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(54) Title: MATERIALS AND METHODS FOR TREATING CANCERS WHICH EXPRESS FOLATE RECEPTORS

(57) Abstract: The invention provides antibody conjugates comprising an antibody binding domain specific for CD3, a folate receptor ligand capable of binding to a folate receptor and a photocleavable moiety which inhibits binding of the antibody binding domain to CD3. The antibody conjugates may be used in methods of treating cancer, wherein one or more of the cells of the cancer express folate receptor. The invention also provides a method of killing a cancer cell by contacting the cancer cell with an antibody conjugate of the invention, irradiating the conjugate to cleave the photocleavable moiety from the conjugate and contacting the cancer cell with a T cell.

Materials and Methods for Treating Cancers Which Express Folate Receptors

Field of the invention

The present invention relates to cancer therapy, and in particular to the provision of materials and methods for treating cancers expressing folate receptors.

Background to the invention

A large number of cancer cell types are known to express receptors for folate. This has led to suggestions that folate may be used to target therapeutic agents to cancer cells. Examples of such agents include antibody conjugates comprising folate moieties conjugated to antibodies directed against the T cell receptor (10; US 5,547,668) in order to activate T cells in the vicinity of the cancer cells.

Summary of the invention

The present inventors consider that the antibody conjugates proposed in the prior art suffer from a number of drawbacks.

As well as being expressed at high levels in certain cancers, folate receptors are also expressed on a number of healthy tissue types. These vary slightly between species, and in humans include kidney and also lung (8). Therapeutic agents which are directed to their targets by folate may thus also be directed to healthy tissues expressing the folate receptor, potentially leading to damage to those tissues.

If antibodies capable of activating T cells are administered remote from the site of the cancer, then will encounter, bind to, and activate T cells en route to the cancer. Thus, such antibodies are capable of causing systemic T cell activation, despite the presence of targeting moieties intended to direct them to the cancer cells. Administration must therefore be precisely directed to the site of the cancer in order to minimise the risk of such side-effects. However, not all cancers are

readily accessible or lend themselves to such precise administration. Even when treating cancers which are accessible, it is possible that at least some of the antibody conjugate will diffuse away from the site of treatment and cause undesirable T cell activation elsewhere in the body.

The invention provides an antibody conjugate comprising:
an antibody binding domain specific for CD3;
a folate receptor ligand; and
a photocleavable moiety which inhibits binding of the antibody binding domain to CD3.

By "conjugate" is meant a single molecule which comprises structurally distinct and recognisable components. Thus the conjugates described herein contain a molecule comprising an antibody binding domain, covalently linked to a photocleavable moiety and a folate receptor ligand.

Thus the antibody binding domain is inhibited from binding to CD3 until the photocleavable moiety is cleaved from the conjugate by application of radiation of a suitable wavelength. Consequently, wherever the conjugate is administered in the subject's body, it is inhibited from activating T cells until irradiated. Thus the risk of inducing systemic T cell activation is substantially reduced or eliminated entirely.

The folate receptor ligand is not inhibited from binding to the folate receptor. Thus the conjugate's affinity for folate enables it to be localised to sites or tissues expressing folate receptor (such as a cancer to be treated) before irradiation is applied to activate the anti-CD3 binding function.

Binding of the conjugate to healthy tissues expressing folate receptor is therefore unlikely to lead to damage, since the conjugate will be unable to activate T cells at that location unless specifically irradiated.

Conjugation of folate or other folate receptor ligands to the antibody may adversely affect binding of the antibody binding domain to CD3, and may also affect solubility of the conjugate. Therefore it may be desirable that the number of folate receptor ligand residues per conjugate molecule is controlled to be as low as realistically feasible, while still ensuring that most or substantially all conjugate molecules carry at least one folate receptor ligand residue. Thus the conjugate may contain between 1 and 10 folate receptor ligand moieties per conjugate molecule, for example, between 1 and 5 folate receptor ligand moieties per conjugate molecule, e.g. 1, 2, 3, 4 or 5 folate receptor ligand moieties per molecule. The skilled person will appreciate that, in a population of conjugate molecules, it may not be possible to ensure that each and every molecule has the same number of folate receptor ligand moieties and that some variation is likely to occur. Thus a population of such antibody conjugates may have an average of between 1 and 10 folate receptor ligand moieties per conjugate molecule, for example an average of between 1 and 5 folate receptor ligand moieties per conjugate molecule, e.g. an average of 1, 2, 3, 4 or 5 folate receptor ligand moieties per conjugate molecule.

The folate receptor ligand may comprise folate or an ester of folate, for example an NHS-ester of folate. Alternatively it may comprise an analogue of folate having affinity for a folate receptor.

The antibody conjugate may have one, two or more binding domains specific for CD3. Thus it may comprise a single chain Fv region, a Fab fragment, a Fab' fragment or a F(ab')2 fragment. In certain embodiments the antibody conjugate comprises 2 identical antibody binding domains specific for CD3.

The antibody conjugate may additionally comprise an antibody Fc region. For example, the antibody conjugate may comprise two Fab regions and a Fc region. The presence of the Fc region may confer certain advantages. For example it may increase the half

life of the antibody conjugate in serum. It may also help to stimulate the immune response against the tumour to a greater extent than would be achieved using an equivalent conjugate lacking an Fc region. Without wishing to be bound by any particular theory, this effect may be mediated by binding of the Fc region to Fc receptors on cells of the immune system (such as B cells) and thus stimulating or activating those cells. Such stimulation or activation may result, for example, in immunostimulatory cytokine production which may contribute to the anti-cancer immune response.

The Fc region may be inhibited from binding to Fc receptors by a photocleavable moiety. The photocleavable moiety will generally be of the same type as that used to inhibit the antibody binding domain from binding to CD3. This inhibition serves to prevent or reduce immune cell activation by the Fc region at sites other than where treatment is desired. Systemic immune activation accompanied by undesirable cytokine production has been observed with other antibody-based therapies. This has been addressed by using engineered Fc regions which have reduced or no affinity for Fc receptors in the recipient. This approach can avoid some of the undesirable side-effects while retaining the advantages that the Fc region provides in terms of serum half-life. However it prevents the potentially beneficial effects of Fc-mediated immune activation from being obtained at the site of therapy.

By contrast, the conjugates of the present invention should avoid the side-effects, since their ability to bind Fc receptors is reduced or abrogated until they are activated by irradiation, which may be performed only at the site where therapy is required. Once activated, though, the full immunostimulatory benefit of the Fc region is made available. It is also possible to administer higher doses of the conjugates of the invention than of other folate-antibody conjugates with significantly lower risk of adverse side effects. This in turn means that a higher local concentration of activated conjugate can be achieved at the site where therapy is required.

The or each antibody binding domain of the conjugate may comprise at least one CDR from OKT3 or UCHT-1. In certain embodiments, the or each antigen binding domain may comprise all the CDRs from OKT3 or UCHT-1.

When the antibody conjugate is intended for use in the treatment of humans, the or each antibody binding domain may have human constant regions and/or human framework regions. Where present, the Fc region may also be human. Thus the antibody may be humanised OKT3 or UCHT-1.

Any suitable photocleavable moiety may be used as long as it is capable of inhibiting binding of the antibody binding domain(s) to CD3. A suitable photocleavable moiety is a 2-nitrophenyl moiety, e.g. a nitrophenyl-ethyl moiety, such as 1-(2-nitrophenyl)-ethyl (NPE), as described in more detail below. Other substituents may be present at other positions on the benzene ring as long as the moiety remains photocleavable on application of suitable radiation. This moiety may be linked to the rest of the conjugate by a linker moiety. NPE may have advantages associated with its use in the conjugate as it seems to enhance the binding of folate to its receptor, so increasing the affinity of the conjugate for its target on the surface of tumour cells. When cleaved from the conjugate, it may also have a stimulatory effect on the immune system, enhancing the immune response against the tumour which is stimulated by the antibody binding domain(s) binding to CD3.

The invention also provides an antibody conjugate as described above for use in a method of medical treatment.

The invention also provides an antibody conjugate as described above for use in the treatment of a cancer, wherein one or more cells of the cancer express folate receptor.

The invention also provides an antibody conjugate as described above which has been irradiated to cleave said photocleavable moiety.

The invention also provides a composition comprising an antibody conjugate as described above. Also provided is a composition obtainable by irradiating an antibody conjugate as described above to cleave said photocleavable moiety. The composition therefore also contains the other products of the photocleavage reaction. For example, cleavage of an NPE residue from a protein by UV irradiation yields 2-nitrosophenyl methyl ketone. Such products may have a stimulatory effect on the immune system as described elsewhere in this specification.

The compositions of the invention may be pharmaceutical compositions. Thus they may comprise an antibody conjugate, or the products of the photocleavage reaction as appropriate, in combination with a pharmaceutically acceptable carrier.

The invention also provides use of an antibody conjugate or a composition as described above in the preparation of a medicament for the treatment of a cancer, wherein one or more cells of the cancer express folate receptor.

The invention also provides a method of activating an antibody as described above comprising irradiating the antibody conjugate to cleave said photocleavable moiety from the conjugate.

The invention also provides a method of killing a cancer cell expressing folate receptor, comprising the steps of:
contacting said cancer cell with an antibody conjugate as described above;
irradiating said antibody conjugate to cleave the photocleavable moiety from the conjugate; and
contacting said cancer cell with a T cell.

The method may be performed in vitro or in vivo. It will be appreciated that the cancer cell is preferably contacted with the antibody conjugate (so resulting in binding of the conjugate to the cancer cell) before irradiation, and that contact with the T cell occurs subsequently. The result is that the T cell is specifically activated at the site of the cancer cell by the anti-CD3 portion of the conjugate, which is bound to the cancer cell via the folate receptor ligand.

Thus the invention also provides a method of treating a subject with cancer, wherein the cancer comprises one or more cells which express folate receptor, the method comprising:
administering an antibody conjugate as described herein to the subject, and
administering radiation to the subject to cleave said photocleavable moiety from the conjugate.

The radiation is typically targeted to the site of the tumour. For example, it may be applied using a laser. It may be desirable that no (or substantially no) radiation is applied to healthy tissue surrounding the cancer, for example, to healthy tissue more than 10mm, more than 5mm, or more than 1mm from the cancer. Radiation may be directed to the site of treatment using a fibre-optic cable.

The cancers to be treated using the conjugates of the invention may be, or may comprise, a solid tumour. This allows activation of the conjugate to be accurately targeted to the site of the tumour.

The cancer may be any cancer which expresses folate receptor. It will be possible to determine whether or not any individual cancer is suitable for treatment by determining whether or not it expresses the receptor, e.g. by investigating the folate receptor status of a sample of the cancer, e.g. a biopsy. Suitable methods are well known to the skilled person. For example, determination may make use of antibodies capable of binding

specifically to the folate receptor, or may directly measure the ability of a folate receptor ligand (e.g. folate or an analogue thereof) to bind to cells of the cancer. A suitable radioligand binding assay has been described by Parker et al. (8). Cancers known to have a high frequency of expression of folate receptor include nonmucinous ovarian carcinoma, endometrial carcinoma, kidney carcinoma, lung carcinoma, mesothelioma carcinoma, breast carcinoma, brain carcinoma, bladder carcinoma, pancreatic carcinoma and myeloid leukemia.

The invention will now be described in more detail, by way of example and not limitation, by reference to the accompanying drawings and examples.

The invention further provides a method of preparing an antibody conjugate as described herein comprising the steps of:
providing a molecule comprising an antibody binding domain specific for CD3;
reacting said molecule with a folate receptor ligand, or a derivative thereof;
and reacting the resulting conjugate with a compound containing a photocleavable group or bond, such that a photocleavable moiety is conjugated to said molecule and inhibits binding of the antibody binding domain to CD3.

Detailed Description of the Invention

Antibodies

The antibody may have the "classical" native antibody structure consisting of two complete heavy chains covalently associated via disulphide bonds to two complete light chains, appropriately folded to form two Fab regions and a Fc region linked by hinge regions, optionally with glycosylation. However it is well known that fragments of a whole antibody can perform the function of binding antigens. The minimal region capable of binding to the cognate antigen is therefore referred to here as an "antibody binding domain". The term "antibody" is therefore used herein to encompass any molecule comprising such a minimal antibody binding

domain, except where the context demands otherwise. Examples of fragments containing antibody binding domains are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S. et al., Nature 341, 544-546 (1989)) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form a molecule capable of binding antigen (i.e. an antibody binding domain) (Bird et al, Science, **242**, 423-426, 1988; Huston et al, PNAS USA, **85**, 5879-5883, 1988).

The conjugates described herein possess at least one antibody binding domain capable of binding to CD3. It may be desirable that they comprise two (or more) such binding domains (e.g. two Fab regions). This may facilitate cross-linking of CD3 on the surface of the cell of the immune system, which may be optimal for cell activation. Typically each or every antibody binding domain of the conjugate is specific for CD3 and the conjugate does not comprise antibody binding domains specific for any other antigen.

It may be desirable for the conjugate to comprise an Fc region. For example, the conjugate may comprise two Fab regions and a Fc region. Indeed, it may comprise a molecule having classical antibody structure as described above.

The Fc region or antibody may be IgG. It is believed that antibodies having IgG2a isotype may be more effective at T cell activation than antibodies of other IgG isotypes. Therefore the antibody may be IgG2a.

Antibodies against human proteins are typically derived from non-human species, and consequently their administration to humans

may provoke an undesirable immune response against the antibody itself. It is possible to reduce immunogenicity by making chimeric antibodies, which contain constant regions from human antibodies fused to the variable regions from the non-human antibody. A more sophisticated approach to "humanise" antibodies involves grafting the CDRs from the non-human antibody to human antibody framework regions. It is also now possible to generate fully human antibodies or fragments thereof by in vitro synthesis and screening (e.g. by phage display) or by producing antibodies from animals (e.g. mice) transgenic for human antibody genes.

Thus the antibody present in the conjugate may be chimeric (i.e. comprise human constant regions and variable regions from a non-human antibody). Alternatively, it may be humanised (i.e. comprise human framework regions, with CDRs from a nonhuman antibody). Whatever the form of the antibody, it may comprise one or more CDRs from OKT3 or UCHT-1, e.g. one, two, three, four, five, or all of the CDRs from OKT3 or UCHT-1. The CDR sequences for OKT3 are provided in US 6,750, 325.

The antibody may be humanised OKT3 (e.g. as described in US 6,750,325) or humanised UCHT-1.

Although OKT3 and UCHT-1 are mentioned particularly, other antibodies against CD3 are known, and the invention may equally make use of these or other antibodies against CD3. Antibodies against human CD3 include 7D6, 12F6, 38.1, 89b1, 131F26, BL-A8, BW239/347, BW264/56, CD3-4B5, CLB-T3/3, CRIS-7, F111-409, G19-4.1, HIT3a, ICO-90, IP30, Leu-4, LY17.2G3, M-T301, M-T302, MEM-57, MEM-92, NU-T3, OKT3 (US 4,658,019), OKT3D, SMC2, T3, T3 (2Ad2), T3 /2Ad2A2, T3 /2AD, T3 (2ADA), T3 /2T8-2F4, T3 /RW2-4B6, T3 /RW2-8C8, T10B9, T101-01, UCHT1, VIT3, VIT3b, X35-3, XXIII.46, XXIII.87, XXIII.141, YTH12.5, and YTH12.5. See the Human Leucocyte Differentiation Antigens (HLDA) Antibody Database for more details.

The antibody may also be a human antibody directed against CD3.

CD3 is a complex comprising at least 5 different polypeptide chains, designated γ, δ, ε, and ζ, which forms part of the T cell receptor complex expressed on the surface of T cells. One CD3 complex comprises one γ chain, one δ chain, and two of each of ε and ζ. In vivo, engagement of the T cell receptor by a suitable antigen presenting complex on the surface of another cell induces signalling via the CD3 complex which results in activation of the T cell. However it is well established that certain antibodies against CD3 are capable of activating and inducing proliferation of T cells. OKT3 and UCHT-1, mentioned above, are well-known examples of such agonist anti-CD3 antibodies, and are both directed against human CD3.

Photocleavable moieties

By a "photocleavable moiety" is meant a chemical moiety which is linked to the rest of the conjugate molecule by a photocleavable group or bond, i.e. a group or bond which is selectively cleavable by application of radiation of a chosen frequency or wavelength. Any frequency of radiation may be suitable, including infra-red (IR) radiation, visible light, ultra-violet (UV) radiation, microwaves, gamma rays, etc. depending on the type of blocking moiety. UV radiation is particularly convenient. For example, the photocleavable moiety may comprise a 2-nitrophenyl moiety, which may also be substituted at other positions around the benzene ring. For example, a 1-(2-nitrophenyl)ethyl (NPE) residue or moiety may be used, which is cleavable by UV radiation.

Laser irradiation may be particularly suitable for therapeutic methods, as its delivery can be very closely controlled. Thus it can be used to target radiation very precisely to a site of diseased tissue (e.g. a tumour) without affecting surrounding healthy tissue. LED radiation may also be used. Whatever the source of radiation, optical fibres may be used to direct the radiation precisely to the required site. This may be especially useful for treatment of tumours located deep within tissues as

these may otherwise be inaccessible to radiation unless more invasive surgery is used to open up the site or healthy tissue is also exposed to the radiation.

Thus the antibody is inhibited from binding to its cognate antigen by a photocleavable moiety. Such photocleavable moieties are well known in the art. Antibodies can be suitably derivatised by means of appropriate reagents which couple to hydroxy or amino residues. Thus phosgene, diphosgene or DCCI (dicyclohexyl carbodiimide) may be used to generate photocleavable esters, amides, carbonates and the like from a wide variety of alcohol. Nitrophenyl derivatives, particularly 2-nitrophenyl alcohol derivatives, may be used in this context. Substituted arylalkanols may be used, such as nitrophenyl methyl alcohol, 1-nitrophenylethan-1-ol, and substituted analogues. The nitro group is preferably present at the 2-position of the benzene ring. The alcohol may thus be activated by reaction of the hydroxyl group with a linker compound containing a first reactive group capable of reacting with a hydroxyl group, and a second reactive group capable of reacting with a group on the surface of a protein, such as an amine group (e.g. a lysine side chain) or a carboxylic acid group (such as an aspartic acid or glutamic acid side chain). Suitable linker compounds thus include phosgene, diphosgene and DCCI as described above.

Thompson et al. (Biochem. Biophys. Res. Com. 201, 1213-1219 (1994) and Biochem. Soc. Trans. 225S, 23 (1995)) describe reversible inhibition of protein function by derivatisation of 1-(2-nitrophenyl)-ethanol with diphosgene to give a reactive acyl chloride, which then reacts with a lysine side chain to yield an NPE derivative. See also refs. 1, 2 and 14. Further photocleavable moieties will be well known to the skilled person, e.g. from "Biological Applications of Photochemical Switches", H. Morrison (ed.), Bioorganic Photochemistry Series, Volume 2, J. Wiley & Sons. (see especially Chapter 1, section 4, pages 34 to 50). Other suitable photocleavable moieties include 1-(2-nitrophenyl)diazoethane (L. Bédouet et al., Recovery of the

oxidative activity of caged bovine haemoglobin after UV photolysis, BBRC,320 (2004) 939-944), 2-nitrophenylglycine (M. Endo et al, Design and synthesis of photochemically controllable caspase-3, Angew. Chem. Int. Ed 2004, 43, 5643-5645), 6-nitroveratryl (M. Endo et al, Design and synthesis of photochemically controllable restriction endonumclease *Bam*HI by manipulation of the salt-bridge network in the dimmer interface, J.Org. Chem, 2004, 69, 4292-4289), *o*-nitrobenzyl and 4-hydroxyphenacyl.

Methods for conjugating photocleavable moieties to antibody conjugates are also described in WO96/34892.

The photocleavable moiety is preferably present in sufficient density to completely abrogate binding of the antibody binding domain to CD3. Preferably it also inhibits (e.g. reduces or substantially prevents) binding of the Fc region (if present) to Fc receptors in the recipient subject.

Thus there may be, for example, 20 to 70 photocleavable moieties per conjugate molecule, e.g. 30 to 50 photocleavable moieties per conjugate molecule. A population of such antibody conjugates may have an average of between 20 and 70 photocleavable moieties per conjugate molecule, for example an average of between 30 and 50 photocleavable moieties per conjugate molecule.

Folate receptor ligands

Folate (pteroyl-L-glutamate) is a water-soluble B-vitamin also known as folacin, vitamin Bc and vitamin B9. Humans and other mammals express a number of proteins which bind to folate and transport it into cells. For example, in humans, alpha and beta folate receptors have been identified, each of which can occur in several isoforms (e.g. as a result of differential glycosylation) (13). The group of these proteins is referred to in this specification as "folate receptors". Thus a folate receptor is considered to be any protein expressed on the surface of a cell (such as a cancer cell) which binds folate in preference to other

structurally unrelated molecules with which the cell would naturally come into contact.

Folate is a preferred folate receptor ligand for use in the conjugates described herein. A number of analogues of folate are known which can bind to folate receptors, and so can be also be used as folate receptor ligands. These include 5-methyltetrahydrofolate, methotrexate, lometrexol, ralitrexed, pemetrexed, ZD9331, CB3717 and quinazoline and cyclopenta[g]quinazoline compounds including ICI 198583, CB3900, CB30523, CB30901, 6S-CB300464, 6S-CB300638 (see ref. 15) and derivatives thereof. However any suitable molecule having affinity for a folate receptor may be used in the conjugates described in this specification. Preferably the folate receptor ligand has a higher affinity for a folate receptor than for any other protein it may encounter when administered to a subject, e.g. a 10-fold, 10-fold or 1000-fold higher affinity than for other proteins, or even greater.

The folate receptor ligand is typically not a protein or peptide (i.e. a substance composed of two or more amino acids linked by peptide bonds). Rather, it is typically a small molecule, e.g. a molecule having a molecular weight below 1000, below 750, or below 500. It is typically structurally related to folate, and may comprise a pteroyl group or a derivative thereof.

For introduction into the conjugates described, the folate receptor ligand may be linked to the rest of the conjugate molecule by a linker compound. For example, the folate receptor ligand may be derivatised by reaction with a linker compound which carries a suitable reactive group capable of reaction with a group on the surface of the antibody, e.g. an amino acid side chain, such as an amine group (e.g. a lysine side chain) or a carboxylic acid group (such as an aspartic acid or glutamic acid side chain), as described above for the photocleavable moiety.

Alternatively, the folate receptor ligand may simply be reacted with the antibody directly, optionally after derivatisation to create a suitable reactive group capable of reacting with a group on the surface of a protein, such as an amino acid side chain, e.g. an amine group (e.g. a lysine side chain) or a carboxylic acid group (such as an aspartic acid or glutamic acid side chain). For example, the carboxylic acid groups of folate may be converted to reactive esters (e.g. NHS esters) which can then be linked to lysine side chains of the antibody.

Sample methods for conjugation of folate to antibodies are described below, and in US 5,547,668.

Typically the folate receptor ligand will be conjugated to the antibody binding domain before the resulting conjugate is derivatised with the photocleavable moiety. However the photocleavable moiety does not prevent binding of folate (or the analogue thereof) to the folate receptor.

Pharmaceutical compositions

The antibody conjugates described in this specification will typically be administered to a recipient in the form of pharmaceutical compositions. These compositions may comprise, in addition to the antibody, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes or topical application. Intravenous, intramuscular or subcutaneous administration is likely to be appropriate in many instances, but other methods of administration are possible.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a

solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection (which may or may not be at the site where immune stimulation is desired), the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Administration is preferably in an "effective amount" sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Suitable carriers, adjuvants, excipients, etc. can be found in standard pharmaceutical texts, for example Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins; and Handbook of Pharmaceutical Excipients, 2nd edition, 1994. However the dose administered will be suitable to stimulate immune cells rather than suppress immune responses. In general lower doses of anti-CD3 are believed to stimulate T cells, while higher doses are more likely to suppress immune responses, possibly by depletion of T cells. See Bluestone, J A, Science 242:569 (1988), and US 6,406,696.

The skilled person will be capable of ascertaining a suitable dose for any given situation.

The compositions and methods described herein are preferably used for treatment of mammals, more preferably primates (e.g. humans, apes or monkeys), domestic animals (e.g. feline, canine, etc.), laboratory animals (e.g. rodents, lagomorphs etc.) or livestock animals (e.g. bovine, equine, porcine, etc.).

The conjugate of the invention may also capable of providing benefit when irradiated *ex vivo* or *in vitro* immediately before administration. Therefore in some embodiments it maybe desirable to activate the antibody binding domain's capability to bind CD3 *ex vivo* or *in vitro* immediately before administration to the subject, although this is not presently preferred.

Examples

Preparation and testing of photo-activatable anti-CD3-folate conjugate

Antibodies and cell lines

The CD3+ T-cell line H9 was obtained from ECACC. The UCHT1 secreting hybridoma (IgG2a subclass) was obtained from Cancer Research UK. The FR-expressing murine ovarian cell line M5076 (11) was also used.

Coupling of folate residues to the UCHT1 anti-human CD3 antibody

10mg of folic acid was first dissolved in 1ml dry DMSO. Its acid groups were then converted to N-hydroxysuccinimide (NHS) esters by the addition of 6mg NHS (in 500µl DMF) followed by 5mg of Dicyclohexylcarbodiimide (DCC, in 500µl DMF). This 2ml mixture was gently shaken for 6h at room temperature then aliquots (30-75µl) of the mixture were added per ml to UCHT1 (1.0-1.6mg/ml) dissolved in 0.1M Sodium Bicarbonate. The NHS esters react with the antibody amine residues to form antibody-folate complexes. After an o/n incubation (gently shaken) the antibody-folate mixtures were dialysed three times against 5l of 25mM Phosphate

Buffer pH7.5 containing 0.9% NaCl to remove uncoupled folate molecules. Aggregates were removed by centrifugation at 13,000rpm for 10 min in a MSE Micro Centaur microfuge and the absorbance (OD at 280nm) and protein concentration of the clear supernatant (against BCA standard) measured. On subtraction of the known OD 280nm of uncoupled antibody (OD 1.37 at 1mg/ml) and knowing the OD 280nm of 0.1mg/ml folate to be approx. 4.4, the amount of folate and hence the number of residues per antibody molecule could be estimated.

Coating of the Folated-UCHT1 with 2-nitrobenzyl groups

11mg NPE was dissolved in 250µl of dioxan. To this 6µl pyridine and 8µl Diphosgene were added. An immediate white precipitate of NPE-chloroformate was obtained. After a further 10min the excess reagents were removed by evaporation in a flow of nitrogen and 1.0ml fresh dioxan was added. Aliquots (5-20µl) of this 1000µl white crystaline suspension were then added to 1ml aliquots of Folated-UCHT1 (0.5-1.0mg/ml) dissolved in 0.1M Sodium Bicarbonate. After an o/n incubation (gently shaken in the dark) the antibody-NPE mixture was dialysed three times against 5l of 0.9% NaCl/25mM Phosphate Buffer pH7.5 to remove uncoupled NPE molecules. Aggregates were removed by centrifugation at 13,000rpm for 10 min in a microfuge and the absorbance (OD at 280nm) and protein concentration of the clear supernatant (against BCA standard) measured. On subtraction of the OD 280nm of uncoupled protein and knowing the OD 280nm of 50µg/ml NPE to be approx. 1.0, the amount of NPE and hence the number of residues per UCHT1 molecule could be determined

T-cell binding assay

The human T-cell line, H9, was grown in RPMI-1640 media supplemented with 10ml FCS and 1ml P/S per 100ml bottle. The cells were maintained by splitting them at a 1 to 10 ratio into fresh medium every 4 to 5 days. To perform an assay a flask of cells (10ml) was centrifuged for 8min at 500g and the cells were resuspended at approx. 10^6 cells/ml in fresh medium. 10µl aliquots of the diluted antibody conjugates (all diluted to

0.05mg/ml) were added to 250ul aliquots of the cell suspension and were left to bind for 30min at 4°C. The cells were then washed 3 times with 1ml PBS followed by centrifugation for 2 min at 2000rpm. The cells were then resuspended in 200µl of a second layer Goat anti mouse FITC antibody (5µl/ml in PBS). After a further 30min incubation the cells were again washed 3 times, resuspended in 500µl PBS and their fluorescence (5000 gated cells) was measured using a Becton Dickinson Flow cytometer.

Cancer cell binding assay

The mouse cancer cell line M5076 was grown up in suspension in folate restricted (25% folate containing media, 75% folate free media) DMEM media containng 10% FCS in order to get it to up-regulate FR on its surface. To perform an assay the cells were collected by centrifugation for 5min at 500g were resuspended at approx 1.5 x 10^6 cells/ml in fresh medium. 10µl aliquots of the diluted antibodies (all diluted to 0.05mg/ml) were added to 250µl aliquots of the cell suspension and were left to bind for 30min at 4°C. The cells were then washed 3 times with 1ml PBS followed by centrifugation for 2 min at 2000rpm. The cells were then resuspended in 200µl of a second layer Goat anti mouse FITC antibody (5µl/ml in PBS). After a further 30min incubation the cells were again washed 3 times, resuspended in 500µl PBS and their fluorescence (5000 cells) was measured using a Becton Dickinson Flow cytometer.

ELISA assay for antibody bound folate determination

In an attempt to more accurately measure the amount of folate coupled to our antibody conjugates an ELISA procedure was developed. Each well of a 96-well ELISA plate was coated with 100µl of a 3µg/ml solution of an anti-idiotype MAb (this can only react with antigen-free antibody) in carbonate buffer (pH 9.6) at 4°C overnight. Any uncoated areas of the well surface were blocked by the addition of 100µl of 0.5% BSA solution in the same carbonate buffer, for 1 hour at room temperature. After washing the plate with Tris-Triton replicate aliquots of 50µl of folate standards and pre-diluted samples (1/400) were added to the plate

in TBT buffer (Tris-Triton , 50mMTris-HCl pH7.4/ 0.05% Triton containing 0.2% BSA), followed by 50µl of a 5 µg/ml solution of the blocking anti-folate MAb. The plate was washed after a 15 minute incubation period, then 100µl of alkaline phosphatase-labelled anti-folate antibody was added to each well for a further 15 minute incubation period. After a final wash 100µl of a 2mg/ml solution of p-Nitrophenylphosphate (pNPP) substrate in carbonate buffer (pH 10.3) was added to each well, and absorbance at 405nm was read on an ELISA plate reader after 10-15 minutes.

Electrophoresis

Samples were separated by native electrophoresis (no reducing agent or detergent) in 8% polyacrylamide gels using a discontinuous buffer system. Bovine serum albumin (BSA) was used as a standard.

Results

Coupling of folic acid to UCHT1

In initial experiments 50µl of folate-NHS esters was added per ml (up to 5ml) of UCHT1. The number of folate residues that coupled to the antibody depended on the initial antibody concentration. If higher concentrations (1.5-1.6mg/ml) of the antibody were used the final yield of folated-UCHT1 was approx 1.1mg/ml and there were between 1.9 and 3.6 folate residues bound to each UCHT1 molecule. If lower concentrations, 0.6mg/ml, were used up to 12 residues of folate could be coupled with a final yield of 0.5mg/ml. After numerous couplings it was found that if 30ul of Folate-NHS was added per ml of UCHT1 (at 1mg/ml) then coupling was very reproducible at 3.0-3.3 residues folate per UCHT1 molecule, although if dialysis was continued for and extra 3-4 days (5-6 extra changes of buffer) this value reduced to 1.4-1.7 folate residues per UCHT1 molecule. These figures are an average value, therefore native electrophoresis in 8% polyacryamide gels was carried out in an attempt to determine the heterogeneity of the UCHT1 folation. No detergent was used so the antibody migrates according to its natural charge. When folate residues couple to the antibody's amine groups the antibody's net negative

charge increases so it migrates faster through the gel. The folated samples also run as a more diffuse band due to the heterogeneity of coupling. The folate-UCHT1 conjugate (3.6 res) migrates faster than the folate-UCHT1 conjugate (1.9res), exactly as would be predicted, with no unconjugated UCHT1 being present in either sample (data not shown). This was a good confirmation of the accuracy of our spectral data.

We were also able to confirm that the antibody was folated using our anti-folate ELISA. The two UCHT-1-Folate conjugates which gave values of folate of 5.1µg/mg UCHT1(1.9res) and 9.7µg/mg UCHT1(3.6res) by spectrophotometry (and were separated by electrophoresis) had values of 16µg/mg and 53.6µg/mg in the ELISA. The higher values are almost certainly due to the fact that the anti-folate antibody used in the assay will bind with a higher avidity to the antibody-folate conjugates than to free folate. All folate antibodies have to be raised against protein-folate conjugates as free folate is an essential vitamin and is not immunogenic.

Coating of Folated-UCHT1 with NPE

When the UCHT1-folate conjugates were coated with NPE, two factors proved to be significant; the UCHT1-folate concentration and the freshness of the dioxan in which the NPE was dissolved. The dioxan has to be very fresh, if the same bottle is used over several weeks then the number of coupled NPE residues gradually declines. After numerous preliminary experiments it was found that if 10ul of the NPE-chloroformate suspension was added per ml of UCHT1-Folate (at 0.75mg/ml in bicarbonate) then the coating with NPE was fairly reproducible. Yields varied from 0.2 and 0.4 mg/ml with between 48 and 27 residues of NPE on each UCHT1 molecule. Yields decreased as the number of coupled NPE groups. If 15ul of NPE-chloroformate was added to the same conc. of antibody, then approx. 60 NPE residues coupled to the antibody but yields decreased to 0.07-0.10mg/ml.

Anti-CD3 biological activity of the UCHT1 conjugates

The ability of the various UCHT1 conjugates to bind to the CD3 expressing H9 T-cell line was measured using flow cytometry (1). As irradiation was needed to re-activate the NPE-coated conjugates we investigated whether UV irradiation damages uncoated UCHT1, but found that UV-A irradiation has very little effect on the binding of UCHT1 to H9 cells.

The ability of a folated-UCHT1 and NPE-coated-folated-UCHT1 to bind to the T-cells was then measured. These samples contained 3.6 Folate residues per UCHT1 molecule and 48 NPE residues per each NPE-coated antibody. The final concentration of the NPE-coated UCHT1-Folate conjugate was 0.36mg/ml. We found that the binding of the UCHT1 antibody was reduced to approximately half of its original activity when it was folated.

When this conjugate was coated with NPE its activity reduced to virtually background levels but after UV-irradiation the conjugate regained approx 25% of its binding capability. The mean values of the fluorescence for each of the peaks being 5, 64, 31, 6 and 18 respectively. Similar results have now been obtained on over 40 occasions using both UCHT1 and OKT3 (1) (a second anti-human CD3 antibody).

Binding of the UCHT1-Fol and NPE-UCHT1-Fol conjugates to cancer cells.

The folated-antibody conjugate had to be able to bind to cancer cells both before and after the NPE coating, as well as after UV-irradiation. To check this, we used a murine ovarian tumour cell line, M5076, in a flow cytometry binding assay. The M5076 cells were continuously grown in folate restricted DMEM medium at 25% normal folate levels to induce them to express FR on the cells. At lower concentrations of folate the cells died quite rapidly. Binding of UCHT1 and two UCHT-Folate conjugates to the M5076 cells (13 days after they had been switched to folate restricted media) was compared to an unrelated control antibody. The mean values of the fluorescence peaks were 11, 14, 34 and 29 for the Control IgG, UCHT1, Fol-UCHT1(1.9res) and Fol-UCHT1(3.6res)

samples respectively. In another assay, 8 weeks later, the fluorescence peaks were 18, 30, 56 and 53 possibly reflecting increased numbers of FR on the cells.

When the folated-UCHT1 conjugates were coated with NPE they were still able to bind to the M5076 cells. Indeed binding to the M5076 cells appeared to increase. In two separate assays using the Folate-UCHT1(3.6 res) sample the mean fluorescence of the peaks were i) 25, 32 and 53 and ii) 29, 47 and 68 for the UCHT1, UCHT-Folate and NPE-UCHT1-Folate samples respectively. This increase in binding has been a consistent finding with numerous NPE-coated Folate-UCHT1 constucts. On treatment with UV light the values reduced back to the uncoated UCHT1-folate values. Surface folate was also still accessible in the folate ELISA. The UCHT1-Folate, NPE-UCHT1-Folate and NPE-UCHT1-Folate(+ UV light) samples had apparent folate values of 43, 44 and 39 µg/mg UCHT1. The Folated-UCHT1 conjugates could therefore still bind to the FR expressing cancer cells even after they were coated with NPE to inhibit their anti-CD3 binding activity.

Other NPE-anti-CD3-Folate conjugates

Further batches of NPE-coated UCHT1-folate conjugates were also prepared. The anti-human CD3 antibody OKT3 was also conjugated to folate and coated with NPE.

Briefly, a UCHT1-folate (1.4res) was coated with NPE residues. 24ul of NPE-chloroformate suspension in dioxan was reacted with 2.4ml UCHT1-folate at 0.75mg/ml. The yield was 0.21mg/ml. 48 NPE residues coupled per each UCHT1-folate molecule.

The ability of this conjugate to bind to a human CD3 expressing T-cell line was then measured using Flow cytometry:

Control	UCHT1	UCHT1-Fol	UCHT1-Fol-NPE	UCHT1-Fol-NPE after UV
2	74	44	4	34

A UCHT1-folate (2.1res) was also coated with NPE residues. 20ul of NPE-chloroformate suspension in dioxan was reacted with 2ml UCHT1-folate at 0.75mg/ml. The yield was 0.27mg/ml. The number of NPE residues coupled per each UCHT1-folate molecule was 27 NPE residues per antibody molecule.

The ability of this conjugate to bind to a human CD3 expressing T-cell line was then measured using Flow cytometry:

Control	UCHT1	UCHT1-Fol	UCHT1-Fol-NPE	UCHT1-Fol-NPE after UV
2.3	84	57	5.0	23

T-cell and M5076 binding capabilities of 2 further NPE-UCHT1-folate constructs are shown below.

Antibody Conjugate	Mean T-cell fluorescence		Mean Cancer Cell Binding
IgG Control	2.0	2.2	20
UCHT1	134	111	28
UCHT1-Folate	93	nd	nd
NPE-UCHT1-Folate	8	6	68
NPE-UCHT1-Folate +UV	35	32	46

The binding of a UCHT1-Folate conjugate with 9 coupled folate residues and 33 coupled NPE residues to each antibody molecule. Results of two T-cell binding assays are included. nd-not determined.

Antibody Conjugate	Mean T-cell fluorescence		Mean Cancer Cell Binding
IgG Control	2	2	28
UCHT1	98	163	36
UCHT1-Folate	64	110	57
NPE-UCHT1-Folate	4.7	6.8	76
NPE-UCHT1-Folate +UV	25	46	nd

The binding of a UCHT1-Folate conjugate with 2.4 coupled folate residues and 44 coupled NPE residues to each antibody molecule.

Results of two T-cell binding assays are included. nd-not determined.

OKT3-Folate

200ul of folate-NHS esters were reacted with 5ml of OKT3. The resulting OKT3-folate conjugate had an OD_{280} of 2.2 and a protein concentration of 1.15mg/ml. Uncoated OKT3 has an OD of 1.3 at 1mg/ml. The OD through folate in the conjugate was therefore 2.22-1.50=0.72 which corresponds to 16.3ug of folate.. Given the relative molecular weights of folate and antibody (approx 440 and 165,000) 2.67ug of folate is needed to cover 1mg of antibody with 1 residue of folate.

There were therefore an average of 16.3/2.67x1.15 = 5.3 folate residues on each OKT3 molecule.

OKT3-Folate-NPE

The OKT3-folate was then coated with NPE residues. 50ul of NPE-chloroformate suspension in dioxan was reacted with 2ml of OKT3-folate at 0.6mg/ml. After centrifugation and dialysis the NPE-OKT3-Folate (0.1mg/ml) was found to be coated with approx 31NPE residues per antibody.

The binding of the conjugate to H9 T-cells was studied by flow cytometry.

Control	OKT3	OKT3-Fol	OKT3-Fol-NPE	OKT3-Fol-NPE after UV
2.3	139	121	7.1	77
2.2	153	129	8.8	71

The ability of the conjugate to bind to folate expressing M5076 ovarian cancer cells was then also studied by Flow cytometry.

Control	OKT3	OKT3-Fol	OKT3-Fol-NPE	OKT3-Fol-NPE after UV
20	32	nd	94	nd

This OKT3-Fol-NPE conjugate was able to bind very strongly bind to the cancer cells.

The above results demonstrate that a folated UCHT1 conjugates can still bind to CD3+ human T-cells although the binding activity of the UCHT1 antibody to CD3 is slightly reduced. The folated-UCHT1 conjugates can then be reversibly inhibited using an NPE coat to generate a photo-activatable cancer targeting conjugate. These conjugates can bind to FR expressing cancer cells both before and after irradiation, whilst T-cell binding only occurs after irradiation with UV-A light. Final yields were between 0.1 and 0.4 mg/ml depending on the extent the conjugates were coated with NPE. Fewer coupled NPE residues gave higher yields but on some occasions this did not fully deactivate the UCHT1 antibody. This is the least reproducible step in the synthesis, and probably reflects the fact that the NPE-chloroformate is a suspension in the dioxin rather than completely dissolved in it.

Anti-tumour activity of photo-activatable folate-anti-CD3 conjugates in vivo

Preparation of tumour tissue

C57BL6 mice were purchased at 8 weeks old and were acclimatised to their new surroundings for 1 week. Frozen M5076 tumour pieces were thawed from liquid nitrogen storage. These were diced as finely as possible in 199 medium and 50µL of diced tumour was injected subcutaneously into each animal using a fine gauge needle. After approx 3 weeks the tumours were excised and either used fresh in subsequent experiments or frozen in liquid nitrogen.

Treatment with conjugate

These experiments utilised mice heterozygous for human CD3. 6 pairs of double positive homozygous C57BL6/humanCD3+ transgenic mice (12) were obtained and these were bred to establish a stock colony. However these mice have very few T-cells. Therefore, for experimental work, normal C57BL6 female mice were bred with double positive transgenic males to generate heterozygote +/- mice each having one copy of the human CD3 transgene. These mice

had normal numbers of T-cells which all expressed many copies of the human CD3 marker.

A small area on the flank of each heterozygous transgenic mouse was shaved using hair clippers. M5076 tumour pieces (fresh or thawed from liquid nitrogen storage) were diced as finely as possible in 199 medium and 50µL of diced tumour was injected subcutaneously with a fine gauge needle into each animal under the shaved area.

4 days after injection of the tumour, animals were injected with 50ul of medium alone, or medium containing 10µg of NPE-anti-CD3-folate conjugate at the same site as the tumour was injected.

After injection irradiation was applied to the skin for 15 minutes with a hand held lamp (VL-206BL UV-A (2 x 6*W* tubes) which has a total UV-A irradiance of approx. 16m*W* cm^{-2}) from a distance of 2-3 cm above the skin.

Animals were normally sacrificed 21-25 days after injection with tumour, and sometimes later if they appeared healthy. Body weight was determined. Subcutaneous tumours (which on rare occasions had also spread intra-peritoneally) were excised and weighed. Livers were examined for presence of metastatic tumours and weighed to obtain an indication of tumour growth and/or immune infiltration where the appearance was normal. "Liver score" was assigned by an independent observer who was given the livers blind, and was based on weight, colour, visible colonies and tissue integrity, 5 being the lowest normal grading and 20 the worst.

Control tumours excised from each experiment were diced and used for subsequent experiments to minimise the numbers of mice required.

Results

Results are shown for three separate experiments.

Days	Body(g)	Tumour(mg)	Liver(mg)	Liver appearance	Score
Control					
22	27.5	470	1630	Mottled colonies	12
22	27.5	550	1620	Large colonies	13
22	27.1	520	2440	Obvious colonies	18/19
22	23.7	430	990	Light but colonies	8/9
23	26.4	380	1610	Mottled mushy	11
23	21.8	460	1910	Riddled	17/18
UCHT1-Fol-NPE					
23	24.8	140	1330	Normal but pale	7
23	26.0	180	1340	Normal	6
23	26.1	220	1320	Large Colonies	15
23	25.8	420	1730	Riddled	16
25	24.7	90	1240	Normal	6

All animals in this experiment were female. This experiment utilised the UCHT1-folate-NPE conjugate described above having 1.4 folate residues per conjugate molecule.

Days	Body(g)	Tumour(mg)	Liver(mg)	Liver appearance	Score
Control					
21	36.5	250	1850	Bad colour	8
21	35.1	570	1680	Bad colour, mottled	10
21	33.0	340	2320	Riddled	18
21	37.2	330	1460	Bad colour; mottled kidneys	8/9
21	36.6	480	2060	Horrid	19
UCHT1-Fol-NPE					
21	30.3	120	1820	Slight mottle?	7/8
21	32.1	260	1870	Normal	6
21	34.3	230	1930	Slight mottle?	7/8
21	28.5	40	1470	Normal	5
21	31.3	210	1500	Palest	6
21	27.5	110	1470	Normal	5

All animals in this experiment were male. This experiment and the subsequent one utilised the UCHT1-folate-NPE conjugate described above having 2.1 folate residues per conjugate molecule.

Days	Body(g)	Tumour(mg)	Liver(mg)	Liver appearance
Control				
25	24.1	390	1490	Lots of colonies
25	25.5	210	900	Pale
25	22.7	260	800	Pale
25	25.5	110	1250	Normal
25	25.7	210	1200	OK?
25	24.8	150	1200	OK?
25	28.7	180	1310	OK?

UCHT1-Fol-NPE				
25	21.4	80	870	Normal
25	21.2	120	840	Normal
25	25.4	110	1330	Pale
25	22.0	70	910	Normal
25	26.7	70	1400	Normal
25	26.1	220	1210	Normal
25	20.9	50	680	Normal
25	24.1	80	1130	Normal

All animals in this experiment were female.

The results show that, after irradiation, conjugates were effective in reducing the size of tumours established at the site of injection, and either capable of preventing metastasis to the liver or of eliminating any metastases which had already taken place.

While the invention has been described in conjunction with the exemplary embodiments described above, many equivalent modifications and variations will be apparent to those skilled in the art when given this disclosure. Accordingly, the exemplary embodiments of the invention set forth are considered to be illustrative and not limiting. Various changes to the described embodiments may be made without departing from the spirit and scope of the invention. All documents cited herein are expressly incorporated by reference.

References

1. CH Self, AC Self, JA Smith. DJ Self, S Thompson, Light-directed activation of human T-cells, *ChemMedChem*, 2007, **2**(11), 1587-90.

2. S Thompson, R Stewart, JA Smith, CH Self, Light activation of anti-CD3 in vivo reduces the growth of an aggressive ovarian carcinoma, *ChemMedChem*, 2007, **2**(11), 1591-3.

3. S.Thompson, J.A. Spoors, M-C. Fawcett and C.H. Self, Photocleavable nitrobenzyl-protein conjugates, *Biochem. Biophys. Res. Comm.*, 1994, **201**, 1213-1219.

4. Self, C.H. & Thompson, S. Light activatable antibodies: Models for remotely activatable proteins, *Nature Medicine*, **2**, 817-820 (1996).

5. Baeuerle PA, Kufer P, Lutterbuse R. Bispecific antibodies for polyclonal T-cell engagement. *Curr Opin Mol Therapeut* 2003; **5**: 413-419.

6. Lum LG, Davol PA. Retargeting T cells and immune effector cells with bispecific antibodies. *Cancer Chemother and Biological response modifiers* 2005; **22**: 273-291.

7. S Thompson, M-C Fawcett, CH Self. The construction of a functional photoactivatable cancer targeting bispecific antibody conjugate. ChemMedChem 2007, **2**, 1162-1164.

8. N Parker, MJ Turk, E Westrick, JD Lewis, PS.Low, CP Leamon. Folate receptor expression in carcinomas and normal tissues determined by a quantitative radiological binding assay. Anal. Biochem, 2005, 338, 284-293.

9. Y Lu, PS Low. Immunotherapy of folate receptor expressing tumors: review of recent advances and future prospects. J. Control. Release, 2003,91,17-29.

10. EJ Roy, U Gawlick, BA Orr, DM Kranz. Folate mediated targeting of T-cells to tumours. Adv. Drug Delivery Rev.2004, 56, 1219-1231.

11. Gates SB, Mendelsohn LG, Shakelford KA, Habeck LL, Kursar JD, Gosset LS, Worzalla JF, Shih C, Grindey GB. Characterisation of folate receptor from normal and neoplastic murine tissue: Influence of dietry folate on folate receptor expression. Clin Cancer Res. 1996, 2, 1135-1141.

12. Weetall M et al , T-cell depletion and graft survival induced by anti-human CD3 immunotoxins in Human CD3e transgenic mice. Transplantation, 2002, 73, 1658-1666.

13. Ross, JF et al., Cancer 73(9):2432-43, 1994.

14. Thompson, S. et al., Photochem. Photobiol. Sci, 2002,. 5, 326-330.

15. Jackman AL et al., 2004, Advanced Drug Delivery Reviews 56, 1111-1125.

Claims

1. An antibody conjugate comprising:
an antibody binding domain specific for CD3;
a folate receptor ligand capable of binding to a folate receptor; and
a photocleavable moiety which inhibits binding of the antibody binding domain to CD3.

2. An antibody conjugate according to claim 1 having between 1 and 5 folate receptor ligand moieties per conjugate molecule.

3. An antibody conjugate according to claim 1 or claim 2 comprising 2 identical antibody binding domains.

4. An antibody conjugate according to any one of claims 1 to 3 wherein the or each antibody binding domain comprises at least one CDR from OKT3 or UCHT-1.

5. An antibody conjugate according to claim 4 wherein the or each antibody binding domain comprises all the CDRs from OKT3 or UCHT-1.

6. An antibody conjugate according to any one of claims 1 to 5 wherein the or each antibody binding domain has human constant regions or human framework regions.

7. An antibody conjugate according to claim 4 or claim 5 wherein the antibody is humanised OKT3 or UCHT-1.

8. An antibody conjugate according to any one of the preceding claims, further comprising an antibody Fc region.

9. An antibody conjugate according to any one of the preceding claims wherein the photocleavable moiety is NPE.

10. An antibody conjugate according to claim 8, wherein the Fc region is inhibited from binding to an Fc receptor by a second photocleavable moiety.

11. An antibody conjugate according to claim 10, wherein the second photocleavable moiety is NPE.

12. An antibody conjugate according to any one of the preceding claims for use in a method of medical treatment.

13. An antibody conjugate according to any one of claims 1 to 12 for use in the treatment of a cancer, wherein one or more cells of the cancer express folate receptor.

14. An antibody conjugate according to claim 13 wherein the cancer comprises a solid tumour.

15. An antibody conjugate according to claim 13 or claim 14 wherein the cancer is ovarian cancer.

16. A composition comprising an antibody conjugate according to any one of the preceding claims.

17. A pharmaceutical composition comprising an antibody conjugate according to any one of claims 1 to 15 and a pharmaceutically acceptable carrier.

18. Use of an antibody conjugate according to any one of claims 1 to 15 in the preparation of a medicament for the treatment of a cancer, wherein one or more cells of the cancer express folate receptor.

19. Use according to claim 18 wherein the cancer comprises a solid tumour.

20. Use according to claim 19 wherein the cancer is ovarian cancer.

21. A method of activating an antibody conjugate according to any one of claims 1 to 15 comprising irradiating said antibody conjugate to cleave said photocleavable moiety from the conjugate.

22. A method of killing a cancer cell expressing folate receptor, comprising:
contacting said cancer cell with an antibody conjugate according to any one of claims 1 to 15;
irradiating said antibody conjugate to cleave the photocleavable moiety from the conjugate; and
contacting said cancer cell with a T cell.

23. A method of treating a subject with cancer, wherein the cancer comprises one or more cells which express folate receptor, the method comprising
administering an antibody conjugate according to any one of claims 1 to 15 to the subject, and
administering radiation to the subject to cleave said photocleavable moiety from the conjugate.

24. A method according to claim 23 wherein the cancer comprises a solid tumour.

25. A method according to claim 24 wherein said radiation is targeted to the site of the tumour.