Targeted Treatment

The use of antibody-drug conjugates has become particularly prevalent in cancer therapy, although their development can be problematic in their execution. However, aptamers can be used as alternative affinity ligands to overcome these drawbacks

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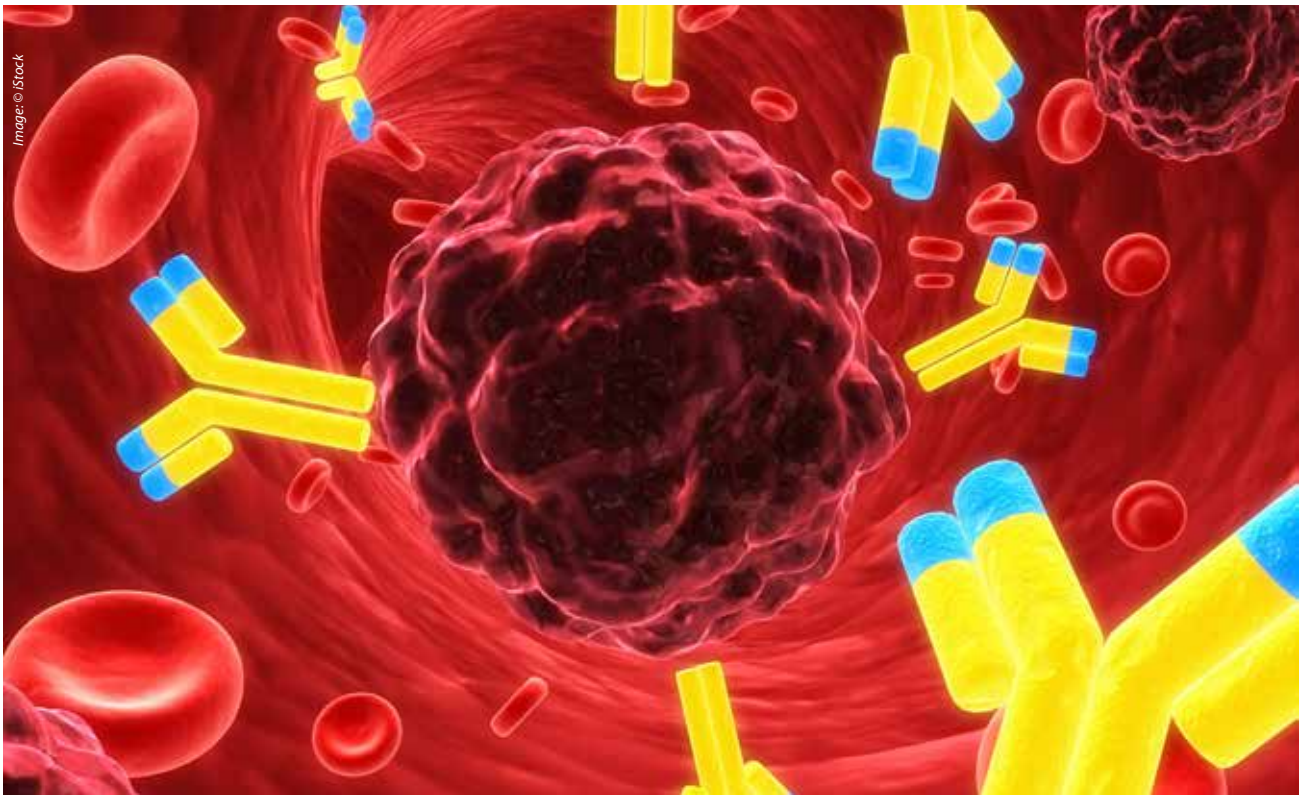

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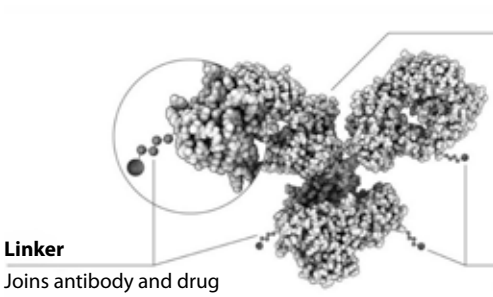
The goal of therapy is to treat infected/diseased tissues and leave healthy tissues unharmed. Unfortunately, therapeutics often have side effects due to off-target interactions, as is evident with chemotherapeutics used to treat cancer. In conditions with high mortality rates (such as cancer), patients are often willing to accept side effects to maximise the effect of treatment on the cancer. However, these off-target effects could be reduced if the drug can be more effectively delivered to the diseased tissues. Examples of this targeting have been achieved through the use of antibody-drug conjugates (ADCs) (see Figure 1, page 39).

With the obvious benefits of this targeting approach, it may be surprising to learn that, to date, only four ADCs have received FDA approval – the first of which (Gemtuzumab ozogamicin) was approved in 2001 for the treatment of acute myelognous leukaemia. Several factors have limited ADC progress, which largely revolve around issues with the antibody component. Firstly, antibodies themselves are part of the immune system, and even minimised fragments used in more recent ADC developments are still potentially immunogenic. This can lead to immune-related side effects. Another issue with ADCs is their relatively poor tissue penetration in solid tumours, although this is less problematic for smaller, recombinant antibody fragments. This problem can also be compensated for by linking highly potent drugs, however, this can lead to elevated risk of vascular leak syndrome (2-3).

Isolating targeting antibodies requires painstaking research to identify and produce a suitable cell surface antigen. This antigen should be uniquely expressed in the diseased tissue or at least expressed in significantly elevated levels, relative to healthy tissue. One example is HER2 – a cell surface receptor, present in approximately 15-30% of breast cancers and targeted with the ADC Trastuzumab emtansine. Once the target protein is identified and produced, a meticulous process of antibody generation and screening is required to isolate the best performing monoclonal antibodies. As these antibodies are typically isolated from rodents, 'humanising' the antibody is necessary to limit recognition of the ADC by the patient's immune system and subsequent clearance from the blood stream (4).



Antibody
- Targets cancer cells with high specificity
- Targeted at a well-defined tumour antigen with limited expression in healthy tissues

Linker
Joins antibody and drug
- Can be cleavable or noncleavable
- Good stability in the circulation
- Timely and targeted release of the cytotoxin

Cytotoxic agent
Destroys cancer cells
- Highly potent
- Permits linker attachment
- Defined mechanism of action

Source: Mehrling T and Soltis D

Figure 1: General structure of an ADC, highlighting the three core components (antibody, linker, and cytotoxic agent) and their respective properties/function

Suitable antibodies must then undergo an extensive programme of 'reformatting' to create the final ADC. This is, by no means, a trivial task. Several factors need to be carefully optimised, including:

- The nature of the drug conjugate, its mode of action, potential off-target effects, etc
- The linker used to attach the drug needs to be stable in the circulation (to prevent 'early release' of the conjugate), but labile enough to efficiently release the payload once the target is reached. It is also important that the linker does not inactivate the drug
- The position of drug attachment must not hinder the interaction between the antibody and antigen. This conjugation typically utilises either cysteine or lysine residues, which limits the number of drug molecules that may be attached and the ADC's efficacy
- Finally, the antibody:drug ratio (ADR) must be optimised to deliver an appropriate amount of drug without hindering the antibody interaction. Overloading with the drug can reduce the efficacy of the ADC (5). This can be mitigated to an extent by engineering the antibody to include specific sites for drug conjugation, as well as optimising the ADR (6). However, the point of attachment can still affect the ADC stability and pharmacokinetics (7)

To reduce the time taken for ADC development, variants of each component (antibody, drug, and linker) are assessed in parallel and optimised in extensive screens to identify the best candidates. These then serve as a platform for subsequent refinement to identify the optimal configuration for the final ADC molecule.

When considering the scale of R&D, refinement, re-engineering, and reformatting required throughout ADC development, perhaps it is more surprising that four ADCs have made it to the clinic. An alternative approach would clearly be beneficial.

In recent years, a number of antibody alternative technologies have been developed to overcome the limitations associated with antibodies. Among the most promising are nucleic acid-based affinity ligands, called aptamers. Unlike antibodies, clinical trials have demonstrated that aptamers have low inherent immunogenicity, making them excellent candidates for therapeutic development (8). They are isolated from degenerate combinatorial libraries, using *in vitro* selection methods. These animal-free selection methodologies allow aptamers to be isolated against a wider variety of targets, including whole cells, tissues, and even microorganisms (9). In targeted drug delivery, this gives aptamers a significant advantage, as they can be isolated against a number of cell (or tissue) surface proteins in parallel, in a hypothesis-free manner, without the need to identify, isolate, or characterise the target protein(s). This bypasses a significant portion of the ADC development pipeline. As aptamers are readily prepared by synthetic chemistry approaches, aptamer drug conjugate (ApDC) development is also simpler (10).

Due to the advantages of aptamers through the *in vitro* isolation processes, synthetic nature, low batch-to-batch

"Aptamers have an additional advantage as they are typically 5-10-fold smaller than antibodies, allowing greater tissue penetration and cellular uptake"

Aptamer name	Company/sponsor	Target	Indication	Clinical stage
Macugen (Pegaptanib)	Eyetech Pharmaceuticals/Pfizer	VEGF-165	AMD diabetic retinopathy	Approved Phase 3
E10030	National Eye Institute	PDGF-B	Von Hippel-Lindau Syndrome	Phase 2
ARC1905	Ophthotech Corp Archemix Corp	C5	Age-related macular degeneration	Phase 1
Zimura	Ophthotech Corp	C5	Idiopathic polypoidal choroidal vasculopathy	Phase 2
NU172	ACRA Biopharma Archemix Corp	Thrombin	Acute coronary artery bypass surgery	Phase 2
REG-1 (RB006/RB007)	Regado Biosciences Archemix Corp	Factor IXa	Percutaneous coronary intervention	Phase 2
NOX-A12	NOXXON Pharma	SDF-1α	Lymphoma	Phase 2
NOX-A12	NOXXON Pharma	SDF-1α	Multiple myeloma	Phase 2
NOX-A12	NOXXON Pharma	SDF-1α	Metastastic colorectal cancer and Pancreatic cancer	Phase 1/Phase 2
NOX-A12	NOXXON Pharma	SDF-1α	Autologous stem cell transplantation	Phase 1
NOX-E36	NOXXON Pharma	CCL2	Type 2 diabetes and diabetic nephropathy	Phase 2
NOX-E36	NOXXON Pharma	CCL2	Renal impairment	Phase 2
NOX-H94	NOXXON Pharma	Hepcidin	Anaemia	Phase 2
AS1411 (AGRO001)	Antisoma Archemix Corp	Nucleonin	Acute myeloid leukaemia	Phase 2

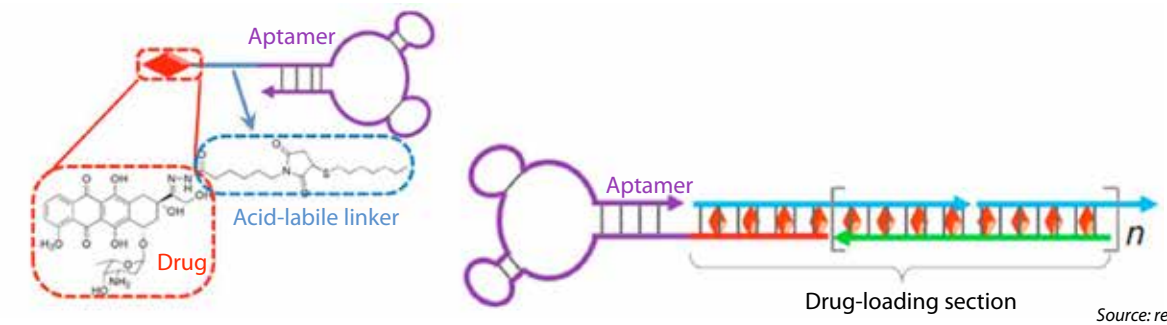
Source: reference 11

Table 1: Aptamer-based therapeutics in clinical trials as of May 2018

variability, broad array of targets, and wide-ranging applications, they have been rapidly adopted by researches from academia and industry alike. Several commercial organisations have also emerged to exploit the technology, including Aptamer Group (UK), AptiSci (South Korea), BasePair Biotechnologies (US), Noxxon Pharma (Germany), and SomaLogic (US), producing aptamers for diagnostic, therapeutic, or research applications. Aptamer selection has also lead to an FDA-approved therapeutic (Macugen™). Several other aptamer-based therapies are currently in preclinical development or clinical trial phases (see Table 1).

The Advantages of Aptamers

Hypothesis-free, *in vitro* selection of cells or tissue targeting aptamers, coupled with synthetic production processes, greatly simplify ApDC development. A comparable range of drug conjugates, linker chemistries, etc are available for use with aptamers. Unlike antibodies, the site of attachment to the aptamer can be controlled, eliminating the possibility that the conjugate will hinder target binding (see Figure 2, page 42). It is also possible to append the aptamer with a nucleic acid 'tail' (or other branched linker molecule).



Source: reference 10

Figure 2: ApDCs can be prepared in a similar format to ADC by attaching the drug(s) to the aptamer through a linker (left). Aptamers may also be appended with an oligonucleotide tail, which may be loaded with compounds capable of intercalating with DNA (right)

These may be loaded with the required number of drug conjugates, greatly simplifying aptamer:drug ratio optimisation (see Figure 2).

It is also possible to prepare the aptamer library with the drug and linker conjugate in place, then directly select the aptamer with the drug *in situ*. This ensures that the resulting ApDC will recognise its target.

Aptamers have an additional advantage as they are typically 5-10-fold smaller than antibodies, allowing greater tissue penetration and cellular uptake. The relative simplicity of re-engineering an existing aptamer to create an ApDC has been highlighted in several examples designed using pre-existing aptamers known to target a specific cell line. In one example, an aptamer against prostate-specific membrane antigen (PSMA) was adapted to specifically deliver the cytotoxic agent doxorubin to a PSMA positive cell line (LNCaP), but not to a PSMA negative cell line (PC-3) (12).

Doxorubicin was also covalently attached to an anti-tyrosine kinase 7 aptamer. The conjugate was released upon reaching the acidic environment of the endosome, in the target leukemic cells (13). Another aptamer (against a Burkitt's lymphoma cell line) was conjugated to the photodynamic ligand, chlorin e6 (14). The aptamer delivered chlorin to the target cells, which were then killed upon exposure to specific wavelengths of light. Several other conjugate approaches are described in a review by Zhu *et al* (10).

Overcoming Aptamer Limitations

Although aptamers show numerous advantages in speed and simplicity of ApDC development, clinical studies have highlighted limitations that must be overcome to expand aptamer use as therapeutics (8). Aptamers' sizes may give them increased tissue and cell penetration; it also means that, without additional modification, they are readily degraded and cleared from the body (15). Methods have been employed to optimise the pharmacokinetics, eg PEGylation, but this can be counter-productive (16-17). Therefore, alternative approaches are being explored.

While ADCs and ApDCs have considerable potential to reduce off-target effects and improve the efficacy of new and existing drugs, the relative simplicity and speed of ApDC development will likely lead to an increase in the clinical application of this technology. To date, most reported ApDCs have been developed by re-engineering existing aptamers. Next generation ApDCs, developed specifically for purpose, are required and highlight a need for novel aptamer selection strategies to overcome current limitations.

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About the authors

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Dr David Bunka is the Chief Technical Officer at Aptamer Group and holds a PhD in molecular biology from the University of Leeds, UK. He spent 12 years developing high throughput automated aptamer selection methods at the university and built up a sold international reputation in the field. Since 2003, David has isolated >300 aptamers against a wide variety of targets including: small molecule antibiotics, food contaminants, disease-associated proteins, several cancer-associated cell lines, viruses, and patient tissue samples. Since joining the group officially in 2012, David has authored several aptamer papers in peer reviewed journals, including invited review articles and a book chapter in 2012 for the Royal Society of Chemistry entitled "Therapeutic Uses of Nucleic Acid Aptamer Conjugates".
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Dr Arron Tolley is the Chief Executive Officer of Aptamer Group and holds a PhD in molecular biology and biophysics from the University of Leeds, UK, and a BSc in molecular medicine. Arron has several years' experience raising aptamers against complex cellular targets and model disease systems. He has developed several aptamer panels against model cell lines associated with oesophageal adenocarcinoma. Arron still plays an active role in the R&D side of the group and is credited with the invention and development of a new aptamer based biomarker discovery platform (Aptasort) to be offered to the life sciences industry through the aptamer group.
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