1 **Clinical Applications of Aptamers and Nucleic Acid Therapeutics in Haematological**

2 **Malignancies**

3 Sarah Shigdar[1*,] Alister C Ward[1], Abhijit De[2], Chaoyong James Yang[3], Mingqian Wei[4] and

4 Wei Duan[1, 5*]

5 1. School of Medicine, Deakin University, Pigdons Road, Waurn Ponds, Victoria, 3217,

6 Australia

7 2. Advanced Centre for Teaching Research Education in Cancer, Tata Memorial Centre,

8 Sector 22, Kharghar, Navi Mumbai-410210, Maharastra, India.

9 3. State Key Laboratory of Physical Chemistry of Solid Surfaces, The Key Laboratory for

10 Chemical Biology of Fujian Province, Key Laboratory of Analytical Science, Department of

11 Chemical Biology, College of Chemistry and Chemical Engineering, Xiamen University,

12 Xiamen 361005, China

13 4. Division of Molecular and Gene Therapies, School of Medical Science, Griffith University,

14 Gold Coast Campus, Southport, Qld 4215, Australia.

15 5. College of Laboratory Medicine, Dalian Medical University, Dalian 116044, Liaoning Province,
16 China.
17

18 Running title: Nucleic Acid Therapeutics and Haematological Malignancies

19 *Corresponding authors: Sarah.Shigdar@deakin.edu.au or

20   Wei.Duan@deakin.edu.au

21 **Summary**

22 Haematological malignancies result from a heterogeneous mix of genetic mutations and

23 chromosome aberrations and translocations. Targeted therapies, such as the anti-CD20

24 antibody rituximab, or the BCR-ABL inhibitor imatinib, have proven to be effective

25 treatments in the management of some of these malignancies, though relapsing or refractory

26 disease is still common. Nucleic acid-based therapies have also entered the clinical arena,

27 providing an alternative, complementary approach. The forerunner of these therapies were

28 the antisense oligonucleotides, but their scope has expanded to include siRNA, microRNA,

29 decoy oligonucleotides and aptamers. These can be used either as mono-therapeutics, in

conjunction with current chemotherapy regimens, or in combination with each other to improve therapeutic efficacy. Not only can these nucleic acid-based therapies silence target genes, they also have the potential of restoring gene function. While challenges remain in delivering effective doses of nucleic acid *in vivo*, these are steadily being met, suggesting an optimistic future in the treatment of haematological malignancies. This review summarises the application of nucleic acid-based therapeutics, particularly aptamers, in the diagnosis and treatment of haematological malignancies.

Keywords: Aptamers; nucleic acid-based therapeutics; oligonucleotides; RNAi; targeted therapeutics.

1   **Introduction**

2   Cancer treatment strategies continue to evolve, with new drugs reaching the marketplace each

3   year and patient survival data increasing steadily. Treatments are now based not only on the

4   histopathological diagnosis of the lesion, but also on its underlying molecular basis. The use

5   of non-specific radio- and chemotherapy that impacts on both healthy and cancerous cells is

6   gradually being replaced by a more targeted, and therefore less toxic, treatment strategies.

7   Traditionally, this has involved small chemicals or protein antibodies that target the proteins

8   underlying the development of the malignancies. However, there remains only a limited

9   number of 'targeted' treatments, and with patients still developing resistance to such

10   therapies, there is a pressing need for alternative targeted therapies. As an alternative, nucleic

11   acid approaches have been developed, which target the gene, the mRNA, or in the case of

12   aptamers, the encoded protein, and represent a promising strategy for combating cancer.

13   Table 1 provides an illustration of some of the nucleic acid drugs, along with a typical

14   antibody and a small molecule drug, used in clinics for the treatment of haematopoietic

15   malignancies.

16

17   **Watson-Crick Base Pairing Approaches**

18   **Antisense Oligonucleotides**

19   The first nucleic acid-based approaches were based on the discovery that a short strand of

20   complementary nucleic acid could inhibit the production of its specific gene product

21   (Paterson *et al* 1977, Gewirtz *et al* 1998). These antisense oligonucleotides (ASOs) represent

22   short single stranded sequences, usually 18-21 bases long, that bind to the complementary

23   sequence of a specific mRNA through Watson-Crick base pairing (Fig 1A). Following

24   binding, the ASO prevents translation in one of two ways. The first way is by binding to the

25   mRNA and interfering with its interactions with ribosomes without affecting mRNA stability.

26   The second way is by causing the degradation of the mRNA through endogenous nucleases,

27   such as RNase H, which are activated by dsRNA, leading to cleavage of the mRNA and

28   release of the ASO (Fig 2) (Vidal *et al* 2005, De Rosa *et al* 2010).

29

1   There have been numerous clinical trials using ASOs for a variety of disease processes, such

2   as infection, inflammation, cardiovascular disease and cancer. Not all have been effective, but

3   several are showing promise. One of these, Genasense (oblimersen) (Genta Inc), targets the

4   anti-apoptotic gene *BCL-2*, which is over-expressed in a wide variety of tumour types and is

5   associated with increased resistance to radiation and chemotherapy (Klasa *et al* 2002). In cell

6   lines and animal models, this ASO has been shown to decrease tumour Bcl-2 expression and

7   promote apoptosis (Vogl & Gewirtz 2008). Phase II/III clinical trials have been conducted

8   with oblimersen (through intravenous and subcutaneous routes of administration) in patients

9   with chronic lymphocytic leukaemia (CLL), acute myeloid leukaemia (AML), multiple

10   myeloma, non-Hodgkin's lymphoma and melanoma (Klasa *et al* 2002, Bhindi *et al* 2007,

11   Rayburn & Zhang 2008). Oblimersen has also been used in a number of combinations: with

12   dexamethasone for the treatment of relapsed or refractory multiple myeloma (phase III)

13   (NCT00017602); with fludarabine and cyclophosphamide (phase III: NCT00024440), or

14   fludarabine and rituximab in previously treated CLL patients (phase I/II: NCT00078234); or

15   with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone in patients with

16   stages II to IV diffuse large B-cell lymphoma (phase I: NCT00736450) (phase II:

17   NCT00080847). When combined with fludarabine/cyclophasphamide in a phase III trial,

18   oblimersen has shown a significant 5-year survival benefit (O'Brien *et al* 2009).

19

20   A number of clinical trials are being conducted with ASOs directed to other genes. These

21   include an ASO targeting *C-MYB*, a proto-oncogene encoding a nuclear binding protein that

22   plays a major role in cell cycle regulation in haematopoietic cells. This ASO has been

23   combined with chemotherapy and bone marrow transplantation in chronic myeloid leukaemia

24   (CML) patients (phase II: NCT00002592), and also in patients with advanced haematological

25   malignancies (phase I: NCT00780052) to purge the *ex vivo* bone marrow stem cells. It has

26   been shown to suppress *C-MYB* mRNA levels in approximately half of the patients studied

27   and to lead to complete cytogenetic remission post-transplantation (Vogl & Gewirtz 2008). A

28   second gene associated with apoptosis inhibition is the X-linked inhibitor of apoptosis

29   (*XIAP*). The overexpression of *XIAP* inhibits apoptosis caused by chemotherapy, radiation

30   and growth-factor deprivation through inhibition of caspase activity and its overexpression in

31   AML is associated with a poor clinical outcome. In xenograft models, the ASO inhibited

32   XIAP protein expression and enhanced chemotherapeutic activity (Vogl & Gewirtz 2008). In

33   addition, a phase I/II study testing the effectiveness of this ASO   combined with

chemotherapy for refractory or relapsed AML patients has recently been completed (NCT00363974), with a positive clinical response reported and limited side effects (peripheral neuropathy in < 4% patients) (Aegera Therapeutics) (Schimmer *et al* 2009). Enzon Pharmaceuticals Inc. have also tested the effectiveness of an ASO targeting the gene encoding the hypoxia-inducible factor-1α (anti-*HIF-1α*, EZN-2968). *HIF-1α* has been associated with cancer pathogenesis, with an increased level of the protein being associated with an increased risk of metastasis and/or a poorer prognosis in most solid tumours (Evens *et al*). This ASO was tested in patients with advanced solid tumours or lymphomas (Hodgkin's and non-Hodgkin's lymphoma) in a phase I clinical trial (NCT00466583) and was found to be well tolerated with prolonged stable disease observed in several patients though dose escalation studies are still ongoing (Patnaik *et al* 2009). Finally, clinical trials targeting Philadelphia chromosome positive CML, AML, CLL, acute lymphocytic leukaemia (ALL) and myelodysplastic syndrome (MDS) with a liposomal-delivered ASO against growth factor receptor bound protein-2 (L-Grb-2) (Bio-Path Holdings Inc.) have commenced. These aim to prevent specific protein translation and therefore inhibit the growth of leukaemic cells (NCT01159028). A phase II trial is also planned which will combine an anti-p53 ASO (Cenersen) (Eleos Inc.) to sensitise AML stem cells with conventional chemotherapy (idarubicin and cytarabine) in order to improve the complete response rate in AML patients 55 years of age or older who have previously shown no response (NCT00967512).

RNAi

Since its discovery in 1998, RNA interference (RNAi) has emerged as an alternative to anti-sense oligonucleotides for switching off cancer-causing genes (Fire *et al* 1998). The short double-stranded RNAs are processed by Dicer and incorporated into an RNA-induced silencing complex (RISC). The sense strand is cleaved during the formation of this complex and the anti-sense strand guides the RISC to the complementary target mRNA, which is cleaved by RISC (Fig 2) (Bhindi *et al* 2007). Several types of RNAi are commonly employed, including short-interfering RNA (siRNA) (Fig 1B), short-hairpin RNA (shRNA) and micro RNA (miRNA) (Fig 1C). All these are able to inhibit the conversion of mRNA into protein (Rayburn & Zhang 2008). One key advantage of RNAi is that each double-stranded RNA can be used multiple times once inside the cells to degrade its target mRNA.

Therefore, siRNA has been shown to be more effective at low nanomolar doses (10 to 200 nM) in vitro, whereas ASOs require much higher doses (200 to 900 nM) to be effective (Ramon & Malvy 2010).

Short-interfering RNAs seem particularly useful in targeting the chromosomal aberrations frequently seen in leukaemias and lymphomas (reviewed in Hexner & Gewirtz 2005). For example, *in vitro* studies have used siRNA to target the *BCR-ABL* oncogene generated by the Philadelphia chromosome (Ph) translocation t(9;22), associated with CML and Ph$^+$ ALL. When primary haematopoietic cells from CML patients were transfected with this siRNA, *BCR-ABL* mRNA levels were down-regulated (Huang & Yang 2010). In addition to providing an effective growth inhibitory effect, induction of apoptosis was also observed. Similarly, the targeting of MLL-AF4 (the result of a t(4;11) translocation associated with a poor prognosis in infant ALL) resulted in reduced mortality in a SCID mouse xenograft model, suggesting that targeted therapy of this translocation may lead to highly specific and effective treatment of this form of leukaemia (Thomas *et al* 2005). Finally, an siRNA against the *AML1/ETO* fusion gene resulting from the t(8;21) translocation prevalent in AML M2 led to a robust downregulation of the corresponding mRNA (Peer 2010). To date, however, no human clinical trials have been published using siRNAs for the treatment of leukaemias or lymphomas.

MicroRNAs (miRNA) are naturally occurring RNAs that are cleaved from 70-100 nucleotide hairpin precursors to a mature 18-25 nucleotide sequence by a complex protein system that includes Dicer, as well as RNase III Drosha (Fig 1C). Mature miRNA exert their regulatory effects either by messenger silencing or translation inhibition (Li *et al* 2009, Marcucci *et al* 2011). Most miRNAs do not silence their own loci, but instead silence other genes, which is attributable to the nuclear processing of the miRNA precursor RNAs, coupled with nuclear exclusion of mature effector miRNAs (Carthew & Sontheimer 2009). The role of miRNA in the pathogenesis of leukaemias and lymphomas and their utility in providing prognostic information as well as their potential for targeted therapies is increasingly being recognised (Yendamuri & Calin 2009, Auer 2011, Marcucci *et al* 2011). The binding of miRNA conforms to the same Watson-Crick base pairing rules as ASOs and siRNA, which provides a simple strategy for designing anti-miRNA oligonucleotides (AMOs) that can block the

interactions between miRNA and their target mRNAs through competitive binding (Li *et al* 2009). For example, the miRNAs mir-let7b and mir-9 are down-regulated in leukaemic patients with favourable cytogenetic profiles, such as t(8;21), t(15;17) or inv(16), and upregulated in patients with a poorer prognosis. These miRNAs could be targeted with AMOs with the aim of improving the prognosis in the latter cohort (Marcucci *et al* 2011).

However, miRNAs can also act as tumour suppressors, as they are generally down-regulated during tumour development. It has, therefore, been suggested that restoring such tumour-suppressive miRNAs would have a detrimental effect on tumour growth, with recent studies supporting this hypothesis. Two miRNAs that are often deleted in CLL are mir-15 and mir-16, both of which target the anti-apoptotic gene *BCL-2*. Transfection of a construct expressing both of these miRNAs into CLL cell lines resulted in a reduction in Bcl-2 protein levels, as well as an increase in apoptosis (Li *et al* 2009). In addition, mir-181a overexpression has been associated with a higher complete remission rate, longer overall survival and a trend for longer disease-free survival in cytogenetically-normal AML patients. Therefore, delivering mir-181a directly to leukaemia cells, or using agents capable of increasing endogenous levels of mir-181a, may provide a therapeutic benefit (Marcucci *et al* 2011). It has also been suggested that combining miRNAs that target oncogenes, with miRNAs that restore tumour suppressor ability would provide a more favourable clinical outcome (Yendamuri & Calin 2009). We await with anticipation the results of relevant antiviral studies and clinical trials.

Decoy Oligonucleotides

Decoy oligonucleotides (DOs) are short double-stranded DNA molecules that competitively bind specific transcription factors with high affinity and specificity (Fig 1D) (Bhindi *et al* 2007). This leads to reduced occupancy of the transcription factor DNA-binding site in the promoter region of target genes, thereby reducing their expression (Fig 2) (De Rosa *et al* 2010). Successful design of DOs only requires knowledge of the consensus binding sequences of a particular transcription factor (Mann 2005, Penolazzi *et al* 2006). One example is the NF-κB pathway, which is constitutively activated in most malignant haematological disorders (Panwalkar *et al* 2004). DOs directed against this family of

transcription factors have been used to target a number of disorders as well as cancer cell lines (Keutgens *et al* 2006, Laguillier *et al* 2007, Ramon & Malvy 2010). Indeed, when a DO targeting the nonsymmetric NF-κB binding site was used in an animal model, it induced apoptosis in osteoclasts, indicating a potential for treatment of tumour-associated osteolytic metastases (Penolazzi *et al* 2006), a condition commonly associated with multiple myeloma (Mundy 2002). Moreover, *in vitro* studies using a lymphoblastoma cell line showed enhanced apoptosis and reduced cell growth when targeted with an NF-κB DO (Lesage *et al* 2003). STAT3 is known to be activated in a number of haematological malignancies and a DO targeting STAT3 has recently been shown to have no adverse toxicity (Sen *et al* 2009). Indeed, a STAT3 DO has been tested in phase I clinical trials in patients with relapsed or refractory non-Hodgkin's lymphoma or multiple myeloma (NCT00511082) and advanced leukaemia of MDS (NCT01029509). Interestingly, a complex DO has been designed in which multiple *cis* elements have been engineered into a single oligonucleotide to target NF-κB, E2F, and STAT3 which could inhibit tumour growth in a murine model (Gao *et al* 2006). Recently, a STAT5 DO has been shown to suppress leukaemic cell growth and induce apoptosis in a *BCR-ABL*-positive cell line, potentially leading to a novel therapeutic approach in the treatment of imatinib-resistant CML (Wang *et al* 2011). Once again, there is great promise, but much more is required before these agents appear in the clinic.

Shape-Specific Recognition

Aptamers

Aptamers are also nucleic acids (DNA or RNA), but differ from the previously mentioned nucleic acid species in that they bind to their target(s) in a manner similar to antibodies, with their tertiary and quaternary structure – rather than primary sequence – being important (Fig 1E) (Rayburn & Zhang 2008). As such, they are also referred to as chemical antibodies. Through internal base pairing, aptamers form complex shapes in association with their target molecule with a high affinity (Fig 2). While unmodified RNA aptamers are more susceptible to hydrolysis, they do provide a much higher level of structural diversity than DNA aptamers (Hermann & Patel 2000, Orava *et al* 2010). Aptamers can be generated completely *in vitro* and then rapidly produced by chemical synthesis in a form that is relatively stable and easy to modify (Das *et al* 2009). Moreover, due to their low or non-immunogenic nature, small size and simple chemical structure, aptamers have advantages over monoclonal antibodies, which

are inherently immunogenic due to their protein nature (Warzocha & Wotowiec 1997, Rayburn & Zhang 2008). Aptamer binding can inhibit the biological activity of its target, including blocking either the catalytic site in the case of enzymes, or the ligand recognition site in the case of receptors, or induce loss-of-function conformational changes (Missailidis & Hardy 2009, Ulrich & Wrenger 2009). The latter effect is quite common, possibly because the active site on the protein contains more exposed heteroatoms for hydrogen bonding and other interactions. Alternatively, aptamers may have a limited number of possible interactions with a target protein, and so aptamers that fit into a crevice on the protein, such as its active site, could be more likely to be selected (Keefe *et al* 2010).

Aptamers are produced by a process known as the systematic evolution of ligands by exponential enrichment (SELEX) (Ellington & Szostak 1990, Tuerk & Gold 1990), which can produce either DNA or RNA species that bind specifically to the target of interest. This method begins with a large random library of either DNA or RNA sequences, with fixed sequences at either end to allow for exponential amplification via PCR between selection cycles. The library is incubated with the target molecule of interest and unbound or weakly bound species are washed off, before either PCR or reverse transcription PCR is performed on the binding species, thus generating a restricted pool of species for further selection. By means of a Darwinian selection process, after 6–15 cycles a small pool of high affinity binders are produced (see Fig 3). These can then be modified, either for imaging, or improved half-life for therapeutic strategies (Stoltenburg *et al* 2007). Several variations on the SELEX cycle have been developed with one modification using live cells for selection to generate aptamers targeting leukaemic cells. In this case, no prior knowledge of the target is required though suitable negative cells lines are required for this type of selection (Phillips *et al* 2008).

The anti-nucleolin aptamer, AS1411, was the first nucleic acid based aptamer approved for Phase I clinical testing for the treatment of cancer in humans. Nucleolin is an abundant cell surface receptor that has been associated with survival, growth and proliferation of cells, nuclear transport, transcription, packing and transport of rRNA, replication and recombination of DNA. It is also associated with a poor clinical prognosis for some cancer types (Vorhies & Nemunaitis 2007). Indeed, nucleolin overexpression has previously been shown to stabilise the expression of Bcl-2 in CLL cells (Otake *et al* 2007). Using an *in vivo*

xenograft model, treatment with AS1411 resulted in initial cytostasis, followed by induction of apoptotic markers and cell death, while AS1411 in combination with gemcitabine, has shown enhanced anti-tumour activity than either AS1411 or gemcitabine alone (Vorhies & Nemunaitis 2007).

An increasing number of pre-clinical studies using aptamers to target haematological malignancies are being reported. For example, the CD33 aptamer has been shown to be efficiently internalised by CD33^{+} myeloid cell lines and has the potential to deliver chemotherapeutic drugs to CD33^{+} adult and paediatric AML patients, as well as the 15-25% of ALL patients that are CD33^{+} (Orava *et al* 2010). Aptamers that specifically target AML cells have also been successfully generated, with recognition of AML-M2, AML-M5 and biphenotypic B myelomonocytic leukaemia (Sefah *et al* 2009). The same group have also generated aptamers targeting T-ALL cells that not only bind specifically to cells from leukaemic patients, but were also able to distinguish molecular differences among patients with the same diagnosis (Shangguan *et al* 2007). Another aptamer generated by this group that was unstable at 4°C was modified to bind to cell surface B-cell receptor (BCR), which is exclusively expressed on B-cells and most B-cell lymphomas and on neoplastic B-cells, making this a potentially highly useful therapeutic or drug delivery agent (Tang *et al* 2007, Mallikaratchy *et al* 2010).

Challenges Faced by Nucleic Acid Delivery

There remains a number of challenges associated with nucleic acid-based approaches. These include off-target effects, issues regarding efficacy, side effects and site-specific delivery (Rayburn & Zhang 2008). Nucleic acid-based strategies also need to combat instability and unfavourable pharmacokinetic profiles *in vivo* (Gewirtz 2007). Nucleic acids are substrates of endo- and exonucleases present not only in the cell, but also in the extracellular space and are rapidly cleared from the blood. Selecting for a target using unmodified bases leads to the production of aptamers with half-lives of minutes to hours before rapid clearance by the kidneys.

1   Modifying the nucleic acids sufficiently to produce a longer half-life is the first requirement

2   for effective therapeutic delivery, and several modifications have been developed which

3   increase their serum half-life (De Rosa *et al* 2010). These can include base, sugar or

4   phosphate substitutions, preventing exo- and endonuclease degradation, or capping of the 3'

5   or 5' ends of the nucleic acid to prevent exonuclease activity (Wilson & Keefe 2006). Given

6   that these modifications can have an effect on the tertiary structure of the nucleic acid,

7   modifications need to be made prior to SELEX selection in the case of aptamers so as not to

8   affect their affinity or specificity to their target. Alternatively, the attachment of high

9   molecular mass polyethylene glycol (PEG) to the 5' end of the aptamer can increase the

10   blood circulation time of the aptamer (Keefe *et al* 2010). Indeed, systemic delivery of RNAi

11   has been achieved through the use of modifications that protects them from nuclease

12   degradation and allows effective biodistribution (Dykxhoorn 2009).

13

14   Another issue with the use of nucleic acids, due to their macromolecular and polyanionic

15   nature, is their inefficient internalisation by cells (Phillips *et al* 2008). In cultured cells, this

16   has been overcome by the use of transfection methods, such as electroporation, although

17   these approaches are not easily translatable to *in vivo* use (Bates *et al* 2009). Nucleic acids

18   can be actively transported across the membrane in a temperature dependent, saturable and

19   structurally specific manner. At high to moderate concentrations nucleic acids enter the cells

20   by fluid-phase endocytosis, whereas at low concentrations they enter via an absorptive

21   endocytosis. Moreover, once inside the endocytic pathway, the concentration may fall

22   dramatically if, due to lysosomal enzyme degradation, they are not released from the

23   endosomes and only a small percentage gain access to the cytoplasm. This suggests that high

24   doses, along with frequent and local administration, would be required to generate an

25   effective response (De Rosa *et al* 2010). In addition, most cells do not passively take up

26   RNAi, necessitating the need for assisted delivery (Gewirtz 2007). Therefore, aptamers,

27   which can be specifically optimised to be internalised into the cell via receptor-mediated

28   endocytosis, represent a valuable addition to the field of nucleic acid-based therapeutics.

29   Indeed, during the selection process, target binding aptamers can be preferentially selected

30   which are internalised. One way of achieving this is to conduct cell SELEX at 37°C to

31   promote internalisation and selecting for the internalised species, an approach used with the

32   human B-cell lymphoma cell line, Ramos, to produce aptamers which show a more efficient

uptake by lymphocytes from CLL patients than those from a non-selected random library (Wu *et al* 2003).

Off-target effects have also been associated with the use of ASOs, RNAi and DOs, especially for systemic delivery. However, more problematic is the off target silencing that occurs from RNAi degradation products, an effect which is increasingly being recognised as a source of unwanted medicinal side effects (Gewirtz 2007). The same toxicity concerns that compromise the use of siRNA apply to the use of miRNA as well. However, the fact that miRNA regulates multiple gene functions adds yet another layer to the complexity of using this for therapy. One miRNA, mir-155, effectively induces tumourigenesis, although its systemic delivery leads to a detrimental modulation of innate immune responses. These effects would necessitate packaging the miRNA into targeted nanoparticles, or viral vectors. It has been shown that lipid-based delivery of miRNA is efficient but induces an inflammatory response, while biodegradable polymers have a less pronounced effect on the inflammatory response, but also shorter effects and reduced delivery efficiency (Li *et al* 2009). This method of encapsulating nucleic acids inside nanoparticles has been used to deliver an ASO targeting the R2 subunit of ribonucleotide reductase, which has been shown to contribute to chemoresistance in AML cells. The nanoparticles were functionalised through the use of transferrin, shown to be overexpressed on cancer cells, including AML cells. This has been used on both cell lines (Kasumi-1 and K562 cell lines) and in pretreatment of unselected bone marrow blasts from AML patients. This ASO has been combined with cytarabine in a phase I trial with promising results, though the intracellular delivery required optimisation to attain a more efficient R2 down-regulation (Jin *et al* 2010).

It's Not Just a Nucleic Acid: Aptamer Conjugates

Systemic delivery would be advantageous and provide more clinical appeal, in spite of the above mentioned limitations. It is now becoming more common to link the siRNA to compounds, such as ligands or peptides to achieve target specificity and nuclease resistance, and thereby eliminate some of the non-targeted effects (Bhindi *et al* 2007). However, systemic delivery to leukocytes is thought to be even more challenging than to organs and tissues due to their dispersal throughout the body(Peer 2010). Aptamers represent a unique

alternative in their ability to specifically target cancer cells. Aptamers that target both extracellular ligands, as well as intracellular proteins have been developed. Those that target the extracellular domains of transmembrane receptor proteins can facilitate the entry of RNAi into cells via receptor mediated endocytosis (Fig 2(ii)) (Vorhies & Nemunaitis 2007, Syed & Pervaiz 2010). What makes aptamers such a perfect therapeutic is the difference between them and other nucleic acid therapeutics. Aptamers, by themselves, are not necessarily effective therapeutics. They can block their target function, and, if internalising aptamers are selected, they can prove to be much more effective as therapeutics through the direct conjugation of drugs or attaching nanoparticles as drug delivery devices. The choice of a suitable therapeutic target is governed by the need to target cancerous cells while leaving healthy cells intact. This is where both aptamers and RNAi come into their element. RNAi can be used to target disease-specific sequences within the cell, while the aptamer can guide the siRNA to the abnormal cell, thus minimising off-target effects. Aptamers have been directly conjugated to siRNA and exhibited a reduction in gene expression similar to that seen when the siRNA was administered to cells using oligofectamine (Chu *et al* 2006). This has benefits when considering the ability of RNAi to target the T315I point mutation in *Abl* which promotes imatinib-resistance. Through the use of gene silencing, it is possible to reverse this resistance (Hexner & Gewirtz 2005). If an aptamer were to be generated against an internalised cell surface marker present on CML cells, such as CD33, it would be possible to directly target these imatinib-resistant cells. This possibility highlights an additional advantage with wide ranging potential. To target the aberrant gene, such as *ABL*, or its mRNA, and thus prevent the abnormal protein, often resistant to protein-specific drugs, from being expressed could effectively control the disease (Hexner & Gewirtz 2005). More than 50% of all T-ALL express activating NOTCH-1 mutations, as well as nodal and cutaneous ALCL (Aifantis *et al* 2008, Zhao 2010). Preclinical evidence using siRNA targeting Notch-1 in a mouse xenograft model showed a reduction in tumour growth and a prolonged survival. This indicates a potential role for the aptamer targeting T-ALL conjugated to the siRNA silencing Notch-1. One approach yet to be attempted could be to combine the aptamer KH1C12 generated by Sefah and colleagues, which targets the AML cell line HL60, with either oblimersen, or an siRNA targeting bcl-2, and treating AML patients with all trans retinoic acid (ATRA), a combination shown to increase the effectiveness of ATRA (Hu *et al* 2008, Sefah *et al* 2009).

Aptamers can also be either directly conjugated to an active chemotherapeutic drug or used in the coating of a vesicle such as a liposome encapsulating the drug (Das *et al* 2009). Indeed, the attachment of a targeting ligand against internalising receptors or antigens at the surface of liposomes has been shown to increase their transfection efficiency *in vitro*, and through the selection of lipid-based carriers that are not positively charged, increased *in vivo* delivery (Sapra *et al* 2005). Moreover, siRNA could be encapsulated into a liposome coated with the aptamer, an approach already used in the Tan lab to deliver targeted drug therapy using their sgc8 aptamer (Fig 2 (iii)) (Kang *et al* 2010). One aptamer that binds to its target but does not affect cell function is the CD30 aptamer (Zhang *et al* 2009). This aptamer has since been conjugated to a nanocomplex carrying ALK targeting siRNA, and has been shown to be efficiently internalised by ALCL cells *in vitro* (Zhao *et al* 2011). The t(2;5) translocation, resulting in the NPM/ALK fusion protein, is present in approximately 75% of all paediatric cases of ALCL. Studies using siRNAs spanning the NPM/ALK fusion site has shown a remarkable downregulation of NPM/ALK as assessed by immunohistochemistry (Damm-Welk *et al* 2003, Peer 2010). If this were to be combined with a CD30 aptamer, there is a potential for treatment of systemic ALCL that currently has a poor prognosis (Benner *et al* 2009). Another aptamer that has been used to functionalise nanoparticles is that targeting T-ALL, with this complex being rapidly internalised into the cell and facilitating delivery of small molecular weight drugs (Kang *et al* 2010). This aptamer, sgc-8, has been covalently conjugated to anthracycline chemotherapeutic agent doxorubicin, leading to rapid internalisation and escape from the endosomal compartment in an *in vitro* study (Huang *et al* 2009).

What can we expect from the future?

The studies described highlight the great potential of nucleic acids as targeted therapeutics. But what lies over the horizon? It has already been noted that combining nucleic acids with conventional chemotherapy can reverse some of the drug resistance that has a detrimental effect on patient prognosis and survival. Within the last five years, a number of trials have been submitted to the U.S National Institute of Health. These include the investigation of specific up- or down-regulation of miRNA in AML (miRNA 34a and miRNA194 (NCT01057199) and miR34a, miR538e, miR193e, miR198 (NCT01298414)); CD9 targeted miRNA deregulation in TEL/AML-1 positive ALL (NCT01282593); global expression

patterns of deregulated miRNA in ALL or AML (NCT 00896766 and NCT00898092/NCT01229124, respectively); or the role of miRNA expression in determining clinical response to chemotherapy drugs in T-cell lymphoma, refractory AML or MDS patients, or in multiple myeloma patients (NCT01129180, NCT00624936, NCT00907452, and NCT00639054, respectively). With these trials lasting anything from three months to five years, a wealth of data regarding the molecular pathways relating to drug resistance may soon be available, expanding the options for treatment significantly. With liposomal-encapsulated doxorubicin, a commonly used chemotherapeutic, as well as ASOs and one aptamer, already approved (Macugen) by the US FDA, it is a short step to putting these together. siRNA or ASO could be packaged into liposomes or other nanocarriers that specifically target cancerous cells through the use of internalising aptamers. Indeed, one of the outstanding advantages with aptamers is the ability to rapidly select and optimise a particular aptamer to a newly identified target, a process taking months rather than years. This would allow the integration of results of current clinical trials into new selection strategies and thus produce more effective second or third generation therapeutics. It is also becoming more widely recognised that while targeting one single oncogene may inhibit growth of cells or even induce apoptosis, advanced cancers have numerous pathways by which to evade treatment. Therefore, it is likely that it will be necessary to target multiple oncogenes or pathways to eradicate the cancer and lead to long-lasting disease-free survival (Rayburn & Zhang 2008). Indeed, one approach to silencing has been to use a cocktail of siRNA targeting various sites within the gene of interest (Hexner & Gewirtz 2005). Additionally, Gao *et al.* has suggested using complex DOs to replace the traditional drug cocktail, which would have the benefit of removing the undesirable drug interactions often seen with the complicated treatment regimens necessary to treat aggressive disease (Gao *et al* 2006). Again, the limitation to systemic delivery can be overcome through the use of functionalised nanocarriers. Moreover, the strategy is not solely restricted to gene silencing – through the use of specific miRNAs, there is potential to up-regulate specific proteins, such as those that would have an apoptotic effect on diseased cells.

Conclusions

Nucleic acid therapeutics are set to make a major contribution to the treatment of malignant disease. This is a field of increasing research priority and relevance. Indeed, the National

Institute of Health in the US has funded numerous projects in the field of aptamers: it awarded nearly $6 million in grants funding RNAi and miRNA in 2009 and recently another $1.6 million to research the role of miRNAs in cancer. Given the successes seen with siRNA and ASOs against specific gene aberrations, and the ability of aptamers to specifically target cancer cells, it is now timely to start combining these nucleic acid therapeutics to achieve even more effective treatments. The problem of off-target effects can be reduced by hiding non-specific nucleic acids inside nanoparticles that can be degraded once inside the cell to release their contents, or directly conjugating these to aptamers to reduce the off-target effects. Specific targeting will mean that nucleic acids targeting a critical pathway in malignancy that is shared by normal cells, or that had significant off-target effects, can now get a second chance as therapeutic treatments.

Acknowledgements

References

Aifantis, I., Raetz, E. & Buonamici, S. (2008) Molecular pathogenesis of T-cell leukaemia and lymphoma. *Nature Reviews Immunology,* **8,** 380-390.

Auer, R.L. (2011) The coming of age of microRNA for B cell lymphomas. *Histopathology,* **58,** 39-48.

Bates, P.J., Laber, D.A., Miller, D.M., Thomas, S.D. & Trent, J.O. (2009) Discovery and development of the G-rich oligonucleotide AS1411 as a novel treatment for cancer. *Experimental and Molecular Pathology,* **86,** 151-164.

Benner, M.F., Jansen, P.M., Meijer, C.J. & Willemze, R. (2009) Diagnostic and prognostic evaluation of phenotypic markers TRAF1, MUM1, BCL2 and CD15 in cutaneous CD30-positive lymphoproliferative disorders. *British Journal of Dermatology,* **161,** 121-127.

Bhindi, R., Fahmy, R.G., Lowe, H.C., Chesterman, C.N., Dass, C.R., Cairns, M.J., Saravolac, E.G., Sun, L.Q. & Khachigian, L.M. (2007) Brothers in arms: DNA enzymes, short interfering RNA, and the emerging wave of small-molecule nucleic acid-based gene-silencing strategies. *American Journal of Pathology,* **171,** 1079-1088.

Carthew, R.W. & Sontheimer, E.J. (2009) Origins and mechanisms of miRNAs and siRNAs. *Cell,* **136,** 642-655.

Chu, T.C., Twu, K.Y., Ellington, A.D. & Levy, M. (2006) Aptamer mediated siRNA delivery. *Nucleic Acids Research,* **34,** e73.

Damm-Welk, C., Fuchs, U., Wossmann, W. & Borkhardt, A. (2003) Targeting oncogenic fusion genes in leukemias and lymphomas by RNA interference. *Seminars in Cancer Biology,* **13,** 283-292.

Das, M., Mohanty, C. & Sahoo, S.K. (2009) Ligand-based targeted therapy for cancer tissue. *Expert Opinion in Drug Delivery,* **6,** 285-304.

De Rosa, G., De Stefano, D. & Galeone, A. (2010) Oligonucleotide delivery in cancer therapy. *Expert Opinion in Drug Delivery,* **7,** 1263-1278.

Dykxhoorn, D.M. (2009) RNA interference as an anticancer therapy: a patent perspective. *Expert Opinion on Therapeutic Patents,* **19,** 475-491.

Ellington, A.D. & Szostak, J.W. (1990) In vitro selection of RNA molecules that bind specific ligands. *Nature,* **346,** 818-822.

Evens, A.M., Sehn, L.H., Farinha, P., Nelson, B.P., Raji, A., Lu, Y., Brakman, A., Parimi, V., Winter, J.N., Schumacker, P.T., Gascoyne, R.D. & Gordon, L.I. (2010) Hypoxia-inducible factor-1 {alpha} expression predicts superior survival in patients with diffuse large B-cell lymphoma treated with R-CHOP. *Journal of Clinical Oncology,* **28,** 1017-1024.

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E. & Mello, C.C. (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature,* **391,** 806-811.

Gao, H., Xiao, J., Sun, Q., Lin, H., Bai, Y., Yang, L., Yang, B., Wang, H. & Wang, Z. (2006) A single decoy oligodeoxynucleotides targeting multiple oncoproteins produces strong anticancer effects. *Molecular Pharmacology,* **70,** 1621-1629.

Gewirtz, A.M. (2007) On future's doorstep: RNA interference and the pharmacopeia of tomorrow. *Journal of Clinical Investigation,* **117,** 3612-3614.

Gewirtz, A.M., Sokol, D.L. & Ratajczak, M.Z. (1998) Nucleic acid therapeutics: state of the art and future prospects. *Blood,* **92,** 712-736.

Griffiths-Jones, S. (2004) The microRNA registry. *Nucleic Acids Research,* **32,** D109-111.

Hermann, T. & Patel, D.J. (2000) Adaptive recognition by nucleic acid aptamers. *Science,* **287,** 820-825.

Hexner, E.O. & Gewirtz, A.M. (2005) RNA interference for treating haematological malignancies. *Expert Opinion on Biological Therapy,* **5,** 1585-1592.

Hu, H., Zhang, Y. & He, D. (2008) Study of Bcl-2 siRNA enhancement of sensitivity of HL-60 cells to all trans retinoic acid. *Chinese Journal of Clinical Oncology,* **5,** 118-121.

Huang, X. & Yang, Y. (2010) Gene therapy for leukemia and lymphoma. In: *Molecular Pathology of Hematolymphoid Diseases* (ed. by Dunphy, C.H.), Vol. 4, pp. 81-89. Springer US.

Huang, Y.F., Shangguan, D., Liu, H., Phillips, J.A., Zhang, X., Chen, Y. & Tan, W. (2009) Molecular assembly of an aptamer-drug conjugate for targeted drug delivery to tumor cells. *Chembiochem,* **10,** 862-868.

Jin, Y., Liu, S., Yu, B., Golan, S., Koh, C.G., Yang, J., Huynh, L., Yang, X., Pang, J., Muthusamy, N., Chan, K.K., Byrd, J.C., Talmon, Y., Lee, L.J., Lee, R.J. & Marcucci, G. (2010) Targeted delivery of antisense oligodeoxynucleotide by transferrin conjugated pH-sensitive lipopolyplex nanoparticles: a novel oligonucleotide-based therapeutic strategy in acute myeloid leukemia. *Molecular Pharmacology,* **7,** 196-206.

Kang, H., O'Donoghue, M.B., Liu, H. & Tan, W. (2010) A liposome-based nanostructure for aptamer directed delivery. *Chemical Commununications (Cambridge),* **46,** 249-251.

Keefe, A.D., Pai, S. & Ellington, A. (2010) Aptamers as therapeutics. *Nature Reviews Drug Discovery,* **9,** 537-550.

Keutgens, A., Robert, I., Viatour, P. & Chariot, A. (2006) Deregulated NF-kappaB activity in haematological malignancies. *Biochemical Pharmacology,* **72,** 1069-1080.

Klasa, R.J., Gillum, A.M., Klem, R.E. & Frankel, S.R. (2002) Oblimersen Bcl-2 antisense: facilitating apoptosis in anticancer treatment. *Antisense and Nucleic Acid Drug Development,* **12,** 193-213.

Konopleva, M., Tari, A.M., Estrov, Z., Harris, D., Xie, Z., Zhao, S., Lopez-Berestein, G. & Andreeff, M. (2000) Liposomal Bcl-2 antisense oligonucleotides enhance proliferation, sensitize acute myeloid leukemia to cytosine-arabinoside, and induce apoptosis independent of other antiapoptotic proteins. *Blood,* **95,** 3929-3938.

Kurreck, J. (2009) RNA interference: from basic research to therapeutic applications. *Angewandte Chemie International Edition English,* **48,** 1378-1398.

Laguillier, C., Hbibi, A.T., Baran-Marszak, F., Metelev, V., Cao, A., Cymbalista, F., Bogdanov, A., Jr. & Fagard, R. (2007) Cell death in NF-kappaB-dependent tumour cell lines as a result of NF-kappaB trapping by linker-modified hairpin decoy oligonucleotide. *FEBS Letters,* **581,** 1143-1150.

Lesage, D., Metelev, V., Borisova, O., Dolinnaya, N., Oretskaya, T., Baran-Marszak, F., Taillandier, E., Raphael, M. & Fagard, R. (2003) Specific covalent binding of a NF-kappaB decoy hairpin oligonucleotide targeted to the p50 subunit and induction of apoptosis. *FEBS Letters,* **547,** 115-118.

Li, C., Feng, Y., Coukos, G. & Zhang, L. (2009) Therapeutic microRNA strategies in human cancer. *AAPS Journal,* **11,** 747-757.

Mallikaratchy, P.R., Ruggiero, A., Gardner, J.R., Kuryavyi, V., Maguire, W.F., Heaney, M.L., McDevitt, M.R., Patel, D.J. & Scheinberg, D.A. (2010) A multivalent DNA aptamer specific for the B-cell receptor on human lymphoma and leukemia. *Nucleic Acids Research*.

Mann, M.J. (2005) Transcription factor decoys: a new model for disease intervention. *Annuals of the New York Academy of Sciences,* **1058,** 128-139.

Marcucci, G., Mrozek, K., Radmacher, M.D., Garzon, R. & Bloomfield, C.D. (2011) The prognostic and functional role of microRNAs in acute myeloid leukemia. *Blood,* **117,** 1121-1129.

Missailidis, S. & Hardy, A. (2009) Aptamers as inhibitors of target proteins. *Expert Opinion on Therapeutic Patents,* **19,** 1073-1082.

Mundy, G.R. (2002) Metastasis to bone: causes, consequences and therapeutic opportunities. *Nature Reviews Cancer,* **2,** 584-593.

O'Brien, S., Moore, J.O., Boyd, T.E., Larratt, L.M., Skotnicki, A.B., Koziner, B., Chanan-Khan, A.A., Seymour, J.F., Gribben, J., Itri, L.M. & Rai, K.R. (2009) 5-year survival in patients with relapsed or refractory chronic lymphocytic leukemia in a randomized, phase III trial of fludarabine plus cyclophosphamide with or without oblimersen. *Journal of Clinical Oncology,* **27,** 5208-5212.

Orava, E.W., Cicmil, N. & Gariepy, J. (2010) Delivering cargoes into cancer cells using DNA aptamers targeting internalized surface portals. *Biochimica et Biophysica Acta,* **1798,** 2190-2200.

Otake, Y., Soundararajan, S., Sengupta, T.K., Kio, E.A., Smith, J.C., Pineda-Roman, M., Stuart, R.K., Spicer, E.K. & Fernandes, D.J. (2007) Overexpression of nucleolin in chronic lymphocytic leukemia cells induces stabilization of bcl2 mRNA. *Blood,* **109,** 3069-3075.

Panwalkar, A., Verstovsek, S. & Giles, F. (2004) Nuclear factor-kappaB modulation as a therapeutic approach in hematologic malignancies. *Cancer,* **100,** 1578-1589.

Paterson, B.M., Roberts, B.E. & Kuff, E.L. (1977) Structural gene identification and mapping by DNA-mRNA hybrid-arrested cell-free translation. *Proceedings of the National Academy of Science U S A,* **74,** 4370-4374.

Patnaik, A., Chiorean, E.G., Tolcher, A., Papadopoulos, K., Beeram, M., Kee, D., Waddell, M., Gilles, E. & Buchbinder, A. (2009) EZN-2968, a novel hypoxia-inducible factor-1α (HIF-1α) messenger ribonucleic acid (mRNA) antagonist: Results of a phase I, pharmacokinetic (PK), dose-escalation study of daily administration in patients (pts) with advanced malignancies. *Journal of Clinical Oncology,* **27,** Suppl; abstr 2564.

Peer, D. (2010) Induction of therapeutic gene silencing in leukocyte-implicated diseases by targeted and stabilized nanoparticles: A mini-review. *Journal of Controlled Release*, 63-68.

Penolazzi, L., Magri, E., Lambertini, E., Calo, G., Cozzani, M., Siciliani, G., Piva, R. & Gambari, R. (2006) Local in vivo administration of a decoy oligonucleotide targeting NF-kappaB induces apoptosis of osteoclasts after application of orthodontic forces to rat teeth. *International Journal of Molecular Medicine,* **18,** 807-811.

Phillips, J.A., Lopez-Colon, D., Zhu, Z., Xu, Y. & Tan, W. (2008) Applications of aptamers in cancer cell biology. *Analytica chimica acta,* **621,** 101-108.

Ramon, A.L. & Malvy, C. (2010) Anticancer Oligonulceotides. In: *Macromolecular Anticancer Therapeutics* (ed. by Reddy, L.H. & Couvreur, P.), pp. 539-568. Springer, New York.

Rayburn, E.R. & Zhang, R. (2008) Antisense, RNAi, and gene silencing strategies for therapy: mission possible or impossible? *Drug Discovery Today,* **13,** 513-521.

Sapra, P., Tyagi, P. & Allen, T.M. (2005) Ligand-targeted liposomes for cancer treatment. *Current Drug Delivery,* **2,** 369-381.

Schimmer, A.D., Estey, E.H., Borthakur, G., Carter, B.Z., Schiller, G.J., Tallman, M.S., Altman, J.K., Karp, J.E., Kassis, J., Hedley, D.W., Brandwein, J., Xu, W., Mak, D.H., LaCasse, E., Jacob, C., Morris, S.J., Jolivet, J. & Andreeff, M. (2009) Phase I/II trial of AEG35156 X-linked inhibitor of apoptosis protein antisense oligonucleotide combined with idarubicin and cytarabine in patients with relapsed or primary refractory acute myeloid leukemia. *Journal of Clinical Oncology,* **27,** 4741-4746.

Sefah, K., Tang, Z.W., Shangguan, D.H., Chen, H., Lopez-Colon, D., Li, Y., Parekh, P., Martin, J., Meng, L., Phillips, J.A., Kim, Y.M. & Tan, W.H. (2009) Molecular recognition of acute myeloid leukemia using aptamers. *Leukemia,* **23,** 235-244.

Sen, M., Tosca, P.J., Zwayer, C., Ryan, M.J., Johnson, J.D., Knostman, K.A., Giclas, P.C., Peggins, J.O., Tomaszewski, J.E., McMurray, T.P., Li, C., Leibowitz, M.S., Ferris, R.L., Gooding, W.E., Thomas, S.M., Johnson, D.E. & Grandis, J.R. (2009) Lack of toxicity of a STAT3 decoy oligonucleotide. *Cancer Chemotherapy and Pharmacology,* **63,** 983-995.

Shangguan, D.H., Cao, Z.H.C., Li, Y. & Tan, W.H. (2007) Aptamers evolved from cultured cancer cells reveal molecular differences of cancer cells in patient samples. *Clinical Chemistry,* **53,** 1153-1155.

Stoltenburg, R., Reinemann, C. & Strehlitz, B. (2007) SELEX-A (r)evolutionary method to generate high-affinity nucleic acid ligands. *Biomolecular Engineering,* **24,** 381-403.

Syed, M.A. & Pervaiz, S. (2010) Advances in aptamers. *Oligonucleotides,* **20,** 215-224.

Tang, Z., Shangguan, D., Wang, K., Shi, H., Sefah, K., Mallikratchy, P., Chen, H.W., Li, Y. & Tan, W. (2007) Selection of aptamers for molecular recognition and characterization of cancer cells. *Analytical Chemistry,* **79,** 4900-4907.

1 Thomas, M., Gessner, A., Vornlocher, H.P., Hadwiger, P., Greil, J. & Heidenreich, O. (2005) Targeting
2   MLL-AF4 with short interfering RNAs inhibits clonogenicity and engraftment of t(4;11)-
3   positive human leukemic cells. *Blood,* **106,** 3559-3566.
4 Tuerk, C. & Gold, L. (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands
5   to bacteriophage T4 DNA polymerase. *Science,* **249,** 505-510.
6 Ulrich, H. & Wrenger, C. (2009) Disease-specific biomarker discovery by aptamers. *Cytometry A,* **75,**
7   727-733.
8 Vidal, L., Blagden, S., Attard, G. & de Bono, J. (2005) Making sense of antisense. *European Journal of*
9   *Cancer,* **41,** 2812-2818.
10 Vogl, D.T. & Gewirtz, A.M. (2008) Nucleic acid therapies for cancer treatment. In: *Molecular*
11   *Targeting in Oncology* (ed. by Kaufman, H.L., Wadler, S. & Antman, K.), pp. 413-429. Humana
12   Press.
13 Vorhies, J.S. & Nemunaitis, J.J. (2007) Nucleic acid aptamers for targeting of shRNA-based cancer
14   therapeutics. *Biologics,* **1,** 367-376.
15 Wang, X., Zeng, J., Shi, M., Zhao, S., Bai, W., Cao, W., Tu, Z., Huang, Z. & Feng, W. (2011) Targeted
16   blockage of signal transducer and activator of transcription 5 signaling pathway with decoy
17   oligodeoxynucleotides suppresses leukemic K562 cell growth. *DNA and Cell Biology,* **30,** 71-
18   78.
19 Warzocha, K. & Wotowiec, D. (1997) Antisense strategy: biological utility and prospects in the
20   treatment of hematological malignancies. *Leukemia and Lymphoma,* **24,** 267-281.
21 Wilson, C. & Keefe, A.D. (2006) Building oligonucleotide therapeutics using non-natural chemistries.
22   *Current Opinion in Chemical Biology,* **10,** 607-614.
23 Wu, C.C., Castro, J.E., Motta, M., Cottam, H.B., Kyburz, D., Kipps, T.J., Corr, M. & Carson, D.A. (2003)
24   Selection of oligonucleotide aptamers with enhanced uptake and activation of human
25   leukemia B cells. *Human Gene Therapy,* **14,** 849-860.
26 Yano, J., Hirabayashi, K., Nakagawa, S., Yamaguchi, T., Nogawa, M., Kashimori, I., Naito, H., Kitagawa,
27   H., Ishiyama, K., Ohgi, T. & Irimura, T. (2004) Antitumor activity of small interfering
28   RNA/cationic liposome complex in mouse models of cancer. *Clinical Cancer Research,* **10,**
29   7721-7726.
30 Yendamuri, S. & Calin, G.A. (2009) The role of microRNA in human leukemia: a review. *Leukemia,* **23,**
31   1257-1263.
32 Zhang, P., Zhao, N., Zeng, Z., Feng, Y., Tung, C.H., Chang, C.C. & Zu, Y. (2009) Using an RNA aptamer
33   probe for flow cytometry detection of CD30-expressing lymphoma cells. *Laboratory*
34   *Investigation,* **89,** 1423-1432.
35 Zhao, N., Bagaria, H.G., Wong, M.S. & Zu, Y. (2011) A nanocomplex that is both tumor cell-selective
36   and cancer gene-specific for anaplastic large cell lymphoma. *Journal of Nanobiotechnology,*
37   **9,** 2.
38 Zhao, W.L. (2010) Targeted therapy in T-cell malignancies: dysregulation of the cellular signaling
39   pathways. *Leukemia,* **24,** 13-21.
40 Zhou, J. & Rossi, J.J. (2010) Cell-specific aptamer-mediated targeted drug delivery. *Oligonucleotides*.
41
42

43

44

45

46

1   Figure 1 Simplified structures of nucleic acid-based therapeutics.

2   Antisense oligonucleotides (A) (Konopleva *et al* 2000), siRNA (B) (Yano *et al* 2004),

3   microRNA (C) (Griffiths-Jones 2004) and decoy oligonucleotides (D) (Mann 2005) bind via

4   Watson-Crick base pairing while aptamers (E) bind in a shape-fitting manner (Zhao *et al*

5   2011).

6

7

8

9

10

11

12

13

14

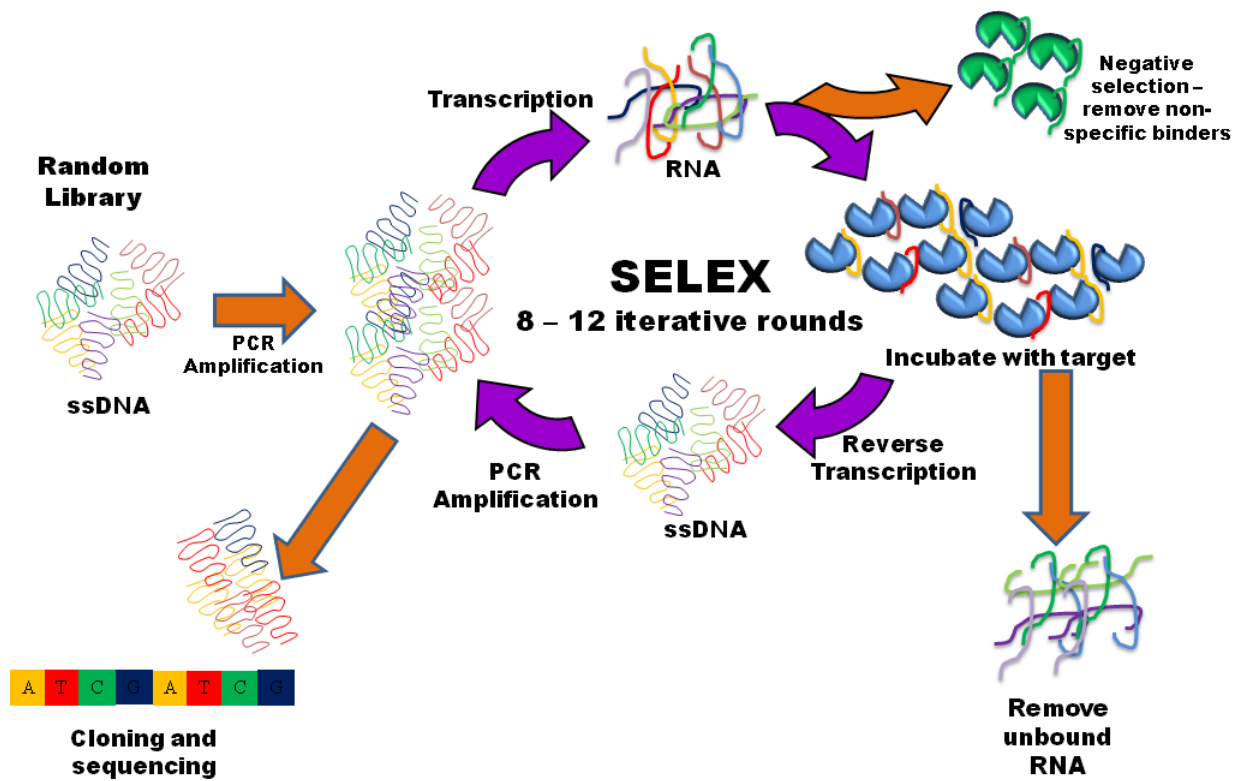Figure 2: Simplified schematic of oligonucleotide delivery to the cell.

Oligonucleotides can either be delivered as free molecules (i), conjugated directly to an aptamer (ii), or within a functionalised nanocomplex (iii). Once inside the cell, decoy oligonucleotides (DO) block transcription factors (TF) from starting the transcription of genomic DNA (iv) to mRNA. Antisense oligonucleotides (ASO) bind to their target mRNA and block translation of mRNA to protein (v). siRNA is first processed via DICER and is incorporated into the RNA-induced silencing complex (RISC) (vi). During this process the sense strand is separated from the anti-sense strand. The sense strand is degraded while the anti-sense strand guides the RISC to its target mRNA, which is then cleaved. miRNA are processed in a similar manner to siRNA. Adapted from several sources (Mann 2005, Vidal *et al* 2005, Kurreck 2009, Jin *et al* 2010, Zhou & Rossi 2010).
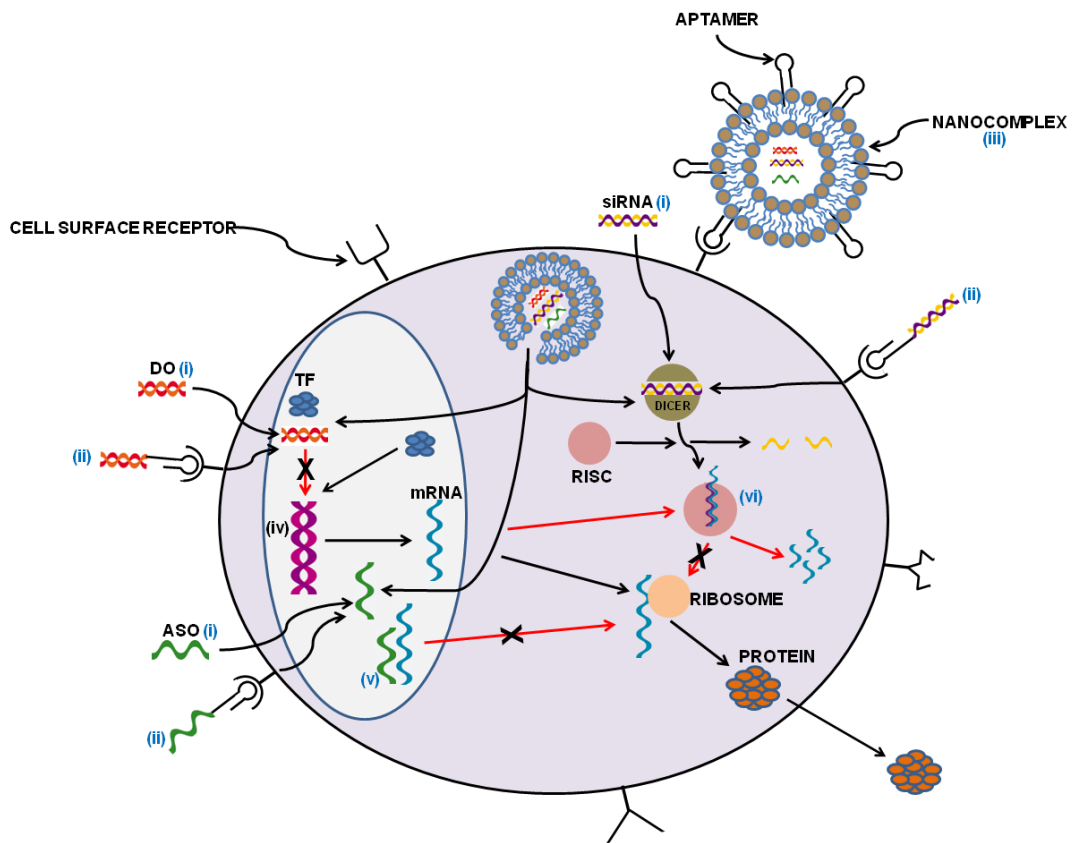
1   Figure 3 Schematic Representation of the Systemic Evolution of Ligands by EXponential

2   Evolution (SELEX) Process.

3

4

5

6