Light-activated antibodies in the fight against primary and metastatic cancer

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The very cytotoxic potency of therapeutic antibodies used in the fight against cancer makes their specific tumour targeting of crucial importance. Unfortunately, in practice, this is often not achieved and can lead to dangerous side-effects. A way of greatly reducing such side-effects is to make the antibodies region-specific to the areas bearing tumour. This can now be achieved by rendering them light dependent so they are only active where illuminated. There are many ways of employing such light-enhanced targeting in very many locations within the body. When it is applied to direct killer T-cells to ovarian primary tumours, not only is primary tumour growth markedly reduced but also a dramatic reduction of metastatic growth is observed in the liver.

A fact that is well understood by all immunodiagnostic companies and critical users (such as hospital laboratories) that use antibodies in relatively simple *in vitro* systems is that antibodies are not as specific as we would like (or many believe). Various diagnostic assays have variable levels of background and both false positives and false negatives are seen. Such unwanted reactions are both more important and more likely when antibodies are used in therapeutic applications in the much more complex environment of the human body. Crucial issues, such as circulatory persistence, sequestration and target fidelity all add to the major problem of cross-reactivity and determine whether a therapeutic antibody will have an acceptable level of side-effects. In an ideal world, a therapeutic antibody would specifically target cancer tumours while leaving normal tissues unaffected [1] and, although a great deal of progress has been made in developing these molecules as clinical therapeutic agents [2–4], it has proved very difficult indeed to obtain native antibodies with the desired level of specificity. Very few, if any, antibodies can be said to be truly tumour specific [5–8]. Whereas antibodies can be humanized to prevent their clearance from the circulation [9,10], it is unlikely that similar recombinant engineering [11] will provide a global answer to improving specificity. That is, of course, only part of the challenge. Even a specific antibody might reside in the wrong place in the body. Therapeutic means to focus the antibody cytotoxic activity to only those sites where the tumour resides are crucial.

Cancer-targeting antibodies can be administered unmodified [1–4,9,11], as a toxic load carrier [2,5,10], or as part of a bispecific conjugate [10,12,13]. In all three of these procedures, too high a level of specific or non-specific cross-reactions with normal tissues can give rise to unacceptable side-effects. In early attempts to minimize these problems, a novel two-step strategy, antibody-directed enzyme prodrug therapy (ADEPT), was developed. Here, bispecific complexes were created in which the cancer-targeting antibody was coupled to an enzyme [10,14]. A few days or weeks later, a harmless inactive prodrug was administered. The prodrug is then converted to a potent cytotoxic form by the antibody-targeted enzyme. The delay between the administration of the antibody–enzyme conjugate and the prodrug is necessary to allow both for weakly bound antibody conjugate to desorb from normal tissues and for persisting circulatory antibody to be sequestered by the bodies' clearance mechanisms. Unfortunately, this procedure does little to reduce the side-effects caused by the enzyme that is specifically targeted to normal tissues expressing the same antigen.

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The tumour-directed enzyme load can also decrease considerably during the waiting time, although this might be reduced by glycosylation of the antibody to speed its removal.

In other attempts to increase specificity, it was proposed that bispecific and other multivalent antibody conjugates could be employed [15,16]. Cancer-targeting diabodies and triabodies can bind to more than one antigen on the cancer tissue, hence increasing their specificity. Alternatively, the cancer-targeting antibody can be linked to a second antibody that subsequently binds a cytotoxic agent or cell. This has allowed bispecific antibodies to be developed to directly target the body's immune response (T-cells) to tumours [12,13,17–19]. One part of the bispecific antibody reacts with a specific tumour antigen, while the other reacts with a Cluster of Differentiation antigen (usually CD3) on the T-cell surface. In theory, the conjugate is injected, flows through the body and binds to the tumour, then T-cells bind to the other reactive site, are targeted to the tumour and kill it. In practice, the conjugates can bind to peripheral T-cells before they reach the tumour, hindering the conjugates' migration to the tumour. This not only leads to peripheral T-cell depletion but also, in some circumstances, can cause very dangerous cytokine storms [20,21]. The further and inescapable problem of the specificity of the anti-tumour antibody still remains, as it does in all antibody-targeting procedures.

Focus on the tumour site

True specificity enhancement requires a positive focus on the tumour site to maximize the toxicity only to cancerous tissues. This can be done in a limited fashion by isolated limb perfusion [22,23] to localize treatment to specific limbs, but photo-thermal [24–28] and photodynamic therapies (PDT) [29–31] have previously been the most promising localizing procedures because they both use laser light to target treatment to tumours. Early photo-thermal treatments involved the injection of tumours with a laser-absorbing dye (often indocyanine green [24]). Cytotoxic heating occurred on localized laser illumination with a specific wavelength, but adjacent normal tissue was also affected. It was proposed that incidental damage to normal tissues could be further reduced by linking the dyes to tumour-targeting antibodies [24]. In very recent studies [25–28], dyes have been superseded with purpose-built gold nanoshells (either hollow or on a silica core). These nanoshells are easily coupled to tumour-targeting antibodies and can be tailored to absorb light much more efficiently, with a precise wavelength in the near-infrared region.

PDT is another light-mediated treatment [29–32], in which a photosensitizing drug produces oxidative damage to tissues when it is irradiated with light. In early studies, damage to normal tissues could be excessive and patients had to stay out of sunlight. Second-generation and third-generation photosensitizers are now being developed [32] that can only be activated with light at very precise wavelengths, but biological distribution can still be a problem. The coupling of these modern photosensitizers to tumour-targeting antibodies [29] holds the promise of delivering an efficient form of photo-immunotherapy in the near future.

The two techniques discussed above use light to burn out the tissue, thermally or chemically, in a specific region once the antibody-targeted drug has bound, by the activation of the chemical targeted by this process. The technique discussed in the rest of this review, light-specific therapeutic antibody targeting, uses the different approach of using light directly to increase the specificity of the antibody. Antibodies can be modified so that they can only bind to their antigen after irradiation with UV light to remove a photo-labile coating of 2-nitrobenzyl groups that has been used to inactivate the antibody. When the inactive antibody is illuminated with UV-A light at a wavelength of 360 nm, the antibody is reactivated only when and – more importantly – where it is required [33,34]. Such an approach can be readily used in easily accessible areas such as the skin, eye and mouth and those exposed during operative procedures. Given the technological advances made with keyhole and other endoscopic approaches, many other areas of the body will also be amenable to treatment by this simple procedure.

Inhibition of unmodified antibodies

The procedure can be carried out on all types of therapeutic antibodies, whether as individual entities or in cytotoxic conjugates and so on. The simplest form is with the antibody on its own. Such an antibody can be inactivated, injected and then reactivated when and, critically, where it is required by irradiation from an external (lamp) or internal (fibre optic) source (Figure 1). Here, the antibody can either be a direct tumour-targeting antibody, such as Herceptin, or an immunoregulating antibody, such as UCHT1.

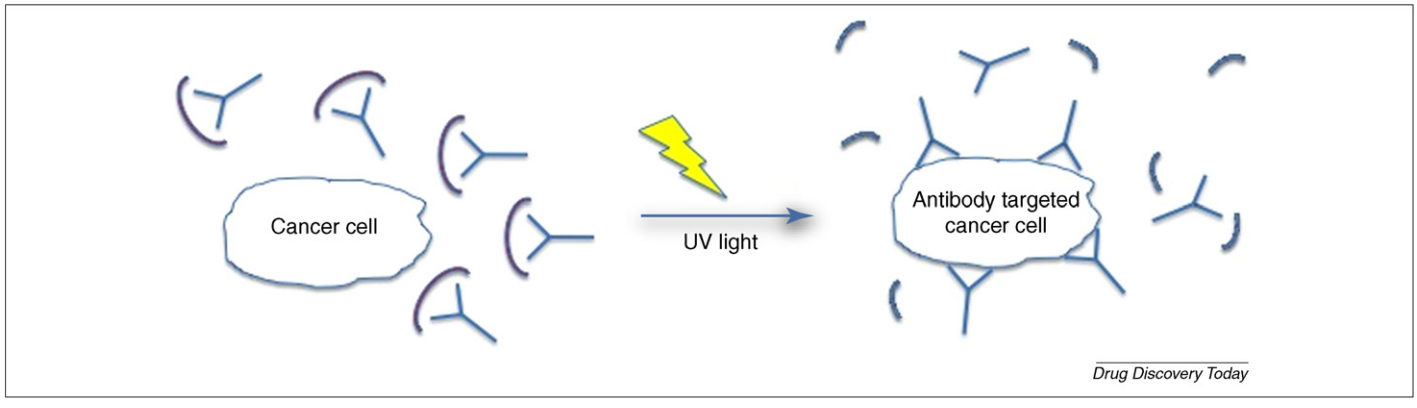

FIGURE 1

The principle behind the new light-mediated targeting strategy. Antibodies are prevented from binding by the NPE coating until it is removed by localized irradiation with UV light.

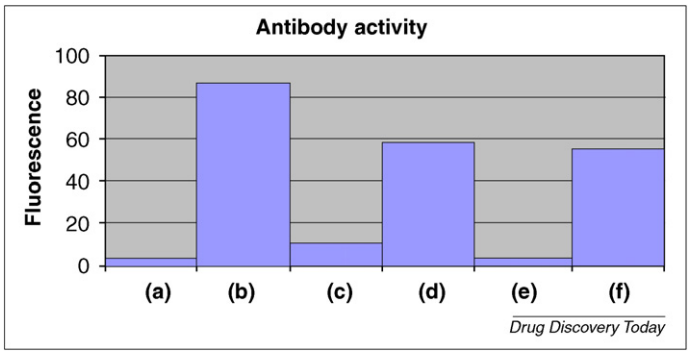

FIGURE 2

The T-cell-binding characteristics of various UCHT1 anti-human CD3 preparations as measured by flow cytometry. **(a)** Irrelevant control antibody. **(b)** Unmodified UCHT1. **(c)** UCHT1 coated with 20 μl NPE. **(d)** Sample **c** after 10 min UV irradiation. **(e)** UCHT1 coated with 30 μl NPE. **(f)** Sample **e** after 10 min UV irradiation. The fluorescence value given is the mean value for the single fluorescent peak given by 5000 gated H9 cells. For more experimental details, see Refs. [35–37].

The anti-cancer antibody would not be able to target the tumour until it had been irradiated. Similarly, the immunoregulating antibody, UCHT1, could not bind to and activate T-cells in the area around the tumour until it had been irradiated. Figure 2 shows flow cytometry experiments demonstrating how the activity of UCHT1, an anti-human CD3 antibody, which binds to and activates human T-cells, can be inhibited then restored with UV irradiation [35–37]. The anti-CD3 antibody is added to a human T-cell line (H9), which expresses CD3 molecules on its cell surface. After a 30 min incubation period and washing, a fluorescent Fluorescein isothiocyanate labeled-anti-mouse antibody is added to quantitate how much anti-CD3 antibody has bound to its target cell. The irrelevant control IgG antibody does not bind to the H9 cells (Figure 2a), but the unmodified UCHT1 binds very well (Figure 2b). When the UCHT1 antibody is coated with 20 μl of 1-(2-nitrophenyl)ethanol (NPE) residues using diphosgene, its activity reduces considerably to approximately 6% (Figure 2c) of its initial activity; on UV irradiation, approximately 80% of the initial activity (Figure 2d) is regained. If, however, a coating of 30 μl NPE is used, the activity reduces to almost background levels (Figure 2e) but is, again, mostly regained by irradiation with UV light (Figure 2f). This work led to the possibility that the immune response could be switched on whenever and wherever it was required in the body, by regional illuminations. When a similar murine anti-CD3 antibody (145-2C11) was inactivated and co-injected into mice with ovarian tumour cells, very invasive tumour growth was obtained. If, however, the conjugate was irradiated with UV light from a hand-held lamp, through a shaved patch of skin, tumour growth was reduced markedly [38]. This demonstrated that the inactivation and reactivation procedure worked *in vivo* as well as *in vitro*.

Antibody conjugates targeting drugs and/or toxins

A second type of targeting conjugate is a cancer-targeting antibody directly linked to a toxin [5,10]. In this type of conjugate, the anti-tumour antibody can be reversibly inhibited to prevent it from binding unless it is illuminated. An even more attractive alternative would be to reversibly inhibit the activity of the toxin. This should be possible whenever the toxin is a protein, such as ricin [39,40]. The cancer-targeting antibody would be free to target the cancer as best it could, but the toxic part of the conjugate would be inactive until irradiated. Such a conjugate would be much more specific with light-mediated targeting added to the tumour-antigen specific targeting. Non-specific and specific targeting of normal tissues expressing the same antigens would not then matter because the toxic portion of the conjugate would be inactive until irradiated.

Bispecific conjugates targeting enzymes

The third type of antibody conjugate that can be administered is an antibody–enzyme conjugate, as used in ADEPT therapy. ADEPT therapy can also gain from the beneficial effects of photo-reversible inactivation, as discussed above. The enzyme might be able to be directly inhibited, as has been shown previously with chymotrypsin [41]. Alternatively, if the enzyme cannot be directly inhibited, then a therapeutic bispecific conjugate can be designed, in

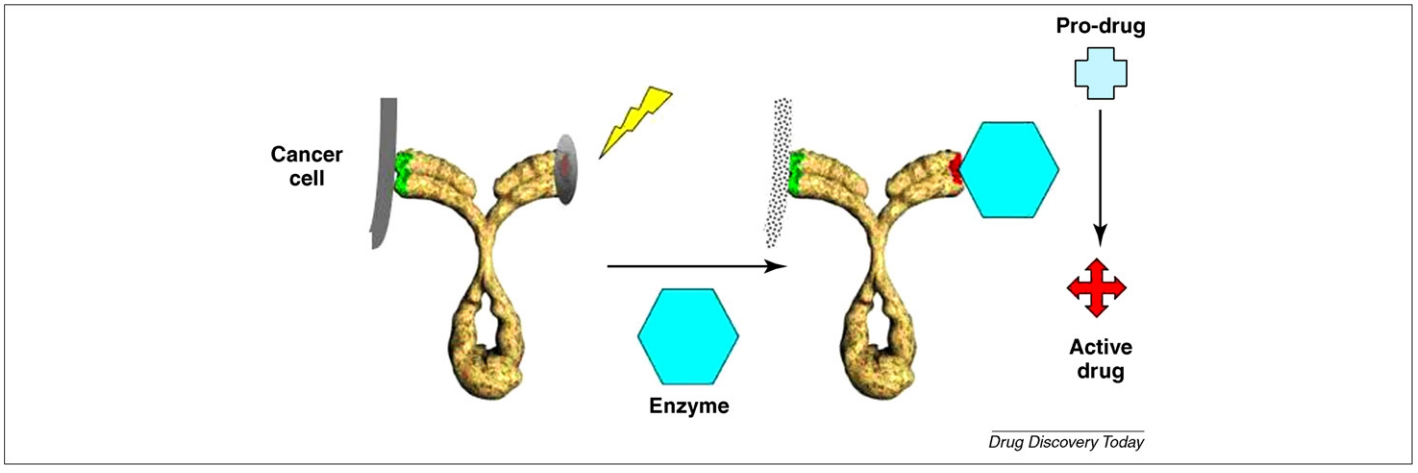

FIGURE 3

This diagram shows a bispecific antibody with one binding site (left arm) targeting a cancer cell and the other enzyme binding arm unavailable. After irradiation with UV light, the enzyme binds to the antibody. On administration of inactive prodrug, it is converted to the active form by the enzyme and kills the attached cancer cell.

which one antibody binding site targets a 'tumour-specific antigen' and the other site targets an enzyme [42] (Figure 3). The conjugate can be administered and left to bind to tumour antigens. After antibody treatment, enzyme can be administered but will only be capable of binding to tumour tissues in regions that have been illuminated. After a time delay, to allow for the clearance of excess unbound enzyme, the inactive prodrug can be injected and will be converted to its active form only where there is active enzyme.

Bispecific antibody conjugates that retarget the immune response

The final and, in our view, most attractive forms of antibody conjugates that could greatly benefit from photo-reversible inactivation are those that retarget the patient's own immunoregulatory cells directly to the surface of a tumour [12,13,17,19,36,37]. In such bispecific antibody conjugates, a tumour-targeting site is normally linked to an antibody that specifically binds to T-cell surface antigens. The main benefit of this type of conjugate is that toxic drugs or enzymes are never introduced into the patient in any form. The patient's own immunoregulatory cells are used to attack the tumour. The conjugate binds to the tumour and T-cells, hence targeting the T-cells to the tumour. If only the T-cell targeting antibody is initially inactive, the cancer-targeting portion is free to react with tumour and normal tissues through specific and non-specific binding. The effectively toxic T-cell binding is then only reactivated in tumour-bearing areas that are illuminated with UV-A light. The conjugate reacts with tissues in a highly ordered manner (Figure 4): first, it interacts with the tumour (Figure 4a), and then, after illumination (Figure 4b), its second binding site (Figure 4c), which is targeted to interact with the T-cells (Figure 4d), is activated. This strategy is intended to prevent cytokine storms and other very dangerous side-effects that can occur with the use of T-cell-activating antibodies [20,21]. The addition of light-specific T-cell targeting to the specificity already conferred by the anti-tumour antibody results in much higher overall specificity of targeting to tumour tissues with fewer harmful side-effects.

This type of cancer-targeting conjugate has already been synthesized [36]. The T-cell-targeting anti-human CD3 antibodies OKT3 and UCHT1 were folated to create bispecific cancer-targeting conjugates; a large proportion of tumours, such as breast and ovarian cancers, overexpress folate receptors [43]. The anti-CD3 activity of the bispecific complexes was then inhibited by coating with 1-(2-nitrophenyl)ethanol residues. The efficacy of such conjugates was confirmed by examining the effects of irradiating the conjugates in transgenic mice that express human CD3 on their T-cell surfaces. Both the primary tumour burden and metastasis of an aggressive ovarian carcinoma were markedly reduced when a pre-established tumour was irradiated through a shaved patch of skin in comparison to that found in non-irradiated mice [37].

The effect on metastasis of the tumour to the liver was particularly exciting and is probably a consequence of the T-cell response being upregulated next to the primary subcutaneous tumour. Such an effect could lead to the development of exciting new treatment regimes. Treatment of accessible secondary metastatic deposits could result in the regression of inaccessible primary and other unknown secondary tumour deposits. The regression of

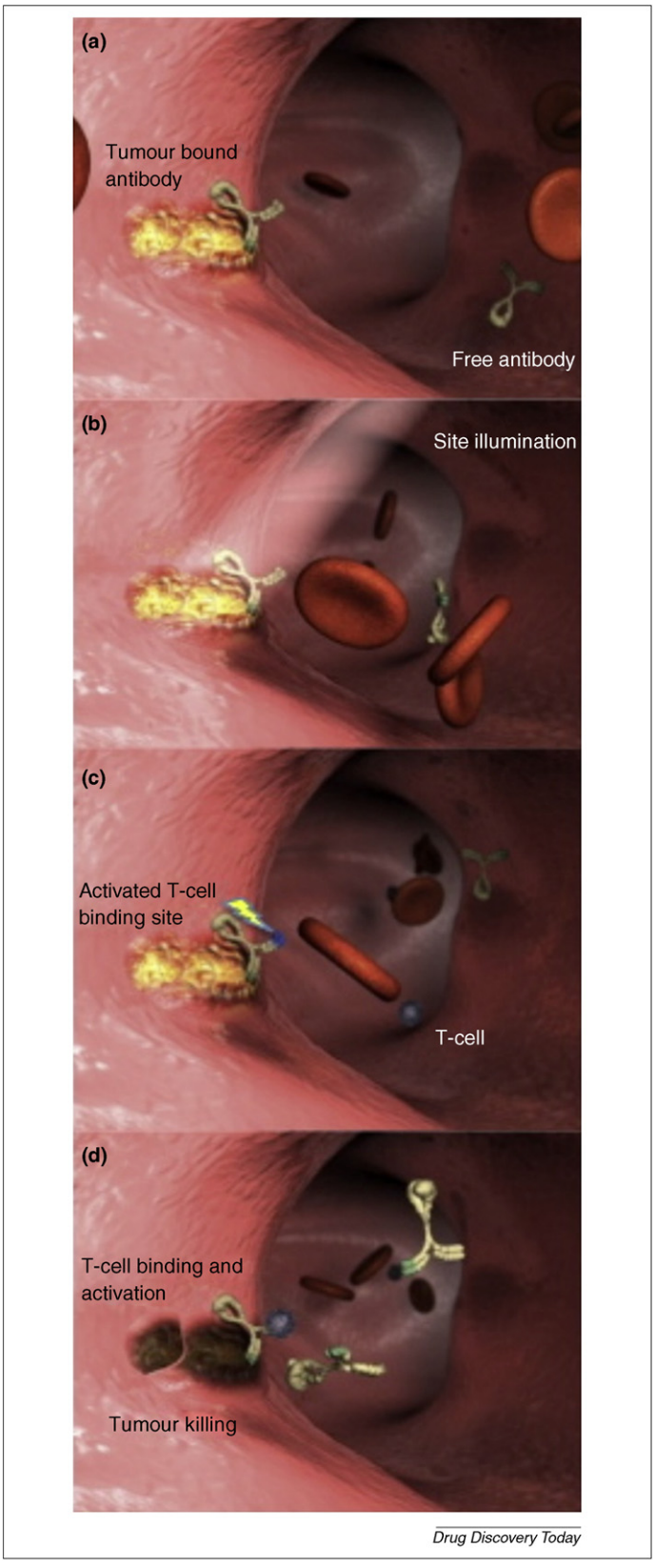

FIGURE 4

A photo-activatable bispecific antibody bound to a tumour **(a)**. On irradiation **(b)**, the T-cell-binding portion of the conjugate reactivates **(c)**, binds T-cells to the tumour and kills it **(d)**.

tumours at a secondary site (the lungs) has been reported when murine subcutaneous tumours were treated with PDT [44]. This effect was explained as a consequence of the PDT fortuitously upregulating the immune response around the primary tumour. If the immune response is deliberately upregulated using photo-activatable bispecific anti-CD3 conjugates then an associated immunization effect should be much more pronounced.

Concluding remarks

One only has to look at the history of vaccination to see that, when harnessed correctly, the immune system is a most powerful weapon in the fight against disease. Control over immune system modulation and targeting has been the holy grail of researchers since the advent of immunology. Whereas great strides have been made in our ability to modulate isolated immune cells, to do so in a targeted way in a clinical situation currently remains problematical. A situation, we hope, that the procedures outlined in this review will go towards solving.

By 2005, only 12 monoclonal antibodies out of 206 that had been studied in clinical trials [4] had been approved for clinical marketing. Many more might be able to return to clinical practice if their activity is restricted to tumour-bearing areas using this light-specific strategy. The mode of delivery of the light needs also to be considered because many sources have limited tissue penetration [30]. In this regard, it is important to note the impressive progression of laparoscopic technologies to access enclosed spaces. The deployment of light-emitting optical fibre probes to many areas of the body, therefore, should be possible. In classical surgery, once an area is debulked of resectable tumour, this light-dependent technology might then enable the surgeon to essentially sterilize the area free of remaining tumour cells simply by illuminating the accessed site. Minimally invasive procedures will be required to treat an increasingly ageing population because they will reduce both the side-effect profiles and the rehabilitation time of patients. With the increased use of screening programmes, detection of early-stage cancers is likely to increase, accentuating the need for effective localized treatments.

In our view, the photo-activation technology described here will improve the targeting of therapeutic conjugates to tumours by increasing their effective specificity. When used in conjunction with improvements in recombinant antibody production techniques and rapidly evolving light-delivery systems, it should be possible to build a wealth of extremely effective, highly tumour-specific therapies in the near future.

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