



For reprint orders, please contact: reprints@futuremedicine.com

Designing two-in-one antibodies

Evaluation of: Bostrom J, Shang-Fan Y, Kan D *et al.*: Variants of the antibody Herceptin® that interact with HER2 and VEGF at the antigen binding site. *Science* 323, 1610–1614 (2009). The longstanding held notion that one antibody equals one antigen and, hence, one function has been challenged in recent years. Improved technology in antibody production, especially the accumulation of sequence data of immunoglobulin genes and the advent of PCR have made it possible to clone antibody gene repertoires. The current paper provides further challenge to the notion of one antibody = one antigen by developing 'two-in-one' antibodies with an antigen-binding site that binds two distinct proteins with high affinity. A therapeutic variant antibody of Herceptin® (Genentech, CA, USA) was isolated that binds the human EGF receptor (HER)2 and also to VEGF. This development may represent a breakthrough discovery and may have significant implications in the therapy of malignant, infectious, allergic and autoimmune disorders.

KEYWORDS: autoimmunity ■ malignancy ■ monoclonal antibody

Ever since the discovery of antibodies in the 19th Century, extensive research has been undertaken to provide an explanation on how a relatively limited number of antibodies can protect against a vast and almost infinite number of invading antigens [1]. Proposed potential explanations include the notion that one antibody may be capable of binding more than one antigen through the selection of specific binding sites on pre-existing antibody conformations, and in this manner antibodies with multiple specificities can be achieved. In addition, it has been suggested that multispecificity may evolve and play a role in the efficient antibody repertoire for immune protection with one antibody performing more than one task [2].

Summary of methods and results

With this in mind, Bostrom *et al.* set out to explore whether dual specific antibodies can be derived from a monospecific antibody with the following approach: a repertoire of Herceptin® (Genentech, CA, USA) antibody variants with mutations in the light chain (LC) complementarity determining regions (CDRs) were generated, and Fabs that can bind a new protein antigen while maintaining human EGF receptor (HER)2 binding were identified [3]. This approach was based on the understanding that modifications of the LC sequence can modulate the binding specificity of antibodies.

The framework regions of Herceptin variable domains (V_H and V_L) belong to subtypes that are prevalent in the human antibody repertoire.

Thus, mutations within the antigen-binding site of Herceptin that confer a second specificity may indicate a potential for a dual specific antibody to evolve from the natural repertoire. In addition, since Herceptin is a validated therapeutic for breast cancers that overexpress HER2, recruitment of a second binding specificity to Herceptin may add to it a distinct pharmacological activity.

The investigators subsequently constructed the repertoire of Herceptin LC variants by randomizing a subset of solvent-exposed LC CDR positions to mimic the natural diversity in amino acid composition and length. Using this approach, more than 100 specific clones containing three to 17 amino acid substitutions and/or insertions compared with Herceptin were generated against VEGF, death receptor (DR)5, and the complement-binding fragment of IgG (Fc). Some clones lost binding affinity for HER2 and switched binding to the new antigen, whereas others maintained the ability to bind HER2 and thus were dual specific.

Representative VEGF- and DR5-binding clones were expressed as Fab and IgG proteins to assess their specificity and affinity. VEGF and DR5 are therapeutic targets of known structure. The dual specific antibodies bound the new antigens (i.e., VEGF or DR5) with high affinity while preserving HER2 binding in the low nanomolar affinity range. One antibody, bH1, exhibited the highest dual affinity for the two completely different protein antigens VEGF ($K_d = 300$ nM) and HER2 ($K_d = 26$ nM).

**Ignacio Garcia Valladares
& Luis R Espinoza[†]**

[†]Author for correspondence:
LSU Health Sciences Center at
New Orleans, 1542 Tulane
Avenue, New Orleans, LA,
70112-2822, USA
Tel.: +1 504 896 1440
Fax: +1 504 421 1293
luisrolan@msn.com

A series of experiments was then conducted to ascertain the molecular basis of the dual specificity. First of all, the crystal structure of the bH1 Fab bound to the extracellular domain of HER2 and to the VEGF receptor-binding domain was determined. The structure of the bH1/HER2 binding domains (bH1 Fv and domain IV of HER2) superimposes onto the Herceptin/HER2 complex, showing that the Herceptin-binding epitope on HER2 is retained. In the structure of the bH1/VEGF complex, the Fab recognizes an epitope that overlaps with the binding sites of the VEGF receptors and other VEGF-binding antibodies. Furthermore, bH1 blocks the interaction between VEGF and its receptors.

Comparison of the bH1/VEGF and the bH1/HER2 complexes shows an extensive overlap of the VEGF- and HER2-binding sites on bH1. In the HER2 complex, the LC and HC CDRs contribute approximately equal antigen contact area, whereas in the VEGF complex, the LC CDRs constitute nearly 70% of the buried surface. The capability of CDR-L1 to rearrange appears to be necessary for the dual specificity of bH1. Similar conformational flexibility of CDR-L1 has been reported to play a role in antigen recognition of natural antibodies.

Shotgun scanning mutagenesis studies performed to assess the energetic contributions of individual CDR residues to VEGF and HER2 binding revealed that energetically the VEGF-binding interaction is mediated primarily by the LC CDRs, whereas HER2 binding is dominated by HC CDRs.

Discussion & significance

Bostrom and colleagues also examined whether the dual binding specificity can translate into dual activity *in vivo* [3]. In order to facilitate the study of the *in vivo* activity of bH1, they generated versions of bH1 with high affinity and specificity for HER2 and VEGF. The potencies of the generated bH1 variants correlated with their relative affinities. The bH1-44 variant inhibited the growth of HUVEC and BT474 cells with potencies similar to bevacizumab or Herceptin. Next, they examined the two unique activities of bH1-44 *in vivo* using a mouse xenograft tumor model known to be responsive to treatment by anti-VEGF antibodies or Herceptin. Results demonstrated that the VEGF- and/or HER2-binding component of bH1-44 inhibited tumor growth.

Therefore, bH1-44 appears to have the pharmacological activity of a VEGF blocking antibody and a Herceptin-like antibody, although it

is not yet known whether bH1-44 is as effective as Herceptin or anti-VEGF when administered separately or in combination.

Future perspective

The notion of antibody conformational diversity is not a new concept, and as mentioned earlier this was initially proposed by Pauling in the 1940s as the mechanism underlying the generation of antibody diversity [4]. In addition, there have been multiple attempts in the past to generate antibody molecules with multiple binding moieties. Foote and Milstein have shown that antibody conformational isomerism may not be that rare, and that its frequency may be as high as 20% of antibodies raised to some haptens [5]. It was the work of James *et al.* and others, however, that conclusively demonstrated the presence of cross-reactivity of antibodies, and thus confirmed the notion that antibodies can use conformational isomerism to recognize two quite different antigens [6,7].

Bostrom *et al.* generated the two-in-one antibody by developing an antibody with a binding site that can interact with HER2 and VEGF through unique elements. When affinity-matured, the antibody inhibits both HER2 and VEGF activity *in vitro* and *in vivo*. The production of a vast array of therapeutic antibodies has been facilitated in the past several years with the establishment of antibody libraries as a result of the accumulation of sequence data of immunoglobulin genes and the advent of PCR technology, which have made it possible to clone antibody gene repertoires [8,9].

The potential, both as a research tool and as a therapeutic modality, is enormous and open to discussion [10]. A variety of chronic disorders, including malignant, infectious and autoimmune conditions, are difficult to treat and often require the use of multiple agents, alone or in combination. The latter approach is already being attempted in some cancer patients. Herceptin, which targets cell proliferation mediated by HER2, and bevacizumab, which targets tumor angiogenesis mediated by VEGF, are being used in combination in breast cancer patients [11].

Autoimmune disorders characterized by complex pathophysiology and clinical manifestations are another group of clinical conditions that may benefit from the use of two-in-one antibodies. At present, autoimmune disorders, particularly rheumatoid arthritis and systemic lupus erythematosus, are being treated with single biologic agents directed at specific pathogenic pathways, but one may envision the use of two-in-one antibodies directed at multiple targets in the not too distant future.

Financial & competing interests disclosure

This work was supported by a grant from the Louisiana Board of Regents, LO, USA. Ignacio Garcia Valladares was supported by a fellowship from the University of Guadalajara, Jalisco, Mexico. The authors have no other relevant

affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

Executive summary

- Evidence is presented describing the development of an antibody with an antigen-binding site that recognizes two distinct proteins with high affinity.
- Isolation of a variant of Herceptin®, a therapeutic monoclonal antibody that binds the human EGF receptor (HER)2 and was able to interact with VEGF.
- This observation has great therapeutic potential for a variety of malignant, infectious, allergic and autoimmune disorders.

Bibliography

- 1 Foote J: Isomeric antibodies. *Science* 299, 1327–1328 (2003).
- 2 Mariuzza RA: Multiple paths to multispecificity. *Immunity* 24, 429–438 (2006).
- 3 Bostrom J, Shang-Fan Y, Kan D *et al.*: Variants of the antibody Herceptin® that interact with HER2 and VEGF at the antigen binding site. *Science* 323, 1610–1614 (2009).
- 4 Pauling L, Campbell DH: The production of antibodies *in vitro*. *Science* 95, 440–441 (1942).
- 5 Foote J, Milstein C: Conformational isomerism and the diversity of antibodies. *Proc. Natl Acad. Sci. USA* 91, 10370–10374 (1994).
- 6 James LC, Roversi P, Tawfik DS: Antibody multispecificity mediated by conformational diversity. *Science* 299, 1362–1367 (2003).
- 7 Sethi DK, Agarwal A, Manivel V *et al.*: Differential epitope positioning within the germline antibody paratope enhances promiscuity in the primary antibody response. *Immunity* 24, 429–438 (2006).
- 8 Chen W, Zhu Z, Feng Y *et al.*: Construction of a large phage-displayed human antibody domain library with a scaffold based on a newly identified highly soluble, stable heavy chain variable domain. *J. Mol. Biol.* 382, 779–789 (2008).
- 9 Nahary L, Benhar I: Design of a human synthetic combinatorial library of single-chain antibodies. *Methods Mol. Biol.* 525, 61–80 (2009).
- 10 Parren PWHI, Burton DR: Two-in-one designer antibodies. *Science* 323, 1567–1568 (2009).
- 11 Bernard-Marty C, Lebrum F, Awada A, Piccart MJ: Monoclonal antibody-based targeted therapy in breast cancer status and future directions. *Drugs* 66, 1577–1591 (2006).