

A Conjugation Platform for the Targeted Delivery of Anticancer Agents

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A novel technology whereby mAb fragments (sc-Fvs) are bioengineered with extra lysine moieties enables the multiple loading of anticancer agents – providing a unique and simple conjugation platform for targeted drug delivery and photodynamic therapy.

Monoclonal antibodies (mAbs) and their derivatives currently constitute the fastest growing class of therapeutic molecules (1-3). Over the past 25 years, more than 30 immunoglobulins (IgGs, the favoured class of mAbs) and related agents have been approved, mainly for the treatment of cancers and inflammatory diseases. In oncology, mAbs are often combined with cytotoxic drugs to enhance their therapeutic efficacy. Alternatively, small anti-neoplastic molecules can be chemically conjugated to mAbs (called antibody-drug conjugates or ADCs), and used both as carriers, to increase the drug's half-life in the body, and as targeting agents for improved drug selectivity.

The concept of combining cytotoxic drug molecules with mAbs that target tumour cells has long been a major research goal for antibody engineers. Success has proved elusive with many high-profile failures. However, companies like Immunogen and Seattle Genetics have been developing more stable,

cleavable linkers that release the drug at its destination (disulfide-based linkers, hydrazone linkers and tumour-specific protease linkers).

ADCs (also called immuno-conjugates) consist of a recombinant mAb covalently bound by a synthetic linker to a given cytotoxic chemical. The main objective is to combine the pharmacological potency of 'small' cytotoxic drugs (300 to 1,000 Da) and the pharmacokinetic and pharmacodynamics of mAbs (Figure 1) for tumour-associated antigen targets.

Anti-neoplastic drugs – such as doxorubicin, daunomycin, vinca-alkaloids and taxoids – have demonstrated their ability to kill cancer cells, but generally with limited selectivity and highly toxic effects on normal cells, yielding only marginal therapeutic indices. On the other hand, approved antibody drugs – such as rituximab, trastuzumab, cetuximab, bevacizumab and panitumumab – have demonstrated their therapeutic utility in malignancies, but only in combination with small cytotoxic drugs to achieve significant clinical efficacy. The use of unmodified mAbs as single agents is sub-optimal; currently, they can only extend survival expectation by a few months. In addition, many antibodies suffer from drug resistance due to mutations in cell-signalling pathways. Indeed, many strategies are being investigated including

enhancement of intrinsic mAb-linked effector functions by glycoengineering, and the use of bispecific antibodies, polyclonal antibodies and conjugates.

Covalent conjugation of mAbs and drugs with synthetic chemical linkers is not a recent concept. In the 1960s, the use of ADCs in animal models was described in the literature, and in the 1980s, clinical trials with murine IgG-based ADCs were conducted. There were many issues and failures due to poor choice of antibody and inferior linker/drugs.

The main method for conjugating drugs to mAbs has been via the thiol side-chains of cysteine residues (Cys-SH). Though affording a certain degree of stoichiometric control during conjugation, this protocol yields only low drug-loading ratios, due mainly to the large mAb-drug conjugate becoming notoriously insoluble at higher drug loadings and loss of binding affinity (1,2).

PhotoBiotics' interest in the use of much smaller mAb fragments (sc-Fvs) to covalently attach anti-cancer drugs started at the beginning of the 21st century, as a way of specifically targeting photosensitisers to tumours,

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thereby improving the success of photodynamic therapy (PDT). Antibody fragments (Figure 1) have benefits such as more rapid tumour penetration and blood clearance that could result in higher potencies and lower side effects, especially for solid tumours that are notoriously difficult to treat.

Photodynamic Therapy

PDT treats localised lesions within the body – for example, tumours and the ocular condition age-related macular degeneration (AMD) – using photosensitisers (PS) to catalyse lesion destruction via irradiation with visible light (4). This involves initial administration of a PS that marginally over-accumulates in the lesion compared with surrounding healthy tissue. Exposure to cold laser light of an appropriate wavelength excites the PS, which then mediates the conversion of molecular oxygen into reactive oxygen species (ROS) – for example, hydroxyl radicals, superoxide anions and singlet oxygen. ROS irreversibly damage a lesion's cellular components (proteins, lipids and DNA) or its blood supply, resulting in cell death.

An important feature of photocatalysed singlet oxygen production is that it returns the PS to its electronic ground state. This means that in principle (provided a sufficient supply of oxygen), a single PS molecule can efficiently generate many times its own concentration of singlet oxygen, making this an efficient cytotoxic drug.

At normal light doses, PDT results in the necrosis of a lesion such as a tumour. However, at lower light doses, PDT triggers apoptosis or programmed cell death (as opposed to the random cell death of necrosis). It appears that

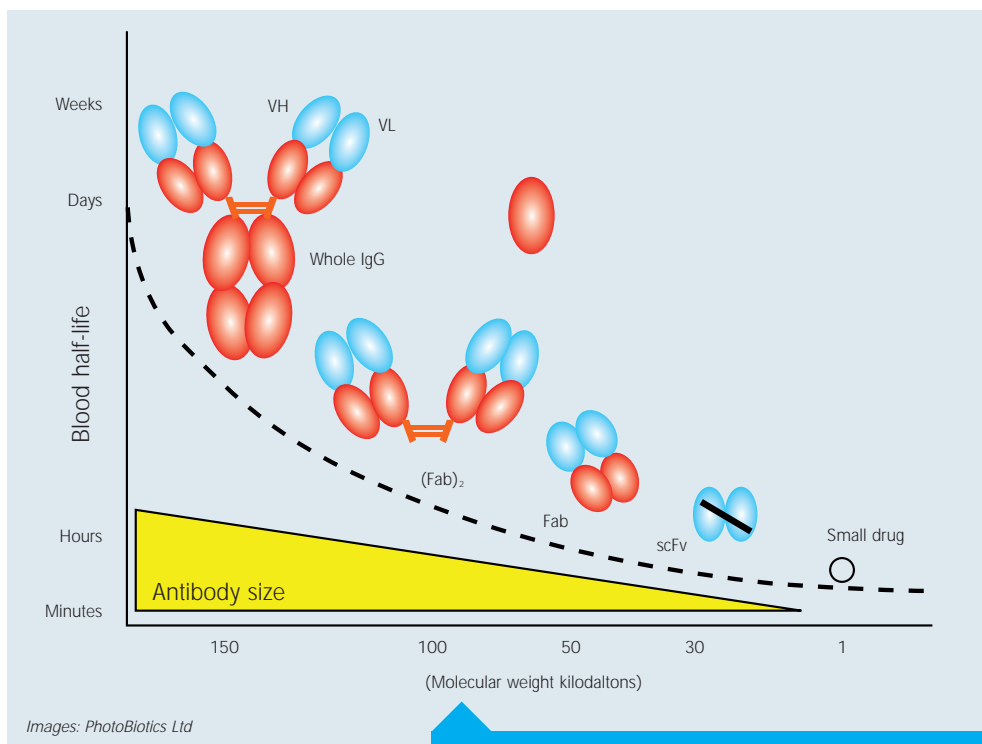


Figure 1: Scheme of different antibody formats from whole IgG to single-chain Fv. The smaller fragments can penetrate tumours faster, clear more rapidly and have less cross-reaction, whereas whole IgGs remain in the blood for weeks. The binding regions (variable domains) are red and the constant domains are blue.

apoptosis might be induced in tumours following PDT, but this outcome seems to depend upon how the treatment is done, what cell line or type of tumour is used, the charge on the sensitiser and how the PS is distributed within the tumour cell.

Thus for example, PDT damage to the tumour cell membrane seems to delay or prevent apoptosis. It is also still unclear which mode of cell death is most beneficial. Certainly, the sub-lethal (for the tumour) PDT doses that trigger apoptosis seem to prevent side reactions and inflammatory responses in healthy tissue. On the other hand, lethal PDT doses that induce tumour necrosis stimulate the body's systemic immune response against the remaining tumour, especially at deeper levels within the body. This latter point is key as it implies that PDT can eradicate metastases, and there is clinical evidence to support this.

Though increasingly successful in the clinic, current PDT does have its shortcomings. Chief among these is post-operative systemic

photosensitivity, sometimes for up to several weeks after treatment, caused by the PS being deposited in the skin. This has led to intensive research principally to improve the photophysical performance of PSs, so that they can address deeper-lying tissues. Thus a 'catechism' has arisen of what might constitute the perfect PDT sensitiser; it must be:

- A pure compound with a reproducible synthesis
- Activated at wavelengths >600nm in order to ensure better absorption of tissue-penetrating red light, and capable of sensitisation by an external light source
- Non-toxic/inert in the absence of light
- Sufficiently long-lived in its triplet excited state to be able to photosensitise the production of ROS, particularly singlet oxygen
- Able to localise specifically in lesions
- Able to clear rapidly from the body after it has done its work,

reducing the time the patient is photosensitive in normal sunlight

- Sufficiently soluble in the body's tissue fluids, or be capable of formulation, so that it can be injected and carried around the body to the lesion

Most research in PDT to date has tended to concentrate on maximising the first four points above, whereas the latter three are proving more difficult to achieve using single PS molecules. Thus, new 'second generation' PDT sensitisers have been developed with much improved drug-light interactions – the most commercially successful of these being verteporfin, developed by QLT in Canada for AMD and marketed under the commercial name Visudyne by Novartis.

Targeting PDT

Concerning the last three points above, mAbs might be thought to offer the ideal tumour-targeting system for PDT, and indeed the attachment of various PSs to mAbs has been studied intensively over the past 20 years (4). The drawbacks of many of these approaches are that they involve factors such as complicated syntheses of the conjugates, physiological transport barriers resulting in slower pharmacokinetics/dynamics and loss of activity by either the mAb (reduced affinity) or – as has been repeatedly demonstrated – the attached PSs having compromised photophysical performance leading to poor PDT efficiency.

Thus, although PSs conjugated to whole mAbs have been known to target a range of cancers or pathogens, experience so far has shown that coupling large numbers of PS molecules to whole antibodies results in many technical problems. These include impaired antibody binding and reduced

solubility of the resulting ADCs. The low PS direct-loading ratios on mAbs reported to date render such an approach impractical at present for PDT applications.

Some researchers have achieved a degree of targeting using alternative carrier moieties, such as branched carbohydrate or short poly-lysine peptide chains; these mimic natural ligands and in turn are linked to the mAb. However, this process requires multiple conjugation steps that have resulted in limited reproducibility, adding to the complexity of commercial manufacture. Apart from managing to achieve only low PS loading ratios, a further disadvantage of such carriers is that PSs can end up being in such close proximity to each other that their excited states are quenched – severely reducing the energy available for cell-damaging ROS generation.

OptiLink Technology Platform

At PhotoBiotics, we are developing a novel technology platform, OptiLink, initially with the intention to maximise the targeting and efficacy of PDT. At first, this was to be achieved by attaching a short helical peptide chain with covalently bound PS molecules to a much smaller antibody fragment (sc-Fv). However, early on it was found that an altogether more efficient, relatively facile and novel approach was to bioengineer sc-Fvs with extra lysine moieties in their primary protein structures. This allowed multiple drug molecules to be conjugated to the lysine-NH₂ side-chains. Such sc-Fv-PS conjugates were called photo-immuno-conjugates or PICs.

Quite counter-intuitively, OptiLink PICs yield far higher drug loadings than have previously been achieved with whole mAbs, without the risk of drug-scFv-conjugate insolubility.

In addition, the spacing of the lysine moieties eradicates any unwanted interactions that might inhibit activity of the attached PS molecules, thus maximising their potency while preserving the PICs' targeting and binding abilities. Other advantages of bioengineering smaller PICs include much improved pharmacokinetics/dynamics over mAbs, easier drug-conjugate manufacturing, solubility and formulation, and a general applicability to most mAbs/scFvs, thereby improving their versatility.

The OptiLink technology platform also vastly improves the LD50s of already-existing commercially-available photosensitisers (5). As an example, a PIC was prepared using verteporfin (the PS used in Visudyne for treating AMD) covalently bound to a target-cell internalisable anti-HER2 scFv derived from the mAb C6.5 and bioengineered by phage display. (HER2 is highly over-expressed on many epithelial cancers of relevance to PDT therapy.) The result was a PIC that showed a 13-fold increase in specificity and a 7-fold increase in PDT potency compared with verteporfin alone.

Towards Targeted Light-Activated Cancer Therapy

Using the OptiLink platform technology, we have created a range of PICs – the lead being ProstaLite for the targeted light-activated treatment of prostate cancer. So far, ProstaLite has demonstrated:

- Excellent *in vitro* cell-kill results
- Virtually zero phototoxicity against non-target cell lines
- Selective targeting of cancer cells in two different animal models of prostate tumours *in vivo*, with much higher selectivity for cancer cells than the PS on its own
- Significant tumour regression and cure in animal models

- Fewer side effects as *in vivo* clearance is far superior to free photosensitiser, resulting in high therapeutic indices (6)

Prosalite therefore confirms the reasoning behind the OptiLink technology platform – namely, that the combination of an sc-Fv with effective PS spacing ensures an optimum targeted PDT effect. The technology is, however, highly versatile with potential applications beyond PDT. Thus, besides PS molecules, it is possible to covalently link existing chemotherapeutic agents to lysine-bioengineered scFvs via cleavable linkers attached to the lysine moieties, and we are actively pursuing this strategy.

Targeted Contrast Agents for MRI

OptiLink technology can also be used to synthesise targeted magnetic resonance imaging (MRI) diagnostic agents. The contrast agents currently used in MRI – for example, those containing the paramagnetic metal ion gadolinium III – lack sensitivity because they cannot be targeted specifically to organs in the body under investigation (7). After imaging, they accumulate in the kidneys where their gadolinium content may lead to nephrotoxicity. OptiLink can overcome these problems.

Inserting paramagnetic metal ions into photosensitiser molecules converts them from light-activated cancer therapeutic moieties into potential MRI contrast agents. After conjugating them to an scFv via OptiLink technology, the resulting multiply-loaded scFv becomes a targeted contrast agent for MRI imaging. Initial tests demonstrate that, even with the less magnetically susceptible metal ion manganese (III), the multiply-loaded scFv has a contrast signal at least

comparable with a standard MRI (Omniscan) contrast agent containing Gd(III) (see Figure 2).

Theranostic Potential

Ultimately, the OptiLink platform will enable multiple loading of scFv targeting moieties with both therapeutic and MRI contrast agents, providing a possible pipeline of novel targeted theranostic (8) or 'see-and-treat' agents. This will allow virtually simultaneous imaging and treatment of tumours – improving the efficacy and tolerability of cancer treatment, significantly shortening tumour treatment times and improving quality of life for cancer patients.

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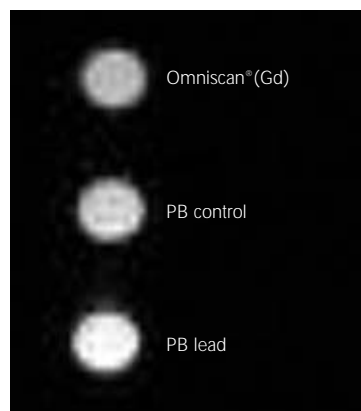


Figure 2: T1-weighted MR-imaging of a PhotoBiotics MRI agent compared with control samples at the same concentration. The PhotoBiotics sample produces a brighter image. The advantages here are that the scFv multiply-loaded MRI contrast agent is targetable and has potentially more favourable pharmacokinetics/dynamics compared with standard contrast agents; the OptiLink platform ensures that the individual paramagnetic entities on the scFv do not magnetically interfere with one other, thus significantly enhancing their performance.



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