2004;104:948–55.
[47] Benjamin RJ, Cobbold SP, Clark MR, Waldmann H. Tolerance to rat monoclonal antibodies. Implications for serotherapy. J Exp Med 1986;163:1539–52.
[48] Isaacs JD, Watts RA, Hazleman BL, et al. Humanised monoclonal antibody therapy for rheumatoid arthritis. Lancet 1992;340:748–52.
[49] Goldstein NI, Giorgio NA, Jones ST, Saldanha JW. Antibody and antibody fragments for inhibiting the growth of tumours. WO9640210; 1996.
[50] Anderson DR, Rastetter WH, Hanna N, Leonard JE, Newman RA, Reff ME. Therapeutic application of chimeric and radiolabeled antibodies to human B lymphocyte restricted differentiation antigen for treatment of B cell lymphoma. WO9411026; 1994.
[51] Waldmann H, Clark MR, Winter GP, Riechmann L. Antibodies to the antigen CAMPATH-1. US5846534; 1998.
[52] Baca M, Wells JA, Presta LG, Lowman HB, Chen Y. Anti-VEGF antibodies. EP1325932; 1998.
[53] Carter PJ, Presta LG. Immunoglobulin variants. US5821337; 1998.
[54] Jakobovits A, Yang X-D. Human monoclonal antibodies to epidermal growth factor receptor. WO9850433; 1998.

Biographies

Charlotte Magdelaine-Beuzelin, PharmD, lecturer in Immunology at the Université Francois-Rabelais de Tours, EA 3853 IPGA (Immuno-Pharmaco-Genetic of therapeutic Antibodies) and the Immunology department of the Hospital of Tours, France. She is preparing her PhD and is specialized in analysis of antibody immunogenicity.

Quentin Kaas, PhD, has developped IMGT/3Dstructure-DB (http://imgt.cines.fr), the database specialized on the analysis of the three-dimensional structures of the immunoglobulins, the T cell receptors and of the major histocompatibility complex. He is presently a postdoc in the University of Queensland, Brisbane, Australia, where he focuses his research on protein structure prediction methods and on molecular dynamics of protein/membrane interactions.

Vanessa Wehbi has worked to the data collection about recombinant monoclonal antibodies for IPGA (Immuno-Pharmaco-Genetics of therapeutic Antibodies), Tours, France. She is at present PhD student of biology at the

Department of Reproductive and behavioural Physiology, INRA-CNRS (UMR 6175), Université François-Rabelais de Tours, Haras Nationaux, Tours Research Centre, Nouzilly, France.

Marc Ohresser, PhD, is a Research Engineer at the Université Francois-Rabelais de Tours, EA 3853 IPGA (Immuno-Pharmaco-Genetic of therapeutic Antibodies), France. He is specialized in molecular biology and protein modelling.

Roy Jefferis, PhD in chemistry moved into the Medical School to began his studies of the structure and function of antibody molecules, in health and disease. These studies led to a DSc (Doctor of Science) in immunology and FRCPath (Fellow of the Royal College of Pathologists) and more than 250 publications. In recent years there has been an emphasis on the effector functions of IgG antibodies and particularly the differential activity of multiple glycoforms. The studies are particularly relevant to the production and application of monoclonal antibody therapeutics.

Marie-Paule Lefranc, PhD, is Professor Classe Exceptionnelle at the University Montpellier II, Senior member of the Institut Universitaire de France, Chair of Immunogenetics and Immunoinformatics, Head of the Laboratoire d'ImmunoGénétique Moléculaire (LIGM) that she created with Gérard Lefranc, in 1982 at Montpellier, the lab being now located within the Institut de Génétique Humaine, UPR CNRS 1142. She has authored over 210 scientific publications in international journals on the molecular immunogenetics of immunoglobulins, T cell receptors, antibody engineering, and in human genetics and immunoinformatics. She received the ROSEN prize of Cancerology in 1988. She is director of IMGT®, the international ImMunoGeneTics Information System® (http://imgt.cines.fr), that she founded in 1989, at Montpellier, France.

Hervé Watier, MD, PhD, is professor of Immunology at the Université François-Rabelais de Tours, France. He is leading the research team IPGA, "Immuno-Pharmaco-Genetic of therapeutic Antibodies" whose aim is to identify immunological, pharmacological and genetic factors influencing the variability of clinical effects of recombinant monoclonal antibodies, in order to optimise their therapeutic use if they are already registered and to help design the drugs of the future.