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Chapter 2
Standardized Sequence and Structure Analysis of Antibody Using IMGT®

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2.1 Introduction

IMGT®, the international ImMunoGeneTics information system® (http://www.imgt.org) (Lefranc et al. 2009), was created in 1989 at Montpellier, France (CNRS and Université Montpellier 2), to standardize the immunogenetics data and to manage the huge diversity of the antigen receptors, immunoglobulins (IG) or antibodies and T cell receptors (TR) (Lefranc and Lefranc 2001a, b). IMGT® is the international reference in immunogenetics and immunoinformatics, and its standards have been approved by the World Health Organization–International Union of Immunological Societies (WHO–IUIS) Nomenclature Committee (Lefranc 2007, 2008). It provides a common access to standardized and integrated data from genome, proteome, genetics and three-dimensional (3D) structures (Lefranc et al. 2005a). IMGT® comprises six databases (for sequences, genes and 3D structures), 15 online tools and Web resources (more than 10,000 HTML pages) (Lefranc et al. 2009) (Fig. 2.1). The accuracy and the consistency of the IMGT® data are based on IMGT-ONTOLOGY, the first ontology for immunogenetics and immunoinformatics (Giudicelli and Lefranc 1999; Lefranc et al. 2004; Duroux et al. 2008).

IMGT® provides the informatics frame and knowledge environment for a standardized analysis of the antibody sequences and 3D structures, in the context of antibody engineering (single chain Fragment variable (scFv), phage displays, combinatorial libraries) and antibody humanization (chimeric, humanized and human antibodies).

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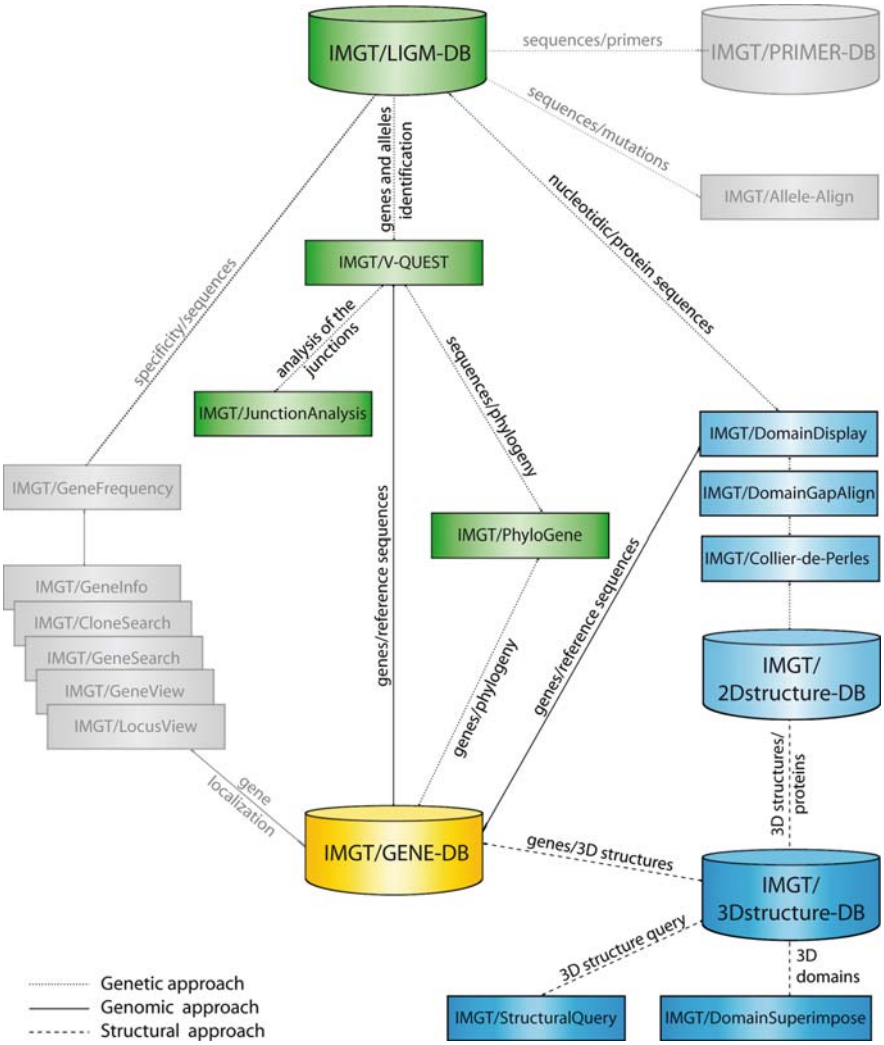


Fig. 2.1 IMGT®, the international ImMunoGeneTics information system® (http://www.imgt.org) (Lefranc et al. 2009). IMGT® databases and tools for sequence and structure analysis of antibody are shown. Other tools are in *pale grey*. The IMGT Repertoire and other Web resources are not shown

In this chapter, the IMGT Scientific chart rules necessary for a standardized analysis of antibody sequences and structures are summarized, with a focus on the IMGT Collier de Perles, the IMGT® flagship that bridges the gap between sequences and 3D structures. We describe the IMGT® tools that support the analysis from nucleotide sequence to 2D structure: IMGT/V-QUEST (Brochet et al. 2008) and the

integrated IMGT/JunctionAnalysis (Yousfi Monod et al. 2004) software, which are widely used for sequence analysis (Lefranc 2004; Giudicelli and Lefranc 2005, 2008). We then describe IMGT® components that support the IMGT® approach from amino acid sequence to 3D structure: the IMGT/DomainGapAlign and IMGT/Collier-de-Perles tools, the IMGT/2Dstructure-DB (for antibodies for which 3D structures are not yet available), the IMGT/3Dstructure-DB (Kaas et al. 2004) (for crystallized antibodies) and the associated tools, IMGT/StructuralQuery and IMGT/DomainSuperimpose.

2.2 IMGT Scientific Chart Rules

2.2.1 IMGT-ONTOLOGY Concepts for Sequence and Structure

In order to manage the immunogenetics data, the IMGT Scientific chart rules (http://www.imgt.org/textes/IMGTScientificChart/) have been implemented, based on IMGT-ONTOLOGY (Giudicelli and Lefranc 1999; Lefranc et al. 2004; Duroux et al. 2008). Four main axioms "IDENTIFICATION", "CLASSIFICATION", "DESCRIPTION" and "NUMEROTATION" have generated the concepts of identification (IMGT® standardized keywords), classification (IMGT® nomenclature), description (IMGT® standardized labels), and numerotation (IMGT unique numbering) which are used in the IMGT® databases, tools and Web resources (Lefranc et al. 2009, 2005a; Duroux et al. 2008). As an example, the functionality, an important concept of identification, is defined for the germline and conventional genes: functional, ORF (open reading frame) or pseudogene, and for the rearranged sequences: productive or unproductive. The IMGT® gene names (Lefranc and Lefranc 2001a, b; Lefranc 2000a, b), part of the concepts of classification, were approved by the Human Genome Organisation (HUGO) Nomenclature Committee (HGNC) in 1999 (Wain et al. 2002) and have been entered in Entrez Gene (Maglott et al. 2007) at the National Center for Biotechnology Information (NCBI) (USA) and in Vega (Wilming et al. 2008) at the Wellcome Trust Sanger Institute (UK) with direct links to IMGT/LIGM-DB (Giudicelli et al. 2006), the IMGT® nucleotide sequence database, and to IMGT/GENE-DB (Giudicelli et al. 2005a), the IMGT® gene database. The IMGT® standardized labels, part of the concepts of description, are recognizable as written in capital letters (Fig. 2.2). Their definitions are available on the IMGT® Web site (http://www.imgt.org). The IMGT unique numbering (Lefranc 1997, 1999; Lefranc et al. 2003, 2005b, c), a key concept of numerotation, has become the standard for the description of the V type domain (Lefranc et al. 2003), C type domain (Lefranc et al. 2005b) and G type domain (Lefranc et al. 2005c). The IMGT unique numbering is valid for nucleotide (codon) sequence, amino acid sequence, 2D structure and 3D structure.

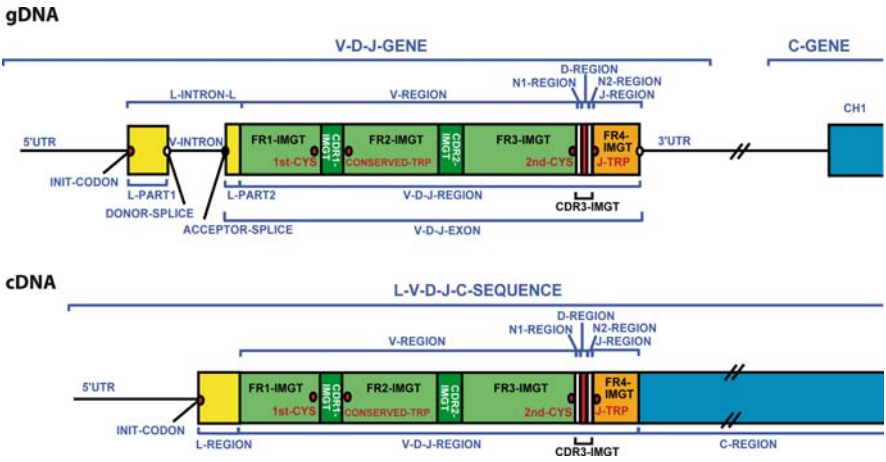


Fig. 2.2 IMGT® standardized labels. The molecular organization of an IGH rearranged sequence in genomic DNA (gDNA) and complementary DNA (cDNA) is shown as an example. In gDNA, the V-D-J-GENE comprises two exons: L-PART1 (L for leader) and the V-D-J-EXON. The V-D-J-EXON codes L-PART2 and the V-D-J-REGION. The V-D-J-REGION corresponds to the VH domain. In cDNA, the L-V-D-J-C-SEQUENCE comprises the complete coding region (L-REGION, V-D-J-REGION and C-REGION). IMGT/V-QUEST (Brochet et al. 2008) analyses the nucleotide sequences of the light chain V-J-REGION and heavy chain V-D-J-REGION, whereas IMGT/JunctionAnalysis (Yousfi Monod et al. 2004) analyses specifically the JUNCTION (the JUNCTION corresponds to the CDR3-IMGT with the anchor positions 2nd-CYS 104 and J-TRP or J-PHE 118 included). IMGT/DomainGapAlign analyses the amino acid sequences of the VH or VL (V-KAPPA or V-LAMBDA) domains as well as those of the C domains, which correspond to the C-REGION (C-KAPPA, C-LAMBDA) or to part of it (for example, CH1, CH2 and CH3 of IG-Heavy-Gamma chains)

2.2.2 *IMGT Collier de Perles*

IMGT Collier de Perles (Ruiz and Lefranc 2002; Kaas and Lefranc 2007; Kaas et al. 2007) is a graphical two-dimensional (2D) representation of domain, based on the IMGT unique numbering, that bridges the gap between sequence and 3D structure (Lefranc et al. 2008) (Fig. 2.3). Conserved amino acids from frameworks (FR-IMGT) of the V and C domains always have the same number whatever the receptor type (IG, TR or other IgSF), the chain type, the domain (V or C), and the species they come from e.g. cysteine 23 (B-STRAND), tryptophan 41 (C-STRAND), hydrophobic amino acid 89 (E-STRAND) and cysteine 104 (F-STRAND) (Lefranc et al. 2003, 2005b). In a V domain, complementarity determining region (CDR-IMGT) lengths (loops BC, C'C", FG) are crucial information shown between brackets and separated by dots, for example [8.10.12]. In FR-IMGT, the hydrophobic amino acids (hydropathy index with positive value) and tryptophan (W) found at a given position in more than 50% of sequences are displayed with a blue background colour. The IMGT Colliers de Perles can be displayed on two layers in order to get a graphical representation closer to the 3D structure (Fig. 2.3).

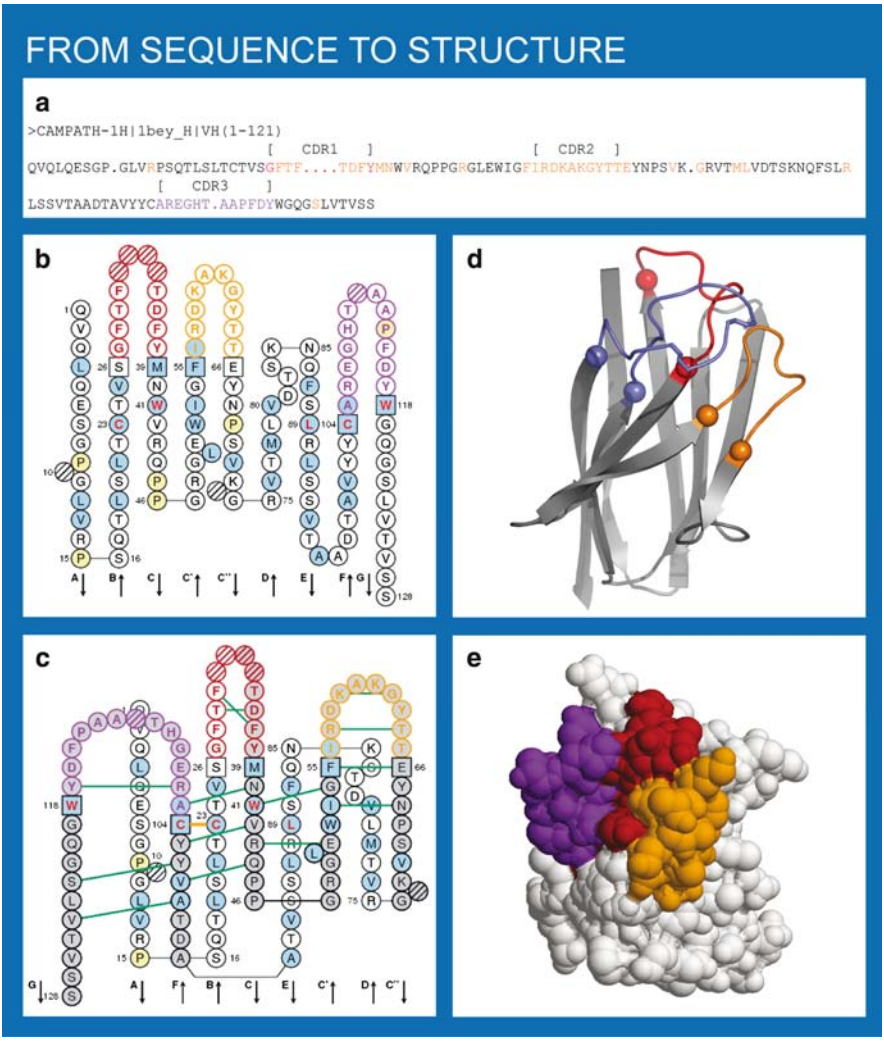


Fig. 2.3 From sequence to structure. The VH domain of the alemtuzumab antibody is shown as an example illustrating the IMGT approach from sequence to three-dimensional (3D) structure (IMGT/3DstructureDB and PDB code: 1bey). (**a**) VH amino acid sequence (http://www.imgt.org). (**b**) IMGT Collier de Perles on one layer. (**c**) IMGT Collier de Perles on two layers. Hydrogen bonds between the amino acids of the C, C′, C″, and F and G strands and those of the CDR-IMGT are shown. (**d**) Ribbon 3D representation. (**e**) Spacefill 3D representation. The CDR1-IMGT, CDR2-IMGT and CDR3-IMGT regions are coloured in red, orange and purple, respectively (IMGT Color menu). The CDR-IMGT lengths are [8.10.12]. Anchor positions are shown as squares in B and C (26 and 39, 55 and 66, 104 and 118), and as spheres in D. Hydrophobic amino acids (hydropathy index with positive value) and tryptophan (W) found at a given position in more than 50% of analysed IG and TR sequences are shown in blue in B and C

The IMGT Colliers de Perles are used in antibody engineering and antibody humanization (Pelat et al. 2008), and for the evaluation of the immunogenicity of therapeutic monoclonal antibodies (Magdelaine-Beuzelin et al. 2007). The information is particularly useful:

1. To precisely define the CDR1-IMGT, CDR2-IMGT and CDR3-IMGT to be grafted in antibody humanization design based on CDR grafting.
2. To localize the amino acids of the CDR-IMGT loops that may be involved in the contacts with the antigen (see Sect. 4.4.2).
3. To identify potential immunogenic residues at given positions in chimeric or humanized antibodies (Magdelaine-Beuzelin et al. 2007).
4. To visualize the repartition of stereotypic patterns (Stamatopoulos et al. 2007).
5. To compare the physicochemical properties of amino acids at given positions to the IMGT Collier de Perles statistical profiles for the human expressed IGHV, IGKV and IGLV repertoires (Pommié et al. 2004) or to the closest V allele IMGT Collier de Perles.
6. To give the possibility to structurally analyse amino acid sequences even in the absence of 3D structures, as demonstrated in IMGT/2Dstructure-DB (see Sect. 4.3).
7. To bridge the gap between linear amino acid sequences and 3D structures, as illustrated by the display of hydrogen bonds for crystallized V type domains (Fig. 2.3) and C type domains (IMGT Collier de Perles on two layers in IMGT/3Dstructure-DB (Kaas et al. 2004) (see Sect. 4.4.1).

2.3 From Nucleotide Sequence to 2D Structure: IMGT/V-QUEST

2.3.1 IMGT/V-QUEST Search

An IMGT/V-QUEST search consists of two easy steps:

– The user selects the antigen receptor (IG or TR) and the species on the IMGT/V-QUEST Home page.
– On the next page, the user submits up to 50 nucleotide sequences in FASTA format. By clicking on “Start”, the analysis is done automatically with the default parameters (Brochet et al. 2008; Giudicelli and Lefranc 2008).

Prior to launching the search, the user may customize the result display options in “Selection for result display”. They can export the results in text and choose the number (Nb) of nucleotides per line in alignments. They can select between two options:

1. “Detailed view” for the display of the results of each analysed sequence individually (with a choice of 14 different result displays) (detailed in Brochet et al. 2008; Giudicelli and Lefranc 2008).

2. "Synthesis view" for the display of the alignments of sequences that express the same V gene and allele (with a choice of eight different result displays) (detailed in Brochet et al. 2008; Giudicelli and Lefranc 2008).

For sophisticated queries or for unusual sequences, the user can modify the default values in "Advanced parameters" (Brochet et al. 2008; Giudicelli and Lefranc 2008). The customizable values are:

1. "Selection of IMGT reference directory set" used for the V, D, J genes and alleles identification and alignments ("F+ORF", "F+ORF+in frame P", "F+ORF including orphons", "F+ORF+in frame P including orphons", where F is functional, ORF is open reading frame and P is pseudogene). This allows the user to work with only relevant gene sequences (for example, orphon sequences are relevant for genomic but not expressed repertoire studies). The selected set can also be chosen either "With all alleles" or "With allele *01 only".
2. "Search for insertions and deletions". In that case, the number of submitted sequences in a single run is limited to 10.
3. "Parameters for IMGT/JunctionAnalysis": Nb of D-GENEs allowed in the IGH, TRB and TRD junctions and Nb of accepted mutations in 3'V-REGION, D-REGION and 5'J-REGION (default values are indicated per locus in the IMGT/V-QUEST Documentation).
4. "Parameters for Detailed View": "Nb of nucleotides to exclude in 5' of the V-REGION for the evaluation of the nb of mutations" (to avoid, for example, to count primer specific nucleotides), and/or "Nb of nucleotides to add (or exclude) in 3' of the V-REGION for the evaluation of the alignment score" (for example in case of low or high exonuclease activity).

2.3.2 IMGT/V-QUEST Output

2.3.2.1 "Detailed View"

The top of the "Detailed view" result page indicates the number of analysed sequences with links to individual results. Each individual result comprises the user sequence displayed in FASTA format (a sequence submitted in antisense orientation is shown as complementary reverse sequence, that is in V gene sense orientation), a "Result summary" table followed, if all parameters were selected, by the 14 different result displays (detailed in Brochet et al. 2008; Lefranc 2004; Giudicelli and Lefranc 2005, 2008).

1. The "Result summary" provides a crucial feature that is the evaluation of the user sequence functionality performed by IMGT/V-QUEST: productive (if no stop codon and in frame junction) or unproductive (if stop codons and/or out of frame junction). It also summarizes the main characteristics of the analysed sequence which include:

- The names of the closest "V-GENE and allele" and "J-GENE and allele" with the alignment score and the percentage of identity,
- The name of the closest "D-GENE and allele" with the D-REGION reading frame,
- The three CDR-IMGT lengths (shown between brackets, for example [8.8.13]) which characterize a V domain,
- The amino acid (AA) JUNCTION sequence.
  IMGT/V-QUEST provides warnings that appear, as notes in red to alert the user, if potential insertions or deletions are suspected in the V (sequences with less than 85% of identity and/or with different CDR1-IMGT and/or CDR2-IMGT lengths compared to the closest germline V-REGION), or if other possibilities for the J gene and allele are identified.
  If the option "Search for insertions and deletions" was selected, the detection and detailed description of insertions and/or deletions are shown in the "Result summary" first row to capture the user attention. Moreover insertions appear as capital letters in the FASTA sequence.

2. Below the "Result summary" are shown the following result displays:

   - The alignments for the V-, D- and J-GENE (detailed in Brochet et al. 2008; Lefranc 2004; Giudicelli and Lefranc 2005, 2008) with the alignment score and the identity percentage with the five closest genes and alleles and, for the V, the length of the V-REGION taken into account for the score evaluation.
   - "Results of IMGT/JunctionAnalysis" (detailed in Yousfi Monod et al. 2004; Giudicelli and Lefranc 2008) with, if selected, the list of eligible D genes and alleles which match more than four nucleotides (nt) with the junction, allowing the user to visualize the result among other close solutions.
   - Different displays of the V region:
     - "V-REGION alignment",
     - "V-REGION translation" (Fig. 2.4),
     - "V-REGION protein display".
   - Different displays of mutations affecting the V region:
     - "V-REGION mutation table" that lists the mutations (nt and AA) of the analysed sequence compared to the closest V-REGION allele. They are described for the V-REGION and for each FR-IMGT and CDR-IMGT, with their positions, and for the AA changes according to the IMGT AA classes (Pommié et al. 2004). For example c16>g, Q6>E (++−) means that the nt mutation (c>g) leads to an AA change at codon 6 with the same hydropathy (+) and volume (+) but with different physicochemical properties (−) classes (Pommié et al. 2004). It is the first time that such qualification of amino acid replacement is provided. This has led to identify 4 types of AA changes: very similar (+++), similar (++−, +−+), dissimilar (−−+, −+−, +−−) and very dissimilar (−−−).

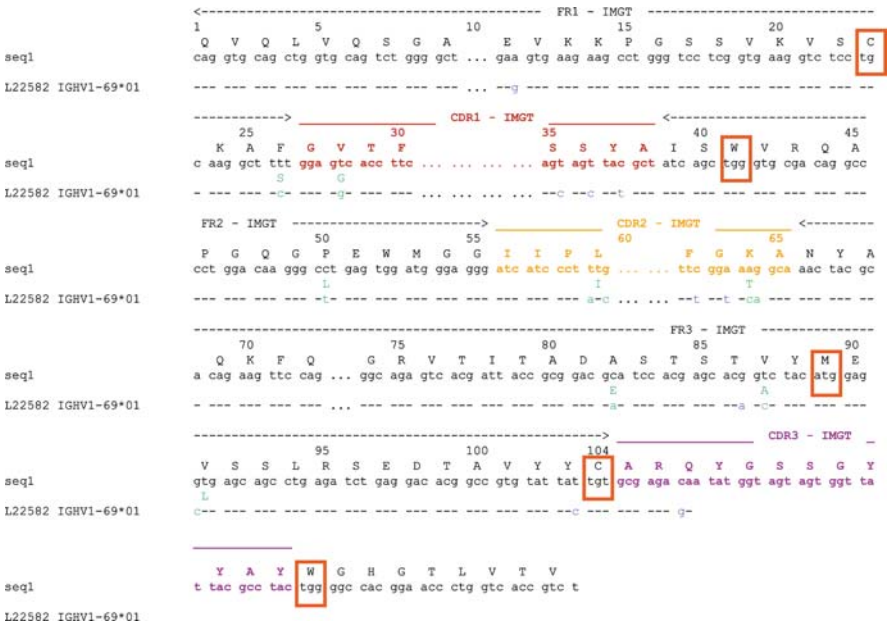


Fig. 2.4 "V-REGION translation". The FR1-IMGT and CDR-IMGT are delimited according to the IMGT unique numbering (Lefranc et al. 2003). Mutations and amino acid changes for nonsilent mutations are shown by comparison with the closest germline V (IGHV1-69*01). The seq1 accession number is DQ100777 from the IMGT/LIGM-DB database (Giudicelli et al. 2006). V-REGION translation is one of the 14 different result displays from "Detailed view" results of IMGT/V-QUEST (see Sect. 3.2.1). Other result displays are detailed in (Brochet et al. 2008; Yousfi Monod et al. 2004; Lefranc 2004; Giudicelli and Lefranc 2005, 2008) and in the IMGT/V-QUEST Documentation (http://www.imgt.org)

  - "V-REGION mutation statistics" that evaluates the number of silent and nonsilent mutations and the number of transitions and transversions of the analysed nucleotide sequence, and the number of AA changes of its translated sequence.
  - "V-REGION mutation hot spots" that shows the patterns and localization of hot spots in the closest germline V-REGION. The identified hot spot patterns are (a/t)a and (a/g)g(c/t)(a/t), and the complementary reverse motifs are t(a/t) and (a/t)(a/g)c(c/t) (see: Lefranc M-P. and Lefranc G. Somatic hypermutations, in IMGT Education, http://www.imgt.org).

- "IMGT Collier de Perles" either as a link to the IMGT/Collier-de-Perles tool (see Sect. 4.2) or as a direct representation integrated in IMGT/V-QUEST results (see Sect. 2.2).
- "Sequences of V-, V-J- or V-D-J-REGION ("nt" and "AA") with gaps in FASTA and access to IMGT/PhyloGene for V-REGION ("nt")" that provides the analysed sequence with IMGT gaps, in FASTA format and on one line, and a link to IMGT/PhyloGene (Elemento and Lefranc 2003).

– "Annotation by IMGT/Automat" (Giudicelli et al. 2003, 2005b) that uses the results of the analysis to provide a full automatic annotation of the user sequences for the V-J-REGION or V-D-J-REGION.

2.3.2.2 "Synthesis View"

The aim of "Synthesis view", a novel IMGT/V-QUEST result, is to facilitate the comparison of sequences that express the same V gene and allele: it allows to compare the localization of the mutations and the composition of their junctions. The "Synthesis view" comprises a "Summary table" (Fig. 2.5) and eight different displays (if all were selected) (see details in Brochet et al. 2008; Giudicelli and Lefranc 2008). The "Summary table" shows, for each sequence, the name of the closest V gene and allele, the evaluation of the sequence functionality, the V score and percentage of identity, the name of the closest J and D genes and alleles, the D-REGION reading frame, the three CDR-IMGT lengths, the AA JUNCTION and the JUNCTION frame. Warnings appear to alert the user on potential insertions or deletions in the V or on other possibilities for the J gene and allele. In such cases it is strongly recommended to check the individual results of these sequences in "Detailed view".

The originality of "Synthesis view" is also to provide alignments of sequences which, in a given run, are assigned to the same V gene and allele. "Alignment for V-GENE", "V-REGION alignment" and "V-REGION translation" are based on the same characteristics as those of "Detailed view". In addition, the hot spot positions are underlined in the germline V-REGION (for an easy comparison with the mutation localizations) and the name of the closest J gene allele is indicated at the 3' end of each sequence. The "V-REGION protein display" shows amino acid sequences aligned with the closest V-REGION allele. This protein display is also provided with AA colours according to the IMGT AA classes (Pommié et al. 2004) or with only the AA changes displayed. The "V-REGION most frequently occurring AA per position and per FR-IMGT and CDR-IMGT" table is given for each alignment to highlight the position of conserved AA in sequence batches. The "Results of IMGT/JunctionAnalysis" are displayed per locus (for example, for the IG sequences, IGH, IGK and IGL) (Fig. 2.5).

2.4 From Amino Acid Sequence to 3D Structure

2.4.1 IMGT/DomainGapAlign

IMGT/DomainGapAlign analyses amino acid domain sequences by comparison with the IMGT reference directory sets (translation of the germline V and J genes and of the C gene domains from IMGT/GENE-DB (Giudicelli et al. 2005a)). These

reference amino acid sequences can be displayed by querying IMGT/DomainDisplay (Fig. 2.1). Several amino acid sequences can be analysed simultaneously, provided that they belong to the same domain type. IMGT/DomainGapAlign identifies the closest germline V-REGION and J-REGION alleles (for V domain) and the closest C-DOMAIN alleles (for C domain). IMGT/DomainGapAlign displays the V region amino acid sequences of the user aligned with the closest V and J regions (Fig. 2.6), or the closest C domain, with IMGT gaps and delimitations of the FR-IMGT and CDR-IMGT according to the IMGT unique numbering (Lefranc et al. 2003, 2005b). For instance, the V-REGION and J-REGION of the alemtuzumab VH domain is identified as having 73 and 92.9% identity with the *Homo sapiens* IGHV4-59*01 and IGHJ4*01, respectively. If several closest alleles are identified, the user can select the display of each corresponding alignment (for example IGHJ4*02 and IGHJ4*03) (Fig. 2.6). The amino acid sequence is displayed, using the IMGT Color menu, with the delimitations of the V-REGION, J-REGION, and for VH domains, N-AND-D-REGION. The complete IMGT Collier de Perles (including CDR3-IMGT and FR4-IMGT) of the analysed VH or VL domain (V-D-J region or V-J region, respectively) is also available (Fig. 2.6). The number of amino acid differences in the FR-IMGT has been used to evaluate the potential immunogenicity of nonhuman primate antibodies (Pelat et al. 2008) and therapeutic monoclonal antibodies (Magdelaine-Beuzelin et al. 2007). The framework of a VH domain comprises 91 positions (25, 17, 38 and 11 positions for FR1-, FR2-, FR3- and FR4-IMGT, respectively), whereas the framework of a VL domain comprises 89 positions (26, 17, 36, 10 positions for FR1-, FR2-, FR3- and FR4-IMGT, respectively) (Magdelaine-Beuzelin et al. 2007). Thus the framework of the alemtuzumab VH is 84.61% (77/91) identical to the framework constituted by the closest human germline IGHV4-59*01 and IGHJ4*01, with 14 different amino acids changes (Pommié et al. 2004), whereas the framework of the trastuzumab VH is 90.10% (82/91) identical to the framework constituted by the closest human germline IGHV3-66*01 and IGHJ6*01, with nine different amino acids (Magdelaine-Beuzelin et al. 2007).

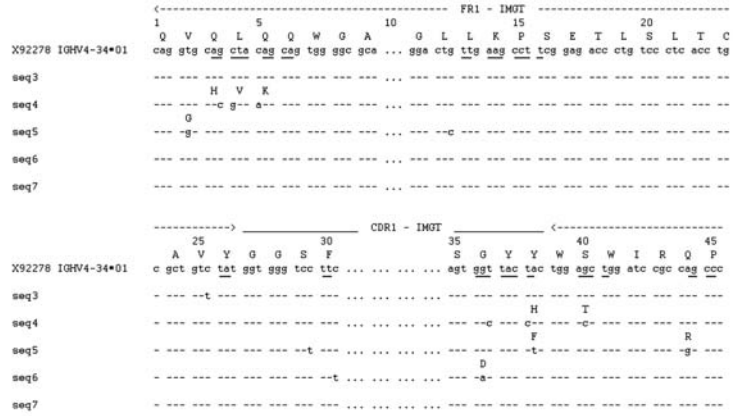
2.4.2 IMGT/Collier-de-Perles Tool

The IMGT/Collier-de-Perles tool, on the IMGT® Web site at http://www.imgt.org, allows the user to draw IMGT Colliers de Perles, on one or two layers, starting from their own domain amino acid sequences. Sequences have to be gapped according to the IMGT unique numbering (using for example IMGT/DomainGapAlign). IMGT/Collier-de-Perles tool can be customized to display the CDR-IMGT according to the IMGT Color menu or the amino acids according to their hydropathy, volume or IMGT physicochemical classes (Pommié et al. 2004).

a Summary table

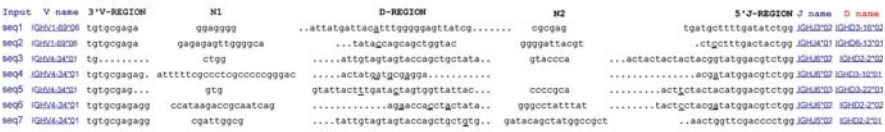
Sequence ID	V-GENE and allele	Functionality	V-REGION score	V-REGION identity % (nt)	J-GENE and allele	D-GENE and allele	D-REGION reading frame	CDR-IMGT lengths	AA JUNCTION	JUNCTION frame
seq1	IGHV1-69*06	Productive	1050	95,63% (219/229 nt)	IGHJ3*02	IGHD3-16*02	2	[8.8.21]	CARGGDYDYIWGSYRASDAFDIW	in-frame
seq2	IGHV1-69*06	Productive	1300	94,79% (273/288 nt)	IGHJ4*01	IGHD6-13*01	1	[8.8.21]	CARERVGAYTSSWYGDYVSFDYW	in-frame
seq3	IGHV4-34*01	Productive	1411	99,30% (283/285 nt)	IGHJ6*02	IGHD2-2*02	3	[8.7.20]	CWIVVVPAAIVPNYYYYGMDVW	in-frame
seq4	IGHV4-34*01	Productive	1294	95,09% (271/285 nt)	IGHJ6*02	IGHD3-10*01	2	[8.7.20]	CARDFSPSPPGHYDARNDMDVW	in-frame
seq5	IGHV4-34*01	Productive	1285	94,74% (270/285 nt)	IGHJ6*03	IGHD3-22*01	2	[8.7.21]	CARWYYFDTSGYYPRNFYYMDVW	in-frame
seq6	IGHV4-34*01	Productive	1258	93,68% (267/285 nt)	IGHJ6*02	IGHD2-2*02	3	[8.7.25]	CARGHKTAIREPPTIGPIYYSYDMDVW	in-frame
seq7	IGHV4-34*01	Productive	1420	100,00% (285/285 nt)	IGHJ5*02	IGHD2-2*01	3	[8.7.25]	CARGDWRIVVVPAAVDTAMAANWFDPW	in-frame

b Sequences aligned with IGHV4-34*01: V-REGION translation



c Results of IMGT/JunctionAnalysis:

Analysis of the JUNCTIONS



Translation of the JUNCTIONS

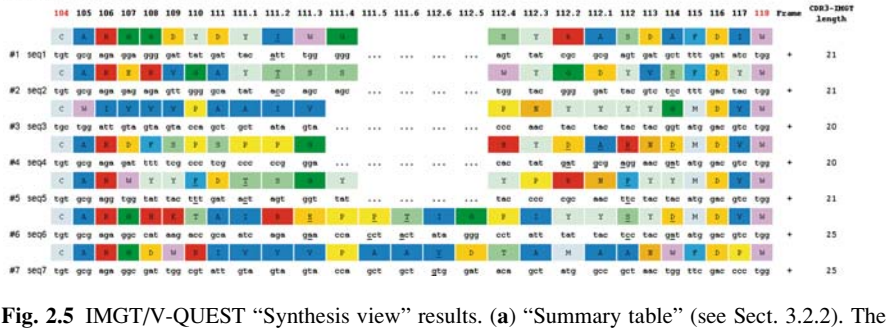


Fig. 2.5 IMGT/V-QUEST "Synthesis view" results. (**a**) "Summary table" (see Sect. 3.2.2). The accession numbers are from IMGT/LIGM-DB (Giudicelli et al. 2006). (**b**) Sequences aligned with IGHV4-34*01. Part of the "V-REGION translation" display is shown. Sequences of the set, which have been identified as using IGHV4-34*01 (closest germline gene and allele), are aligned, with nucleotide (nt) mutations and amino acid changes shown by comparison with IGHV4-34*01. Dashes indicate identical nucleotides. Dots represent gaps according to the IMGT unique

2.4.3 IMGT/2Dstructure-DB

In a further effort to bridge the gap between sequence and 3D structure, a new extension of IMGT/3Dstructure-DB, designated as IMGT/2Dstructure-DB, was recently created to describe and analyse amino acid sequences of antibodies for which no 3D structures are available. These amino acid sequences are analysed and managed with the IMGT® criteria of standardized nomenclature, description and numerotation. IMGT/2Dstructure-DB uses the IMGT/3Dstructure-DB informatics frame and interface (see Sect. 4.4) which allow to analyse, manage and query antibodies as polymeric receptors made of several chains, in contrast to the IMGT/LIGM-DB sequence database that analyses and manages IG chains, individually. The current IMGT/2Dstructure-DB entries include sequences of antibodies ("-mab") and sequences of fusion proteins for immune applications (FPIA) ("-cept") from the WHO International Nonproprietary Names (INN) programme (http://www.who.int/medicines/services/inn/en/). Queries can be made on the INN name or the INN code (for example INN: 8005 for alemtuzumab). The IMGT/2Dstructure-DB cards provide standardized IMGT information on chains and domains and IMGT Colliers de Perles on one or two layers as described later (see Sect. 4.4); however, the information on experimental structural data (hydrogen bonds in IMGT Collier de Perles on two layers, Contact analysis) is only available in the corresponding IMGT/3Dstructure-DB cards, if the antibodies have been crystallized.

2.4.4 IMGT/3Dstructure-DB

2.4.4.1 IMGT/3Dstructure-DB Card

The "IMGT/3Dstructure-DB card" is the core unit of IMGT/3Dstructure-DB (detailed in Kaas et al. 2004). Indeed, there is one card per IMGT/3Dstructure-DB entry and this card provides access to all data related to that entry. This card has been used as model for the IMGT/2Dstructure-DB card (Sect. 4.3). The section "Chain details" of the IMGT/3Dstructure-DB card comprises information first on

numbering (Lefranc et al. 2003). Underlined nucleotides in the IGHV4-34*01 correspond to mutation hot spot positions. (**c**) "Results of IMGT/JunctionAnalysis". Results shown per locus (here IGH) comprise "Analysis of the JUNCTIONs" and "Translation of the JUNCTIONs" with amino acids coloured according to the 11 IMGT physicochemical classes (Pommié et al. 2004). Additional results, not shown in the figure, are provided on the IMGT® Web site. They include the number of mutations in the 3'V-REGION (Vmut), D-REGION (Dmut) and 5'J-REGION (Jmut), the ratio of the number of g+c nucleotides to the total number of N region nucleotides (Ngc) and the molecular mass and pI of the junction amino acid sequence (Brochet et al. 2008; Yousfi Monod et al. 2004; Lefranc 2004; Giudicelli and Lefranc 2005, 2008)

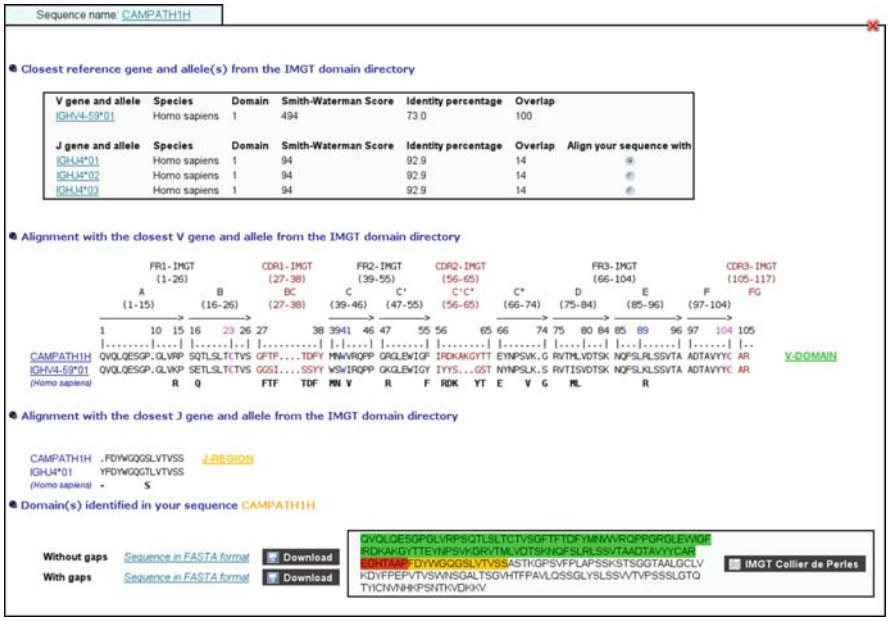


Fig. 2.6 IMGT/DomainGapAlign results for a VH domain. The V-REGION and J-REGION of the alemtuzumab VH domain is identified as having 73 and 92.9% identity at the amino acid level with the *Homo sapiens* IGHV4-59*01 and IGHJ4*01, respectively. The user can display the alignment of the sequence with IGHJ4*02 and IGHJ4*03 that have the same score as IGHJ4*01. Amino acid differences are indicated below the V and J alignments. IMGT/DomainGapAlign displays the V region amino acid sequence of the user with IMGT gaps and delimitations of the FR-IMGT and CDR-IMGT according to the IMGT unique numbering (Lefranc et al. 2003). The VH domain sequence is displayed with the V-REGION in green, N-AND-D-REGION in red and the J-REGION in yellow according to the IMGT Color menu. The complete IMGT Collier de Perles (including CDR3-IMGT and FR4-IMGT) is available for the analysed V-J or V-D-J region by clicking on the button

the chain itself, then per domain (Fig. 2.7). Chains and domains are described with standardized IMGT® labels.

1. The information for each chain includes:
   - Chain ID (for example 1bey_H),
   - Chain length in amino acids (for example 219),
   - IMGT chain description with the delimitations of the different domains (for example VH+CH1 = VH(1-121) + CH1(122-210),
   - Chain sequence with delimitations of the regions and domains, highlighting of AA that are different from the closest genes and alleles, and links to *Sequence in FASTA format* and to *Sequence in IMGT format*.
2. The information for each V domain, as an example, includes:
   - IMGT domain description (for example VH),

Fig. 2.7 IMGT/3Dstructure-DB card. "IMGT/3Dstructure-DB card" is available for each entry of the database. "Chain details" section for the VH-CH1 chain (1bey_H) of the alemtuzumab Fab is shown. Chains and domains are described with standardized IMGT labels (see Sect. 4.4.1). Similar result displays are provided for IMGT/2Dstructure-DB cards (see Sect. 4.3). However, in those cases, information on experimental structural data (hydrogen bonds in IMGT Collier de Perles on two layers, Domain contacts) is only available in the corresponding IMGT/3Dstructure-DB cards, if the antibodies have been crystallized

- IMGT gene and allele name with the percentage of identity for the V (for example IGHV4-59*01 (73.00%) (Human)) and a link to *Alignment details*
- IMGT gene and allele name with the percentage of identity for the J (for example IGHJ4*01 (92.90%)(Human) as well as other alleles giving the same percentage of identity), and a link to *Alignment details*,
- 2D representation: links to *IMGT Collier de Perles on one layer or IMGT Collier de Perles on two layers*,
- Contact analysis: a link to *Domain contacts (overview)*,
- CDR-IMGT lengths (for example [8.10.12]),
- Sheet composition (for example [A'BDE][A"CC'C"FG]),
- The domain amino acid sequence with CDR-IMGT delimitations and highlighting of AA that are different from the closest V and J genes and alleles,
- Link to *IMGT/DomainGapAlign results*.

2.4.4.2 IMGT/3Dstructure-DB Contact Analysis

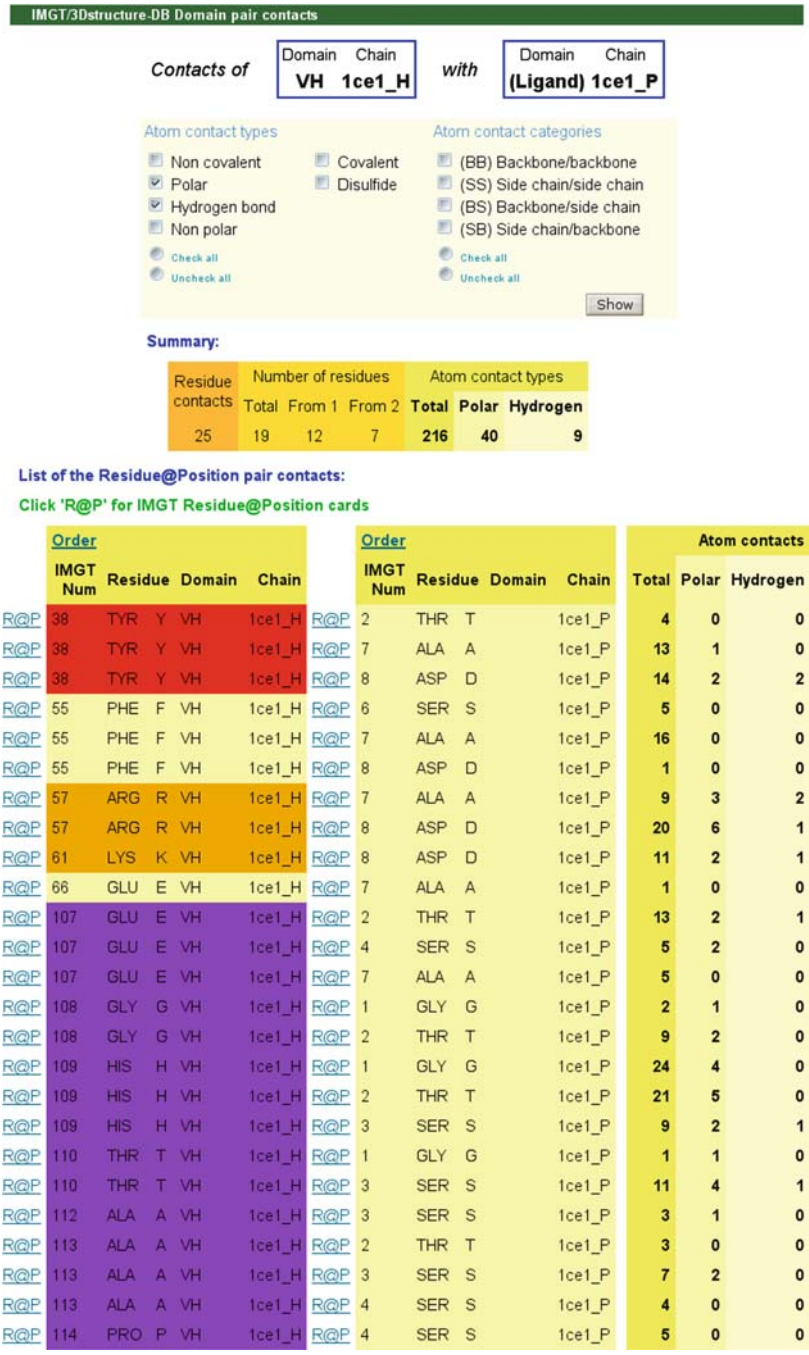
The IMGT/3Dstructure-DB Contact analysis (detailed in Kaas et al. 2004) provides extensive information on the contacts between domains and/or chains and on the internal contacts in an IMGT/3Dstructure-DB entry. This information can be obtained at different levels.

1. Domain contacts (overview),
2. Domain pair contacts ("DomPair") that provides information on the contacts between a pair of partners (for example VH domain of 1ce1_H chain with the ligand (1ce1_P chain)) (Fig. 2.8),
3. IMGT Residue@Position card ("R@P") that provides structural information and contacts for a given residue at a given position, or IMGT Residue@Position. An IMGT Residue@Position is defined by the IMGT position numbering, the residue name, the IMGT domain description and the IMGT chain ID (for example 28 – PHE (F) – VH – 1ce1_H). The IMGT Residue@Position cards can be accessed directly from the amino acid sequences of the IMGT/3Dstructure-DB card or from the IMGT Colliers de Perles, by clicking on one AA.

Contacts at each level can be queried by atom contact types (Noncovalent, Polar, Hydrogen bond, etc.) and/or atom contact categories ((BB) Backbone/backbone, (SS) Side chain/side chain, etc.) (Kaas et al. 2004).

2.4.5 IMGT/StructuralQuery and IMGT/DomainSuperimpose

IMGT/DomainSuperimpose allows to superimpose the 3D structures of two domains from IMGT/3Dstructure-DB. IMGT/StructuralQuery (Kaas et al. 2004)



IMGT/3Dstructure-DB Domain pair contacts

Contacts of Domain: VH, Chain: 1ce1_H *with* Domain: (Ligand), Chain: 1ce1_P

Atom contact types: Non covalent, Polar, Hydrogen bond, Non polar, Covalent, Disulfide; Check all / Uncheck all

Atom contact categories: (BB) Backbone/backbone, (SS) Side chain/side chain, (BS) Backbone/side chain, (SB) Side chain/backbone; Check all / Uncheck all

Show

Summary:

Residue contacts	Number of residues			Atom contact types		
	Total	From 1	From 2	Total	Polar	Hydrogen
25	19	12	7	216	40	9

List of the Residue@Position pair contacts:

Click 'R@P' for IMGT Residue@Position cards

Order						Order				Atom contacts		
	IMGT Num	Residue	Domain	Chain		IMGT Num	Residue	Domain	Chain	Total	Polar	Hydrogen
R@P	38	TYR Y	VH	1ce1_H	R@P	2	THR T		1ce1_P	4	0	0
R@P	38	TYR Y	VH	1ce1_H	R@P	7	ALA A		1ce1_P	13	1	0
R@P	38	TYR Y	VH	1ce1_H	R@P	8	ASP D		1ce1_P	14	2	2
R@P	55	PHE F	VH	1ce1_H	R@P	6	SER S		1ce1_P	5	0	0
R@P	55	PHE F	VH	1ce1_H	R@P	7	ALA A		1ce1_P	16	0	0
R@P	55	PHE F	VH	1ce1_H	R@P	8	ASP D		1ce1_P	1	0	0
R@P	57	ARG R	VH	1ce1_H	R@P	7	ALA A		1ce1_P	9	3	2
R@P	57	ARG R	VH	1ce1_H	R@P	8	ASP D		1ce1_P	20	6	1
R@P	61	LYS K	VH	1ce1_H	R@P	8	ASP D		1ce1_P	11	2	1
R@P	66	GLU E	VH	1ce1_H	R@P	7	ALA A		1ce1_P	1	0	0
R@P	107	GLU E	VH	1ce1_H	R@P	2	THR T		1ce1_P	13	2	1
R@P	107	GLU E	VH	1ce1_H	R@P	4	SER S		1ce1_P	5	2	0
R@P	107	GLU E	VH	1ce1_H	R@P	7	ALA A		1ce1_P	5	0	0
R@P	108	GLY G	VH	1ce1_H	R@P	1	GLY G		1ce1_P	2	1	0
R@P	108	GLY G	VH	1ce1_H	R@P	2	THR T		1ce1_P	9	2	0
R@P	109	HIS H	VH	1ce1_H	R@P	1	GLY G		1ce1_P	24	4	0
R@P	109	HIS H	VH	1ce1_H	R@P	2	THR T		1ce1_P	21	5	0
R@P	109	HIS H	VH	1ce1_H	R@P	3	SER S		1ce1_P	9	2	1
R@P	110	THR T	VH	1ce1_H	R@P	1	GLY G		1ce1_P	1	1	0
R@P	110	THR T	VH	1ce1_H	R@P	3	SER S		1ce1_P	11	4	1
R@P	112	ALA A	VH	1ce1_H	R@P	3	SER S		1ce1_P	3	1	0
R@P	113	ALA A	VH	1ce1_H	R@P	2	THR T		1ce1_P	3	0	0
R@P	113	ALA A	VH	1ce1_H	R@P	3	SER S		1ce1_P	7	2	0
R@P	113	ALA A	VH	1ce1_H	R@P	4	SER S		1ce1_P	4	0	0
R@P	114	PRO P	VH	1ce1_H	R@P	4	SER S		1ce1_P	5	0	0

Fig. 2.8 IMGT/3Dstructure-DB Contact analysis results. IMGT/3Dstructure-DB Domain pair contacts between the VH domain of alemtuzumab (1ce1_H) and the CD52 peptide ligand

allows to retrieve the IMGT/3Dstructure-DB entries containing a V, C or G domain, based on specific structural characteristics of the intramolecular interactions: phi and psi angles, accessible surface area, type of atom contacts, distance in angstrom between amino acids, Residue@Position contacts and, for V domain, CDR-IMGT length or pattern.

2.5 Conclusion

IMGT®, the international ImMunoGeneTics information system®, http://www.imgt.org has developed standard tools for the analysis of the antibody sequences and structures that describe the main characteristics required for research, clinical studies, antibody engineering and antibody humanization. IMGT® tools are constantly improved following the results of basic and clinical research. New alleles are annotated in IMGT® and integrated in the IMGT reference directory as soon as they are confirmed experimentally and publicly available in the generalist databases. Rules for sequence and structure analysis have been improved through scientific collaboration and with the constant feedback of clinicians and scientists. IMGT/V-QUEST has been recommended by the European research Initiative on chronic lymphocytic leukaemia CLL (ERIC) for the analysis of the IGHV gene mutational status in CLL (Ghia et al. 2007). IMGT® standards are approved by the WHO-IUIS subcommittee for IG and TR (Lefranc 2007, 2008). Through its efforts for standardization, IMGT® aims to answer the needs of the researchers, clinicians and biotechnology scientists, and to maintain an international information system of high quality for immunogenetics and immunoinformatics.

2.6 Availability and Citation

Authors who use IMGT® databases and tools are encouraged to cite this study and to quote the IMGT® Home page, http://www.imgt.org. Online access to IMGT® databases and tools is freely available for academics and under licences and contracts for companies.

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Fig. 2.8 (continued) (1ce1_P) are shown. "Polar" and "Hydrogen bonds" were selected prior to display, in "Atom contact types". Amino acids belonging to the CDR1-IMGT, CDR2-IMGT and CDR3-IMGT are coloured in red, orange and purple, respectively. The positions 55 and 66 are anchor positions. Clicking on R@P gives access to the IMGT Residue@Position cards (see Sect. 4.4.2)

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