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Short Communication Open Access

Design of *in silico* Trifunctional Antibody (hIgG1-FC: mouse-antiHER2× human-B7.1) Gene Cassettes and Expression Vectors: The Stage Prior to Production

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Abstract

The specific gene cassettes and expression vectors for tri-functional antibody (hIgG1-FC: mouse-antiHER2× human-B7.1) were designed *in silico*. The *in silico* tri-functional antibody has been derived from human IgG1, human B7.1, and mouse antiHER2 antibody. The variable domains of IgG1 framework were substituted by Cha21 (mouse-antiHER2 scFV) VH-VL, and B7.1 IgV-like domains. The domain data of human IgG1 heavy and light chain-constant regions, human B7.1 IgV-like, and cha21 variable regions were obtained from UniprtoKB and PDB databases. The 3D structure of domains was trimmed and modeled by pyMol software. To ensure the presence of antigen binding residues, complementarity determining regions, and framework regions in variable domain structure, the researchers analyzed the cha21-VH and VL nucleotide sequences using Paratome and IMGT softwares, respectively. In this study, two *in silico* expression vectors were designed. The vector forms were ready *in silico* to be synthesized and transfected into CHO cells in order to be developed; the cells expressing the chimeric tri-functional antibody which enables the eradication of HER2-expressing cancer cells using apoptotic induction, T-cell activation and accessory cell activation by antiHER2, B7.1, and Fc domains, respectively.

Keywords: AntiHER2 antibody; Bladder cancer; Breast cancer; Cholangiocarcinoma; Choriocarcinoma; Pancreatic cancer; Ovarian cancer

Introduction

Cancer is a heterogeneous disease with miscellaneous clinical performance, demanding a wide variety of treatment modalities. Making advances in cancer treatment has been linked to the detection of suitable targets for therapy. Receptor tyrosine kinase (RTKs) is one of the most attractive approaches for developing targeted therapy in cancer. These receptors are widely deregulated in cancer cells compared with normal cells and attributed to more aggressive tumor growth and worse prognosis. Human epidermal growth factor receptor (HER) family consists of four different high affinity tyrosine kinase receptors including EGFR (HER 1 or ErbB-1), HER2/c-neu (ErbB-2), Her 3 (ErbB-3), and Her 4 (ErbB-4) [1,2]. The HER2 over-expression, as a part of neoplastic progression, has been implicated in tumor proliferation, division, and angiogenesis [3].

In the absence of a known ligand, hetero-dimerization of this receptor with other family members, particularly with EGFR which results in effective activation of intracellular signaling pathways, promotes cell proliferation and survival [3-5]. Since these cell surface receptors (HER2 and EGFR) are amplified and over-expressed in a large number of tumor types, they are considered as ideal targets for selective molecular therapy of cancer [1-5]. Monoclonal antibodies' (mAbs) development against the extra-cellular domain of these receptors is an approach which is, currently, being taken to prevent their signaling. In recent years, various antibodies including humanized, chimeric, and more recently single-domain mAbs have been successfully developed against these receptors [1-5].

Systems biology (SB)-molecular modeling- helps prioritize the suitable targets to eradicate specific cancer cells, hence, ultimately, design effective biopharmaceutical drugs to treat cancer patients. In this regard, this study was designed to assess the biologic activity of an *in silico* tri-functional antibody (hIgG1-FC: mouse-antiHER2× human-B7.1). Tri-functional monoclonal antibodies (Triomab) belong to the first generation of bispecific antibodies (bsAbs) targeting two different epitopes [6,7], such as ertumaxomab which, in turn, targets tumoral HER2/Neu antigen (by antiHER2 domains), T-cell CD3 receptor epitopes (by anti-CD3 domains), and accessory cells such as natural killer cells (NK cells), contributing to destroy cells, (by antibody Fc) through antibody-dependent as well as complement-dependent induction of cytotoxicity (ADCC and CDCC, respectively) [8-10]. Completing the phase I and II of clinical trials related to ertumaxomab and catumaxomab (Triomabs) [11,12] and the effective low dose range (microgram dose range versus milligram dose, i.e., high dose administration) in cancer therapy [7,10,13,14] have triggered the incentive of designing *in silico* specific expression vectors for the production of this class of antibodies. As functional diversity for mAb and triomab gets equal to 4/6 (x_1/n_1) and 6/6 (x_2/n_2), respectively, p gets equal to 10/12 $(x_1+x_2)/(n_1+n_2)$ resulting z and $|z|$ scores to get equal to -1.55 and 1.55, respectively, which is less than z scores equal to 1.96 (α=0.05) and 2.576 (α=0.01). Hence, the null hypothesis regarding its equivalence to total functional potential mAb and triomab, is not rejected. Therefore, the substitution of two VH-VL domains by two human B7.1 IgV-like domains doesn't reduce antibody functional

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potential. As triomabs are, physicochemically, enable to bind three different cell types compared with mAbs' ability to bind two different cell types, it can be concluded that, the anti-cancer efficacy of triomab is more than mAb. Theoretically, there is a very low synergism between triomab and mAb - when χ^2 and r are equal to 0.1 and 0.067, respectively. There is a weak, linear, direct, and partial correlation between mAb and triomab, which demonstrates that the domain substitutions in triomab neither reduce the total Ab functional potential nor require the addition of accessory mAb (Table 1).

Tri-functional monoclonal antibodies (TMAs) are produced by Quadroma. These cells are formed by the fusion of two hybridomasomatic cells; each parental hybridoma produces a monoclonal antibody specific for one of the two antigens. Trioma cells are formed by the fusion of a hybridoma and a lymphocyte; each produces antibodies against one of the two antigens. The light and heavy chains of both parental types will be synthesized in Quadroma and Trioma cells [15].

The antibody physicochemical potential and its apoptotic induction are notable criteria in antibody based cancer therapy. In this study, two effective constructs were designed to promote the triomab production. Two triomab expression vectors were designed in order to express equimolar concentrations of secretory Ab chains and promote producing process. After the transfection of CHO cell with these *in silico* expression vector synthetic forms, a trifunctional antibody was expressed that can recognize HER2, and CD28 receptors, and utilize T cells and accessory cells.

Material and Methods

1. Sequence Data and BLAST: The data of human IgG1 scaffold(Accession: 1HZH-HandK), cha21-scFV (anti-HER2, Accession:3H3B), human B7.1 (CD80, Accession: 1I8L-B), Newcastle disease virus (NDV) furin-cleavage (Accession: PIZA05N277), Foot-Mouth disease virus (FMDV) 2A peptide(Accession: P03305), EF1-HTLV promoter (Accession: KC176267), CMV-HTLV promoter (Accession: KC176268), EM2KC promoter(Accession: AB902850), SV40-β globin poly A(Accession: JO2400), Keima-red fluorescent protein (KRFP, Accession: 2WHU-D), green-fluorescent protein (GFP, Accession: P42212), Zeocin resistance gene (Accession: EDN63766), Blasticidin-S resistance gene(Accession: P0C2PO) and colE1 (from pBR322, Accession: JO1566) and pUC57 replication origin(Accession: Y14837) sequences were obtained from NCBI-GenBank database. The above sequences were analyzed by BLAST.

2. Antibody VH-VL Analysis Using IMGT and Paratome Softwares: The framework regions (FRs) and complementarity determining regions (CDRs) of antiHER2 antibody VH-VL were determined using IMGT software. Also, antigen binding residues (ABRs) were discriminated using paratome software.

3. PDB Database, pyMol: The above-mentioned sequence data were received from PDB database. The images of 3D protein structures were made or produced. They were selected using pyMol, then the model structures were constructed for triomab.

4. Snapgene Software: The *in silico* expression vectors of tri-functional antibody (hIgG1-FC: mouse-antiHER2×human-B7.1) were designed and depicted by Snapgen software.

	Involved Domains	Binding Targets	SUM
Triomab	6 (5.45)	6 (6.54)	12
mAb	4 (4.54)	6 (5.45)	10
SUM	10	12	22

Table 1: Correlation between Triomab and mAb based on Domains and Functional sites of Antibodies. There are the relevant explanations at the above context (χ^2 & r are equal to 0.1 and 0.067, respectively).

Results

Sequence alignment

IMGT analysis (ImMunoGeneTics)

To obtain the CDRs and FR regions according to IMGT, the researchers applied the IMGT-gap tool (Retrieved from http://www.imgt.org/3Dstructure-B/cgi/DomainGapAlign.cgi, last accessed on 23 May, 2012). Cha21 antibody nucleotide sequence was entered in the IMGT-QUEST online tool. The system analyzed the sequence and displayed the CDR and FR regions. The results confirmed the presence of mouse antibody variable domain framework (Table 2).

Paratome analysis

ABRs of anti-Her2 antibody were identified according to its amino acid sequence. This prediction was achieved based on a set of consensus regions obtained from a structural alignment of a non-redundant set of the anti-Her2 antibody-antigen complexes. Given a query sequence, the server identified the regions in the query antibody that correspond to the consensus ABRs. The results confirmed that the antibody contained ABRs and was able to recognize antigen epitopes (Tables 3 and 4).

In silico model for the tri-functional antibody

As combining structural biology and bioinformatics helps to surely design an expected multifunctional construct, analyzing the experimental 3D models, the researchers selected the necessary functional domains and utilized them for modeling the chimeric tri-functional antibody. Homology modeling was performed $(PS)^2$ Server, then stereochemical quality of the relevant structures was assessed by using PROCHECK program (Tables 5 and 6). The modeling 3D structures of antigen (HER2)-antibody (antiHER2) interaction, anti-HIV human IgG1 and human B7.1 were obtained from PDB database and trimmed by pyMol software (Figures 1-4). The analysis showed that there was a significant interaction between anti-HER2 antibody and HER2 antigen. Also, the interaction of B7.1 with CTLA-4 confirmed the interaction of B7.1 with CD28 receptor.

In silico two gene-cassettes and expression vectors' design

Two triomab gene cassettes were designed. The first cassette included theEF1-HTLV promoter, insulin ribosome binding site (IRBS), IL-2 signal sequence (I2SS), cha21-VH, hIgG1-CHS, NDV furin-cleavage sequence (FCS), FMDV-2A sequence (2AS), I2SS, cha21-VL, hIgG1- kappa-CL, SV40/β-globin poly A (Sβ-PA), and TGA stop codon. The gene cassette was merged into an expression vector-A. This vector contained the bacterial replication origin (ColE1), the resistance gene for zeocin and the GFP reporter gene.

The second cassette included the EF1-HTLV promoter, IRBS, I2ss, human B7.1 IgV-like sequence (hB7VS), hIgG1-Kappa-CL, SV-PA, and TGA stop codon. This cassette was merged into an expression vector-B. This vector contained the resistance gene for blasticidin-S and the Keima red fluorescence protein (KRFP) reporter gene.

Through co-transfection of these vectors into CHO cells, secretory hIgG1-FC: antiHER2×B7.1 triomab will be co-transcribed and co-expressed.

Max Score	Total Score	Query cover (%)	E-Value	Identity (%)	Accession	Entry	Unitropt KB	PDB ID
946	946	100	0.0	100	1 HZH-H	IgG1	Po1857	1 HZH-H
946	946	100	0.0	100	1 HZH-K	IgG1	Po1834	1 HZH-K
522	522	100	0.0	100	3H3B-D	Cha21	-	3H3B
539	539	100	0.0	100	2GJJ	Sca21	-	2GJJ
602	602	100	0.0	100	1I8L-B	B7.1	P33681	1I8L-B
4875	4875	100	0.0	100	P03305	FMDV-2Apeptide	P03305	-
11049	11049	100	0.0	100	Kc176267	EF1-HTLV Promoter	-	-
12022	12022	100	0.0	100	Kc176268	CMV-HTLV Promoter	-	-
13900	14032	100	0.0	100	AB902850	EM2KC	-	-
9683	9951	100	0.0	100	Jo2400	SV40-Bglobin polyA	-	-
503	503	100	8e-179	100	2WHU-D	KRFP	Q1JV70	2WHU-D
496	496	100	4e-176	100	P42212	GFP	P42212	1BFP
212	212	100	2e-68	100	EDN63766	Zeocin Resistance Gene	A6ZNA1	-
264	264	100	3e-88	100	P0C2PO	Blasticidin-S Resistance Gene deaminase	POC2Po	1NN57
12273	12273	100	0.0	100	JO1566	CoLE1	-	-
5005	5005	100	0.0	100	Y14837	PUC57 Replication Origin	-	-
691	691	100	0.0	100	PIZA05N277	NDV	-	-

Table 2: The obtained sequences were searched using BLAST. This analysis confirmed the relevant sequence alignment (Below).

Label	Location/Qualifiers
V-J-REGION	IVLTQTPSSLPVSVGEKVTMTCKSSQTLLYSNNQKNYLAWYQQKPGQSPKLLISWAFTRK SGVPDRFTGSGSGTDFTLTIGSVKAEDLAVYYCQQYSNYPWTFGGGTRLEIK
V-REGION	allele="Musmus IGKV8-30*01 F", gene="Musmus IGKV8-30" IVLTQTPSSLPVSVGEKVTMTCKSSQTLLYSNNQKNYLAWYQQKPGQSPKLLISWAFTRK SGVPDRFTGSGSGTDFTLTIGSVKAEDLAVYYCQQYSN
FR1-IMGT	AA_IMGT:IVLTQTPSSLPVSVGEKVTMTCKSS
CDR1-IMGT	AA_IMGT:QTLLYSNNQKNY
FR2-IMGT	AA_IMGT:LAWYQQKPGQSPKLLIS
CDR2-IMGT	AA_IMGT:WAF
FR3-IMGT	AA_IMGT:TRKSGVPDRFTGSGSGTDFTLTIGSVKAEDLAVYYC
CDR3-IMGT	AA_IMGT:QQYSNYPWT
FR4-IMGT	AA_IMGT:FGGGTRLEIK

Table 3a: Annotation by IMGT/Automat: After submitting nucleotide sequences of anti-HER2 Ab VL-region using IMGT tool, the data were analyzed and the FRs and CDRs of the VL-region were identified.

Label	Location/Qualifier
V-D-J-REGION	QQSGPEVVKTGASVKISCKASGYSFTGYFINWVKKNSGKSPEWIGHISSSYATSTYNQKF KNKAAFTVDTSSSTAFMQLNSLTSEDSAVYYCVRSGNYEEYAMDYWGQGTSVTVS
V-REGION	allele="Musmus IGHV1-39*01 F", gene="Musmus IGHV1-39" QQSGPEVVKTGASVKISCKASGYSFTGYFINWVKKNSGKSPEWIGHISSSYATSTYNQKF KNKAAFTVDTSSSTAFMQLNSLTSEDSAVYY
FR1-IMGT	AA_IMGT:QQSGPEVVKTGASVKISCKAS
CDR1-IMGT	AA_IMGT:GYSFTGYF
FR2-IMGT	AA_IMGT:INWVKKNSGKSPEWIGH
CDR2-IMGT	AA_IMGT:ISSSYATS
FR3-IMGT	AA_IMGT:TYNQKFKNKAAFTVDTSSSTAFMQLNSLTSEDSAVYYC
CDR3-IMGT	AA_IMGT:VRSGNYEEYAMDY
FR4-IMGT	AA_IMGT:WGQGTSVTVS

Table 3b: Annotation by IMGT/Automat: After submitting nucleotide sequences of anti-HER2 Ab VH-region using IMGT tool, the data were analyzed and the FRs and CDRs of the VH-region were identified.

Discussion

Increasing the immunological effector functions of antibodies is a way to improve the efficacy of antibody-based cancer therapy. Bispecific antibodies (BsAbs), which act via antibody-dependent cell-mediated cytotoxicity (ADCC), have represented a significant advance in cancer immunotherapy. They are powerful tools for immunological treatment of malignant tumors because of their ability to bind two different antigens at the same time [11].

T-cell requires two signals from antigen presenting cells (APCs) for activation and proliferation. The first signal is provided by the recognition of MHC/peptide complexes on the APC by cell surface T-cell receptors (TCR). The second signal is induced by the interactions of co-stimulatory protein, especially B7 proteins (CD80 and CD86), on the APC with their complementarity receptors, CD28, on the surface of the T cells. The B7 family consists of some Ig-like molecules, including B7.1 and B7.2 [12].

Nowadays, many different types of BsAbs have been recognized

>paratome_1_Anti_HER2_VL_CL (light chain)
IVLTQTPSSLPVSVGEKVTMTCKSSQTLLYSNNQKNYLAWYQQKPGQSPKLLISWAFTRKSGVPDRFTGSGSGTDFTLTI
GSVKAEDLAVYYCQQYSNYPWTFGGGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

ABR1	**ABR2**	**ABR3**
QTLLYSNNQKNYLA	**LLISWAFTRKS**	**QQYSNYPW**

>paratome_2_Anti_HER2_VH_CHs (heavy chain)
QQSGPEVVKTGASVKISCKASGYSFTGYFINWVKKNSGKSPEWIGHISSSYATSTYNQKFKNKAAFTVDTSSSTAFMQLN
SLTSEDSAVYYCVRSGNYEEYAMDYWGQGTSVTVSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA
LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

ABR1	**ABR2**	**ABR3**
YSFTGYFIN	**WIGHISSSYATSTY**	**VRSGNYEEYAMDY**

Table 4: Paratome Analysis: the Ag binding sites (ABRs) of antiHER2 Ab VL & VH regions were identified using Paratome tool.

Target	Template	Length	Identity (%)	Score	E-value	Gaps	Method
Anti-HER2 antibody-IgG1 heavy chain	1hzhH	457	83	575	1e-165	1%	Composition-based stats
Anti-HER2 antibody-kappa light chain	1za6A	220	88	280	1e-76	-	Composition-based stats
B7.1-IgG1 heavy chain	1hzhH	457	77	384	1e-107	4%	Composition-based stats
B7.1-kappa light chain	1y0lA	216	60	175	5e-45	2%	Composition-based stats

Table 5: Protein 3D Structure Prediction Using $(PS)^2$ Server: Prediction of the tertiary protein structures was performed by using automated homology modeling $(PS)^2$ Server. The Server revealed the most conserved homologies.

PDB Code	Model	Length	Identity (%)	Aminoacid Overlap	z-Score	Protein Name
1hzhH	X-ray 2.70A°	457	85.8	452	1919.2	Crystal structure of the intact human IgG b12 with broad and activity against primary HIV=1 isolates
1za6(A)	X-ray 2.80A°	220	88.6	219	961.0	The structure of an antitumor cha21-domain- deleted huamanized antibody
1hzh(H)	X-ray 2.70A°	457	85.4	403	1706.0	Crystal structure of the intact human IgG b12 with broad and activity against primary HIV=1 isolates
4d9r(L)	X-ray 2.42A°	214	61.2	206	618.7	Inhibiting alternative pathway complement activation by targ exosite on factor d

Table 6: *PROCHECK Analyses:* Stereochemical quality assessment of the predicted protein structures was performed by using PROCHECK program. The analyses confirmed high quality of the protein structures in stereochemistry.

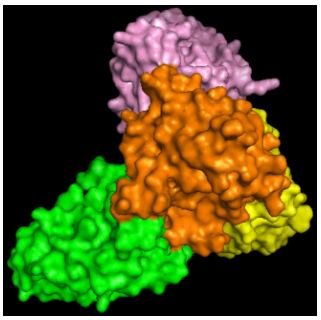

Figure 1: Anti-HER2 (cha 21, PDB code 3H3B) scFV and HER2 receptor Interaction. Chains A (yellow) and B (orange) are HER2 receptor residues 1-192. Chains C (green) and D (pink) are anti-HER2 Variable domains.

which bind to tumor cells and T-cells, and act via T-cell-mediated lysis. The BsAbs drugs, Catumaxumab and Ertumaxumab, have been approved by Food and Drug Administration (FDA) [11,12]. This study presented the researchers' effort in designing the *in silico* expression model of BsAbs which recognizes both HER2 and CD28, and utilizes NK cells. The researchers, therefore, intended to conduct and activate T-cells against tumor cells via an Ab- fusion protein with the specificity of a HER2 receptor. The localized B7.1 costimulatory molecule within the Ab could assist the activation of a systemic antitumor immune response. The autocleavage expressed polypeptide chains from these trifunctional-antibody gene cassettes are composed of the fusion-protein furin-cleavage site of Newcastle Disease (NDV)-velogenic strains [14,15] and Foot-mouth-disease virus (FMDV) 2A peptide [16]. Therefore, with this strategy, the researchers were able to attain equimolar concentrations of antibody chains instead of distributing Ab-chain sequences among several vectors or utilizing Quadroma and Trioma cells that express unequal molar concentrations of Ab chains [13]. On the other hand, the merged I2ss into the designed gene cassettes is led to secrete Ab chains in suitable conformation.

Quadroma and Trioma cells express more than 10 Ab variants. Theoretically, this design reduces Ab variants obtained from quadroma and trioma cells. Therefore, the trifunctional antibody expression and purification will be facilitated utilizing this method compared with quadroma and trioma technology. MAbs are typically generated in mice (murine Abs). When murine Abs were used by human, they could be identified as foreign elements by the immune system. They can initiate an allergic reaction, and can be, quickly, removed from the patient's circulation. These problems enabled the researchers to repeat the antibody administration for treatment purposes. Recent advances

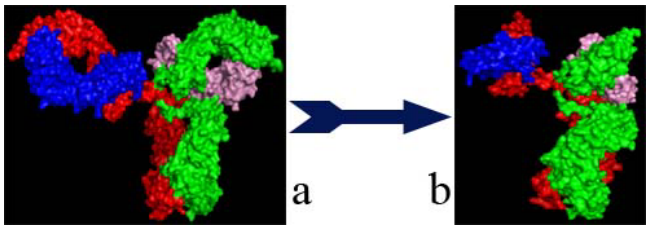

Figure 2: Human IgG1 against HIV-1 (PDB code 1HZH) and Trimming steps. a) the length of heavy (H & K, red and green) and light (L a M, blue and pink) chains are 457 & 215 aminoacid residue, respectively. b) 3D structure of hIgG1 constant domains by removing VH & VL domains (pyMol software).

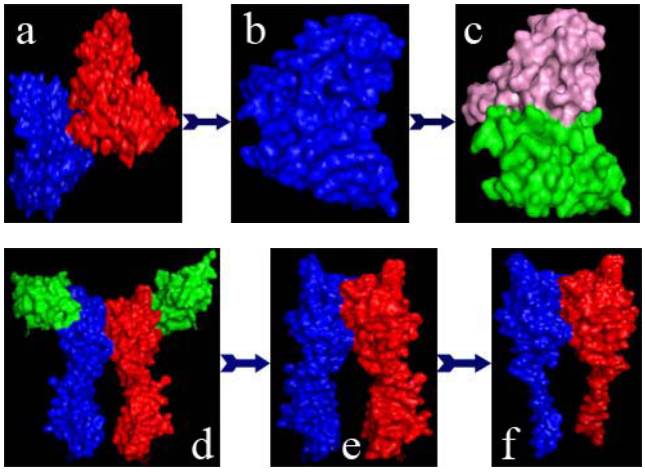

Figure 3: 3D structure of anti-HER2 engineered antibody (sca 21 or cha 21) and B7.1 (CD80), along with the trimming steps (pyMol software). a) sca 21 (PDB code 2GJJ) contains two chains. Chain-A (red) and Chain B (blue) contain variable domains. b) Chain A was removed and Chain B was retained. c) VL & VH domains were colored pink and green, respectively. Aminoacid residues 6-116 (pink) and 143-257 (green) are VL & VH domains, respectively. d) B7.1 & CTLA-4 (PDB code 1I8L) interaction. e) CTLA-4 domains (green, C & D chains) were removed. A (blue) and B (red) chains of B7.1 contain aminoacid residues 35-242. f) Aminoacid residues 136-242 were removed and aminoacid residues 35-135 (interacting with extracellular variable-like domain) were retained.

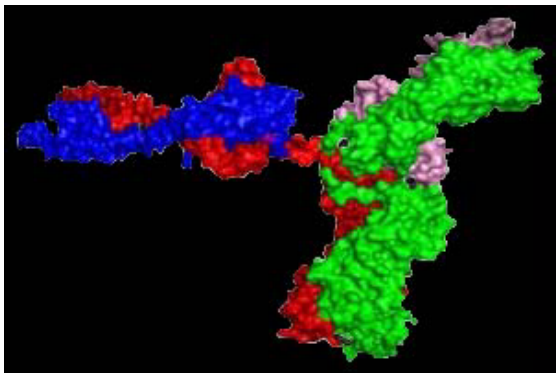

Figure 4: The Image of Tri-Functional Antibody. The model of tri-functional antibody containing B7.1 and IgG1 light chain constant domain (blue), B7.1 and IgG1 heavy chain constant domains (red), light chain variable domain of antiHER2 antibody, and IgG1 light chain constant domain (pink), and heavy chain variable domain of antiHER2 antibody and IgG1 heavy chain constant domains (green).

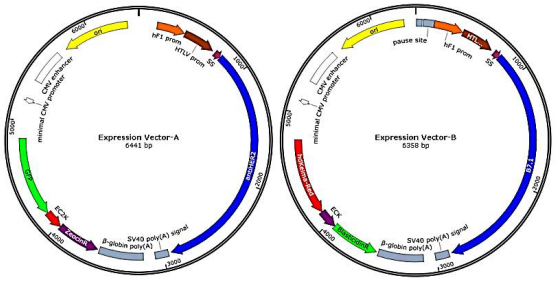

Figure 5: This figure illustrates the expression vectors A and B. Trifunctional antibody gene cassette including anti-HER2 and B7.1, and their expression vectors were designed using Snapgene software.

in genetic engineering techniques increase the hope for diminution of immunogenicity of the murine antibodies by generating the chimeric or humanized antibodies. Here, a novel biopharmaceutical-production process was developed, and the great part of its product (antibody) was humanized (~83.3%). On the other hand, the developed product helps to minimize possible human immune response against foreign molecules. In this work, the B7.1-IgV-like domain was used instead of anti-CD3 Ab due to its advantageous. It can direct CD28 cytotoxic T cells to kill tumor cells compared with the accessory cell-mediated killers such as macrophages, natural killer cells (NKC), and dendritic cells (DC) [5-8].

The Fc domains of antibody which have been utilized in this design help to create cytotoxic T-cell synergism effect through accessory cell activation.

B7.1 can promote T-cell activation pathway through secondary signal induction. Activated T-cells have both killing and cytotoxic effects on cancer cells, and inducing effect on the expression of CTLA-4, to inactivate the cell; therefore, undesirable systemic inflammatory reactions do not occur. Using this design, the researchers promoted the production process, increased antibody efficacy, reduced produced antibody variant diversity, and increased antibody half-life. 3D model analysis of the interaction of B7.1 and CTLA-4 indicated that the B7.1 can interact with CD28 receptor. Therefore, IgV-like nucleotide sequence of B7.1 was utilized in expression vector-B design.

The GFP-Zeocin and Keima-Red-Blasticidin reporter -genes have been contrived within the expression vectors to indicate the presence of each vector within either a CHO cell or similar cells. The *in silico* drug 3D structure modeling that has been performed by pyMol software can help to depict the final product from.

As the major destruction of tumor cells overexpressing HER2/Neu antigen (Ag), such as breast, pancreatic, bladder, ovarian, choriocarcinoma and cholangiocarcinoma cancer cells, is performed by targeting them through HER2 Ag [17-19], the nucleotide sequences of VH-VL-domains of cha21 antiHER2 scFV were selected, then analyzed by IMGT and Paratome tools. IMGT confirmed the presence of mouse antibody variable domain framework. Paratome confirmed that the antibody contained ABR pattern and enabled the recognition of antigen epitopes. Therefore, the nucleotide sequence has been utilized in trifunctional antibody expression vector-A design.

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