Dhaka Univ. J. Biol. Sci. **30**(3 CSI): 393-403, 2022 (June)
DOI: https://doi.org/10.3329/dujbs.v30i3.59032

INTERACTIONS OF EPITOPIC VARIANTS OF EPIDERMAL GROWTH FACTOR RECEPTOR WITH THERAPEUTIC ANTI-EGFR ANTIBODIES

SU MAHDIYAH[1], T AHSAN[1,2], K FATEMA, SS SHOILY AND AA SAJIB*

Department of Genetic Engineering & Biotechnology, University of Dhaka, Dhaka-1000, Bangladesh

Key words: EGFR, Cetuximab, Necitumumab, Nimotuzumab, Panitumumab, Therapeutic antibody

Abstract

Epidermal growth factor receptor (EGFR) plays important roles in cancerous transformation of epithelial cells in many solid cancers. Due to the pivotal role of EGFR in cellular proliferation and metastasis, it is a promising molecular target for the treatment of various cancers. One of the major treatment approaches uses anti-EGFR monoclonal antibodies (mAbs) targeted to the extracellular domain of the receptor to competitively block the binding of its ligands. Cetuximab, necitumumab, nimotuzumab, and panitumumab are such approved mAbs which are commercially available and used to treat multiple types of cancers. The response rates to these expensive therapeutics in various cancers range from nearly 9% to 91%. Hence, the objective of this study was to indentify whether any of the missense single nucleotide polymorphisms (SNPs) in the EGFR gene impart any structural and functional impact on the receptor's interaction with these antibodies. We used X-ray crystallographic structures (from Protein Data Bank) of the F_{ab} fragments of these therapeutic antibodies in complex with EGFR to analyze the effects of the missense mutations on the antigen-antibody interactions. We also assessed the potential association of the destabilizing variants with pathogenicity and disease susceptibility. EGFR H433Q (rs1171743336), S464T (rs746763556), S492G (rs1057519760) and S492R (rs1057519860) variants appear to weaken interactions between EGFR and cetuximab, which is the most widely used anti-EGFR therapeutic antibody. Other epitopic variants do not appear to affect interactions between EGFR and relevant mAbs (necitumumab, nimotuzumab, and panitumumab). Prior to treatment of the EGFR mediated conditions with cetuximab, screening of variants that destabilize antibody-EGFR interaction may be considered as a companion diagnostic test for avoiding unresponsiveness and improving therapeutic outcomes.

Introduction

The epidermal growth factor receptor (EGFR, also known as HER1 in human) is a tyrosine kinase that acts as a transmembrane receptor for extracellular growth factors belonging to the epidermal growth factor (EGF) family(1). EGFR is the first member of the

*Author for correspondence: <abu.sajib@du.ac.bd>. [1]Equal contribution. [2]Department of Mathematics and Natural Sciences, BRAC University, Dhaka-1212, Bangladesh.

ErbB receptor tyrosine kinase family, which includes several other members- HER2 (ERBB2), HER3 (ERBB3) and HER4 (ERBB4)[2]. These receptors are notable for their important roles in cancerous transformation of epithelial cells[3]. EGFR has an extracellular receptor domain for ligand binding, a hydrophobic transmembrane domain, an intracellular receptor tyrosine kinase (RTK) domain, and a C-terminal domain[4]. Binding of ligand causes homo or hetero dimerization of the receptors and autophosphorylation of the tyrosines on the RTK domain, which triggers signaling cascades including the MAP kinase pathway, PI3 kinase-Akt pathway, as well as CDK and cyclin activation for progression through G1 to S phase of cell cycle, resulting in activation of biological processes such as cell proliferation, cell division, mitosis, ductal development of mammary glands, etc[5].

Overexpression of EGFR results in hyperactive cellular signaling pathways dictating more aggressive growth and invasive properties, which is evident in many solid cancers including breast cancer, ovarian cancer, non–small-cell lung cancer (NSCLC), head-and-neck cancer, renal cancer, and colon cancer[2]. Due to the pivotal role of EGFR in mediating cellular proliferation and metastasis, it is a promising molecular target in the treatment of various cancers. One of the major approaches uses anti-EGFR monoclonal antibodies (mAbs) targeted to the extracellular domain of the receptor to competitively block the binding of its ligands like epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α)[6]. Four such monoclonal antibodies (cetuximab, necitumumab, nimotuzumab, and panitumumab) are approved by FDA and commercially available. These antibodies are currently used to treat multiple types of cancers[7].

Cetuximab is a chimeric (mouse/human) mAb used to treat colorectal cancer as well as head and neck cancer in patients with wild type KRAS gene[8]. Nimotuzumab and panitumumab are humanized and fully human mAbs, respectively, which also works in patients with wild type KRAS gene[9,10]. Necitumumab is a recombinant human mAb used for treatment of previously untreated squamous metastatic NSCLC[11]. These monoclonal antibodies, specially cetuximab, panitumumab, and necitumumab, are widely prescribed. In fact, cetuximab (brand name Erbitux®) is one of the top best-selling drugs globally. Despite their high sales, these therapeutic antibodies are quite expensive and 'non-cost-effective'[12]. In contrast to their price-point, the response rates to these therapeutics in various cancers range from as low as 9%[13] to 91%[14]. While some of the non-responsiveness can be explained from presence of specific KRAS and BRAS mutations and some other factors[6], these cannot fully explain such high rates. Therefore, there must be some inherent differences in the receptor itself that may cause such variability. Hence, the objective of this study was to identify whether any of the missense single nucleotide polymorphism (SNPs) in the EGFR gene has any structural and functional impact on the receptor's interaction with the antibodies. We used the X-ray

crystallographic structures of the F_{ab} fragments of these therapeutic antibodies (cetuximab, necitumumab, nimotuzumab and panitumumab) complexed with EGFR to analyze the effects of the missense mutations on the antigen-antibody interaction. We also assessed the potential association of the destabilizing variants with pathogenicity and disease susceptibility.

Materials and Methods

Selection of approved anti-EGFR therapeutic monoclonal antibodies: List of approved mAbs for therapeutic applications as EGFR antagonist was collected from the Therapeutic Structural Antibody Database (Thera-SabDab) database[7].

Identification of missense variants in the EGFR epitope: Amino acid residues, that are present on EGFR epitopes targeted with different approved therapeutic antibodies, were retrieved through literature survey[15-18]. Ensembl genome database[19] was used to identify the missense variants within the EGFR epitopes.

Analysis of missense variants' effects on EGFR-mAb interactions: X-ray crystallographic structures of EGFR-Cetuximab (PDB ID: 1YY9), EGFR-Necitumumab (PDB ID: 3B2U), EGFR-Nimotuzumab (PDB ID: 3GKW) and EGFR-Panitumumab (PDB ID: 5SX4) were retrieved from the Protein Data Bank (PDB)[20]. UCSF Chimera 1.14 was used for customizing the X-ray crystallographic structures as follows. Only the A chain (EGFR molecule), D chain (heavy chain of antibody), and C chain (light chain of antibody) of 1YY9 were retained, and these chains were renamed to A, H and L, respectively. The other chains (B, E, F and G) were removed. 3B2U and 5SX4 contain complex structures with multiple epitopes, heavy and light chains. A chain (EGFR molecule), D chain (heavy chain of antibody), and C chain (light chain of antibody) of 3B2U were retained for further analysis and these chains were renamed to A, H and L, respectively. Only the I chain (light chain of antibody), J chain (heavy chain of antibody) and M chain (EGFR molecule) of 5SX4 were kept for further analysis. The EGFR, heavy and light chains were renamed to A, H and L, respectively (where appropriate). 3GKW contains a single light and a heavy chain of the antibody with a single epitope.

mCSM-PPI2[21], SAAMBE-3D[22], MutaBind[23] and BeAtMuSic V1.0[24] servers were used to assess the effects of missense variants on antigen-antibody interactions (based on the $\Delta\Delta G$ values) using the above mentioned structures as input.

Construction of 3-D models: Amino acid sequence of EGFR (UniProt accession number: P00533) was retrieved from UniProt[25]. EGFR has a long signal peptide (24 amino acid residues) at the N-terminal, which was deleted. Amino acid sequences of the heavy and light chains of the approved mAbs were obtained from the Therapeutic Structural Antibody Database (Thera-SAbDab). SWISS-MODEL[26] was used to generate 3-D models of both wild type and mutant EGFR-mAb complexes using the X-ray crystallographic structures as templates.

Investigation of interfaces, interactions, and structures: Missense3D web server[27] was used to assess the effects of selected missense variants on EGFR structure. iCn3D was used to measure distances between selected atoms at the interface and the areas of interacting surfaces between EGFR and mAb chains.

Evaluation of missense variants' pathogenicity: SIFT[28], PolyPhen-2[29], PMut[30] and PredictSNP 1.0[31] were used to predict the pathogenicity associated information of all selected missense variants.

Prediction of the effects of missense variants on EGFR-receptor interactions: Effects of the missense variants on interaction between EGFR and EGF were predicted based on the X-ray crystallographic structure of EGF-EGFR complex (PDB ID: 1IVO) following the principle mentioned above with the EGFR-mAb interactions. 1IVO was retrieved from the PDB[20]. The PDB file contained multiple EGFR and EGF chains. Only the A (EGFR) and C (EGF) chains were retained and renamed to R and L, respectively. Other chains were removed using UCSF Chimera 1.14. This redesigned PDB file was used as an input in mCSM-PPI2[21], SAAMBE-3D[22], MutaBind2[23] and BeAtMuSiC V1.0[24] to predict the effects of selected missense variants on EGF-EGFR interactions.

Results and Discussion

Impact of epitopic variants on interaction between EGFR and anti-EGFR therapeutic antibodies: List of epitopic amino acids that are important for interaction between EGFR and the approved therapeutic monoclonal anti-EGFR antibodies (cetuximab, necitumumab, nimotuzumab, and panitumumab) was retrieved through literature survey (Table 1). Among these, eleven and five missense variants are present in the epitopes for cetuximab and panitumumab, respectively. Two missense variants reside in each of the epitopes for necitumumab and nimotuzumab.

Based on the X-ray crystallographic structures of the EGFR-antibody complexes, the relative change in binding affinity ($\Delta\Delta G = \Delta G_{mutant} - \Delta G_{wild\text{-}type}$) were predicted with mCSM-PPI2, SAAMBE-3D, MutaBind2 and BeAtMuSiC V1.0. It was reported earlier that mutations in epitope resulting in $\Delta\Delta G > 0.5$ kcal/mol may significantly reduce binding affinity in case of antigen-antibody interactions[33]. As the predictions from several tools may help in clarifying the potential impacts of the variants, we considered epitopic variants to be significantly destabilizing if at least three or more of these four computational servers predicted the corresponding $\Delta\Delta G$ to be > 0.5 kcal/mol. Based on this principle, none of the variants in the epitopes for panitumumab, necitumumab and nimotuzumab were predicted to significantly destabilize the interactions between EGFR and the heavy (H) and light (L) chains of the antibodies (Data not shown). Four variants (H433Q [H409Q], S464T [S440T], S492G [S468G] and S492R [S468R]; positions in absence of the 24 amino acid long signal peptide are shown within the square brackets) in the epitope for cetuximab were predicted by at least three of the four tools to significantly

destabilize interaction between EGFR and cetuximab H or L chain (Table 2). Using iCn3D, contacts and interactions between H and L chain of cetuximab and EGFR (wild type and mutated receptors) were observed to assess further impact (Figs 1-3).

Table 1. Epitopic amino acids in EGFR important for interaction with therapeutic antibodies.

Antibody	Epitopic amino acids in EGFR important for interaction with mAbs		Variant	SNP IDs	Consequence
	With signal peptide*	Without signal peptide*			
Cetuximab	376	352	F/L	rs1341708803	Missense variant
	433	409	H/R	rs750713244	Missense variant, splice region variant
	433	409	H/Q	rs1171743336	Missense variant, splice region variant
	442	418	S/N	rs765091640	Missense variant
	464	440	S/T	rs746763556	Missense variant
	464	440	S/A	rs746763556	Missense variant
	467	443	K/N	rs1009449079	Missense variant
	491	467	I/V	rs768500612	Missense variant
	492	468	S/G	rs1057519760	Missense variant
	492	468	S/R	rs1057519860	Missense variant
	497	473	N/K	rs774773441	Missense variant
Necitumumab	433	409	H/R	rs750713244	Missense variant, splice region variant
	433	409	H/Q	rs1171743336	Missense variant, splice region variant
Nimotuzumab	383	359	H/N	rs755972013	Missense variant
	383	359	H/R	rs1169461493	Missense variant
Panitumumab	376	352	F/L	rs1341708803	Missense variant
	413	389	N/Y	rs895496054	Missense variant
	413	389	N/S	rs770466526	Missense variant
	414	390	R/M	rs1424097500	Missense variant
	415	391	T/K	rs1274543317	Missense variant

* 24 amino acid long signal peptide in the N-terminal.

Variants H409Q (rs1171743336) and S440T (rs746763556) were predicted by mCSM-PPI2, SAAMBE3D and BeAtMuSiC to weaken interaction ($\Delta\Delta G > 0.5$) between cetuximab H chain and EGFR. The π stacking between H409 in the wild type EGFR ($EGFR^{WT}$) and

cetuximab H chain-Y101 is lost in case of the variant $EGFR^{H409Q}$ (Fig. 1). The weakening of interaction between cetuximab H chain and EFGR by variant H409Q could result from this loss of interaction as π stacking (π–π interactions) contribute strongly to the stabilization of antigen–antibody complex[34]. In addition, cetuximab H chain-Y102 protrudes into a hydrophobic pocket of domain III and forms hydrogen bond (H-bond) with Q384 and Q408 of EGFR at the center of the EGFR-antibody interface, which augments the packing of the antibody[16]. Mutations in the region near this hydrophobic pocket disrupt binding of EGFR to cetuximab as seen for a double mutant Q408M/H409E, which reduced cetuximab binding to EGFR by 150-fold[16]. Thus, H409Q may be a destabilizing variant that can either reduce cetuximab efficiency or make EGFR resistant to cetuximab.

Table 2. Predicted scores of interaction between the EGFR epitopic variants and cetuximab.

SNP ID	Variant	Antibody Chain	mCSM-PPI2	SAAMBE3D	MutaBind2	BeAtMuSiC
rs1171743336	H433Q	H	*0.515*	*0.71*	0.35	*0.72*
		L	*0.619*	0.3	-0.15	0.07
rs746763556	S464T	H	*0.726*	*0.54*	0.29	*0.55*
		L	0.104	0.39	-0.18	0.28
rs746763556	S464A	H	0.228	*0.51*	*0.85*	0.28
		L	0.185	0.3	0.1	0.18
rs1009449079	K467N	H	0.231	0.46	0.24	0.05
		L	0.407	*0.71*	0.14	0.32
rs1057519760	S492G	H	-0.043	*0.68*	-0.11	-0.41
		L	0.182	*1.14*	*0.61*	*0.67*
rs1057519860	S492R	H	0.083	0.39	-0.42	-0.04
		L	*1.084*	*1.22*	*0.68*	*0.76*

Although no H-bond was detected with iCn3D between EGFR-S440 and cetuximab, in previous studies this polar amino acid was reported to be involved in a H-bond that contributes to the anchoring of cetuximab over the hydrophobic pocket on domain III and reside within H-bonding distance from the carbonyl backbone of cetuximab-Y102 as well as the phenolic OH-group of cetuximab-Y104[16,35]. Interruption of the bond between EGFR-S440 and cetuximab due to substitution by a threonine may be the reason of the destabilizing effect predicted for variant S440T.

SAAMBE-3D, MutaBind2 and BeAtMuSiC predicted S468G (rs1057519760) to reduce binding affinity of L chain of cetuximab to EGFR. S468R (rs1057519860) was predicted to significantly destabilize interaction between cetuximab L chain and EGFR by all the four

tools. Along with a series of interactions, a hydrogen bond between cetuximab H chain-Y104 and EGFR-S468 plays role in the packing of cetuximab over the hydrophobic pocket of EGFR[16].

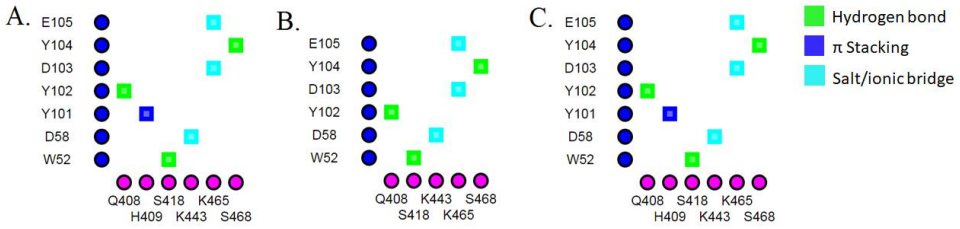

Fig. 1. 2D plots of interactions between cetuximab heavy chain and EGFR wild-type (A), H433Q (B) and S464T (C) structures. EGFR residues (positions are shown without the 24 amino acid long signal peptide) are shown along the horizontal axis and cetuximab heavy chain residues are shown along the vertical axis.

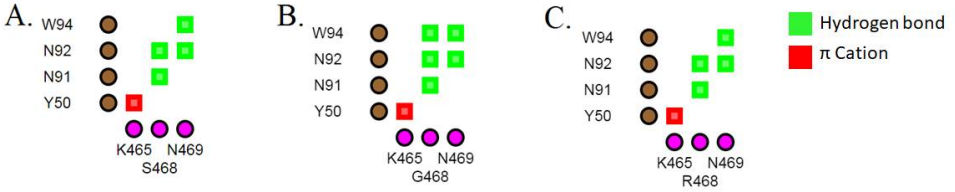

Fig. 2. Interactions between cetuximab light chain and EGFR wild-type (A), S492G (B) and S492R (C) structures. EGFR residues are shown along the horizontal axis and cetuximab light chain residues are shown along the vertical axis.

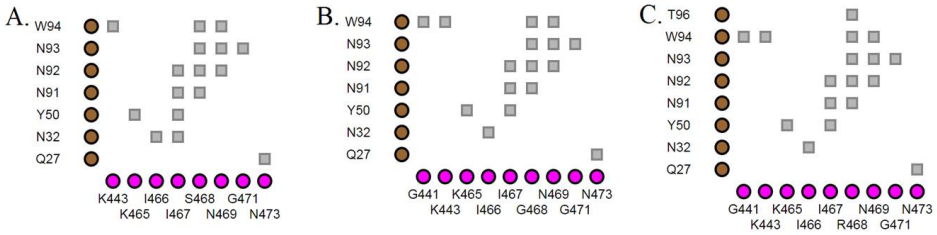

Fig. 3. Contacts between cetuximab light chain and EGFR wild-type (A), S492G (B) and S492R (C) structures. EGFR residues are shown along the horizontal axis and cetuximab light chain residues are shown along the vertical axis.

A hydrogen bond, that is absent between EGFR-S468 (WT) and cetuximab L chain-W94, is present between EGFR-G468 and cetuximab L chain-W94 (Fig. 2). In mutant $EGFR^{S468G}$, the L chain-W94 is in contact (cut-off value 4Å) with Gly441, but no such contact is seen in $EGFR^{WT}$ (Fig. 3). Contact between cetuximab L chain-N32 and Ile467 seen in $EGFR^{WT}$ are lost in case of $EGFR^{S468G}$ (Fig. 3). Variants I467M and G441R in EGFR prevent binding of cetuximab to EGFR[35]. As substitution of Ile467 and Gly441 can result

in prevention of binding, change in their position along with the newly formed H-bond may play role in the predicted destabilization effect of the variant S468G.

In $EGFR^{WT}$, the EGFR-S468 residue is not in contact with cetuximab L chain-T96, and EGFR-I467 is in contact with cetuximab L chain-N32 (Fig. 3). In $EGFR^{S468R}$, cetuximab L chain-T96 is brought in contact with EGFR-R468 and L chain-N32 is moved away by more than 4Å from EGFR-I467 (Fig. 3). So, a conformational change is mediated by variant S468R, which could be the reason of the predicted destabilization effect on binding with cetuximab. Although $EGFR^{S468R}$ is resistant to binding with cetuximab, panitumumab retains the capacity to bind to this mutant form of EGFR[36]. EGFR-R468 variant may serve as a marker for selecting panitumumab as the primary treatment option for patients harboring this variant as well for those unresponsive to cetuximab. None of the variants in the epitope for cetuximab was predicted to be pathogenic by SIFT, PolyPhen-2, PMut and PredictSNP 1.0 (Table 3).

In the absence of bound ligands, monomeric EGFR adopts either an extended (untethered) conformation or a closed (tethered) conformation[16]. Growth factors bind preferentially to the extended conformation. Upon simultaneous binding of growth factors to two sites (within domains I and III) in the extracellular region of EGFR, the dimerization arm, a critical region of domain II required for EGFR dimerization, is exposed. Thus, through dimerization of two ligand-bound monomers, the downstream signaling process is mediated[16]. Both H and L chain of cetuximab interact with high affinity to a site on domain III of EGFR that overlaps with the growth factor binding site and sterically prevents the receptor from adopting the dimerization-competent extended configuration[16,37]. Disruption in the interactions of the cetuximab H and L chains with EGFR via the epitopic variants (H433Q [H409Q], S464T [S440T], S492G [S468G] and S492R [S468R]; positions in absence of the 24 amino acid long signal peptide are shown within the square brackets)– with potential destabilizing effect may lead to reduced response to cetuximab therapy.

Table 3. Influence of EGFR epitopic variants on interaction with EGF.

Variant	Change in binding energy, ΔΔG (kcal/mol)[a]				Pathogenic effect			
	mCSM-PPI2	SAAMBE3D	MutaBind2	BeAtMuSiC	SIFT	PolyPhen-2	PMut	PredictSNP
H433Q	0.402	1.14	0.46	*1.51*	Tolerated	Benign	Neutral	Neutral
S464T	*0.65*	0.47	1.06	0.21	Tolerated	Benign	Neutral	Neutral
S464A	0.238	0.38	0.74	-0.02	Tolerated	Benign	Neutral	Neutral
K467N	0.067	0.4	0.27	0.2	Tolerated	Benign	Neutral	Neutral
S492G	-0.061	*0.68*	0.28	*0.57*	Tolerated	Benign	Neutral	Neutral
S492R	-0.146	0.38	-0.58	0.34	Tolerated	Benign	Neutral	Neutral

[a]Positive values of ΔΔG indicate decreasing affinity.

Effect of EGFR epitopic variants on interaction between EGFR and EGF: The changes in binding affinities between the epitopic variants of EGFR and EGF were assessed using SAAMBE-3D, MutaBind2 and BeAtMuSiC V1.0 (Table 3). None of these variants were predicted by all the three tools to substantially reduce binding affinity ($\Delta\Delta G > 0.5$ kcal/mol) between EGFR and EGF. The binding of EGF to EGFR-Q433 was predicted to be significantly weak ($\Delta\Delta G > 1$ kcal/mol) by mCSM-PPI2 and BeAtMuSiC compared to the interactions between EGF and EGFR-WT. The same two tools predicted epitopic variant EGFR-G492 to destabilize ($\Delta\Delta G > 0.5$ kcal/mol) interactions between EGFR and EGF.

Therefore, prior to treatment of the EGFR mediated conditions with cetuximab, screening of variants that destabilize antibody-EGFR interaction may be considered as a companion diagnostic test for avoiding unresponsiveness or inefficient outcome. Further studies may be focused on identifying the prevalence of these variants (rs1171743336, rs746763556, rs1057519760, and rs1057519860) in Bangladeshi and other populations as well as their causal relationship to responsiveness with cetuximab treatment.

Disclosure statement

The authors have no conflict of interest to declare.

References

1. Normanno N, A De Luca, C Bianco, L Strizzi, M Mancino, MR Maiello, A Carotenuto, G De Feo, F Caponigro and DS Salomon 2006. Epidermal growth factor receptor (EGFR) signaling in cancer. Gene. **366**(1): 2-16.
2. Herbst RS 2004. Review of epidermal growth factor receptor biology. Int J Radiat Oncol Biol Phys. **59**(2 Suppl): 21-26.
3. Nicholson RI, JM Gee and ME Harper 2001. EGFR and cancer prognosis. Eur J Cancer. **37**(Suppl 4): 9-15.
4. Sabbah DA, R Hajjo and K Sweidan 2020. Review on Epidermal Growth Factor Receptor (EGFR) structure, signaling pathways, interactions, and recent updates of EGFR inhibitors. Curr Top Med Chem. **20**(10): 815-834.
5. Voldborg BR, L Damstrup, M Spang-Thomsen and HS Poulsen 1997. Epidermal growth factor receptor (EGFR) and EGFR mutations, function and possible role in clinical trials. Ann Oncol. **8**(12): 1197-1206.
6. Brand TM, M Iida and DL Wheeler 2011. Molecular mechanisms of resistance to the EGFR monoclonal antibody cetuximab. Cancer Biol Ther. **11**(9): 777-792.
7. Raybould MIJ, C Marks, AP Lewis, J Shi, A Bujotzek, B Taddese, CM Deane 2020. Thera-SAbDab: the Therapeutic Structural Antibody Database. Nucleic acids res. **48**(D1): D383-D388.
8. Cohen MH, H Chen, S Shord, C Fuchs, K He, H Zhao, S Sickafuse, P Keegan and R Pazdur 2013. Approval summary: Cetuximab in combination with cisplatin or carboplatin and 5-fluorouracil for the first-line treatment of patients with recurrent locoregional or metastatic squamous cell head and neck cancer. Oncologist. **18**(4): 460-466.

9. Mazorra Z, L Chao, A Lavastida, B Sanchez, M Ramos, N Iznaga and T Crombet 2018. Nimotuzumab: beyond the EGFR signaling cascade inhibition. Semin Oncol. **45**(1-2):18-26.
10. Giusti RM, KA Shastri, MH Cohen, P Keegan and R Pazdur 2007. FDA drug approval summary: panitumumab (Vectibix). Oncologist. **12**(5): 577-583.
11. Fala L 2016. Portrazza (Necitumumab), an IgG1 monoclonal antibody, FDA approved for advanced squamous non-small-cell lung cancer. Am Health Drug Benefits. **9**(Spec Feature): 119-122.
12. Carvalho AC, F Leal and AD Sasse 2017. Cost-effectiveness of cetuximab and panitumumab for chemotherapy-refractory metastatic colorectal cancer. PLoS ONE. **12**(4): e0175409.
13. Lawler M, B Johnston, S Van Schaeybroeck, M Salto-Tellez, R Wilson, M Dunlop and PG Johnston 2020. Colorectal Cancer. In: Abeloff's Clinical Oncology (Sixth Edition). Edited by Niederhuber JE, Armitage JO, Kastan MB, Doroshow JH, Tepper JE: Elsevier.
14. Yoshida K, Y Yatabe, JY Park, J Shimizu, Y Horio, K Matsuo, T Kosaka, T Mitsudomi and T Hida 2007. Prospective validation for prediction of gefitinib sensitivity by epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer. J Thorac Oncol. **2**(1): 22-28.
15. Bagchi A, JN Haidar, SW Eastman, M Vieth, M Topper, MD Iacolina, JM Walker, A Forest, Y Shen, RD Novosiadly and KM Ferguson 2018. Molecular basis for necitumumab inhibition of EGFR variants associated with acquired cetuximab resistance. Mol Cancer Ther. **17**(2): 521-531.
16. Li S, KR Schmitz, PD Jeffrey, JJ Wiltzius, P Kussie and KM Ferguson 2005. Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. Cancer Cell. **7**(4): 301-311.
17. Tundidor Y, CP García-Hernández, A Pupo, Y Cabrera Infante and G Rojas 2014. Delineating the functional map of the interaction between nimotuzumab and the epidermal growth factor receptor. MAbs. **6**(4): 1013-1025.
18. Voigt M, F Braig, M Göthel, A Schulte, K Lamszus, C Bokemeyer and M Binder 2012. Functional dissection of the epidermal growth factor receptor epitopes targeted by panitumumab and cetuximab. Neoplasia. **14**(11): 1023-1031.
19. Yates AD, P Achuthan, W Akanni, J Allen, J Allen, J Alvarez-Jarreta, MR Amode, IM Armean, AG Azov, R Bennett et al 2000. Ensembl 2020. Nucleic acids res. **48**(D1): D682-D688.
20. Berman HM, J Westbrook, Z Feng, G Gilliland, TN Bhat, H Weissig, IN Shindyalov and PE Bourne 2000. The Protein Data Bank. Nucleic acids res. **28**(1): 235-242.
21. Rodrigues CHM, Y Myung, DEV Pires and DB Ascher 2019. mCSM-PPI2: predicting the effects of mutations on protein-protein interactions. Nucleic acids res. **47**(W1): W338-W344.
22. Pahari S, G Li, AK Murthy, S Liang, R Fragoza, H Yu and E Alexov 2020. SAAMBE-3D: predicting effect of mutations on protein-protein interactions. Int J Mol Sci. **21**(7): 2563.
23. Li M, FL Simonetti, A Goncearenco and AR Panchenko 2016. MutaBind estimates and interprets the effects of sequence variants on protein-protein interactions. Nucleic acids res. **44**(W1): W494-501.
24. Dehouck Y, JM Kwasigroch, M Rooman and D Gilis 2013. BeAtMuSiC: Prediction of changes in protein-protein binding affinity on mutations. Nucleic Acids Res. **41**(Web Server issue): W333-339.

25. UniProt Consortium T 2018. UniProt: The universal protein knowledgebase. Nucleic Acids Res. **46**(5): 2699.

26. Waterhouse A, M Bertoni, S Bienert, G Studer, G Tauriello, R Gumienny, FT Heer, TAP de Beer, C Rempfer, L Bordol, R Lepore and T Schwede 2018. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic acids res. **46**(W1): W296-W303.

27. Ittisoponpisan S, SA Islam, T Khanna, E Alhuzimi, A David and MJE Sternberg 2019. Can predicted protein 3D structures provide reliable insights into whether missense variants are disease associated? J Mol Biol. **431**(11): 2197-2212.

28. Ng PC and S Henikoff 2003. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res. **31**(13): 3812-3814.

29. Adzhubei I, DM Jordan and SR Sunyaev 2013. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet. **7**: Unit7.20.

30. López-Ferrando V, A Gazzo, X de la Cruz, M Orozco and JL Gelpí 2017. PMut: a web-based tool for the annotation of pathological variants on proteins, 2017 update. Nucleic Acids Res. **45**(W1): W222-W228.

31. Bendl J, J Stourac, O Salanda, A Pavelka, ED Wieben, J Zendulka, J Brezovsky and J Damborsky 2014. PredictSNP: robust and accurate consensus classifier for prediction of disease-related mutations. PLoS Comp Biol. **10**(1): e1003440.

32. Zhang N, Y Chen, H Lu, F Zhao, RV Alvarez, A Goncearenco, AR Panchenko and Li M 2020. MutaBind2: predicting the impacts of single and multiple mutations on protein-protein interactions. iScience. **23**(3): 100939.

33. Gonzalez TR, KP Martin, JE Barnes, JS Patel and FM Ytreberg 2020. Assessment of software methods for estimating protein-protein relative binding affinities. PLoS ONE. **15**(12): e0240573.

34. Dalkas GA, F Teheux, JM Kwasigroch and M Rooman 2014. Cation-π, amino-π, π-π, and H-bond interactions stabilize antigen-antibody interfaces. Proteins. **82**(9): 1734-1746.

35. Arena S, B Bellosillo, G Siravegna, A Martínez, I Cañadas, L Lazzari, N Ferruz, M Russo, S Misale, I González M Iglesias, E Gavilan, G Corti, S Hobor, G Crisafulli, m Salido, J Sánchez, A Dalmases, J Bellmunt, G De Fabritiis, A Rovira, F Di Nicolantonio, J Albanel, A Bardelli and C Montagut 2015. Emergence of multiple EGFR extracellular mutations during cetuximab treatment in colorectal cancer. Clin Cancer Res. **21**(6): 2157-2166.

36. Montagut C, A Dalmases, B Bellosillo, M Crespo, S Pairet, M Iglesias, M Salido, M Gallen, S Marsters, SP Tsai 2012. Identification of a mutation in the extracellular domain of the Epidermal Growth Factor Receptor conferring cetuximab resistance in colorectal cancer. Nat Med. **18**(2): 221-223.

37. Kaufman NEM, S Dhingra, SD Jois and MDGH Vicente 2021. Molecular targeting of Epidermal Growth Factor Receptor (EGFR) and Vascular Endothelial Growth Factor Receptor (VEGFR). Molecules. **26**(4): 1076.

(Manuscript received: 20 April, 2021; accepted: 27 July, 2021)