mAbs 6:5, 1145–1154; September/October 2014; © 2014 Taylor & Francis Group, LLC

In vitro and in vivo modifications of recombinant and human IgG antibodies

Hongcheng Liu[1,*], Gomathinayagam Ponniah[1], Hui-Min Zhang[2], Christine Nowak[1], Alyssa Neill[1], Nidia Gonzalez-Lopez[1], Rekha Patel[1], Guilong Cheng[1], Adriana Z Kita[1], and Bruce Andrien[1]

[1]Protein Characterization; Alexion Pharmaceuticals Inc.; Cheshire, CT USA; [2]Bioprocess Development; Merck Research Laboratories; Merck; Kenilworth, NJ USA

Keywords: recombinant monoclonal antibodies, endogenous IgG antibodies, posttranslational modifications, pyroglutamate, leader sequence, C-terminal lysine, oligosaccharides

Tremendous knowledge has been gained in the understanding of various modifications of IgG antibodies, driven mainly by the fact that antibodies are one of the most important groups of therapeutic molecules and because of the development of advanced analytical techniques. Recombinant monoclonal antibody (mAb) therapeutics expressed in mammalian cell lines and endogenous IgG molecules secreted by B cells in the human body share some modifications, but each have some unique modifications. Modifications that are common to recombinant mAb and endogenous IgG molecules are considered to pose a lower risk of immunogenicity. On the other hand, modifications that are unique to recombinant mAbs could potentially pose higher risk. The focus of this review is the comparison of frequently observed modifications of recombinant monoclonal antibodies to those of endogenous IgG molecules.

Introduction

Most recombinant monoclonal antibody (mAb) therapeutics are produced in one of three mammalian cell lines, Chinese hamster ovary (CHO), murine NS0 or murine SP2/0. Although, in general, the amino acid sequence of recombinant mAbs are expressed in those cell lines with high fidelity, low levels of variation have been observed. The use of non-human cell lines can introduce post-translational modifications that are not intrinsically present in the human body. Such unnatural modifications may also be introduced during the period between purification and patient administration. The presence of those modifications is a concern due to the possibility of undesired effects such as loss of efficacy and increased immunogenicity.

In this review, we compiled data on modifications that occur in recombinant mAbs with the aim of answering three questions: 1) What modifications occur?; 2) What happens to recombinant monoclonal antibodies with those modifications in vivo?; and 3) Are the same modifications present in endogenous human IgGs? An underlying assumption is that a particular modification should pose a lower risk if it can be removed rapidly in circulation or if it is also present in endogenous IgG. The main categories discussed here are N-terminal modifications, C-terminal modifications, oligosaccharides, degradation of asparagine and aspartate, oxidation of methionine and tryptophan, cysteine-related variants and glycation. For each category, specific modifications will be discussed first for recombinant mAbs and then endogenous IgG antibodies.

*correspondence to: Hongcheng Liu; Email: liuh@alxn.com
Submitted: 06/12/2014; Revised: 07/07/2014; Accepted: 07/09/2014
http://dx.doi.org/10.4161/mabs.29883

N-Terminal Modifications

Cyclization of the N-terminal glutamine (Gln) or glutamate (Glu) to form pyroglutamate (pyroE) and incomplete removal of leader sequence are the two major types of N-terminal modifications. Truncation of the N-terminus resulting in the light chain lacking two amino acids has been reported in a recombinant mAb.[1] So far, however, truncation has not been established as a general modification of recombinant mAbs.

N-Terminal Pyroglutamate

It is common that the first amino acid of the light chain, heavy chain or both is either Gln or Glu, encoded in the genes. Spontaneous cyclization of N-terminal Gln[2-4] and to a lesser degree, N-terminal Glu[5-7] results in the formation of pyroE. The presence of pyroE has no effect on antibody structure[5] and antigen binding.[8] In addition, no difference in in vivo clearance between antibodies with N-terminal Glu compared with antibody with N-terminal pyroE has been observed.[9] One study demonstrated that the levels of pyroE of a recombinant mAb recovered from rat serum after 1 h in circulation did not show much difference compared with the starting material.[10] However, the reaction of cyclization of Gln is expected to continue in circulation because of the non-enzymatic nature of the reaction. Using a synthesized peptide, it was found that Gln was converted to pyroE at a rate of 1.41% per hour in cell culture.[4] Assuming a comparable in vivo rate, conversion of Gln to pyroE will be complete within a day because the majority of the N-terminal Gln of most recombinant mAbs is already cyclized after purification. The conversion from Glu to pyroE of recombinant mAbs

continues in vivo and pyroE naturally exists in endogenous human IgG.[9] Overall, this type of N-terminal modification is not expected to have a substantial effect on efficacy and safety.

Partial Leader Sequence

Incomplete removal of leader sequence has also been observed for recombinant mAbs.[1,8,11,12] Typically, only a portion of the leader sequence remains attached to the antibody instead of the entire leader sequence. The presence of a portion of the leader sequence has no effect on antigen binding,[8,11] structure, FcRn binding, or pharmacokinetics.[11] Signal peptides are composed of a hydrophobic region that is flanked by a polar region often with net positive charge on the N-terminal side and a polar region containing proline (Pro) and glycine (Gly) with small uncharged residues at positions -3 and -1 on the C-terminal side.[13] It is unlikely that the remaining leader sequence of recombinant mAbs will be removed in circulation because the remaining portion of the leader sequence does not have the structural characteristics required for cleavage. The presence of partial leader sequence in recombinant mAbs may likely be due to malfunctions of the cell machinery of the recombinant cell lines that are under stress to produce extremely high levels of proteins. In this sense, endogenous IgG antibodies should not have a partial leader sequence under normal physiological conditions. However, the presence of a partial leader sequence may not be a concern if human leader sequences were used for making the constructs of recombinant mAbs.

C-Terminal Modifications

The heavy chain C-terminal amino acid sequences encoded in the genes are PGK for IgG1, IgG2, IgG3, and LGK for IgG4. The first major modification is the removal of C-terminal lysine (Lys). The second major modification is amidation of Pro, for IgG1, IgG2 and IgG3 and leucine (Leu) for IgG4 with concurrent loss of Gly.

C-Terminal Lysine

C-terminal Lys is usually partially removed during mammalian cell culture.[14-16] Mammalian cell culture generates antibodies containing either zero, one or two C-terminal Lys residues. Removal of C-terminal Lys has no effect on structure,[11] thermal stability,[17] antigen binding and potency,[8,11,18] and FcRn binding and pharmacokinetics in rats.[11] Using a recombinant human IgG2 as a model, it was found that the half-life of the C-terminal Lys is about 62 min after intravenous injection in human.[19] This modification is only present at extremely low level (0.02% of total heavy chain) in endogenous human IgG.[19,20]

C-Terminal Amidation

Amidation of the C-terminal Pro residue was first identified in a recombinant monoclonal IgG1 antibody.[21] Later, another study demonstrated amidation is probably a common modification of mAbs because it was observed in multiple IgG1 and IgG4 molecules.[20] Amidation of IgG2 and IgG3 is also expected because IgG1, IgG2 and IgG3 share the same C-terminal sequence. The level of Pro amidation increased with the increase of copper added to the culture medium.[12] Amidation has no effect on antigen binding and Fc effector functions.[21] Amidation was not detected in endogenous human IgG antibodies.[20] However, because amidation has been commonly observed in biologically active peptides including peptide hormones and neurotransmitters in humans,[22] it is not considered an unnatural modification to the human immune system.

Oligosaccharides

Oligosaccharides are a well-studied modification of antibodies. In addition to glycosylation of the conserved asparagine (Asn) in the CH2 domain, 20–30% of human IgGs include N-linked glycosylation in variable domains.[23] N-linked glycosylation in the variable domains has been reported for recombinant mAbs.[24-26] Atypical glycosylation of recombinant mAbs has also been reported, including O-fucosylation of a serine (Ser) residue in light chain complementary-determining region (CDR)1[27] and N-glycosylation of Asn and Gln residues in non-consensus sequences.[27-29] These atypical glycosylations are only present to an extreme low level. A single fucosylation has been reported in human urinary-type plasminogen activator[30] and N-glycosylation of Asn in non-consensus sequence has also been observed in human endogenous IgG.[28] Therefore, those modifications may not be a concern with regard to immunogenicity.

The major glycoforms of recombinant mAbs expressed in CHO,[31-37] murine NS0,[35,36,38-40] and murine SP2/0 cell lines[25,36] are G0F, G1F and G2F. Some minor species are also common, including low percentages of afucosylated complex, high mannose, sialylated, and hybrid oligosaccharides[8,25,26,32,34,36-44] and low percentage of aglycosylated species.[26,34] The major difference between CHO cell lines and the two mouse cell lines is the presence of immunogenic α1, 3 gal[3,24,25,35,36,40,44] and N-glycolylneuraminic acid (Neu5Gc)[8,25,36,45] in recombinant mAbs expressed using murine cell lines. The presence of oligosaccharides is critical for the structural integrity, stability and functions of IgG molecules, as will be discussed later, but specific structures are also important. For example, lack of core-fucose results in IgG molecules with higher affinity to FcγIII receptor and enhanced ADCC,[46,47] while the presence of a terminal galactose[47,48] or bisecting residue[48] only has a subtle effect on receptor binding and ADCC. Human IgG antibodies share the same major and minor glycoforms with recombinant mAbs.[49,50]

The majority of the oligosaccharides of human and recombinant IgGs include core-fucose. In most cases, the levels of

terminal galactose and bisecting residue are higher in human IgG compared with recombinant IgG molecules. However, aglycosylated antibodies and high mannose are usually present at much higher levels in recombinant mAbs compared with human IgG. In addition, immunogenic α1,3 gal and Neu5Gc are only present in recombinant mAbs when murine cell lines are used for expression.

Aglycosylated Antibodies

Compared with glycosylated antibodies, antibodies without oligosaccharides show conformational changes, decreased stability, increased propensity to aggregate, and almost complete loss of effector functions.[51-54] The effect of aglycosylation on antibody half-life cannot be generalized because there are studies demonstrating shorter half-life[54-57] and normal half-life.[51,52,54] A very low level (0.1%) of aglycosylation has been found in human IgG.[50] Clinical experience with aglycosylated antibodies did not demonstrate increased risk.[58]

High Mannose

High mannose oligosaccharides have been commonly observed in recombinant mAbs at higher levels than in endogenous IgG antibodies. Antibodies with high mannose showed defects in Fc effector functions.[59,60] Although some studies showed no difference in clearance,[51,61] the majority showed a faster clearance of antibodies with high mannose.[37,55,59,60,62] High mannose with greater than five mannose residues can be converted to mannose 5 because of mannosidase activity in circulation.[37] High mannose has been observed in endogenous human IgG at a very low level.[50]

Immunogenic Oligosaccharides

α-1,3 gal is not present in endogenous human IgG antibodies due to the absence of the gene for the synthesizing enzyme, α-1,3-galactosyltransferase.[49,63,64] Therefore, α1,3 gal is foreign to the human immune system. The presence of IgE antibodies specific to α-1,3 gal in the Fab region of cetuximab have been reported to cause hypersensitivity in some patients.[65]

Neu5Gc is not normally present in human IgG[49] because of the lack of CMP-N-acetylneuraminic acid hydroxylase activity due to mutation in the gene.[66] However, Neu5Gc can be metabolically incorporated into human cells because of diet or cell culture medium containing animal derived material.[67,68] Anti-Neu5Gc antibodies have been detected[69-71] or induced because of exposure to Neu5Gc[71] in humans.

Degradation of Asparagine and Aspartate

Deamidation is the major degradation pathway of Asn, which results in the formation of aspartate (Asp) and isoaspartate (IsoAsp). IsoAsp can also be formed from isomerization of Asp, which is another major degradation pathway of recombinant mAbs. Deamidation and isomerization share the same reaction intermediate, succinimide. Deamidation of recombinant mAbs and endogenous IgG antibodies can occur in vivo in monkeys as well as in humans.[72-74] Deamidation and isomerization has been implicated in aging and several age-related diseases, and the existence of the repairing enzyme, protein isoaspartate methyl transferase, further highlights the importance of deamidation and isomerization in vivo.[75]

Deamidation

Deamidation of Asn has been widely reported in recombinant mAbs in either the CDR regions[72,76,77] or in the constant regions.[74,78-80] Increased thermal stability of Fab with Asp and decreased thermal stability of Fab with isoAsp compared with Fab with the original Asn residue were observed for a recombinant mAb as a result of deamidation.[77] Several studies have demonstrated that deamidation in the CDR regions resulted in decreased binding affinity and potency.[72,76,77,81] As expected, deamidation in the constant domain has no effect on antigen binding.[8,81] Deamidation of Asn residues in CDR regions continues in vivo in monkey serum.[72,73] No preferential clearance is suggested by the constant ratio of Asp to isoAsp.[72] Deamidation of Asn residues in the constant region of a recombinant mAb continues in circulation in human[74] and deamidation of the same sites was also observed in endogenous IgG.[74]

Isomerization

Isomerization of Asp to form isoAsp introduces a minimal charge difference. However, isomerization can cause a conformational change because of the introduction of an additional methyl group to the peptide backbone. An ~50% decrease in binding affinity was observed with one Fab with the original Asn and the other one with either isoAsp or succinimide.[82] Isomerization of Asp102 in one of the heavy chain CDR3 resulted in an antibody only 9–21% as potent as the antibody with the original Asp.[76] Isomerization of Asp92 in the light chain CDR3 of a recombinant monoclonal IgG2 antibody deactivated its antigen binding capability.[83] As discussed in the previous section, isoAsp can be formed in vivo in monkey[72,73] and in human[74] in both recombinant mAbs and human endogenous IgG antibodies.[74] Therefore, isoAsp from isomerization is not foreign to the human immune system, indicating a lower risk of immunogenicity.

Succinimide

Although succinimide is unstable, it has been detected in several recombinant mAbs.[73,76,81,82,84-86] The presence of succinimide in the CDR regions of several antibodies from Asp isomerization resulted in decreased antigen binding and

potency.[73,81,82,84,86] Succinimide of a recombinant mAb was rapidly converted to isoAsp and Asp after injection into cynomolgus monkeys.[73] Succinimide is expected to be present in circulation because of in vivo Asn deamidation.[72-74]

Oxidation of Methionine, Tryptophan and Other Residues

Oxidation of recombinant mAbs has been commonly reported, mainly at methionine (Met) residues and less frequently at tryptophan (Trp), histidine (His) and other residues. Two conserved Met residues, Met252 and Met428, in the Fc region are highly susceptible to oxidation.[87-92] Oxidation of Met residues in the Fc region has no effect on antigen binding,[87] but results in a conformational change in the CH2 domain[90,91] and decreased binding to protein A,[89,93] protein G[89] and FcRn.[93-95] It also has a subtle effect on Fc receptors.[94] Decreased half-life was only observed with relatively high levels of oxidation.[95] Oxidation of Trp has only been reported in a few cases.[92,96,97] In one of those studies, oxidation of the single Trp residue in the CDR3 caused a substantial decrease in antigen binding and potency.[92] Oxidation of Trp to form various products during exposure to light or heat causes changes to the color of mAb products.[98] Metal-catalyzed oxidation can also lead to oxidative carbonylation of Arg, Pro, Lys and Thr, especially when those residues are located on the surface of the molecules.[99] The observation of oxidative carbonylation on mAbs in the unstressed drug substance indicates that such reactions can occur during manufacturing because of product contact with metal surface. Direct His oxidation[100] and its further reaction product, His-His cross-linking,[101]are observed when mAbs were exposed to light.

Oxidation of Met and Trp are probably present in endogenous IgG from humans, especially for patients with inflammation.[102,103] Oxidation of proteins including Met and Trp residues has been widely detected in vivo, and may result from aging and several pathological conditions.[104]

Cysteine Related Variants

In the classical view, cysteine (Cys) residues are involved in formation of disulfide bonds with well-defined homogeneous linkage for each subclass of IgG antibodies. However, several variations have been discovered, including alternative disulfide bond linkage, trisulfide bond, thioether linkage, free Cys and racemization. Cysteinylation of Cys residues has also been observed, but only to antibodies with extra Cys residues,[105] which is rare.

Alternative Disulfide Bond Linkage

Alternative disulfide bond linkage was first reported for IgG4. The two inter-heavy chain disulfide bonds of IgG4 exist in equilibrium with the formation of two intra-heavy chain disulfide bonds,[106,107] which can result in the formation of half-molecules. The formation of half-molecules was almost eliminated when the Ser residue in the IgG4 hinge of CPSC was mutated to a Pro residue, thereby making an IgG1 hinge of CPPC.[106-108] Trace amounts of half-molecule were also observed for IgG1.[106] The other consequence of the instability of the IgG4 hinge is the formation of hybrid molecules between two different IgG4 molecules, which can occur in recombinant mAbs incubated with glutathione or injected into mice[109] and naturally in human.[109,110]

In addition to the classical IgG2 disulfide bond structure, termed IgG2A, two additional structures, termed IgG2B and IgG2A/B were discovered.[111] IgG2A has a larger hydrodynamic size than IgG2B,[112] and, in a subset of IgG2, IgG2-A shows higher potency.[112] Incubation of IgG2 in vitro with redox similar to human blood shows a decrease in IgG2A and an increase in IgG2B.[112] A similar conversion from IgG2A to IgG2B was also observed in cell culture medium and in circulation after administration into human body.[113] The exact isoforms are also naturally present in human IgG2.[111,112]

Trisulfide Bonds

Trisulfide bonds were first reported in a recombinant monoclonal IgG2 antibody in the hinge region between the two heavy chains.[114] It was later found that trisulfide bonds are present in all subclasses of recombinant IgGs.[115] Higher percentages of trisulfide bonds were observed between light chain and heavy chain than between the two heavy chains and no trisulfide bonds were associated with intrachain disulfide bonds.[115] The presence of trisulfide bonds has no effect on thermal stability,[114]and antigen binding and potency.[115,116] Trisulfide bonding has also been shown to affect the reduction step for the production of antibody-drug conjugation.[117] Cell culture parameters such as scale and age have a significant effect on the level of trisulfide bonds.[115,118] Trisulfide bonding can be eliminated by incubation with mild reducing reagents.[114,115] Trisulfide bonds were stable in vitro in buffers and in rat serum; however, they are completely converted to disulfide bonds after 24 h in vivo in rat serum.[115] Trisulfide bonds between the light chain and heavy chain were also found in endogenous IgG.[115,119]

Thioether

Thioethers between the light chain and heavy chain were first identified in a recombinant monoclonal IgG1 antibody.[120] Higher pH promotes the formation of a thioether bond.[121] A thioether between the light chain and heavy chain increases at about 0.1%/day for therapeutic antibodies in healthy volunteers.[119] There is no clearance difference between antibodies with and without a thioether.[119] Thioethers naturally exist in human endogenous IgG molecules, 11.0% for IgG1λ and about 5.2% for IgGκ.[119]

Free Cysteine

Low levels of free Cys have been widely observed for recombinant mAbs, especially under denaturing conditions in the range from trace level to 2.3 moles per mole of IgGs[10,122-125] Free sulfhydryl is associated with every single cysteine residue in the IgG molecules.[126,127] In some cases, antibodies with incomplete formation of the intrachain disulfide bond in the heavy chain variable domain were identified as separated peaks.[10,122,126] Higher levels free sulhydryl resulted in decreased thermal stability,[124] formation of covalent aggregates[123] and decreased potency.[122] One study showed a slightly higher antigen binding with no difference in complement-dependent cytotoxicity.[128] Intrachain disulfide bond in the heavy chain variable domain can be rapidly formed from the free sulfhydryl state during in vitro incubation with 5,5′-dithiobis-(2-nitrobenzoic acid), in rat serum and human serum or after circulation in rat serum in vivo.[10] Free cysteine has also been detected in human endogenous IgG antibodies with levels varied in different studies,[123,124,129,130] probably due to difference in methods.

Racemization

Racemization of the heavy chain Cys residue involved in the formation of the inter-heavy/light chain disulfide bond from L-Cys to D-Cys was observed in a recombinant monoclonal IgG1 antibody during storage.[131] It was later found that all Cys residues involved in interchain disulfide bonds of IgG1 and IgG2 can be racemized to D form to some degree under basic conditions.[132] In addition, racemization was also observed in endogenous IgG molecules from human.[132]

Glycation

Glycation is a non-enzymatic reaction between reducing sugars and protein N-terminal amino group or the side chain of Lys residues. Glycation occurs during cell culture, formulation and storage,[133-144] where reducing sugars are used or generated from non-reducing sugars. Glycation susceptibility is altered by the surface accessibility of the Lys residues,[134] and can also be catalyzed by amino acids in close proximity.[136] Glycation can be controlled by optimizing cell culture conditions.[141] A mAb containing 17% glycation did not show any structural difference compared with the main peak with no glycation.[11] However, glycation increases the propensity of aggregation of recombinant mAbs.[143] Several studies demonstrated glycation of Lys in various CDR regions from 10% glycation to about 100% had no effect on antigen binding and potency.[11,138,140] Extensive glycation has no effect on binding to FcγRIIIa and FcRn and protein A.[144] However, those results can only demonstrate that those Lys residues are not critical for various ligand binding. A significant effect is expected if the glycated residues are localized in the binding pockets because of the loss of the positive charge of Lys upon glycation. Acidic species with 17% glycation did not show difference in pharmacokinetics in rats.[11] Glycation of recombinant mAbs increases with the increase of circulation time in human.[144] Glycation has also been detected in endogenous IgG of healthy subjects with a comparable rate as recombinant mAb in circulation.[144] The risk of glycation of recombinant mAbs may be low due to its low level and its presence in vivo. However, it is worthwhile to mention that antibodies targeting glycated IgG has been observed in rheumatoid arthritis patients[145] and the interaction of advanced glycation end product (AGEs) with AGE-specific receptors can stimulate the generation of reactive oxygen species and inflammation.[146]

Low Level of Sequence Variant

A low level of tyrosine (Tyr) to Gln sequence variation that occurred during transfection of a recombinant mAb expressed in CHO was first reported in 1993.[147] Low levels of sequence variants have been more widely reported recently,[148-154] which can be attributed to the advance of modern analytical techniques offering much higher sensitivity. Sequence variants are introduced because of mutation at the DNA level,[153,155] during transfection,[147] or translation.[148,154,155] Sequence variants of recombinant mAbs can be eliminated depending on the specific causes. For example, codon-specific low levels of Ser replaced by Asn can be eliminated by changing the codon from AGC to another Ser codon.[155] Misincorporation of amino acids due to amino acid starvation can be eliminated by providing sufficient amounts of the specific amino acids.[149,150,154] It should be mentioned that low levels of misincorporation can occur naturally,[156] and it should not be a surprise that endogenous IgG molecules also contain low levels of sequence variants. Low levels of sequence variation may never be completely eliminated. In this case, maintaining misincorporation at an extremely low level and ensuring batch-to-batch consistency may be more practical and important for the production of recombinant mAbs.

Others

Several other modifications have been reported, but only in limited cases. Expression of intron sequence results in a recombinant mAb with an additional 24 amino acids between the variable and constant domain of the heavy chain.[3] Homologous recombination between the light chain gene and the heavy chain gene results in an antibody with a minor species of heavy chain containing the light chain variable domain.[157] Reaction of methylglyoxal, a by-product of the tricarboxylic acid cycle, with arginine can result in increased levels of acidic species of mAb.[158]

Modifications and Their Importance

A thorough characterization of mAbs and their major degradation pathways is required for development of mAb therapeutics. Such efforts have provided in-depth understanding of the

Table 1. Common modifications of recombinant mAbs, and their presence in endogenous IgG and importance

Modifications	Endogenous IgG	Importance
N-terminal modifications		
Truncation	?	+
PyroE	Yes	+
Partial leader sequence	Yes	+
C-terminal modifications		
C-terminal Lys	Yes	+
Amidation	No	+
Oligosaccharides		
Aglycosylated species	Yes	+
High mannose	Yes	+++
Neu5Gc	No	++++
α1,3-gal	No	++++
Degradation of Asn and Asp		
Asn deamidation	Yes	+++
Isomerization	Yes	+++
Succinimide	Yes	+++
Oxidation		
Met oxidation	Yes	++
Trp oxidation	Yes	++
His oxidation and cross-link	?	+
Cysteine-related		
Alternative disulfide bond linkage	Yes	++
Trisulfide bond	Yes	++
Thioether	Yes	++
Free cysteine	Yes	++
Racemization	Yes	++
Cystenylation	?	+
Glycation	Yes	++
Low level sequence variants	?	+
Others		
Intron sequence	?	+
Methylglyoxal modification	?	+

++++ indicates the highest level of importance, + indicates the lowest level of importance.

basic structure and function relationships of recombinant and endogenous IgG molecules with regard to various modifications. Undoubtedly, more modifications will be identified due to the application of sensitive analytical techniques such as modern mass spectrometry.[159-161]

The major known modifications in recombinant mAbs and their presence in endogenous IgG molecules are summarized in **Table 1**. We defined the importance of each modification based on an overall evaluation of their prevalence, importance to safety, immunogenicity and efficacy. For example, pyroE is widely observed, but it is not important because it has no effect on structure, stability and function and it is endogenous. On the other hand, degradation of Asn and Asp is considered to be important because it has been widely observed and can affect mAb structure, stability and functions. In addition, although degradation of Asn and Asp has been observed in endogenous IgG molecules, exposure of patients to recombinant mAbs with high levels of those degradation products may still pose a high risk. Although sharing some common features, each mAb is different and should be evaluated on a case-by-case basis. Modifications that are not defined as highly important should also be controlled. Consistency of various modifications from batch to batch is necessary to demonstrate a well-controlled process.

Conclusions

The development of recombinant monoclonal mAbs has evolved from mouse, chimeric, humanized to human antibodies with the goal of reducing immunogenicity. Although significant progress has been made to achieve this goal, significant immunogenicity risks remain even for fully human antibodies. Therefore, the identification and mitigation of non-human modifications of recombinant monoclonal therapeutics is crucial.

By far, the host cell line plays the most important role in introducing non-human modifications. For example, α-1,3-gal and Neu5Gc are the natural products of using murine cell lines, where optimizing cell culture conditions may not be sufficient to eliminate those modifications. However, cell culture conditions could have a bigger effect on several other modifications, such as N-terminal cyclization, C-terminal Lys removal, glycation, trisulfide bonds, and IgG2 isoforms. Various modifications introduced during purification and storage, such as glycation, deamidation, N-terminal cyclization, can also be controlled by lowering temperature and optimizing pH and excipients. Some of the modifications may be eliminated rapidly in circulation, thus lowering the risk of immunogenicity. In general, the human body can better tolerate modifications that are natural compared with those that are not.

Thorough characterization of modifications and risk assessment at an early stage of development based on the understanding of the nature of modifications and the production of batches consistent in quality attributes throughout clinical stage and commercial stage are critical to ensure the supply of efficacious and safe products.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Kotia RB, Raghani AR. Analysis of monoclonal antibody product heterogeneity resulting from alternate cleavage sites of signal peptide. Anal Biochem 2010; 399:190-5; PMID:20074542; http://dx.doi.org/10.1016/j.ab.2010.01.008
2. Werner WE, Wu S, Mulkerrin M. The removal of pyroglutamic acid from monoclonal antibodies without denaturation of the protein chains. Anal Biochem 2005; 342:120-5; PMID:15958188; http://dx.doi.org/10.1016/j.ab.2005.04.012
3. Beck A, Bussat MC, Zorn N, Robillard V, Klinguer-Hamour C, Chenu S, Goetsch L, Corvaïa N, Van Dorsselaer A, Haeuw JF. Characterization by liquid chromatography combined with mass spectrometry of monoclonal anti-IGF-1 receptor antibodies produced in CHO and NS0 cells. J Chromatogr B Analyt Technol Biomed Life Sci 2005; 819:203-18; PMID: 15833284; http://dx.doi.org/10.1016/j.jchromb.2004.06.052
4. Dick LW Jr., Kim C, Qiu D, Cheng KC. Determination of the origin of the N-terminal pyro-glutamate variation in monoclonal antibodies using model peptides. Biotechnol Bioeng 2007; 97:544-53; PMID: 17099914; http://dx.doi.org/10.1002/bit.21260
5. Yu L, Vizel A, Huff MB, Young M, Remmele RL Jr., He B. Investigation of N-terminal glutamate

cyclization of recombinant monoclonal antibody in formulation development. J Pharm Biomed Anal 2006; 42:455-63; PMID:16828250; http://dx.doi.org/10.1016/j.jpba.2006.05.008
6. Liu H, Gaza-Bulseco G, Sun J. Characterization of the stability of a fully human monoclonal IgG after prolonged incubation at elevated temperature. J Chromatogr B Analyt Technol Biomed Life Sci 2006; 837:35-43; PMID:16644295; http://dx.doi.org/10.1016/j.jchromb.2006.03.053
7. Chelius D, Jing K, Lueras A, Rehder DS, Dillon TM, Vizel A, Rajan RS, Li T, Treuheit MJ, Bondarenko PV. Formation of pyroglutamic acid from N-terminal glutamic acid in immunoglobulin gamma antibodies. Anal Chem 2006; 78:2370-6; PMID:16579622; http://dx.doi.org/10.1021/ac051827k
8. Lyubarskaya Y, Houde D, Woodard J, Murphy D, Mhatre R. Analysis of recombinant monoclonal antibody isoforms by electrospray ionization mass spectrometry as a strategy for streamlining characterization of recombinant monoclonal antibody charge heterogeneity. Anal Biochem 2006; 348:24-39; PMID:16289440; http://dx.doi.org/10.1016/j.ab.2005.10.003
9. Liu YD, Goetze AM, Bass RB, Flynn GC. N-terminal glutamate to pyroglutamate conversion *in vivo* for human IgG2 antibodies. J Biol Chem 2011; 286:11211-7; PMID:21282104; http://dx.doi.org/10.1074/jbc.M110.185041
10. Ouellette D, Alessandri L, Chin A, Grinnell C, Tarcsa E, Radziejewski C, Correia I. Studies in serum support rapid formation of disulfide bond between unpaired cysteine residues in the VH domain of an immunoglobulin G1 molecule. Anal Biochem 2010; 397:37-47; PMID:19766583; http://dx.doi.org/10.1016/j.ab.2009.09.027
11. Khawli LA, Goswami S, Hutchinson R, Kwong ZW, Yang J, Wang X, Yao Z, Sreedhara A, Cano T, Tesar D, et al. Charge variants in IgG1: Isolation, characterization, *in vitro* binding properties and pharmacokinetics in rats. MAbs 2010; 2:613-24; PMID:20818176; http://dx.doi.org/10.4161/mabs.2.6.13333
12. Kaschak T, Boyd D, Lu F, Derfus G, Kluck B, Nogal B, Emery C, Summers C, Zheng K, Bayer R, et al. Characterization of the basic charge variants of a human IgG1: effect of copper concentration in cell culture media. MAbs 2011; 3:577-83; PMID:22123059; http://dx.doi.org/10.4161/mabs.3.6.17959
13. Martoglio B, Dobberstein B. Signal sequences: more than just greasy peptides. Trends Cell Biol 1998; 8:410-5; PMID:9789330; http://dx.doi.org/10.1016/S0962-8924(98)01360-9
14. Harris RJ. Processing of C-terminal lysine and arginine residues of proteins isolated from mammalian cell culture. J Chromatogr A 1995; 705:129-34; PMID:7620566; http://dx.doi.org/10.1016/0021-9673(94)01255-D
15. Moorhouse KG, Nashabeh W, Deveney J, Bjork NS, Mulkerrin MG, Ryskamp T. Validation of an HPLC method for the analysis of the charge heterogeneity of the recombinant monoclonal antibody IDEC-C2B8 after papain digestion. J Pharm Biomed Anal 1997; 16:593-603; PMID:9502155; http://dx.doi.org/10.1016/S0731-7085(97)00178-7
16. Rao P, Williams A, Baldwin-Ferro A, Hanigan E, Kroon D, Makowshi M, Meyer E, Numsuwan V, Rubin E, Tran A. C-Terminal Modification Occurs in Tissue Culture Produced OKT3. BioPharm 1991; Nov–Dec:38-43.
17. Liu H, Bulseco GG, Sun J. Effect of posttranslational modifications on the thermal stability of a recombinant monoclonal antibody. Immunol Lett 2006; 106:144-53; PMID:16831470; http://dx.doi.org/10.1016/j.imlet.2006.05.011
18. Antes B, Amon S, Rizzi A, Wiederkum S, Kainer M, Szolar O, Fido M, Kircheis R, Nechansky A. Analysis of lysine clipping of a humanized Lewis-Y specific IgG antibody and its relation to Fc-mediated effector function. J Chromatogr B Analyt Technol Biomed Life Sci 2007; 852:250-6; PMID:17296336; http://dx.doi.org/10.1016/j.jchromb.2007.01.024
19. Cai B, Pan H, Flynn GC. C-terminal lysine processing of human immunoglobulin G2 heavy chain *in vivo*. Biotechnol Bioeng 2011; 108:404-12; PMID:20830675; http://dx.doi.org/10.1002/bit.22933
20. Tsubaki M, Terashima I, Kamata K, Koga A. C-terminal modification of monoclonal antibody drugs: amidated species as a general product-related substance. Int J Biol Macromol 2013; 52:139-47; PMID:23022270; http://dx.doi.org/10.1016/j.ijbiomac.2012.09.016
21. Johnson KA, Paisley-Flango K, Tangarone BS, Porter TJ, Rouse JC. Cation exchange-HPLC and mass spectrometry reveal C-terminal amidation of an IgG1 heavy chain. Anal Biochem 2007; 360:75-83; PMID:17113563; http://dx.doi.org/10.1016/j.ab.2006.10.012
22. Bradbury AF, Smyth DG. Peptide amidation. Trends Biochem Sci 1991; 16:112-5; PMID:2057999; http://dx.doi.org/10.1016/0968-0004(91)90044-V
23. Abel CA, Spiegelberg HL, Grey HM. The carbohydrate contents of fragments and polypeptide chains of human gamma-G-myeloma proteins of different heavy-chain subclasses. Biochemistry 1968; 7:1271-8; PMID:4173998; http://dx.doi.org/10.1021/bi00844a004
24. Huang L, Biolsi S, Bales KR, Kuchibhotla U. Impact of variable domain glycosylation on antibody clearance: an LC/MS characterization. Anal Biochem 2006; 349:197-207; PMID:16360109; http://dx.doi.org/10.1016/j.ab.2005.11.012
25. 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; PMID:17362871; http://dx.doi.org/10.1016/j.ab.2007.01.023
26. Lim A, Reed-Bogan A, Harmon BJ. Glycosylation profiling of a therapeutic recombinant monoclonal antibody with two N-linked glycosylation sites using liquid chromatography coupled to a hybrid quadrupole time-of-flight mass spectrometer. Anal Biochem 2008; 375:163-72; PMID:18249181; http://dx.doi.org/10.1016/j.ab.2008.01.003
27. Valliere-Douglass JF, Brady LJ, Farnsworth C, Pace D, Balland A, Wallace A, Wang W, Treuheit MJ, Yan B. O-fucosylation of an antibody light chain: characterization of a modification occurring on an IgG1 molecule. Glycobiology 2009; 19:144-52; PMID:18952827; http://dx.doi.org/10.1093/glycob/cwn116
28. Valliere-Douglass JF, Kodama P, Mujacic M, Brady LJ, Wang W, Wallace A, Yan B, Reddy P, Treuheit MJ, Balland A. Asparagine-linked oligosaccharides present on a non-consensus amino acid sequence in the CH1 domain of human antibodies. J Biol Chem 2009; 284:32493-506; PMID:19767389; http://dx.doi.org/10.1074/jbc.M109.014803
29. Valliere-Douglass JF, Eakin CM, Wallace A, Ketchem RR, Wang W, Treuheit MJ, Balland A. Glutamine-linked and non-consensus asparagine-linked oligosaccharides present in human recombinant antibodies define novel protein glycosylation motifs. J Biol Chem 2010; 285:16012-22; PMID:20233717; http://>dx.doi.org/10.1074/jbc.M109.096412
30. Buko AM, Kentzer EJ, Petros A, Menon G, Zuiderweg ER, Sarin VK. Characterization of a posttranslational fucosylation in the growth factor domain of urinary plasminogen activator. Proc Natl Acad Sci U S A 1991; 88:3992-6; PMID:2023947; http://dx.doi.org/10.1073/pnas.88.9.3992
31. Ma S, Nashabeh W. Carbohydrate analysis of a chimeric recombinant monoclonal antibody by capillary electrophoresis with laser-induced fluorescence detection. Anal Chem 1999; 71:5185-92; PMID:10575965; http://dx.doi.org/10.1021/ac990376z
32. Raju TS. Electrophoretic methods for the analysis of N-linked oligosaccharides. Anal Biochem 2000; 283:125-32; PMID:10906231; http://dx.doi.org/10.1006/abio.2000.4647
33. Kamoda S, Nomura C, Kinoshita M, Nishiura S, Ishikawa R, Kakehi K, Kawasaki N, Hayakawa T. Profiling analysis of oligosaccharides in antibody pharmaceuticals by capillary electrophoresis. J Chromatogr A 2004; 1050:211-6; PMID:15508314; http://dx.doi.org/10.1016/j.chroma.2004.08.049
34. Kamoda S, Ishikawa R, Kakehi K. Capillary electrophoresis with laser-induced fluorescence detection for detailed studies on N-linked oligosaccharide profile of therapeutic recombinant monoclonal antibodies. J Chromatogr A 2006; 1133:332-9; PMID:16945378; http://dx.doi.org/10.1016/j.chroma.2006.08.028
35. Sheeley DM, Merrill BM, Taylor LC. Characterization of monoclonal antibody glycosylation: comparison of expression systems and identification of terminal alpha-linked galactose. Anal Biochem 1997; 247:102-10; PMID:9126378; http://dx.doi.org/10.1006/abio.1997.2036
36. Maeda E, Kita S, Kinoshita M, Urakami K, Hayakawa T, Kakehi K. Analysis of nonhuman N-glycans as the minor constituents in recombinant monoclonal antibody pharmaceuticals. Anal Chem 2012; 84:2373-9; PMID:22394092; http://dx.doi.org/10.1021/ac300234a
37. Chen X, Liu YD, Flynn GC. The effect of Fc glycan forms on human IgG2 antibody clearance in humans. Glycobiology 2009; 19:240-9; PMID:18974198; http://dx.doi.org/10.1093/glycob/cwn120
38. Lifely MR, Hale C, Boyce S, Keen MJ, Phillips J. Glycosylation and biological activity of CAMPATH-1H expressed in different cell lines and grown under different culture conditions. Glycobiology 1995; 5:813-22; PMID:8720080; http://dx.doi.org/10.1093/glycob/5.8.813
39. Hills AE, Patel A, Boyd P, James DC. Metabolic control of recombinant monoclonal antibody N-glycosylation in GS-NS0 cells. Biotechnol Bioeng 2001; 75:239-51; PMID:11536148; http://dx.doi.org/10.1002/bit.10022
40. Bailey MJ, Hooker AD, Adams CS, Zhang S, James DC. A platform for high-throughput molecular characterization of recombinant monoclonal antibodies. J Chromatogr B Analyt Technol Biomed Life Sci 2005; 826:177-87; PMID:16174568; http://dx.doi.org/10.1016/j.jchromb.2005.08.021
41. Chen X, Flynn GC. Analysis of N-glycans from recombinant immunoglobulin G by on-line reversed-phase high-performance liquid chromatography/mass spectrometry. Anal Biochem 2007; 370:147-61; PMID:17880905; http://dx.doi.org/10.1016/j.ab.2007.08.012
42. Siemiatkoski J, Lyubarskaya Y, Houde D, Tep S, Mhatre R. A comparison of three techniques for quantitative carbohydrate analysis used in characterization of therapeutic antibodies. Carbohydr Res 2006; 341:410-9; PMID:16378604; http://dx.doi.org/10.1016/j.carres.2005.11.024
43. Gennaro LA, Salas-Solano O. On-line CE-LIF-MS technology for the direct characterization of N-linked glycans from therapeutic antibodies. Anal Chem 2008; 80:3838-45; PMID:18426228; http://dx.doi.org/10.1021/ac800152h
44. Stadlmann J, Pabst M, Kolarich D, Kunert R, Altmann F. Analysis of immunoglobulin glycosylation by LC-ESI-MS of glycopeptides and oligosaccharides. Proteomics 2008; 8:2858-71; PMID:18655055; http://dx.doi.org/10.1002/pmic.200700968
45. Flesher AR, Marzowski J, Wang WC, Raff HV. Fluorophore-labeled carbohydrate analysis of immunoglobulin fusion proteins: Correlation of oligosaccharide content with *in vivo* clearance profile. Biotechnol Bioeng 1995;

47:405; PMID:18623415; http://dx.doi.org/10.1002/bit.260470314
46. Shields RL, Lai J, Keck R. O,Connell LY, Hong K, Meng YG, Weikert, S, Presta LG. Lack of fucose on human IgG1 N-linked Oligosaccharide improves binding to human FcγRIII and antibody-dependent cellular toxicity. JBC 2002; 277:26733-40; http://dx.doi.org/10.1074/jbc.M202069200
47. Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, Sakurada M, Uchida K, Anazawa H, Satoh M, Yamasaki M, et al. The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem 2003; 278:3466-73; PMID:12427744; http://dx.doi.org/10.1074/jbc.M210665200
48. Wright A, Morrison SL. Effect of C2-associated carbohydrate structure on Ig effector function: studies with chimeric mouse-human IgG1 antibodies in glycosylation mutants of Chinese hamster ovary cells. J Immunol 1998; 160:3393-402; PMID:9531299
49. Raju TS, Briggs JB, Borge SM, Jones AJ. Species-specific variation in glycosylation of IgG: evidence for the species-specific sialylation and branch-specific galactosylation and importance for engineering recombinant glycoprotein therapeutics. Glycobiology 2000; 10:477-86; PMID:10764836; http://dx.doi.org/10.1093/glycob/10.5.477
50. Flynn GC, Chen X, Liu YD, Shah B, Zhang Z. Naturally occurring glycan forms of human immunoglobulins G1 and G2. Mol Immunol 2010; 47:2074-82; PMID:20444501; http://dx.doi.org/10.1016/j.molimm.2010.04.006
51. Ghirlando R, Lund J, Goodall M, Jefferis R. Glycosylation of human IgG-Fc: influences on structure revealed by differential scanning micro-calorimetry. Immunol Lett 1999; 68:47-52; PMID:10397155; http://dx.doi.org/10.1016/S0165-2478(99)00029-2
52. Hristodorov D, Fischer R, Joerissen H, Müller-Tiemann B, Apeler H, Linden L. Generation and comparative characterization of glycosylated and aglycosylated human IgG1 antibodies. Mol Biotechnol 2013; 53:326-35; PMID:22427250; http://dx.doi.org/10.1007/s12033-012-9531-x
53. Kayser V, Chennamsetty N, Voynov V, Forrer K, Helk B, Trout BL. Glycosylation influences on the aggregation propensity of therapeutic monoclonal antibodies. Biotechnol J 2011; 6:38-44; PMID:20949542; http://dx.doi.org/10.1002/biot.201000091
54. Tao MH, Morrison SL. Studies of aglycosylated chimeric mouse-human IgG. Role of carbohydrate in the structure and effector functions mediated by the human IgG constant region. J Immunol 1989; 143:2595-601; PMID:2507634
55. Goetze AM, Liu YD, Zhang Z, Shah B, Lee E, Bondarenko PV, Flynn GC. High-mannose glycans on the Fc region of therapeutic IgG antibodies increase serum clearance in humans. Glycobiology 2011; 21:949-59; PMID:21421994; http://dx.doi.org/10.1093/glycob/cwr027
56. Wawrzynczak EJ, Cumber AJ, Parnell GD, Jones PT, Winter G. Blood clearance in the rat of a recombinant mouse monoclonal antibody lacking the N-linked oligosaccharide side chains of the CH2 domains. Mol Immunol 1992; 29:213-20; PMID:1542298; http://dx.doi.org/10.1016/0161-5890(92)90102-4
57. Wawrzynczak EJ, Parnell GD, Cumber AJ, Jones PT, Winter G. Blood clearance in the mouse of an aglycosyl recombinant monoclonal antibody. Biochem Soc Trans 1989; 17:1061-2; PMID:2628082
58. Hristodorov D, Fischer R, Linden L. With or without sugar? (A)glycosylation of therapeutic antibodies. Mol Biotechnol 2013; 54:1056-68; PMID:23097175; http://dx.doi.org/10.1007/s12033-012-9612-x
59. Wright A, Morrison SL. Effect of altered CH2-associated carbohydrate structure on the functional properties and *in vivo* fate of chimeric mouse-human immunoglobulin G1. J Exp Med 1994; 180:1087-96; PMID:8064227; http://dx.doi.org/10.1084/jem.180.3.1087
60. Kanda Y, Yamada T, Mori K, Okazaki A, Inoue M, Kitajima-Miyama K, Kuni-Kamochi R, Nakano R, Yano K, Kakita S, et al. Comparison of biological activity among nonfucosylated therapeutic IgG1 antibodies with three different N-linked Fc oligosaccharides: the high-mannose, hybrid, and complex types. Glycobiology 2007; 17:104-18; PMID:17012310; http://dx.doi.org/10.1093/glycob/cwl057
61. Millward TA, Heitzmann M, Bill K, Längle U, Schumacher P, Forrer K. Effect of constant and variable domain glycosylation on pharmacokinetics of therapeutic antibodies in mice. Biologicals 2008; 36:41-7; PMID:17890101; http://dx.doi.org/10.1016/j.biologicals.2007.05.003
62. Alessandri L, Ouellette D, Acquah A, Rieser M, Leblond D, Saltarelli M, Radziejewski C, Fujimori T, Correia I. Increased serum clearance of oligomannose species present on a human IgG1 molecule. MAbs 2012; 4:509-20; PMID:22669558; http://dx.doi.org/10.4161/mabs.20450
63. Joziasse DH, Shaper JH, Jabs EW, Shaper NL. Characterization of an alpha 1—3-galactosyltransferase homologue on human chromosome 12 that is organized as a processed pseudogene. J Biol Chem 1991; 266:6991-8; PMID:1901859
64. Lantéri M, Giordanengo V, Vidal F, Gaudray P, Lefebvre JC. A complete alpha1,3-galactosyltransferase gene is present in the human genome and partially transcribed. Glycobiology 2002; 12:785-92; PMID:12499400; http://dx.doi.org/10.1093/glycob/cwf087
65. Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M, Murphy BA, Satinover SM, Hosen J, Mauro D, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. N Engl J Med 2008; 358:1109-17; PMID:18337601; http://dx.doi.org/10.1056/NEJMoa074943
66. Varki A. Glycan-based interactions involving vertebrate sialic-acid-recognizing proteins. Nature 2007; 446:1023-9; PMID:17460663; http://dx.doi.org/10.1038/nature05816
67. Hashii N, Kawasaki N, Nakajima Y, Toyoda M, Katagiri Y, Itoh S, Harazono A, Umezawa A, Yamaguchi T. Study on the quality control of cell therapy products. Determination of N-glycolylneuraminic acid incorporated into human cells by nano-flow liquid chromatography/Fourier transformation ion cyclotron mass spectrometry. J Chromatogr A 2007; 1160:263-9; PMID:17570377; http://dx.doi.org/10.1016/j.chroma.2007.05.062
68. Ghaderi D, Zhang M, Hurtado-Ziola N, Varki A. Production platforms for biotherapeutic glycoproteins. Occurrence, impact, and challenges of non-human sialylation. Biotechnol Genet Eng Rev 2012; 28:147-75; PMID:22616486; http://dx.doi.org/10.5661/bger-28-147
69. Tangvoranuntakul P, Gagneux P, Diaz S, Bardor M, Varki N, Varki A, Muchmore E. Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid. Proc Natl Acad Sci U S A 2003; 100:12045-50; PMID:14523234; http://dx.doi.org/10.1073/pnas.2131556100
70. Padler-Karavani V, Yu H, Cao H, Chokhawala H, Karp F, Varki N, Chen X, Varki A. Diversity in specificity, abundance, and composition of anti-Neu5Gc antibodies in normal humans: potential implications for disease. Glycobiology 2008; 18:818-30; PMID:18669916; http://dx.doi.org/10.1093/glycob/cwn072
71. Ghaderi D, Taylor RE, Padler-Karavani V, Diaz S, Varki A. Implications of the presence of N-glycolylneuraminic acid in recombinant therapeutic glycoproteins. Nat Biotechnol 2010; 28:863-7; PMID:20657583; http://dx.doi.org/10.1038/nbt.1651
72. Huang L, Lu J, Wroblewski VJ, Beals JM, Riggin RM. *In vivo* deamidation characterization of monoclonal antibody by LC/MS/MS. Anal Chem 2005; 77:1432-9; PMID:15732928; http://dx.doi.org/10.1021/ac0494174
73. Ouellette D, Chumsae C, Clabbers A, Radziejewski C, Correia I. Comparison of the *in vitro* and *in vivo* stability of a succinimide intermediate observed on a therapeutic IgG1 molecule. MAbs 2013; 5:432-44; PMID:23608772; http://dx.doi.org/10.4161/mabs.24458
74. Liu YD, van Enk JZ, Flynn GC. Human antibody Fc deamidation *in vivo*. Biologicals 2009; 37:313-22; PMID:19608432; http://dx.doi.org/10.1016/j.biologicals.2009.06.001
75. Clarke S. Aging as war between chemical and biochemical processes: protein methylation and the recognition of age-damaged proteins for repair. Ageing Res Rev 2003; 2:263-85; PMID:12726775; http://dx.doi.org/10.1016/S1568-1637(03)00011-4
76. Harris RJ, Kabakoff B, Macchi FD, Shen FJ, Kwong M, Andya JD, Shire SJ, Bjork N, Totpal K, Chen AB. Identification of multiple sources of charge heterogeneity in a recombinant antibody. J Chromatogr B Biomed Sci Appl 2001; 752:233-45; PMID:11270864; http://dx.doi.org/10.1016/S0378-4347(00)00548-X
77. Vlasak J, Bussat MC, Wang S, Wagner-Rousset E, Schaefer M, Klinguer-Hamour C, Kirchmeier M, Corvaïa N, Ionescu R, Beck A. Identification and characterization of asparagine deamidation in the light chain CDR1 of a humanized IgG1 antibody. Anal Biochem 2009; 392:145-54; PMID:19497295; http://dx.doi.org/10.1016/j.ab.2009.05.043
78. Wang L, Amphlett G, Lambert JM, Blättler W, Zhang W. Structural characterization of a recombinant monoclonal antibody by electrospray time-of-flight mass spectrometry. Pharm Res 2005; 22:1338-49; PMID:16078144; http://dx.doi.org/10.1007/s11095-005-5267-7
79. Chelius D, Rehder DS, Bondarenko PV. Identification and characterization of deamidation sites in the conserved regions of human immunoglobulin gamma antibodies. Anal Chem 2005; 77:6004-11; PMID:16159134; http://dx.doi.org/10.1021/ac050672d
80. Sinha S, Zhang L, Duan S, Williams TD, Vlasak J, Ionescu R, Topp EM. Effect of protein structure on deamidation rate in the Fc fragment of an IgG1 monoclonal antibody. Protein Sci 2009; 18:1573-84; PMID:19544580; http://dx.doi.org/10.1002/pro.173
81. Yan B, Steen S, Hambly D, Valliere-Douglass J, Vanden Bos T, Smallwood S, Yates Z, Arroll T, Han Y, Gadgil H, et al. Succinimide formation at Asn 55 in the complementarity determining region of a recombinant monoclonal antibody IgG1 heavy chain. J Pharm Sci 2009; 98:3509-21; PMID:19475547; http://dx.doi.org/10.1002/jps.21655
82. Cacia J, Keck R, Presta LG, Frenz J. Isomerization of an aspartic acid residue in the complementarity-determining regions of a recombinant antibody to human IgE: identification and effect on binding affinity. Biochemistry 1996; 35:1897-903; PMID:8639672; http://dx.doi.org/10.1021/bi951526c
83. Rehder DS, Chelius D, McAuley A, Dillon TM, Xiao G, Crouse-Zeineddini J, Vardanyan L, Perico N, Mukku V, Brems DN, et al. Isomerization of a single aspartyl residue of anti-epidermal growth factor receptor immunoglobulin gamma2 antibody highlights the role avidity plays in antibody activity. Biochemistry 2008; 47:2518-30; PMID:18232715; http://dx.doi.org/10.1021/bi7018223
84. Valliere-Douglass J, Jones L, Shpektor D, Kodama P, Wallace A, Balland A, Bailey R, Zhang Y. Separation and characterization of an IgG2 antibody containing a cyclic imide in CDR1 of light chain by hydrophobic interaction chromatography and mass spectrometry. Anal Chem 2008; 80:3168-74; PMID:18355059; http://dx.doi.org/10.1021/ac702245c

85. Chu GC, Chelius D, Xiao G, Khor HK, Coulibaly S, Bondarenko PV. Accumulation of succinimide in a recombinant monoclonal antibody in mildly acidic buffers under elevated temperatures. Pharm Res 2007; 24:1145-56; PMID:17385019; http://dx.doi.org/10.1007/s11095-007-9241-4
86. Yu XC, Joe K, Zhang Y, Adriano A, Wang Y, Gazzano-Santoro H, Keck RG, Deperalta G, Ling V. Accurate determination of succinimide degradation products using high fidelity trypsin digestion peptide map analysis. Anal Chem 2011; 83:5912-9; PMID: 21692515; http://dx.doi.org/10.1021/ac200750u
87. Lam XM, Yang JY, Cleland JL. Antioxidants for prevention of methionine oxidation in recombinant monoclonal antibody HER2. J Pharm Sci 1997; 86:1250-5; PMID:9383735; http://dx.doi.org/10.1021/js970143s
88. Chumsae C, Gaza-Bulseco G, Sun J, Liu H. Comparison of methionine oxidation in thermal stability and chemically stressed samples of a fully human monoclonal antibody. J Chromatogr B Analyt Technol Biomed Life Sci 2007; 850:285-94; PMID: 17182291; http://dx.doi.org/10.1016/j.jchromb.2006.11.050
89. Gaza-Bulseco G, Faldu S, Hurkmans K, Chumsae C, Liu H. Effect of methionine oxidation of a recombinant monoclonal antibody on the binding affinity to protein A and protein G. J Chromatogr B Analyt Technol Biomed Life Sci 2008; 870:55-62; PMID: 18567545; http://dx.doi.org/10.1016/j.jchromb.2008.05.045
90. Liu H, Gaza-Bulseco G, Xiang T, Chumsae C. Structural effect of deglycosylation and methionine oxidation on a recombinant monoclonal antibody. Mol Immunol 2008; 45:701-8; PMID:17719636; http://dx.doi.org/10.1016/j.molimm.2007.07.012
91. Liu D, Ren D, Huang H, Dankberg J, Rosenfeld R, Cocco MJ, Li L, Brems DN, Remmele RL Jr. Structure and stability changes of human IgG1 Fc as a consequence of methionine oxidation. Biochemistry 2008; 47:5088-100; PMID:18407665; http://dx.doi.org/10.1021/bi702238b
92. Wei Z, Feng J, Lin HY, Mullapudi S, Bishop E, Tous GI, Casas-Finet J, Hakki F, Strouse R, Schenerman MA. Identification of a single tryptophan residue as critical for binding activity in a humanized monoclonal antibody against respiratory syncytial virus. Anal Chem 2007; 79:2797-805; PMID:17319649; http://dx.doi.org/10.1021/ac062311j
93. Pan H, Chen K, Chu L, Kinderman F, Apostol I, Huang G. Methionine oxidation in human IgG2 Fc decreases binding affinities to protein A and FcRn. Protein Sci 2009; 18:424-33; PMID:19165723; http://dx.doi.org/10.1002/pro.45
94. Bertolotti-Ciarlet A, Wang W, Lownes R, Pristatsky P, Fang Y, McKelvey T, Li Y, Li Y, Drummond J, Prueksaritanont T, et al. Impact of methionine oxidation on the binding of human IgG1 to Fc Rn and Fc gamma receptors. Mol Immunol 2009; 46:1878-82; PMID:19269032; http://dx.doi.org/10.1016/j.molimm.2009.02.002
95. Wang W, Vlasak J, Li Y, Pristatsky P, Fang Y, Pittman T, Roman J, Wang Y, Prueksaritanont T, Ionescu R. Impact of methionine oxidation in human IgG1 Fc on serum half-life of monoclonal antibodies. Mol Immunol 2011; 48:860-6; PMID:21256596; http://dx.doi.org/10.1016/j.molimm.2010.12.009
96. Yang J, Wang S, Liu J, Raghani A. Determination of tryptophan oxidation of monoclonal antibody by reversed phase high performance liquid chromatography. J Chromatogr A 2007; 1156:174-82; PMID: 17379231; http://dx.doi.org/10.1016/j.chroma.2007.01.140
97. Lam XM, Lai WG, Chan EK, Ling V, Hsu CC. Site-specific tryptophan oxidation induced by autocatalytic reaction of polysorbate 20 in protein formulation. Pharm Res 2011; 28:2543-55; PMID:21656082; http://dx.doi.org/10.1007/s11095-011-0482-x
98. Li Y, Polozova A, Gruia F, Feng J. Characterization of the degradation products of a color-changed monoclonal antibody: tryptophan-derived chromophores. Anal Chem 2014; 86:6850-7; PMID:24937252; http://dx.doi.org/10.1021/ac404218t
99. Yang Y, Stella C, Wang W, Schöneich C, Gennaro L. Characterization of oxidative carbonylation on recombinant monoclonal antibodies. Anal Chem 2014; 86:4799-806; PMID:24731230; http://dx.doi.org/10.1021/ac4039866
100. Amano M, Kobayashi N, Yabuta M, Uchiyama S, Fukui K. Detection of histidine oxidation in a monoclonal immunoglobulin gamma (IgG) 1 antibody. Anal Chem 2014; 86:7536–43
101. Liu M, Zhang Z, Cheetham J, Ren D, Zhou ZS. Discovery and characterization of a photo-oxidative histidine-histidine cross-link in IgG1 antibody utilizing ^{8}O-labeling and mass spectrometry. Anal Chem 2014; 86:4940-8; PMID:24738698; http://dx.doi.org/10.1021/ac500334k
102. Jasin HE. Oxidative modification of inflammatory synovial fluid immunoglobulin G. Inflammation 1993; 17:167-81; PMID:8387962; http://dx.doi.org/10.1007/BF00916103
103. Lunec J, Blake DR, McCleary SJ, Brailsford S, Bacon PA. Self-perpetuating mechanisms of immunoglobulin G aggregation in rheumatoid inflammation. J Clin Invest 1985; 76:2084-90; PMID:3001140; http://dx.doi.org/10.1172/JCI112212
104. Griffiths HR. Antioxidants and protein oxidation. Free Radic Res 2000; 33(Suppl):S47-58; PMID:11191275
105. Gadgil HS, Bondarenko PV, Pipes GD, Dillon TM, Banks D, Abel J, Kleemann GR, Treuheit MJ. Identification of cysteinylation of a free cysteine in the Fab region of a recombinant monoclonal IgG1 antibody using Lys-C limited proteolysis coupled with LC/MS analysis. Anal Biochem 2006; 355:165-74; PMID: 16828048; http://dx.doi.org/10.1016/j.ab.2006.05.037
106. Bloom JW, Madanat MS, Marriott D, Wong T, Chan SY. Intrachain disulfide bond in the core hinge region of human IgG4. Protein Sci 1997; 6:407-15; PMID: 9041643; http://dx.doi.org/10.1002/pro.5560060217
107. Schuurman J, Perdok GJ, Gorter AD, Aalberse RC. The inter-heavy chain disulfide bonds of IgG4 are in equilibrium with intra-chain disulfide bonds. Mol Immunol 2001; 38:1-8; PMID:11483205; http://dx.doi.org/10.1016/S0161-5890(01)00050-5
108. Angal S, King DJ, Bodmer MW, Turner A, Lawson AD, Roberts G, Pedley B, Adair JR. A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody. Mol Immunol 1993; 30:105-8; PMID:8417368; http://dx.doi.org/10.1016/0161-5890(93)90432-B
109. van der Neut Kolfschoten M, Schuurman J, Losen M, Bleeker WK, Martínez-Martínez P, Vermeulen E, den Bleker TH, Wiegman L, Vink T, Aarden LA, et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. Science 2007; 317:1554-7; PMID:17872445; http://dx.doi.org/10.1126/science.1144603
110. Schuurman J, Van Ree R, Perdok GJ, Van Doorn HR, Tan KY, Aalberse RC. Normal human immunoglobulin G4 is bispecific: it has two different antigen-combining sites. Immunology 1999; 97:693-8; PMID:10457225; http://dx.doi.org/10.1046/j.1365-2567.1999.00845.x
111. Wypych J, Li M, Guo A, Zhang Z, Martinez T, Allen MJ, Fodor S, Kelner DN, Flynn GC, Liu YD, et al. Human IgG2 antibodies display disulfide-mediated structural isoforms. J Biol Chem 2008; 283:16194-205; PMID:18339624; http://dx.doi.org/10.1074/jbc.M709987200
112. Dillon TM, Ricci MS, Vezina C, Flynn GC, Liu YD, Rehder DS, Plant M, Henkle B, Li Y, Deechongkit S, et al. Structural and functional characterization of disulfide isoforms of the human IgG2 subclass. J Biol Chem 2008; 283:16206-15; PMID:18339626; http://dx.doi.org/10.1074/jbc.M709988200
113. Liu YD, Chen X, Enk JZ, Plant M, Dillon TM, Flynn GC. Human IgG2 antibody disulfide rearrangement *in vivo*. J Biol Chem 2008; 283:29266-72; PMID: 18713741; http://dx.doi.org/10.1074/jbc.M804787200
114. Pristatsky P, Cohen SL, Krantz D, Acevedo J, Ionescu R, Vlasak J. Evidence for trisulfide bonds in a recombinant variant of a human IgG2 monoclonal antibody. Anal Chem 2009; 81:6148-55; PMID: 19591437; http://dx.doi.org/10.1021/ac9006254
115. Gu S, Wen D, Weinreb PH, Sun Y, Zhang L, Foley SF, Kshirsagar R, Evans D, Mi S, Meier W, et al. Characterization of trisulfide modification in antibodies. Anal Biochem 2010; 400:89-98; PMID: 20085742; http://dx.doi.org/10.1016/j.ab.2010.01.019
116. Aono H, Wen D, Zang L, Houde D, Pepinsky RB, Evans DR. Efficient on-column conversion of IgG1 trisulfide linkages to native disulfides in tandem with Protein A affinity chromatography. J Chromatogr A 2010; 1217:5225-32; PMID:20598700; http://dx.doi.org/10.1016/j.chroma.2010.06.029
117. Cumnock K, Tully T, Cornell C, Hutchinson M, Gorrell J, Skidmore K, Chen Y, Jacobson F. Trisulfide modification impacts the reduction step in antibody-drug conjugation process. Bioconjug Chem 2013; 24:1154-60; PMID:23713462; http://dx.doi.org/10.1021/bc4000299
118. Kshirsagar R, McElearney K, Gilbert A, Sinacore M, Ryll T. Controlling trisulfide modification in recombinant monoclonal antibody produced in fed-batch cell culture. Biotechnol Bioeng 2012; 109:2523-32; PMID:22473825; http://dx.doi.org/10.1002/bit.24511
119. Zhang Q, Schenauer MR, McCarter JD, Flynn GC. IgG1 thioether bond formation in vivo. J Biol Chem 2013; 288:16371-82; PMID:23625924; http://dx.doi.org/10.1074/jbc.M113.468397
120. Tous GI, Wei Z, Feng J, Bilbulian S, Bowen S, Smith J, Strouse R, McGeehan P, Casas-Finet J, Schenerman MA. Characterization of a novel modification to monoclonal antibodies: thioether cross-link of heavy and light chains. Anal Chem 2005; 77:2675-82; PMID:15859580; http://dx.doi.org/10.1021/ac0500582
121. Cohen SL, Price C, Vlasak J. Beta-elimination and peptide bond hydrolysis: two distinct mechanisms of human IgG1 hinge fragmentation upon storage. J Am Chem Soc 2007; 129:6976-7; PMID:17500521; http://dx.doi.org/10.1021/ja0705994
122. Harris RJ. Heterogeneity of recombinant antibodies: linking structure to function. Dev Biol (Basel) 2005; 122:117-27; PMID:16375256
123. Huh JH, White AJ, Brych SR, Franey H, Matsumura M. The identification of free cysteine residues within antibodies and a potential role for free cysteine residues in covalent aggregation because of agitation stress. J Pharm Sci 2013; 102:1701-11; PMID: 23559428; http://dx.doi.org/10.1002/jps.23505
124. Lacy ER, Baker M, Brigham-Burke M. Free sulfhydryl measurement as an indicator of antibody stability. Anal Biochem 2008; 382:66-8; PMID:18675772; http://dx.doi.org/10.1016/j.ab.2008.07.016
125. Zhang W, Czupryn MJ. Free sulfhydryl in recombinant monoclonal antibodies. Biotechnol Prog 2002; 18:509-13; PMID:12052067; http://dx.doi.org/10.1021/bp025511z
126. Chaderjian WB, Chin ET, Harris RJ, Etcheverry TM. Effect of copper sulfate on performance of a serum-free CHO cell culture process and the level of free thiol in the recombinant antibody expressed. Biotechnol Prog 2005; 21:550-3; PMID:15801797; http://dx.doi.org/10.1021/bp0497029

127. Xiang T, Chumsae C, Liu H. Localization and quantitation of free sulfhydryl in recombinant monoclonal antibodies by differential labeling with 12C and 13C iodoacetic acid and LC-MS analysis. Anal Chem 2009; 81:8101-8; PMID:19722496; http://dx.doi.org/10.1021/ac901311y
128. Zhang T, Zhang J, Hewitt D, Tran B, Gao X, Qiu ZJ, Tejada M, Gazzano-Santoro H, Kao YH. Identification and characterization of buried unpaired cysteines in a recombinant monoclonal IgG1 antibody. Anal Chem 2012; 84:7112-23; PMID:22794164; http://dx.doi.org/10.1021/ac301426h
129. Schauenstein E, Dachs F, Reiter M, Gombotz H, List W. Labile disulfide bonds and free thiol groups in human IgG. I. Assignment to IgG1 and IgG2 subclasses. Int Arch Allergy Appl Immunol 1986; 80:174-9; PMID:3710611; http://dx.doi.org/10.1159/000234048
130. Gevondyan NM, Volynskaia AM, Gevondyan VS. Four free cysteine residues found in human IgG1 of healthy donors. Biochemistry (Mosc) 2006; 71:279-84; PMID:16545064; http://dx.doi.org/10.1134/S0006297906030072
131. Amano M, Hasegawa J, Kobayashi N, Kishi N, Nakazawa T, Uchiyama S, Fukui K. Specific racemization of heavy-chain cysteine-220 in the hinge region of immunoglobulin gamma 1 as a possible cause of degradation during storage. Anal Chem 2011; 83:3857-64; PMID:21466225; http://dx.doi.org/10.1021/ac200321v
132. Zhang Q, Flynn GC. Cysteine racemization on IgG heavy and light chains. J Biol Chem 2013; 288:34325-35; PMID:24142697; http://dx.doi.org/10.1074/jbc.M113.506915
133. Andya JD, Maa YF, Costantino HR, Nguyen PA, Dasovich N, Sweeney TD, Hsu CC, Shire SJ. The effect of formulation excipients on protein stability and aerosol performance of spray-dried powders of a recombinant humanized anti-IgE monoclonal antibody. Pharm Res 1999; 16:350-8; PMID:10213364; http://dx.doi.org/10.1023/A:1018805232453
134. Gadgil HS, Bondarenko PV, Pipes G, Rehder D, McAuley A, Perico N, Dillon T, Ricci M, Treuheit M. The LC/MS analysis of glycation of IgG molecules in sucrose containing formulations. J Pharm Sci 2007; 96:2607-21; PMID:17621682; http://dx.doi.org/10.1002/jps.20966
135. Brady LJ, Martinez T, Balland A. Characterization of nonenzymatic glycation on a monoclonal antibody. Anal Chem 2007; 79:9403-13; PMID:17985928; http://dx.doi.org/10.1021/ac7017469
136. Zhang B, Yang Y, Yuk I, Pai R, McKay P, Eigenbrot C, Dennis M, Katta V, Francissen KC. Unveiling a glycation hot spot in a recombinant humanized monoclonal antibody. Anal Chem 2008; 80:2379-90; PMID:18307322; http://dx.doi.org/10.1021/ac701810q
137. Fischer S, Hoernschemeyer J, Mahler HC. Glycation during storage and administration of monoclonal antibody formulations. Eur J Pharm Biopharm 2008; 70:42-50; PMID:18583113; http://dx.doi.org/10.1016/j.ejpb.2008.04.021
138. Quan C, Alcala E, Petkovska I, Matthews D, Canova-Davis E, Taticek R, Ma S. A study in glycation of a therapeutic recombinant humanized monoclonal antibody: where it is, how it got there, and how it affects charge-based behavior. Anal Biochem 2008; 373:179-91; PMID:18158144; http://dx.doi.org/10.1016/j.ab.2007.09.027
139. Kaschak T, Boyd D, Yan B. Characterization of glycation in an IgG1 by capillary electrophoresis sodium dodecyl sulfate and mass spectrometry. Anal Biochem 2011; 417:256-63; PMID:21756870; http://dx.doi.org/10.1016/j.ab.2011.06.024
140. Miller AK, Hambly DM, Kerwin BA, Treuheit MJ, Gadgil HS. Characterization of site-specific glycation during process development of a human therapeutic monoclonal antibody. J Pharm Sci 2011; 100:2543-50; PMID:21287557; http://dx.doi.org/10.1002/jps.22504
141. Yuk IH, Zhang B, Yang Y, Dutina G, Leach KD, Vijayasankaran N, Shen AY, Andersen DC, Snedecor BR, Joly JC. Controlling glycation of recombinant antibody in fed-batch cell cultures. Biotechnol Bioeng 2011; 108:2600-10; PMID:21618472; http://dx.doi.org/10.1002/bit.23218
142. Zhang J, Zhang T, Jiang L, Hewitt D, Huang Y, Kao YH, Katta V. Rapid identification of low level glycation sites in recombinant antibodies by isotopic labeling with 13C6-reducing sugars. Anal Chem 2012; 84:2313-20; PMID:22324758; http://dx.doi.org/10.1021/ac202995x
143. Banks DD, Hambly DM, Scavezze JL, Siska CC, Stackhouse NL, Gadgil HS. The effect of sucrose hydrolysis on the stability of protein therapeutics during accelerated formulation studies. J Pharm Sci 2009; 98:4501-10; PMID:19388069; http://dx.doi.org/10.1002/jps.21749
144. Goetze AM, Liu YD, Arroll T, Chu L, Flynn GC. Rates and impact of human antibody glycation *in vivo*. Glycobiology 2012; 22:221-34; PMID:21930650; http://dx.doi.org/10.1093/glycob/cwr141
145. Ligier S, Fortin PR, Newkirk MM. A new antibody in rheumatoid arthritis targeting glycated IgG: IgM anti-IgG-AGE. Br J Rheumatol 1998; 37:1307-14; PMID:9973155; http://dx.doi.org/10.1093/rheumatology/37.12.1307
146. Ramasamy R, Yan SF, Schmidt AM. Advanced glycation endproducts: from precursors to RAGE: round and round we go. Amino Acids 2012; 42:1151-61; PMID:20957395; http://dx.doi.org/10.1007/s00726-010-0773-2
147. Harris RJ, Murnane AA, Utter SL, Wagner KL, Cox ET, Polastri GD, Helder JC, Sliwkowski MB. Assessing genetic heterogeneity in production cell lines: detection by peptide mapping of a low level Tyr to Gln sequence variant in a recombinant antibody. Biotechnology (N Y) 1993; 11:1293-7; PMID:7764191
148. Yu XC, Borisov OV, Alvarez M, Michels DA, Wang YJ, Ling V. Identification of codon-specific serine to asparagine mistranslation in recombinant monoclonal antibodies by high-resolution mass spectrometry. Anal Chem 2009; 81:9282-90; PMID:19852494; http://dx.doi.org/10.1021/ac901541h
149. Wen D, Vecchi MM, Gu S, Su L, Dolnikova J, Huang YM, Foley SF, Garber E, Pederson N, Meier W. Discovery and investigation of misincorporation of serine at asparagine positions in recombinant proteins expressed in Chinese hamster ovary cells. J Biol Chem 2009; 284:32686-94; PMID:19783658; http://dx.doi.org/10.1074/jbc.M109.059360
150. Khetan A, Huang YM, Dolnikova J, Pederson NE, Wen D, Yusuf-Makagiansar H, Chen P, Ryll T. Control of misincorporation of serine for asparagine during antibody production using CHO cells. Biotechnol Bioeng 2010; 107:116-23; PMID:20506364; http://dx.doi.org/10.1002/bit.22771
151. Yang Y, Strahan A, Li C, Shen A, Liu H, Ouyang J, Katta V, Francissen K, Zhang B. Detecting low level sequence variants in recombinant monoclonal antibodies. MAbs 2010; 2:285-98; PMID:20400866; http://dx.doi.org/10.4161/mabs.2.3.11718
152. Ren D, Zhang J, Pritchett R, Liu H, Kyauk J, Luo J, Amanullah A. Detection and identification of a serine to arginine sequence variant in a therapeutic monoclonal antibody. J Chromatogr B Analyt Technol Biomed Life Sci 2011; 879:2877-84; PMID:21900054; http://dx.doi.org/10.1016/j.jchromb.2011.08.015
153. Fu J, Bongers J, Tao L, Huang D, Ludwig R, Huang Y, Qian Y, Basch J, Goldstein J, Krishnan R, et al. Characterization and identification of alanine to serine sequence variants in an IgG4 monoclonal antibody produced in mammalian cell lines. J Chromatogr B Analyt Technol Biomed Life Sci 2012; 908:1-8; PMID:23122394; http://dx.doi.org/10.1016/j.jchromb.2012.09.023
154. Feeney L, Carvalhal V, Yu XC, Chan B, Michels DA, Wang YJ, Shen A, Ressl J, Dusel B, Laird MW. Eliminating tyrosine sequence variants in CHO cell lines producing recombinant monoclonal antibodies. Biotechnol Bioeng 2013; 110:1087-97; PMID:23108857; http://dx.doi.org/10.1002/bit.24759
155. Guo D, Gao A, Michels DA, Feeney L, Eng M, Chan B, Laird MW, Zhang B, Yu XC, Joly J, et al. Mechanisms of unintended amino acid sequence changes in recombinant monoclonal antibodies expressed in Chinese Hamster Ovary (CHO) cells. Biotechnol Bioeng 2010; 107:163-71; PMID:20506532; http://dx.doi.org/10.1002/bit.22780
156. Loftfield RB, Vanderjagt D. The frequency of errors in protein biosynthesis. Biochem J 1972; 128:1353-6; PMID:4643706
157. Wan M, Shiau FY, Gordon W, Wang GY. Variant antibody identification by peptide mapping. Biotechnol Bioeng 1999; 62:485-8; PMID:9921157; http://dx.doi.org/10.1002/(SICI)1097-0290(19990220)62:4<485::AID-BIT12>3.0.CO;2-E
158. Chumsae C, Gifford K, Lian W, Liu H, Radziejewski CH, Zhou ZS. Arginine modifications by methylglyoxal: discovery in a recombinant monoclonal antibody and contribution to acidic species. Anal Chem 2013; 85:11401-9; PMID:24168114; http://dx.doi.org/10.1021/ac402384y
159. Beck A, Sanglier-Cianférani S, Van Dorsselaer A. Biosimilar, biobetter, and next generation antibody characterization by mass spectrometry. Anal Chem 2012; 84:4637-46; PMID:22510259; http://dx.doi.org/10.1021/ac3002885
160. Beck A, Wagner-Rousset E, Ayoub D, Van Dorsselaer A, Sanglier-Cianférani S. Characterization of therapeutic antibodies and related products. Anal Chem 2013; 85:715-36; PMID:23134362; http://dx.doi.org/10.1021/ac3032355
161. Zhang H, Cui W, Gross ML. Mass spectrometry for the biophysical characterization of therapeutic monoclonal antibodies. FEBS Lett 2014; 588:308-17; PMID:24291257; http://dx.doi.org/10.1016/j.febslet.2013.11.027