WO 01100244 PCT/USOO/I7229

Osol, A. Ed. (1980)), in the farm of lyophilized formulations or aqueous solutions. Acceptable carriers, axcipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and includebtiffa such as phosphate, citrate, and other organic acids;antioxidants including ascorbic acid and methionine; preservatives (such
as octadacyldimathylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzathonium
5 chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechot; resorcinol;
cyclohexanol; 3-pentanal; and m.cresol); low molecular weight (loss than about 10 residues) polypaptidas; proteins,
such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrroffdone; amino acids
such as glycina, glutamine, asparagine, histidina, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or daxtrins; chelating agents such as EDIA; sugars such as sucrose,
inannitol, trehalose or sorbital; salt-forming counter-ions such as sodium; metal complexes (e.g Zn-protein complexes); and/or non-ionic surfactants such as TWEEN, PUJRDNICS or polyethylene glycol (PEG). Preferred lyophilized anti-
ErbB2 antibody formulations are described in WO 97104801, expressly incorporated herein by reference.
The formulation herein may also contain more than one active compound as necessary for the particular
indication being treated, preferably those with complementary activities that do not adversely affect each other. For
15 example, it may be desirable to further provide antibodies or antibody-maytansinoid conjugates which bind to EGFRS
ErbB2 (e.g. an antibody which binds a different epitope on ErbB2), ErbB3, Erb84, or vascular ondothelial factor (VEGF)
in the one formulation. Alternatively, or additionally, the composition may further comprise a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, and/or cardloprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.
20 The active ingredients may also be entrapped in microcapsules prepared, for example, by cuacervatian
techniques or by interfacial polymerization, for example, hydroxymethylcellufose or gelatin-microcapsules and poiy-(mithylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, Oposomes, albumin microsphoras, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed
in Remington’s Pharmaceutical Sciences 18th edition, Osol, A. Ed. (1980).
25 Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations
include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form
of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogets
(far example, poly(2-hydroxyethyl-methacrylate), or poty(vinylalcohotfl, polylactides (U.S. Pet. No. 3,773,919),
copolymers of L-glutamic acid and ethyi-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-
30 glycolic acid copolymers such as the LUPRON DEPOT (injectable microspheres composed of lactic acid-glycolic acid
copolymer and leuprolida acetate), and poly-D-1-).3hydroxybutyric acid.
The formulations to be used fork viva administration must be sterile. This is readily accomplished by
filtration through sterile filtration membranes.

35 E. Treatnijnt with theenti-ErbQ2 antiboiiimavtarisinoid conhieat
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WO 01/00244 PT/USO0/17229

The murine monoclonal antibody 405 was humanized, using a novel "gene conversion mutagenesis" strategy, as described in U.S. Patent No. 5,821.337, the entire disclosure of which is hereby expressly incorporated
by reference. The humanized monoclonal antibody 405 used in the following experiments is designated huMAb4D5-8. This antibody is of lytil isotype.
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HERCEPIINfiDM 1 coniuoaies
1. Purification of HERCEPTINfi
HERCEPTINfi I huMAb4058, rhuMAb HER2, U.S. Patent No. 5,821,337) (1 vial containing 440 mg
10 antibody) was dissolved in 50 ml MES buffer (25 mM MES, 50 mM NaCl, pH 5.6). The sample was loaded on a cation exchange column (Sepharaso 5, 15 cm x 1.7 cm) that had been equilibrated in the some buffer. The column
was then washed with the some buffer (5 column volumes). HERCEPTINfi was eluted by raising the NaCl
concentration of the buffer to 200 mM. Fractions containing the antibody were pooled, diluted to 10 mgjmL, and
dialyzed into a buffer containing 50 mm potassium phosphate, 50 mM NBC], 2 mM EUTA, pH 8.5.
15 2. Modification of HERCEPTINfi with SPP
The purified HERCEPTIN0 antibody was modified with N..succinimidyl4.(2-pyridylthio)pentanoete (SPP) to introduce dithiopyridyl groups. The antibody (376.0 mg, 8 mgfml) in 44.7 ml of 50 mM potassium phosphate buffer
(pH 6.5) containing NaCI (50 mM) and EDIA 0 mM) was treated with SPP (5.3 molar equivalents in 2.3 ml ethanol). After incubation for 90 minutes under argon at ambient temperature, the reaction mixture was gel filtered through a
20 Sephadex 025 column equilibrated with 35 mM sodium cItrate, 164 mM NaCl, 2

EDTA. Antibody containing
fractions were pooled and assayed. The degree of modification of the antibody was determined as described above. Recovery of the modified antibody (HERCEPTINfi-SPP-Py) was 337 mg (89.7%) with 4.5 releasable 2thiopyridina
groups linked per antibody.
3. onjunatinn of HERCEPTINfi.SPPPv with DMi
25 The modified antibody (337.0 tog, 9.5 pinals of releasable 2.thiopyridine groups) was diluted with the above
35 mM sodium citrate buffer, pH 6.5, to a final concentration of 2.5 mg!mL. DM1 (the structure of which is shown in Figure 1)11.7 equivalents, 16.1 J.Lmols) in 30 mM dimathylacetamide (DMA, 3% viv in the final reaction mixture) was
then added to the antibody solution. The reaction proceeded at ambient temperature under argon for 20 hours. The Structure of HERCEPTINfi.DM1 conjugates is illustrated in Figure 2.
30 The reaction was loaded on a Sephacryl $300 gel filtration column (5.0 cm x 90.0 cm, 1.77 1) equilibrated
with 35 mM sodium citrate, 154 mM NaCl, pH 6.5. The flow rate was 5.0 mLlmin and 65 fractions (20.0 ml each)
were collected. A major peak centered around fraction No. 47 (Figure 3). The major peak comprises monomeric HERCEPTINfi-DM1. Fractions 44-51 were pooled and assayed. The number of OM drug molecules linked per

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