

Mechanism of resistance to cetuximab therapy in colorectal cancer

Possible role of antibodies to immunoglobulin allotypes

Janardan P. Pandey

Department of Microbiology and Immunology; Medical University of South Carolina; Charleston, SC USA

The response rate to cetuximab, a chimeric (mouse-human) monoclonal antibody directed against epidermal growth factor receptor (EGFR), in colorectal cancer is low (10–15%), with significant inter-individual variability. Mechanisms proposed thus far to explain resistance to this therapy—such as the emergence of EGFR and KRAS mutations and aberrant ERBB2 signaling—do not account for the total inter-individual variation in treatment responses in *de novo* and in acquired resistance,¹⁻³ which suggests involvement of additional mechanisms. I propose that preexisting antibodies to the GM 3/f allotype, a genetic marker of immunoglobulin (Ig) G1 that is present on cetuximab,⁴ or such antibodies induced in response to administered cetuximab, could contribute to resistance to cetuximab. This novel mechanism, if supported by experimental observations, could provide an urgently needed biomarker for targeting eligible patients for this therapy.

Most manufacturers of mouse-human chimeric antibodies have treated the constant (C) region of human Ig as if it were naturally monomorphic and therefore not immunogenic in humans. The C region of Ig γ chains is highly polymorphic^{4,5} with at least 18 testable specificities (GM allotypes)—4 on $\gamma 1$, 1 on $\gamma 2$ and 13 on $\gamma 3$. With the exception of allelic GM 3 and GM 17 determinants expressed in the Fd region, all other GM alleles are expressed in the Fc region of γ chains. Most GM determinants are highly immunogenic, and the Ig molecules carrying these markers cross the maternal-fetal barrier in both directions, leading to anti-GM antibody production in the mother against the paternal GM markers present in the child,

and in the child against the maternal GM alleles.⁶

Patients with colorectal cancer who lack the GM 3 allotype would be expected to generate antibodies to this determinant if exposed through maternal-fetal incompatibility, allotype-incompatible blood transfusion or infusion of cetuximab. These preexisting or cetuximab-induced anti-GM 3 antibodies and the administered cetuximab could form immune complexes that would be eliminated by phagocytic cells, leading to non-responsiveness. Furthermore, by binding to the GM 3 (arginine) residue, these antibodies could alter the specificity of cetuximab. Contrary to the prevalent belief in immunology that the variable (V) region of Ig is the sole determinant of antibody specificity, several studies have shown that structural variation in the C region affects the expression of certain idiotypes and causes variation in the specificity of variable-region-identical Ig molecules.^{7,8} Amino acid sequence polymorphisms in the CH1 region affect the secondary structure of the antigen-binding site in the V region.⁹ The binding of anti-GM 3 antibodies to the arginine residue in the CH1 domain may also affect the conformation of the CH2 and CH3 domains involved in binding to the Fc γ receptors, thereby influencing the level of antibody-dependent cell-mediated cytotoxicity (ADCC), a major host defense mechanism against tumors and the leading mechanism underlying the clinical efficacy of therapeutic antibodies such as cetuximab. The fact that Ig molecules expressing GM 3 are immunogenic has been known since the discovery of this determinant almost half a century ago.¹⁰

In fact, anti-GM 3 antibodies derived from humans are employed in the hemagglutination-inhibition assay, the most commonly used method for GM allotyping.¹¹

The human C region of the κ light chain in cetuximab could constitute another source of antigenicity in this antibody. Like the γ chains, the κ chain is also polymorphic, characterized by the segregation of three alleles—KM 1, KM 1,2 and KM 3. Over 98% of the people positive for the KM 1 allotype are also positive for KM 2; the KM 1 allele is extremely rare. These alleles are characterized by amino acid substitutions at positions 153 and 191 of κ chain—KM 1: valine 153, leucine 191; KM 1,2: alanine 153, leucine 191; and KM 3: alanine 153, valine 191. Cetuximab expresses the KM 3 allotype,⁴ which can potentially induce anti-KM 3 antibodies in KM 3-lacking cetuximab recipients. These antibodies could also form immune complexes with cetuximab that would be eliminated by phagocytic cells, contributing to nonresponsiveness.

To determine whether or not anti-GM 3 or anti-KM 3 antibodies contribute to cetuximab resistance, these antibodies could be measured in a large sample of cetuximab-treated responders/non-responders. Association of anti-allotype antibodies with nonresponsiveness could lead to individualized therapy: cetuximab treatment would be limited to GM 3-KM 3 positive subjects and construction of two additional anti-EGFR antibodies, GM 17-KM 1,2 and GM 17-KM 3, would provide treatments for the rest of the population. In fact, the latter two constructs could replace cetuximab, as the GM 17

allotype does not appear to be immunogenic in humans (ref. 10 and personal observations).

Similar experiments could be done to determine whether or not anti-allotype antibodies contribute to the resistance to panitumumab, a human anti-EGFR IgG2 antibody. IgG2 expresses a polymorphic determinant, GM 23/n, characterized by methionine at position 282 of γ 2 chain. If panitumumab expresses the GM 23 determinant, it could induce anti-GM 23 antibodies with consequences similar to that of anti-GM 3 antibodies induced by cetuximab.

Apart from potential antigenicity, another very important consideration in the selection of an appropriate C region would be whether or not it can mediate potent ADCC against tumor cells. For IgG1 antibodies, an ideal C region would be able to engage with Fc γ R1IIa expressing either valine or phenylalanine variants on natural killer cells. For IgG2 antibodies, an ideal C region would be able to engage with Fc γ R1IIa expressing either histidine or arginine variants on myeloid effector cells (i.e., neutrophils, monocytes).

Cetuximab-induced anaphylaxis mediated by anti-IgE antibodies to galactose- α -1,3-galactose present on the Fab portion of the chimeric antibody is another significant impediment to this therapy in some individuals.^{12,13} It would be of interest to

determine whether a genetic marker identified on IgE, EM(1),¹⁴ influences the anti-IgE response to this oligosaccharide, just as the IgG2 allotype GM 23 influences the anti-IgG2 responses to polysaccharide antigens.^{4,5} If a significant relationship between EM(1) and anti-IgE responses to the oligosaccharide is established, it could help identify suitable subjects for cetuximab therapy.

In summary, characterization of certain Igallotypes and anti-allotype antibodies in patients treated with mouse-human or fully human antibodies could open a new avenue of investigation toward our understanding of resistance to antibody therapy in general. Additionally, it could help in the design of bio-better versions of antibodies, and may help identify patients who would benefit the most from such immunotherapeutic interventions.

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