IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF DELAWARE

ZYMOGENETICS, INC., a Washington Corporation, Plaintiff, v. BRISTOL-MYERS SQUIBB CO., a Delaware Corporation, and DOES 1 through 100, Defendants.	)	C.A. No. 06-500-SLR **PUBLIC VERSION**

DECLARATION OF NICHOLAS R. J. GASCOIGNE, PH.D. IN SUPPORT OF ZYMOGENETICS, INC.'S OPPOSITION TO BRISTOL-MYERS SQUIBB CO.'S MOTION FOR SUMMARY JUDGMENT OF NONINFRINGEMENT

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I, Nicholas Gascoigne, declare:

1. I am a Professor in the Department of Immunology and Microbial Science at The Scripps Research Institute.

2. In 1983, I received a Ph.D. in Immunology from the University of London.

3. Bristol-Myers Squibb's ("Bristol") Orencia product is comprised of a soluble fusion protein that consists of the extracellular domain of human CTLA-4 joined to the modified Fc (hinge, C_H2 and C_H3 domains) portion of human IgG1. The fusion protein is produced by recombinant DNA technology in a mammalian cell expression system.

4. The technology involved in the production of the soluble fusion protein found in Orencia includes expression of a soluble non-immunoglobulin protein fused to part of the constant region of the immunoglobulin heavy chain. The Orencia protein (CTLA-4Ig) is produced from a DNA construct containing a transcriptional promoter joined to a secretory signal sequence from Oncostatin M, joined in-frame to the extracellular ligand-binding domain of CTLA-4.

NONIMMUNOGLOBULIN

5. CTLA-4 is not an immunoglobulin. CTLA-4 is not an antibody. One skilled in the art understands immunoglobulin to mean antibody.

6. It is my understanding that Bristol has taken a position that CTLA-4 is an immunoglobulin based on its definition that an immunoglobulin would include proteins which contain domains that are immunoglobulin-like, and that have "significant" sequence homology to immunoglobulin. However, this definition confuses two separate concepts. The term Immunoglobulin (Ig) specifically refers to an antibody. Thus if CTLA-4 were an Ig, then there would be no need to call the Orencia fusion protein "CTLA-4Ig" -- "Ig-Ig" would describe it.

7. CTLA-4 and many other proteins are members of the immunoglobulin superfamily (IgSF). Other examples of IgSF proteins include CD4, CD8, PDGF-receptor, and many more. A quote from a paper by Linsley (Linsley, P.S. et al., J. Biol. Chem.

270:15417-15424 (1995)) will serve to address that this distinction between immunoglobulin proper and the IgSF was well understood by Bristol scientists during development of CTLA4Ig/Orencia: "The Ig domains of CD28 and CTLA-4 display little sequence similarity with other members of the IgSF, limited to a few conserved residues characteristic of the Ig fold……." (from p15423). Thus, members of this superfamily share sequence homology to the Ig fold, but not to the immunoglobulins themselves. That is to say, members of the immunoglobulin superfamily *are not* themselves immunoglobulins – only members of the antibody family are immunoglobulins. Even under Bristol's definition, CTLA4 does not share significant sequence homology to immunoglobulin.

8. Members of the IgSF are defined by containing one or more "domains" with a protein structure related to an Ig variable (V) region or constant (C) region. Such domains have a protein folding pattern called a "β-barrel" structure, where two "β-pleated sheets" are held together by a highly conserved disulfide bond.

9. CTLA-4 has a single V-like domain and is included in the IgSF (Brunet, J-F, et al., Nature, 328:267-270 (1987); Williams, A.F. and Barclay, A.N., Annu. Rev. Immunol., 6:381-405 (1988)).

10. The IgSF "includes such functionally important members as the immunoglobulins themselves," as well as numerous other proteins (Brunet, J-F, et al., Nature, 328:267-270 (1987)). A published definition of IgSF is as follows: "Share a globular structure called an immunoglobulin (Ig) domain, which was originally described in antibodies. This domain is ~110 amino acids long with an internal disulphide bond and two layers of β-pleated sheet. Ig domains are called V-like or C-like, with IgV domains having two extra β-strands and a characteristic DxYxGxC motif at the second Ig cysteine. B7 and CD28 are subsets of the Ig superfamily." (from glossary of Sharpe, A.H. and Freeman, G.J., The B7-CD28 superfamily. Nature Rev. Immun., 2:116-126). Bristol is defining an immunoglobulin as a protein that includes domains derived from immunoglobulin-like genes, and that these proteins have significant sequence homology to immunoglobulin. Such a

definition is not scientifically sound. CTLA-4 and CD28 for example, two proteins considered to be within the same subfamily of the superfamily, share only around 30% protein sequence homology. CTLA-4 would share much less homology to the immunoglobulins, and could not be said to be "significantly" immunoglobulin-like even under Bristol's definition.

11. There is some homology however *in those conserved V-like domains* of CTLA-4 with the immunoglobulin V-like domain. But overall protein homology is extremely minimal. Thus it is incorrect to state that CTLA-4 shares a high or substantial degree of homology to the immunoglobulins – it does not. CTLA-4 is a nonimmunoglobulin.

12. Even if one were to accept Bristol's definition of immunoglobulin, CLTA-4 would still be considered a nonimmunoglobulin. There are numerous members of the Ig superfamily, and one member can exhibit quite different structural and functional properties from another member. For example, CTLA-4 and TCR are both members of the IgSF, but are structurally and functionally different. Not every member of the Ig superfamily would qualify as an immunoglobulin. Molecules in the Ig superfamily share a certain degree of homology to immunoglobulins. The T cell receptor does share a higher degree of homology to immunoglobulin than does CTLA-4, for example. Thus one could argue that TCR is more "immunoglobulin-like" than other members of the superfamily. But such a minimally shared homology to a short protein segment or "domain" with immunoglobulin does not equate to such proteins being classified as immunoglobulin. Indeed, CTLA-4 shares little homology with the immunoglobulins (or other members of the superfamily for that matter) outside of the Ig-fold domain, and is certainly not "significantly" homologous to immunoglobulin.

DIMERIZING PROTEIN

13. The patents in this case define dimerizing protein as "A polypeptide chain having affinity for, or binding covalently or non-covalently, to a second polypeptide chain, such that the two chains associate under physiological conditions to form a dimer. The

second polypeptide chain may be the same (forming a homodimer) or a different chain (forming a heterodimer)." I believe this is a scientifically sound definition.

14. The patent claims further define that the dimerizing protein to be made from a portion of the heavy chain of immunoglobulin G (IgG.) Bristol's Orencia is a fusion protein that is made from a portion of the IgG. See Product Insert for Orencia.

15. Bristol's definition of dimerizing protein adopts the above definition, but adds that the dimerizing protein must "drive" the dimerization of the extracellular domain of CTLA-4 in this case, and that the CTLA-4 portion would not be able to form a stable dimer. Even under Bristol's definition, the extracellular domain of CTLA-4 used in Orencia does require the IgG portion to ensure or "drive" dimerization. Bristol acknowledged that the extracellular domain of CTLA-4 could not dimerize on its own ("[t]hus the presence of C120 does not ensure dimerization [of the extracellular domain]."). Linsley, P.S. et al., J. Biol. Chem. 270 at 15423.

16. The part of IgG1 (hinge, C_H2, C_H3) present in the Orencia protein differs from natural IgG1 in that 3 cysteine residues in the hinge region have been mutated to serine residues. There is a fourth mutation in a proline residue in the C_H2 domain. Thus the IgG part of Orencia is not a disulfide-bonded dimer (Orencia is a disulfide-bonded dimer through a linkage between the CTLA-4 domains).

17. However, a dimerizing protein does NOT require covalent linkage to form a dimer. For example, invertase, a dimerizing protein listed in the claims, is not a disulfide-bonded dimer. The IgG1 region of Orencia is indeed a dimer. Bristol explicitly recognizes this, for example in the structural model shown the Biologics License Application (3.2.S.1.2.F01) (BMS001290163). Thus, the first portion of Bristol's definition (that the dimerizing protein associate) is met: the immunoglobulin portion of Orencia is forming a dimer.

18. [redacted]

[redacted]

19. Independent evidence that the C_H2 and C_H3 regions are sufficient to form non-covalent dimers is provided by some earlier data. Prolonged proteolytic degradation of rat IgG2a with papain released two Fc fragments. These were found to be cut carboxy-terminal to the hinge region, i.e. downstream of the disulfide bonds of the hinge region. They were found to be associated as dimers purely through electrostatic interactions (Arend, W.P. et al., J. Immunol. 118:388-394 (1977)). Thus, non-covalent interactions are sufficient to hold the Fc region together as a dimer. Even more extreme, the C_H3 domain of human IgG1 isolated by limited proteolysis remains as a homodimer (Isenman, Biochem. 18:3327 (1979)). Recombinantly produced C_H3 alone is sufficient to form a non-covalently bound homodimer, and the dimerization is of remarkably high affinity. Dimers are present even at 10^{-10} M (Thies, M.J.W., et al., J. Mol. Biol. 293:67-79). All of this information is independent evidence that the part of the immunoglobulin C-region used in Orencia is indeed a dimerizing protein, held together by non-covalent interactions.

20. Orencia is a homodimer. This was shown originally in the first paper describing CTLA4-Ig (Linsley, P.S. et al., J. Exp. Med. 174:561 (1991)). It is expressly stated in the BLA (3.2.S.1.2 Structure) that "it exists as a covalent homodimer…", and is shown as such in (3.2.S.1.2.F01) (BMS001290163).

21. Bristol's definition that the dimerizing protein "drive" the dimerization is also met. Studies by Dr. Linsely and his group (Linsley, P.S. et al., J. Biol. Chem. 270:15417-15424 (1995)) clearly demonstrated that when the immunoglobulin portion of CTLA-4Ig was removed, very little dimerization occurred of the extracellular domain of CTLA-4. When the immunoglobulin is present, 100% of the fusion protein is dimerized. See BLA (BMS001290163). The product insert describing Orencia also states that it is a dimer. Thus, the immunoglobulin portion is a dimerizing protein because it forms a dimer, and it "drives" the formation of the stable CTLA-4Ig dimer.

REQUIRE DIMERIZATION FOR BIOLOGICAL ACTIVITY

22. The patents define biological activity as:

> A function or set of activities performed by a molecule in a biological context (i.e., in an organism or an in vitro facsimile thereof). Biological activities may include the induction or inhibition of extracellular matrix secretion from responsive cell lines, the induction or inhibition of hormone or cytokine secretion, the induction or inhibition of chemotaxis, the induction or inhibition of mitogenesis, the induction or inhibition of differentiation, or the inhibition of cell division of responsive cells. A recombinant protein or peptide is considered to be biologically active if it exhibits one or more biological activities of its native counterpart.

I agree that this definition is scientifically sound.

23. Bristol's definition states that the nonimmunoglobulin (CTLA-4 in this case) need be dimerized to have biological activity, and that the CTLA-4 be unable to dimerize on its own. The second part of this definition is not a tenable position. A protein that must exist in a dimeric state in order to be biologically active must also, accordingly, be able to form a dimer. Bristol must be referring to the portion of CTLA-4 found in CTLA-4Ig in this instance, and not the native form of CTLA-4.

24. CTLA-4 is a receptor requiring dimerization for biological activity. CTLA-4 is a receptor for the proteins B7.1 (CD80) and B7.2 (CD86). Orencia is made from a portion (the extracellular domain of native CTLA-4) and is called CTLA-4Ig. As mentioned, CTLA-4Ig is also a dimer, as shown in the BLA submitted to the FDA (BMS001290163). This is also clear from data published by Bristol (Linsley, P.S., et al., J. Biol. Chem. 270: 15417-15424 (1995)). In this article, they show firstly that natural CTLA-4 is a covalently bound homodimer (Fig. 1). Comparison of the difference between monomer and dimer for their binding avidity, showed the dimer to bind stronger by a 30-90-fold margin (Fig. 5). "Monomeric CTLA-4 had reduced binding activity when compared with dimeric CTLA-4. $CTLA4X_{C120S}$ was 30-90-fold less active at competing for ^{125}I-CTLA4Ig than dimeric CTLA4t". In this same article, it was conclusively shown that the extracellular domain of CTLA-4 failed to dimerize fully on its own, and that the C120 was not sufficient to ensure

dimerization. Thus, even under Bristol's definition, the extracellular domain of CTLA-4 requires assistance to dimerize.

25. In another Bristol article, it was found that the dimer bound faster, and dissociated slower than the monomer (Greene, J.L. et al., J. Biol. Chem. 271:26762-26771). The abstract of this paper states: "Therefore, covalent dimerization of CTLA-4 is required for its high avidity binding".

26. The X-ray crystallographic structures of human extracellular domain CTLA-4 (Schwartz, J.-C. D. et al., Nature 410:604-608 and Stamper, C.C., et al., Nature 410:608-611) demonstrate that the protein is a dimer, and indicate a mechanism for why this is important for biological activity.

27. It is important to note that the portion of CTLA4 that is used in Orencia (the extracellular domain) is derived from the native CTLA-4 found in humans. That is, native CTLA-4 is the source material. It is this native, source material that requires dimerization for biological activity.

28. Even if a native monomeric form were able to bind B7.1 or B7.2, such binding would be insufficient to be considered biological activity. In the biological context, full biological activity would include binding of ligand, and transmission of that signal within the cell. That is how CTLA-4 functions in the human body. Binding of ligand to a monomeric form would not trigger such a signaling process, as it is only the dimeric form of native CLTA-4 that is able to transmit signal. Thus, the studies on monomeric forms of extracellular domains of CTLA-4 do not address the ultimate question of whether or not binding of ligand to a monomer of the native form of CTLA-4 would constitute biological activity.

I declare under penalty of perjury under the laws of the United States that all statements made herein of my own knowledge are true and correct, and that all statements made on information and belief are believed to be true.

Dated: August 17, 2008

Nicholas R. J. Gascoigne, Ph.D.

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CERTIFICATE OF SERVICE

I, Philip A. Rovner, hereby certify that on August 25, 2008, the within document was filed with the Clerk of the Court using CM/ECF which will send notification of such filing(s) to the following; that the document was served on the following counsel as indicated; and that the document is available for viewing and downloading from CM/ECF.

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