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Aptamer delivery of siRNA, radiopharmaceutics and chemotherapy agents in cancer

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ABSTRACT

Aptamers are oligonucleotide reagents with high affinity and specificity, which among other therapeutic and diagnostic applications have the capability of acting as delivery agents. Thus, aptamers are capable of carrying small molecules, nanoparticles, radiopharmaceuticals or fluorescent agents as well as nucleic acid therapeutics specifically to their target cells. In most cases, the molecules may possess interesting therapeutic properties, but their lack of specificity for a particular cell type, or ability to internalise in such a cell, hinders their clinical development, or cause unwanted side effects. Thus, chemotherapy or radiotherapy agents, famous for their side effects, can be coupled to aptamers for specific delivery. Equally, siRNA have great therapeutic potential and specificity, but one of their shortcomings remain the delivery and internalisation into cells. Various methodologies have been proposed to date, including aptamers, to resolve this problem. Therapeutic or imaging reagents benefit from the adaptability and ease of chemical manipulation of aptamers, their high affinity for the specific marker of a cell type, and their internalisation ability via cell mediated endocytosis. In this review paper, we explore the potential of the aptamers as delivery agents and offer an update on current status and latest advancements.

1. Introduction

Aptamers have recently closed their 25 yrs of age, since the first description of the SELEX methodology in 1990 (Ellington and Szostak, 1990; Tuerk and Gold, 1990) and they have had their birthday celebration with special symposia and issues in prestigious journals including Nature. That has been because aptamers have been described of coming of age at their 25 (Sullenger, 2016). Aptamers have been made great progress in those past 25 yrs both in therapy and in diagnostics and imaging application, alone or coupled to a variety of agents. As small oligonucleotide molecules, RNA or DNA, specific for any type of target of interest, with high affinity and selectivity, stable at ambient temperatures, flexible and adaptable to a number of situations and chemical modifications, aptamers are ideal delivery agents, conferring specificity and selectivity to their counterpart molecule. Furthermore, depending the choice of aptamers target, aptamers can enter cells specifically via cell mediated endocytosis, resolving potential problems of other oligonucleotide therapeutics such as siRNA, shRNA and miRNA.

Thus, coupled to a small molecule chemotherapy agent, aptamers confer to that agent specificity for the cancer cell that expresses a particular tumour marker, and prohibits the cell entering in any and all types of cells, thus augmenting its efficacy and reducing its side effects. On the other hand, coupled to an imaging agent, such as a radiopharmaceutical, guides it specifically on the type of tissue desired, reducing the radiation dose necessary and increasing imaging efficiency and definition, and finally offering rapid clearance from the system. In terms of use with nanoparticles, aptamers have the ability to guide the nanoparticle and are an excellent decorating agent for various types of nanoparticles described. Their size, being considerably smaller

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than that of an antibody does not increase the nanoparticle size as much, whereas its coupling efficiency and variety of chemical modifications makes it an ideal delivery agent.

Equally, the market is now ripe to receive novel type of biomedicines, which are operating on a high efficiency of treatment principle, or theragnostic agents that can be equally used as diagnostic agents for identifying a marker, and as therapeutic agents for the inhibition or targeting of that market in therapeutic applications. Thus, according to the Market Report published by Market Research.com, entitled "Aptamers Market – Technology Trend Analysis By Applications – Therapeutics, Diagnostics, Biosensors, Drug Discovery, Biomarker Discovery, Research Applications with Market Landscape Analysis – Global Forecasts to 2018 [Web reference 1], aptamers represent an emergent market, considered as a rival or substitute of antibodies in the scientific sector. Aptamers are ready to grow rapidly in a number of areas of applications, including therapy and diagnostics. The global market of aptamers has been evaluated at US$287 millions in 2013 with a projection of reaching US$2.1 billion in 2018. According to the same report, though aptamers exist in parallel with antibodies in the last quarter of a century, their market is not really utilised yet, not merely a fraction of it. Yet, with the FDA approval of Macugen (pegaptanib), this market has received a new impulse. The list of promising aptamers in the pipeline of clinical trials estimates that these chemical antibodies, as they are often called, could quickly reach or overcome monoclonal antibodies in terms of approval for therapeutic, diagnostic or imaging applications. With technological merits over antibodies, the aptamer market is ready to see a rapid growth in the next 10–15 years [Web reference 1].

In cancer, apart from surgery, the major therapeutic modalities remain chemotherapy, radiotherapy and biotherapy. Aptamers, as a novel modality that is making its reach for the market, has entered successfully in all the three modalities mentioned above. Aptamers have contributed obviously in biotherapy, with a number of aptamer in clinical trials at the moment and others in developmental stages. These have been recently reviewed in a number of publications (Hu and Zhang, 2013; Prakash and Rajamanickam, 2015; Wu et al., 2015). Aptamers like the AS1411, against nucleolin, have reached phase II clinical trials for different cancer indications, as direct inhibitors of a protein associated with cancer. However, in the majority of cases, the selected aptamers have found their best application as delivery agents of another modality, thus being capable of improving current radiotherapy, biotherapy or chemotherapy treatments, alleviating side effects, improving specificity and reducing dose, thus making such treatments more efficient.

In this article, we shall demonstrate some of the applications of aptamers as delivery agents, with focus on oligonucleotide therapeutics, radiopharmaceuticals, small molecules, and nanoparticles, offering examples, primarily from the area of cancer, and an outlook on the potential of this technology to expand and participate in the future generation of therapeutic agents.

2. Aptamer-siRNA chimeras

Previous reviews have described in some extension many targeting therapeutics with aptamers, specially aptamers conjugated with siRNA, miRNA, anti-miRNA and small hairpin-RNA in order to deliver these molecules to cancer cells (Aaldering et al., 2015; Davydova et al., 2011; Kanwar et al., 2011; Keefe et al., 2010; Li et al., 2013; Singh et al., 2010; Thiel and Giangrande, 2010; Xiang et al., 2015; Zhou et al., 2012).

Aptamers offer an obvious ease of coupling with a siRNA molecule. Thus, RNA aptamer may be simply extended to incorporate the part of the siRNA molecule, for example

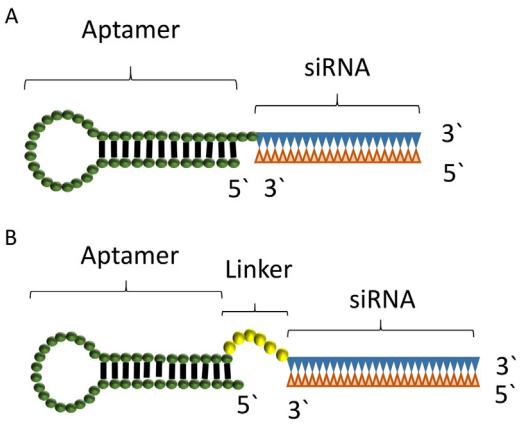

Fig. 1. Schematic of two different aptamer_siRNA coupling approaches (a) Aptamer and siRNA sense strand are co-transcribed and then the complementary siRNA anti sense is anneled. (b) Aptamer and siRNA are connected by a linker. Adapted from Zhou and Rossi (2014).

(Fig. 1a). On the other hand, a linker sequence can and has been used to link the aptamer with the siRNA (Fig. 1b). In other cases, an additional double stranded-stick sequence has been also added after the linker. Finally, more complex structures have been explored, in the use of streptavidin with biotinylated aptamers. This offers the advantage of a tetrameric molecule that can contains two aptamers and two different or identical siRNA molecules, thus offering the advantage of targeting two targets at the same time (Fig. 2). This approach was developed as a proof of principle by Chu (2006), conjugating the anti-PSMA aptamer (A-9) with biotinylated 27-mer lamin A/C or GAPDH siRNAs via a central streptavidin molecule (Chu, 2006). Using two different siRNA molecules, has in turn been successfully employed by Subramanian et al. (2015b) in breast cancer treatment approaches. An additional approach for linking aptamers with siRNA delivery, is with the use of nanoparticles or other nanotechnology reagents. In this case, the siRNA can be protected inside the nanoparticle, whereas the aptamer can act as a decorating agent that leads and specifically binds the nanoparticle to the cancer cell (Namgung and Kim, 2012) (Fig. 3).

Approaches that exploit the RNAi pathways through a therapeutic use of siRNA and miRNA-based drugs coupled with aptamers were widely addressed on the past decade (Esposito et al., 2014). Multiple in vivo and in vitro studies were conducted in different cancer cell lines. Remarkably prostate cancer was the most exhaustively studied cancer type and a number of therapeutics for prostate cancer based on this system are currently in preclinical development.

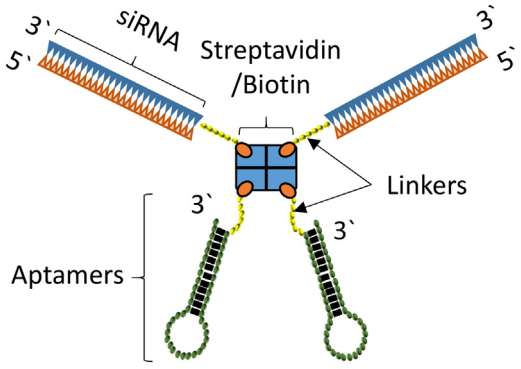

Fig. 2. Schematic of tretrameric molecule containing two aptamers and two siRNA molecules. Adapted from Zhou and Rossi (2014).

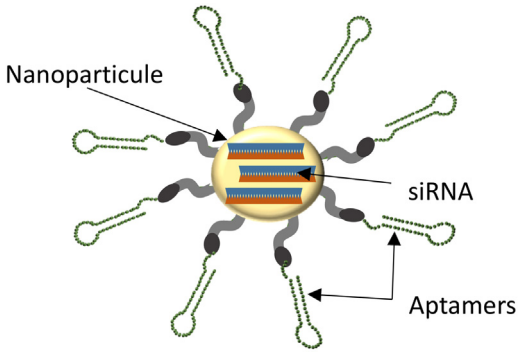

Fig. 3. Schematic of siRNA encapsulated in nanoparticles decorated with aptamers. Adapted from Zhou and Rossi (2014).

In early 2006, an anti-prostate-specific membrane antigen (PSMA) aptamer was conjugated to an siRNA targeting lamin A/C and GAPDH genes using streptavidin as a linker (Chu, 2006). Thus, through the non-covalent approach mentioned above, the conjugates led to the inhibition of gene expression, when compared to similar molecules analyzed in this study.

Furthermore, a chimeric aptA10-siRNA molecule was successfully generated and tested for its ability to bind to PSMA protein and efficiently target polo-like kinase 1 gene (PLK1), leading to PLK1 depletion and consequently cell death (McNamara et al., 2006). This method was proved to mediate targeted delivery of siRNA through a cell-type dependent mechanism (prostate cancer cells expressing PSMA). The A10-PLK1-siRNA was then optimized in an in vivo model by the formation of an alternative chimera using a truncated aptamer, added with polyethyleneglycol (PEG) moiety. This new molecule acquired an increased half-life by the presence of the PEG and it was able to promote regression of PSMA-expressing tumor xenograph (Dassie et al., 2009).

The same AptA10-siRNA constructs against PSMA-positive cells were also used in a multivalent design to silence eukaryotic elongation factor 2 (EEF2) gene, resulting in: specific cytotoxicity and induced apoptosis (Wullner et al., 2008); in a successful in vivo selective radiosensitization method using siRNA against DNA protein kinase (*DNAPK*) gene (Ni et al., 2015); and in an interesting biodegradable construct against androgen receptor (AR) gene which resulted in target knock-down and xenograft tumor regression (Yang et al., 2012).

In another study with the same PSMA receptor as target, Wullner and his team used the xPSM-A10-3 aptamer as a mechanism of delivery of RNAi to promote knockdown of the gene encoding the enzyme "Eukaryotic Elongation Factor 2" (EEF2) (Wullner et al., 2008). This enzyme is important because it participates in the protein biosynthesis of cells and therefore, the non-production of this enzyme due to silencing, resulted in the induction of apoptosis and consequent death of the tumor cells (Wullner et al., 2008). Thus, two siRNA assays were performed targeting EEF2 from two types of tumor cells that have the PSMA receptor on their membrane, human prostate carcinoma cells (LNCaP) and MCF-7 (human mammary adenocarcinoma cells). In both assays it was possible to observe that the specific knockdown of the EEF2 gene led the tumor cells to apoptosis.

The PSMA aptamers have also been used in conjunction with nanostructures, such as the use of nanoconstructors, which are neutral polymers containing siRNA in their interior and aptamers adhered to the outer membrane. Aptamers act as transporters and recognizers of prostate-specific-membrane-antigen (PSMA) receptors for the introduction of the nanoconstructor, which has the role of protecting the siRNA from being degraded in the extracellular environment in prostate tumor cells (Namgung and Kim, 2012). The nanoconstructor produced by Ran Namgung and Won Jong Kin was developed by the hybridization of two sense-conjugated polymers and anti-senses iRNA strand, without the need of the use of cationic condensation reagents, resulting in a minimization of toxicity. In addition, the reduced size of the nanoconstructor allows easy entry into the target cell, which, once in the cytoplasm, is degraded due to reducing conditions that lead to the reduction of the disulfide bonds, releasing the siRNA that is bound to the polymer chain DEXTRAN (DEX -CHO). Thus, the prostate-carcinoma-binding-peptide-aptamer complex (DUP-1), which binds specifically to PSMA, allows the specific entry of siRNA into PC-3 (human prostate cancer cell lines) cells.

Another approach using nanotechnologies was presented by Bagalkot and Gao (2011). In that study, the authors presented a mechanism of siRNA delivery by aptamers specific for the PSMA receptor that occurs in two steps. The first step is characterized by the delivery of the chimeric aptamer attached non-covalently to nanoparticles that have the ability to break the endosome, releasing the siRNA more easily. In addition, the nanoparticle has the ability to neutralize some positive charges on its surface, which helps prevent the formation of non-specific electrostatic bonds. The second step consists of restoring the chimeric aptamer-siRNA maintaining its conformation and accessibility (Bagalkot et al., 2006).

Many others aptamers against cell surface targets were tested using similar mechanisms to that studied on the PSMA-positive cell type studies. In breast cancer, for example, there is a study about targeting human epidermal growth factor receptor 2 (HER2) in a chemo-sensitizing model (Thiel et al., 2012) and STAT3 gene silencing on breast cancer bone metastasis (Mai et al., 2014). Epithelial cell adhesion molecule (EpCAM) tumor marker was accessed for its viability as target through an RNA aptamer (Gilboa-Geffen et al., 2015)·(Subramanian et al., 2015a). When coupled with siRNAs against STMN1 and BIRC5 genes, related to oncogenesis and chemoresistance respectively, these aptamer constructs led to successful silencing of genes expression in breast cancer and retinoblastoma models (Subramanian et al., 2015b). This EpCAM aptamer was submitted to a Locked Nucleic Acid (LNA) modification that consists in adding to the nucleotide an extra bridge connecting the 2′ oxygen and 4′ carbon, and which bestows in the aptamer increased stability, to further increase its potential use. Cytotoxic T lymphocyte–associated antigen 4 (CTLA4) covalently conjugated with anti STAT3 gene siRNA also achieved the proposed goal in malignant CD8+ T cells and ultimately led to tumor growth metastasis inhibition (Herrmann et al., 2014).

Wilner et al. (2012) proposes the use of aptamers as substitutes for a transferrin in various applications, including cell imaging, targeted drug applications and an association with silencing RNA for gene inhibition. The choice of transferrin as a target is related to the fact that the protein is highly expressed in cells with increased proliferation rate, in particular cancerous cells. In addition, its receptor (CD71) is also expressed at high levels in the blood-brain barrier (BBB), functioning as a non-resource transport pathway (Wilner et al., 2012). The aptamers developed in the study bound to the human transferrin receptor and were successfully internalized, which demonstrate their importance and great utility to other cell surface targets that are known to be internalized in a cell or where a function is desired internalization induced by aptamers.

Most recently, Liu et al. described an elegant approach in the first report about bivalent aptamer action in vivo. They developed a chimera containing two siRNA, one against EGFR and the other against survivin, sandwiched between two anti PSMA aptamers. This architecture was designed to simultaneously silence both pathways in prostate cancer cells and xenograft tumors (Liu et al., 2016), simultaneously suppressing tumor growth and inhibiting angiogenesis.

3. Aptamers as delivery agents of radiopharmaceuticals

The challenge in delivering radionuclides to tumor sites to either produce diagnostic images or improved therapeutic approaches involves a number of pharmacokinetics aspects. First of all, the delivering methods have to favor the preferential uptake of radionuclides by tumor cells rather than other tissues. The concept of tumor-to-non target organ radioactive ratio is one of the parameters that dictates the success of the delivering approach. Higher ratios generate a high contrast between tumor and non-tumor tissue. Ideally, after few hours of injection of radionuclides coupled to any tumor driver the radioactivity should be concentrated preferentially in tumor cells/sites. Another aspect is that once in the tumor sites the time of retention of radionuclides have to be optimized in order to allow a proper realization of the desired diagnostic or therapeutic procedure.

Aptamers are definitely arising as a very potent delivery tool in cancer diagnosis and treatment, considering its high target specificity, low cost of production, low immunogenicity and adaptable pharmacokinetics features. The potential application of aptamers in delivering with high specificity different probes to tumor cells is a growing field (Gomes et al., 2010). Aptamers can be applied to guide different probes to target tissues or cells in many imaging techniques such Fluorescence and bioluminescence imaging, magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission tomography (SPECT), computed tomography (CT) and ultrasound (US) (Dougherty et al., 2015; Sun et al., 2016). In the scope of this review we will focus on aptamer delivering radionuclides for imaging in cancer diagnosis, and for tumor therapeutics.

Hicke BJ et al. published the first effort toward the valuable use of radiolabeled aptamers in vivo for tumor target imaging (Hicke et al., 2006). ^{99m}Tc Radiolabeled aptamer TTA1 targeting tenascin-C was intravenously injected in mice bearing glioblastoma (U251) and breast (MDA-MB-435) tumor xenographs (Hicke et al., 2006). Remarkably, variation of radiometal chelators produced a significantly change in the pattern of radiolabeled aptamer uptake and clearance signaling the influence of the radionuclide aptamers coupling methods in the efficiency of its distribution and retention in tissues. The authors obtained an important ratio tumor-to-blood of TTA1, meaning high and rapid blood clearance with high and long tumor retention. These results encouraged the application of this delivery method in producing tumor target imaging. Images of glioblastoma and breast cancer obtained by planar scintigraphy after 18 h revealed a good concentration of radioactivity in both glioblastoma and breast tumor xenographs with almost no radioactivity in the rest of the test animal body. It was the first demonstration of a potential use of aptamers to deliver radionuclides to cancer sites.

One-year later, the Missalidis' group developed a series of mono- and multimeric target radiopharmaceuticals using anti-MUC1 aptamers as a driver to MCF7 tumor xenographs sites. The multimeric cyclen-based conjugate ligand demonstrated a notable penetration in a solid tumor compared with the same characteristic observed using antibodies, despite the formation of a labile complex with ^{99m}Tc (Borbas et al., 2007). Subsequently the same group conjugated two aptamers, one against MUC1 protein core (AptA) and one against tumor glycosylated MUC1 (AptB), in a monomeric form, with MAG2 and labeled them with ^{99m}Tc. Both radiolabeled conjugates were injected in mice bearing MCF7 tumor xenographs and demonstrated a similar pattern in the tumor-blood ratio in test animals, but with high uptake in the intestine. The tumor-to-blood ratio for radiolabeled AptA and AptB were quite similar at 3, 5 and 22 h p.i. with the exception that, by 16 h, AptA showed a much high tumor-to-blood ratio due to the low tumor clearance of this conjugate compared with the AptB. At 22 h p.i. both conjugates had returned at background levels. However, the conjugate containing aptamers against the tumor glycosylated MUC1 (AptB) had previously shown a fast internalization in the tumor cells (Pieve et al., 2009). One limitation revealed in this paper was the clearance of the most of radioactivity of the test animal by 3 h p.i. So in a sequential study the same group aimed to improve the body circulating half-life of the radiolabeled aptamers. Da Pieve et al. (2012) conjugated the AptA aptamer with poly (ethylene glycol) (PEG) and studied its distribution in MCF-7 tumor xenographs bearing mice. In fact, they found that PEGlylation was able to delay the blood and body clearance of the aptamers. In conclusion, the monomeric radiolabeled aptamers of these studies were not able to reach their target with high efficiency probably due to the relatively weak affinity of this aptamer for its target, and these subsequent constructs still remained inferior to the initial tetrameric complexes (Da Pieve et al., 2012).

Da Rocha Gomes et al. (2012) raised an aptamer against the human malignancy marker metalloproteinase 9 (hMMP-9). They synthetized a truncated aptamer F3Bomf presenting a Kd of 20 nM against hMMP-9 but no binding properties against hMMP-7 and hMMP-2. F3Bomf was functionalized with MAG3 with no loss of specificity and then radiolabeled with ^{99m}Tc for imaging studies. 99mTc-MAG3-F3Bomf anti-hMMP-9 aptamer demonstrated a higher specificity for hMMP-9 when compared to immunohistochemistry using labeled antibody against MMP-9 in human glioblastoma sections. The radiolabeled aptamer conjugate was then tested against a panel of human central nervous tumor types expressing MMP-9. Aptamer conjugate generated a stronger signal for all tumor sections than immunohistochemistry did. Both aptamer and antibody revealed an increasing signal in a grade of malignancy dependent manner (Da Rocha Gomes et al., 2012).

Another initiative to develop a radiopharmaceutical based on aptamers was described using a modified aptamer against HER-2 conjugated with hyne as the chelator, tricine as the co-ligant and ^{99m}T as a radiotracer. In vitro binding studies have shown a specific binding to HER2 target in SKOV-3 ovarian cells that over express HER2 but not to MCF-7 cells with low levels of HER2 expression. The Kd of 27 nM was reported, almost 8 folds weaker than the unlabeled aptamer (Varmira et al., 2013). Furthermore, the authors observed a high intestine uptake, similar to that observed by the Missailidis study, and which probably indicated that the radiolabeled aptamer was rapidly removed from the blood by the liver and was subsequently excreted into the intestine. Altogether, although the aptamers in this study showed high efficiency in tumor cell studies, they did not perform equally well in experimental model studies, with high non-target uptake appearing to be a major obstacle to the efficient tumor uptake of the HER2-targeted 99mTc-hynic-RNA aptamer. Thus, the authors suggest that further modifications to the radiolabeled aptamer should be necessary for efficient pharmacokinetic properties. They explain the high liver uptake as one of three possibilities, namely the non-specific binding of the aptamer in the liver, the effect of the hynic chelator with tricine as a co-ligand on the aptamer lipophilicity, or the phosphate groups of the aptamer promoting liver accumulation (Varmira et al., 2013). Taken together with previous observations by the Missailidis group on a study of various chelators, it has been shown that the second suggestion is the most probable, seen as different chelators significantly affected the liver uptake of the aptamer in biodistribution studies (Borbas et al., 2007).

This was further confirmed when Valmira et al., in a subsequent study, described an improved radiolabeled RNA aptamer against HER2 by using *ethylenediamine-N,N′-diacetic acid* (EDDA) instead of tricine as a co-ligant. They reported a higher tumor accumulation of this new version compared with the one using tricine as co-ligant. The uptake of the EDDA-co-ligated 99mTc-HYNIC-RNA

aptamer by the liver and spleen was notably lower. The biodistribution of the new conjugate showed the tumor-non-target ratios after 1 and 4 h p.i higher than 3, meaning a 3 fold increase comparing to the biodistribuition of the tricine conjugated aptamer, and a faster clearance of the radiolabeled aptamer from blood and soft tissues (Varmira et al., 2014). This was attributed to the different chemical nature of the EDDA, as compared with tricine.

At the same year a number of studies where published with the use of aptamers as radiopharmaceutical modalities. In a similar approach to those previously published, Li et al. (2014) used the nucleolin aptamer AS1411 as a delivery agent to copper-64 based radiotherapy and diagnostic imaging agent (Li et al., 2014). AS1411 is a well characterized dimeric, g-quadruplex aptamer against nucleolin that is currently in phase II clinical trials. Li et al. used this aptamer as a targeting moiety, and studied the effect of four chelators in the binding, uptake and biodistribution of AS1411 labelled with 64Cu. This study confirms the above observations of the importance of the chelator on the aptamer mediated delivery of radiopharmaceuticals. Li et al. observed, for example, that TE2A 64Cu-labelled AS1411 showed superior cell internalization and clear tumour uptake from 1 to 24 h p.i. compared to the other ligands, DOTA, DOTA-Bn and NOTA-Bn, making it a more appropriate chelator for diagnostic imaging or tumour-specific radiotherapy.

In that same year, two additional studies were published, with a different approach that aimed to eliminate the chelator issue on aptamer-mediated radiopharmaceutical delivery. Thus, both Wu et al. (2014) and Correa et al. (2014) published studies based on the direct labeling of aptamers with the metal, eviting the use of an appropriate chelator. To do so, Wu et al. (2014) used an EGFR aptamer labelled with 188Re (Wu et al., 2014), whereas Correa et al. (2014) selected aptamers to CEA, which they labelled with 99mTc (Correa et al., 2014). The latter group demonstrated that the directly labelled aptamer had both radiochemical purity and stability, as confirmed by Thin Layer Chromatography. Both the EGFR and the CEA directly labelled aptamers, apart from radiochemical purity and stability, continued to maintain affinity for their target antigen and were shown to bind to EGFR positive glioblastoma and CEA positive tumor cells respectively.

In the subsequent year, Jacobson et al. (2015a,b) published two articles using different approaches to aptamer based targeted radiopharmaceuticals (Jacobson et al., 2015a,b). In the first instance, he used aptamers to protein tyrosine kinase-7 to target colon carcinomas, labelled with 18F in a PET imaging approach (Jacobson et al., 2015a,b), whereas in the other study he used Tenascin-C as a marker for the aptamer and chose PET and SPECT imaging using 18F and 64Cu labeling respectively (Jacobson et al., 2015a,b). In vivo PET imaging using PTK7 aptamer was shown to clearly visualize PTK7 expression in xenographed mice. Furthermore, the 18F labelled aptamer was rapidly cleared from the blood through renal clearance, leaving a high tumor-to-blood and tumor-to-muscle ratio in a promising methodology for aptamers in PET based imaging applications. Similar positive data were also presented with the 18F labelled tenascin-C aptamer, creating a robust PET tracer with fast clearance and high tumor contrast (Jacobson et al., 2015a,b). With the increased presence de PET imaging facilities in hospitals and the smaller half-life of 18F, of nearly two hours (109 min) compared to the 6 h for Tc99m, 12 h for 64Cu and 17 h for Rhenium-188, this may be an interesting imaging alternative. However, the short half-life of these radionuclides makes it more challenging to complete chemical coupling and labelling within the appropriate time considering the radionuclide half-life.

Using a different metal radionuclide, Kryza et al. (2016) selected aptamers against the human matrix metalloproteinase-9 (MMP-9), using chemically modified RNA aptamers, resistant to nuclease degradation. They coupled the aptamers with three different chelators, established the binding properties of the aptamer-chelator complex with MMP-9 in cell lines and using fluorescence imaging in melanoma-bearing mice. The authors labelled the complexes with 99mTc and 111In, using MAG or DOTA as chelators, also demonstrating a high tumor-to-muscle ratio and complex stability.

Finally, earlier this year (2017), Santos et al. published a study of the MUC1 aptamers previously developed by the Missailidis group in a nanoparticle formulation for breast cancer imaging. The aim was to improve on the biological clearance and pharmacokinetic properties of the aptamers, using poly(lactic-co-glycolic acid) nanoparticles, successfully incorporating the MUC1 aptamers on the nanoparticle surface (loaded nanoparticle). This resulted in an aptamer-decorated nanoparticle of about 260 nm mean size, that was further labelled with Tc99 m for SPECT imaging. The authors labelled the decorated and empty nanoparticle, in order to compare the properties of the two and attribute specifically their characteristics. The aptamer-decorated nanoparticle had a high intestine uptake, both small and large, some liver and kidney uptake, and about 5% accumulation on the tumor site. In contrast, the empty nanoparticle had a much higher kidney uptake, but little to no intestine or liver uptake, and no uptake at the tumor site. The presence of MUC1 on the intestine epithelial cells have been described as the most probable cause of the intestine uptake. Although it has been shown that chelators often determine the liver uptake of aptamers, should the nanoparticle played a similar role, liver and intestine uptake should have been observed with the empty nanoparticle, which was not the case. Thus, in this case, it appears that this is a specific property of the MUC1 aptamer, which could limit its potential, though the specific tumor uptake remains promising.

In addition to the various attempts for labelling aptamers with radionuclides for PET or SPECT imaging, there exists a single reference to labeling with alpha-particles to effect specific cancer kill. Thus, PEGylated liposomes were loaded with 225Ac and labelled with an anti-PSMA antibody or the anti-PSMA A10 aptamer and evaluated for selectivity, internalization potential and killing efficacy (Bandekar et al., 2014). The authors concluded that targeted delivery of alpha-particle radiotherapy is a promising approach, but their findings actually showed that the antibody was more efficient than the aptamer in terms of cytotoxicity and lethal dose values (Bandekar et al., 2014). However, this difference could be easily explained, considering the fact that double number of antibodies were in the surface of the liposome compared to the number of aptamers. Thus, it was reported that 17 antibodies were in the surface of each liposome, compared to only 9 aptamers. If we further consider that each antibody has two binding sites, compared to only one per aptamer, this gives some 4 times higher binding capacity to the liposomes decorated with the antibody than with the aptamer, even though these constructs showed some 1.5–1.7 times lower lethal dose values, showing that improving the coupling reaction, aptamer could be equal or better than the antibody as delivery agent.

In terms of imaging, one more methodology has been explored, though is not really using a radionuclide agent, but can be very efficient and should not be overlooked. With only two publications, it remains somewhat overlooked, but it could be one that significant developments can follow. This is MRI imaging, a methodology that has the benefit of not needing radiation to obtain its image. However, when a contrast agent is used, such as gadolinium, the dose is significantly high and uncomfortable for the patient. Aptamer coupling could reduce the dose and increase the contrast in the MRI image. Thus, Wang et al. (2008) were the first to describe an aptamer super paramagnetic iron oxide

nanoparticle (SPION) complex for MRI imaging applications and potential treatment. They conjugated the A10 aptamer against PMSA with SPIONs and analysed it for its ability in MRI imaging of prostate cancer (Wang et al., 2008).

Still using SPIONs, some three years later, Yu et al. (2011) described a thermally cross-linked SPION, coupled to the same PMSA aptamer, but also loaded with doxorubicin, thus permitting MRI imaging and targeted chemotherapy at the same time. In animal models of prostate tumors, they were capable of demonstrating successful MRI imaging of the tumor, and specific delivery of doxorubicin to the cancer cells (Yu et al., 2011).

Two more recent studies have also been published focusing on MRI imaging. In 2014, You et al. described the coupling of a VEGF aptamer coupled to another type of iron oxide nanoparticles, ultrasmall super paramagnetic iron oxide (USPIO) nanoparticles, for specific imaging or cancer in animal models of liver tumor xenografts (You et al., 2014). Finally, in 2015, Zhang et al. used a thermosensitive liposome, loaded with Gd-DTPA as a contrast agent and coupled to the nucleolin AS1411 aptamer. This complex presented increased relaxivity, no cytotoxic effects, higher specificity and biocompatibility, making it thus a particularly interesting MRI agent for tumor imaging and early diagnosis (Zhang et al., 2015).

The lack of immunogenicity and the increased tissue penetration, make aptamers an interesting modality for specific, targeted imaging approaches, such as SPECT and PET, offering high contrast between tumor and non-tumor tissue, as well as rapid clearance, compared to the antibodies that remain for long periods in circulation, a factor that can limit their dose. Yet, some issues still remain, particularly in consideration of the aptamer pharmacokinetic properties and the choice of chelator, when an indirect labelling is used.

4. Aptamers as delivery of chemotherapy agents

The use of aptamers as delivery agents for chemotherapy was one of the early identified applications of the aptamer technology. Standard chemotherapy, such as that targeting rapidly proliferating cells based on DNA binding or inhibition of DNA binding proteins such as topoisomerases, has been used for the treatment of various types of cancer, and is still in use nowadays. However, it is a treatment that has severe side effects that often limit the adherence to the treatment protocol, thus limiting its effectiveness. A chemotherapy agent that would enter specifically only the cancer cell, thus eliminating whatever associated side effects, remained a desired aspect since Paul Ehlrich described the 'magic bullet' concept. In an attempt to fulfil this demand, aptamers have been coupled to chemotherapy agents since the early days of their existence, with the example of the aptamer to neutrophil elastase, coupled to an elastase inhibitor via conjugation of the inhibitor to a probe complementary to the aptamer primer sequence (Charlton et al., 1997).

From there on, a number of studies have been performed, using aptamers coupled to drugs like methotrexate and doxorubicin. Doxorubicin became a model drug for this type of studies, and a number of approaches have been adopted for its delivery. Following the example of the neutrophil elastase inhibitor, various attempts of chemotherapy delivery using aptamers were focused on coupling the chemotherapy agent covalently to the aptamer, using one of the variety of chemical modifications with functional groups available, such as amino and thiol or azide groups. Thus, doxorubicin aptamer complexes have been described using a thiol modified aptamer through an acid-labile acylhydrazone linker, that permits release of the aptamer in the acid environment of the endosome following successful receptor mediated endocytosis (Huang et al., 2009). The low pH environment of the endosome has been explored by another group, that coupled an anti-PSMA aptamer to doxorubicin using a formaldehyde based pH-sensitive covalent linker (Boyacioglu et al., 2013). Finally, in a more recent work, Porciani et al. (2015) have generated a bifunctional construct of the aptamer linked with DOX, but also carrying a decoy oligonucleotide against NFkB in a pancreatic cancer treatment approach (Porciani et al., 2015).

The covalent coupling of aptamers to the chemotherapy reagents offer the advantage that the drug is separate from the aptamer and should not interfere with its structure and binding to its ligand (Fig. 4a). Furthermore, exploring the various linkers, their flexibility, pH sensitivity and even length, one can tailor-make chimeras that would release the active ingredient on target, exploring the specific internalization features. On the other hand, it involves chemical coupling and purification procedures that can eventually impose some technical difficulties. However, a number of authors explored the particular DNA intercalating characteristics of molecules like DOX in order to link it directly with the double stranded part of the aptamer, and deliver directly the complex (Fig. 4b). The potential problems associated with that approach have been the fact that a number of molecules intercalated into an aptamer could cause distortion of its structure and abolish or diminish its target recognition, thus rendering it ineffective. Furthermore, it is important to ensure that the bound intercalator would be released from the aptamer within the cell and be free to exert its function. But, there are no chemical coupling reactions involved, and a simple mixing of the drug with the aptamer would suffice to have an active targeted chimeric drug with high potential.

Once it was established that the intercalation does not abolish aptamer binding and the drug is readily released from the aptamer at the low endosomal/lysosomal pH, this model was broadly

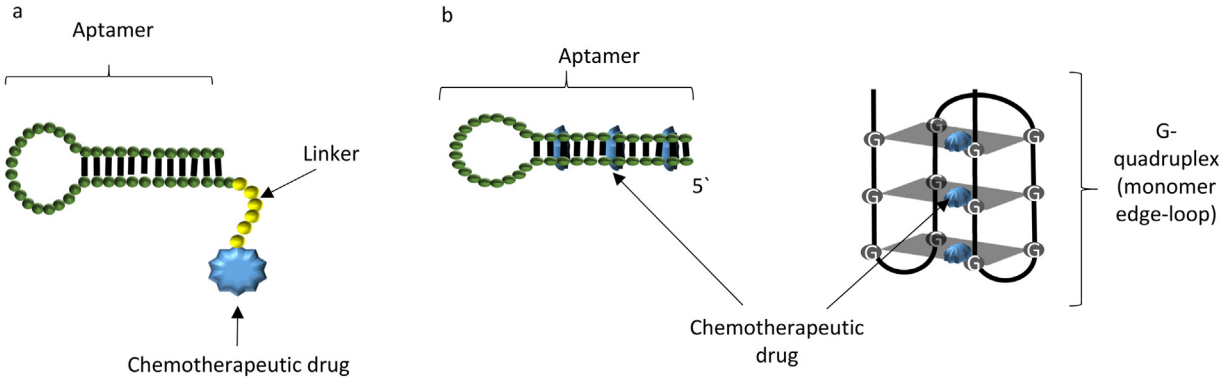

Fig. 4. Schematic of (a) covalent and (b) non-covalent aptamer-chemotherapeutic agent conjugation. Adapted from Bagalkot et al. (2006).

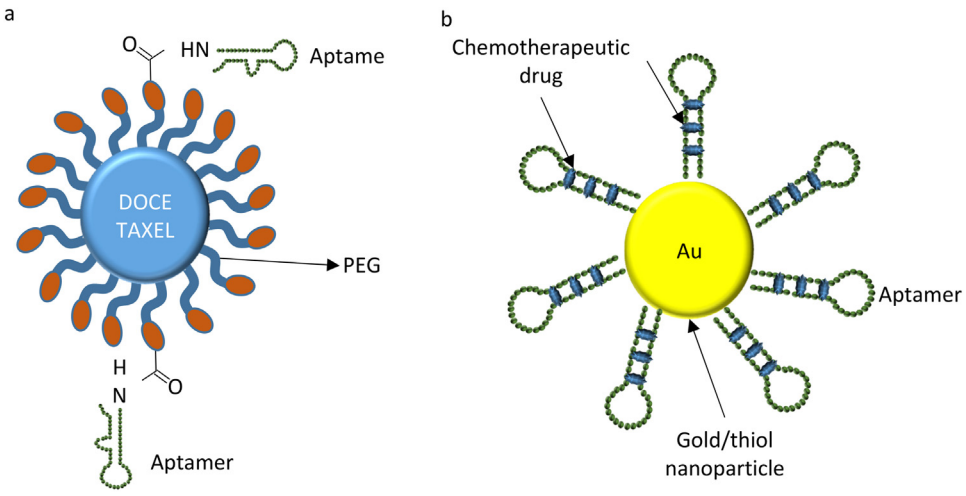

Fig. 5. Schematic (a) encapsulation of a large number of chemotherapeutics (eg. Docetaxel) into a nanoparticle which is then decorated by aptamer for specific delivery, (b) gold/thiol nanoparticle conjugated with aptamers charged with chemotherapeutics (intercalated).

explored with DOX linked non-covalently to aptamers against PSMA for prostate cancer (Bagalkot et al., 2006), anti-epithelial cell adhesion molecule (EpCAM) for retinoblastoma treatment (Subramanian et al., 2012), protein tyrosine kinase 7 (PTK-7) for T-Cell acute lymphoblastic leukemia (Taghdisi et al., 2010), MUC1 mucin for breast cancer targeting (Hu et al., 2012) or the HER2 also for breast cancer (Liu et al., 2012). Furthermore, by incorporating a double stranded G-C rich tail, more doxorubicin molecules could be loaded to the aptamer, further increasing the toxicity of the construct. This was explored in a human hepatocarcinoma cell line specific aptamer by Meng et al. (2012) in the treatment of liver cancer (Meng et al., 2012). Liver cancer was also the target in the AS1411 anti-nucleolin aptamer loaded with DOX (Trinh et al., 2015).

All these doxorubicin approaches have shown toxicity levels similar to those of free doxorubicin, but with reduced general toxicity or cardiotoxicity, due to the elimination of non-specific interactions of DOX because of the specific aptamer delivery. Other successful approaches include delivery of toxins, like gelonin (Chu, 2006), or photodynamic therapies (Ferreira et al., 2009), which also have shown cell specificity and increased toxicity.

Finally, an additional methodology has been used for the delivery of chemotherapy to cancer cells, and that has been using nanotechnology. Nanoparticles are an option that offers various possibilities and advantages and many different setups have been used. Two examples are the delivery of docetaxel in nanoparticles formulated with biocompatible and biodegradable poly(D,L-lactic-co-glycolic acid)-block-poly(ethylene glycol) (PLGA-b-PEG) copolymer and surface functionalized with the A10 anti-PSMA aptamers, showing remarkable efficacy, enhanced cellular toxicity and reduced non-specific toxicity (Farokhzad et al., 2006). This model explores the potential for encapsulation of a large number of chemotherapeutics into a nanoparticle, which is then decorated by aptamer for specific delivery (Fig. 5a). A different model has been adopted by Luo et al. (2011), who used gold nanoparticles to couple thiol modified sgs8c aptamers to their surface and load them with DOX, resulting in about 25 aptamers loaded on the nanoparticle, carrying some 300 DOX molecules, and demonstrating cellular uptake and specific cell kill (Luo et al., 2011) (Fig. 5b). Other nanotechnologies, such as carbon nanotubes, different material nanoparticles and nanospheres offer increased possibilities for this type of drug delivery, and aptamers offer the specificity to the cell target.

5. Conclusions and prospects of the technology

Aptamers remain a very promising technology for specific delivery of non-specific agents. They still face a number of developmental issues, which have not yet permitted them to make a successful transition from the lab to the market. These include their pharmacokinetic properties, which are often affected by the agent they are coupled to for delivery, their susceptibility to nucleases, which often dictate a number of modifications necessary for function, their rapid renal filtration and rapid distribution from the plasma compartment to the tissues, or even the still expensive production in large scale for clinical use. However, they have shown very promising preclinical results, and as delivery agents have demonstrated the ability to drive their load, whether another oligonucleotide, a radiopharmaceutical, a chemotherapy drug or a nanoparticle, specifically to their target site. They are flexible and easy to adapt, with a number of chemical modifications available for coupling and solutions to the above mentioned shortfalls, like protection from nucleases or coupling to PEG or nanoparticles for alteration of size and pharmacokinetic and pharmacodynamic properties. This characteristic permits them applications that were not previously possible and promises greater advances in the near future, with the expansion of the aptamer market and reduction of their production costs, driven by larger demand, and the promise of novel teranostic agents and improved targeted therapies.

References

Aaldering, L.J., Tayeb, H., Krishnan, S., Fletcher, S., Wilton, S.D., Veedu, R.N., 2015. Smart functional nucleic acid chimeras: enabling tissue specific RNA targeting therapy. RNA Biol. 12, 412–425. doi:http://dx.doi.org/10.1080/15476286.2015.1017234.

Bagalkot, V., Gao, X., 2011. siRNA-aptamer chimeras on nanoparticles: preserving targeting functionality for effective gene silencing. ACS Nano 5 (10), 8131–8139. doi:http://dx.doi.org/10.1021/nn202772p.

Bagalkot, V., Farokhzad, O.C., Langer, R., Jon, S., 2006. An aptamer-doxorubicin physical conjugate as a novel targeted drug-delivery platform. Angew. Chem. Int. Ed. 45, 8149–8152. doi:http://dx.doi.org/10.1002/anie.200602251.

Bandekar, a., Zhu, C., Jindal, R., Bruchertseifer, F., Morgenstern, A., Sofou, S., 2014. Anti-prostate-specific membrane antigen liposomes loaded with Ac-225 for potential targeted antivascular alpha-particle therapy of cancer. J. Nucl. Med. 55, 107–114. doi:http://dx.doi.org/10.2967/jnumed.113.125476.

Borbas, K.E., Ferreira, C.S.M., Perkins, A., Bruce, J.I., Missailidis, S., 2007. Design and synthesis of mono- and multimeric targeted radiopharmaceuticals based on novel cyclen ligands coupled to anti-MUC1 aptamers for the diagnostic imaging and targeted radiotherapy of cancer. Bioconjug. Chem. 18, 1205–1212. doi:http://dx.doi.org/10.1021/bc0700741.

Boyacioglu, O., Stuart, C.H., Kulik, G., Gmeiner, W.H., 2013. Dimeric DNA aptamer complexes for high-capacity–targeted drug delivery using pH-sensitive covalent linkages. Mol. Ther. Nucleic Acids 2, e107. doi:http://dx.doi.org/10.1038/mtna.2013.37.

Charlton, J., Sennello, J., Smith, D., 1997. In vivo imaging of inflammation using an aptamer inhibitor of human neutrophil elastase. Chem. Biol. 4, 809–816. doi:http://dx.doi.org/10.1016/S1074-5521(97)90114-9.

Chu, T.C., 2006. Aptamer:toxin conjugates that specifically target prostate tumor cells. Cancer Res. 66, 5989–5992. doi:http://dx.doi.org/10.1158/0008-5472.CAN-05-4583.

Correa, C.R., Barros, A.L.B., De Ferreira, C.D.A., De Goes, A.M., Cardoso, V.N., De Andrade, A.S.R., 2014. Aptamers directly radiolabeled with technetium-99 m as a potential agent capable of identifying carcinoembryonic antigen (CEA) in tumor cells T84. Bioorg. Med. Chem. Lett. 24, 1998–2001. doi:http://dx.doi.org/10.1016/j.bmcl.2014.02.048.

Da Pieve, C., Blackshaw, E., Missailidis, S., Perkins, A.C., 2012. PEGylation and biodistribution of an anti-MUC1 aptamer in MCF-7 tumor-bearing mice. Bioconjug. Chem. 23, 1377–1381. doi:http://dx.doi.org/10.1021/bc300128r.

Da Rocha Gomes, S., Miguel, J., Azéma, L., Eimer, S., Ries, C., Dausse, E., Loiseau, H., Allard, M., Toulmé, J.-J., 2012. 99 m Tc-MAG3-aptamer for imaging human tumors associated with high level of matrix metalloprotease-9. Bioconjug. Chem. 23, 2192–2200. doi:http://dx.doi.org/10.1021/bc300146c.

Dassie, J.P., Liu, X.-Y., Thomas, G.S., Whitaker, R.M., Thiel, K.W., Stockdale, K.R., Meyerholz, D.K., McCaffrey, A.P., McNamara, J.O., Giangrande, P.H., 2009. Systemic administration of optimized aptamer-siRNA chimeras promotes regression of PSMA-expressing tumors. Nat. Biotechnol. 27, 839–846. doi:http://dx.doi.org/10.1038/nbt.1560.

Davydova, A.S., Vorobjeva, M.A., Venyaminova, A.G., 2011. Escort aptamers: new tools for the targeted delivery of therapeutics into cells. Acta Nat. 3, 12–29.

Dougherty, C., Cai, W., Hong, H., 2015. Applications of aptamers in targeted imaging: state of the art. Curr. Top. Med. Chem. 15, 1138–1152. doi:http://dx.doi.org/10.2174/1568026615666150413153400.

Ellington, a D., Szostak, J.W., 1990. In vitro selection of RNA molecules that bind specific ligands. Nature 346, 818–822. doi:http://dx.doi.org/10.1038/346818a0.

Esposito, C.L., Catuogno, S., de Franciscis, V., 2014. Aptamer-mediated selective delivery of short RNA therapeutics in cancer cells. J. RNAi Gene Silencing 10, 500–506.

Farokhzad, O.C., Cheng, J., Teply, B.A., Sherifi, I., Jon, S., Kantoff, P.W., Richie, J.P., Langer, R., 2006. Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. Proc. Natl. Acad. Sci. 103, 6315–6320. doi:http://dx.doi.org/10.1073/pnas.0601755103.

Ferreira, C.S.M., Cheung, M.C., Missailidis, S., Bisland, S., Gariepy, J., 2009. Phototoxic aptamers selectively enter and kill epithelial cancer cells. Nucleic Acids Res. 37, 866–876. doi:http://dx.doi.org/10.1093/nar/gkn967.

Gilboa-Geffen, A., Hamar, P., Le, M.T.N., Wheeler, L.A., Trifonova, R., Petrocca, F., Wittrup, A., Lieberman, J., 2015. Gene knockdown by EpCAM aptamer-siRNA chimeras suppresses epithelial breast cancers and their tumor-initiating cells. Mol. Cancer Ther. 14, 2279–2291. doi:http://dx.doi.org/10.1158/1535-7163.MCT-15-0201-T.

Gomes, S.D.R., Azéma, L., Allard, M., Toulmé, J.-J., 2010. Aptamers as imaging agents. Expert Opin. Med. Diagn. 4, 511–518. doi:http://dx.doi.org/10.1517/17530059.2010.516248.

Herrmann, A., Priceman, S.J., Kujawski, M.I., Xin, H., Cherryholmes, G.A., Zhang, W., Zhang, C., Lahtz, C., Kowolik, C., Forman, S.J., Kortylewski, M., Yu, H., 2014. CTLA4 aptamer delivers STAT3 siRNA to tumor-associated and malignant T cells. J. Clin. Invest. 124 (7), 2977–2987. doi:http://dx.doi.org/10.1172/JCI73174.

Hicke, B.J., Stephens, A.W., Gould, T., Chang, Y.-F., Lynott, C.K., Heil, J., Borkowski, S., Hilger, C.-S., Cook, G., Warren, S., Schmidt, P.G., 2006. Tumor targeting by an aptamer. J. Nucl. Med. 47, 668–678 (47/4/668 [pii]).

Hu, M., Zhang, K., 2013. The application of aptamers in cancer research: an up-to-date review. Future Oncol. 9, 369–376. doi:http://dx.doi.org/10.2217/fon.12.201.

Hu, Y., Duan, J., Zhan, Q., Wang, F., Lu, X., Yang, X., 2012. Novel muc1 aptamer selectively delivers cytotoxic agent to cancer cells in vitro. PLoS One 7 (2), e31970. doi:http://dx.doi.org/10.1371/journal.pone.0031970.

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. doi:http://dx.doi.org/10.1002/cbic.200800805.

Jacobson, O., Weiss, I.D., Wang, L., Wang, Z., Yang, X., Dewhurst, A., Ma, Y., Zhu, G., Niu, G., Kiesewetter, D.O., Vasdev, N., Liang, S.H., Chen, X., 2015a. 18F-Labeled single-stranded DNA aptamer for PET imaging of protein tyrosine kinase-7 expression. J. Nucl. Med. 56, 1780–1785. doi:http://dx.doi.org/10.2967/jnumed.115.160960.

Jacobson, O., Yan, X., Niu, G., Weiss, I.D., Ma, Y., Szajek, L.P., Shen, B., Kiesewetter, D. O., Chen, X., 2015b. PET imaging of tenascin-C with a radiolabeled single-stranded DNA aptamer. J. Nucl. Med. 56, 616–621. doi:http://dx.doi.org/10.2967/jnumed.114.149484.

Kanwar, J.R., Roy, K., Kanwar, R., 2011. Chimeric aptamers in cancer cell targeted drug delivery. Crit. Rev. Biochem. Mol. Biol. 46, 459–477.

Kryza, D., Debordeaux, F., Azéma, L., Hassan, A., Paurelle, O., Schulz, J., Savona-Baron, C., Charignon, E., Bonazza, P., Taleb, J., Fernandez, P., Janier, M., Toulmé, J.J., 2016. Ex vivo and in vivo imaging and biodistribution of aptamers targeting the human matrix metalloprotease-9 in Melanomas. PLoS One 11 (2), e0149387. doi:http://dx.doi.org/10.1371/journal.pone.0149387.

Keefe, A.D., Pai, S., Ellington, A., 2010. Aptamers as therapeutics. Nat. Rev. Drug Discov. 9, 537–550. doi:http://dx.doi.org/10.1038/nrd3249.

Li, X., Zhao, Q., Qiu, L., 2013. Smart ligand: aptamer-mediated targeted delivery of chemotherapeutic drugs and siRNA for cancer therapy. J. Control. Release 171 (2), 152–162. doi:http://dx.doi.org/10.1016/j.jconrel.2013.06.006.

Li, J., Zheng, H., Bates, P.J., Malik, T., Li, X.F., Trent, J.O., Ng, C.K., 2014. Aptamer imaging with Cu-64 labeled AS1411: preliminary assessment in lung cancer. Nucl. Med. Biol. 41, 179–185. doi:http://dx.doi.org/10.1016/j.nucmedbio.2013.10.008.

Liu, Z., Duan, J.-H., Song, Y.-M., Ma, J., Wang, F.-D., Lu, X., Yang, X.-D., 2012. Novel HER2 aptamer selectively delivers cytotoxic drug to HER2-positive breast cancer cells in vitro. J. Transl. Med. 10, 148. doi:http://dx.doi.org/10.1186/1479-5876-10-148.

Liu, H.Y., Yu, X., Liu, H., Wu, D., She, J.-X., 2016. Co-targeting EGFR and survivin with a bivalent aptamer-dual siRNA chimera effectively suppresses prostate cancer. Sci. Rep. 6, 30346. doi:http://dx.doi.org/10.1038/srep30346.

Luo, Y.-L., Shiao, Y.-S., Huang, Y.-F., 2011. Release of photoactivatable drugs from plasmonic nanoparticles for targeted cancer therapy. ACS Nano 5, 7796–7804. doi:http://dx.doi.org/10.1021/nn201592s.

Mai, J., Huang, Y., Mu, C., Zhang, G., Xu, R., Guo, X., Xia, X., Volk, D.E., Lokesh, G.L., Thiviyanathan, V., Gorenstein, D.G., Liu, X., Ferrari, M., Shen, H., 2014. Bone marrow endothelium-targeted therapeutics for metastatic breast cancer. J. Control. Release 187, 22–29. doi:http://dx.doi.org/10.1016/j.jconrel.2014.04.057.

McNamara, J.O., Andrechek, E.R., Wang, Y., Viles, K.D., Rempel, R.E., Gilboa, E., Sullenger, B.A., Giangrande, P.H., 2006. Cell type–specific delivery of siRNAs with aptamer-siRNA chimeras. Nat. Biotechnol. 24, 1005–1015. doi:http://dx.doi.org/10.1038/nbt1223.

Meng, L., Yang, L., Zhao, X., Zhang, L., Zhu, H., Liu, C., Tan, W., 2012. Targeted delivery of chemotherapy agents using a liver cancer-specific aptamer. PLoS One 7, e33434. doi:http://dx.doi.org/10.1371/journal.pone.0033434.

Namgung, R., Kim, W.J., 2012. A highly entangled polymeric nanoconstruct assembled by siRNA and its reduction-triggered siRNA release for gene silencing. Small 8, 3209–3219. doi:http://dx.doi.org/10.1002/smll.201200496.

Ni, X., Zhang, Y., Zennami, K., Castanares, M., Mukherjee, A., Raval, R.R., Zhou, H., DeWeese, T.L., Lupold, S.E., 2015. Systemic administration and targeted radiosensitization via chemically synthetic aptamer-siRNA chimeras in human tumor xenografts. Mol. Cancer Ther. 14, 2797–2804. doi:http://dx.doi.org/10.1158/1535-7163.MCT-15-0291-T.

Pieve, C. Da, Perkins, S., Missailidis, S., 2009. Anti-MUC1 aptamers: radiolabelling with 99mTc and biodistribution in MCF-7 tumour-bearing mice. Nucl. Med. Biol. 36, 703–710. doi:http://dx.doi.org/10.1016/j.nucmedbio.2009.04.004.

Porciani, D., Tedeschi, L., Marchetti, L., Citti, L., Piazza, V., Beltram, F., Signore, G., 2015. Aptamer-mediated codelivery of doxorubicin and NF-κB decoy enhances chemosensitivity of pancreatic tumor cells. Mol. Ther. Nucleic Acids 4, e235. doi:http://dx.doi.org/10.1038/mtna.2015.9.

Prakash, J., Rajamanickam, K., 2015. Aptamers and their significant role in cancer therapy and diagnosis. Biomedicines 3, 248–269. doi:http://dx.doi.org/10.3390/biomedicines3030248.

Singh, Y., Murat, P., Defrancq, E., 2010. Recent developments in oligonucleotide conjugation. Chem. Soc. Rev. 39, 2054. doi:http://dx.doi.org/10.1039/b911431a.

Subramanian, N., Raghunathan, V., Kanwar, J.R., Kanwar, R.K., Elchuri, S.V., Khetan, V., Krishnakumar, S., 2012. Target-specific delivery of doxorubicin to retinoblastoma using epithelial cell adhesion molecule aptamer. Mol. Vis. 18, 2783–2795.

Subramanian, N., Kanwar, J.R., Athalya, P., Janakiraman, N., Khetan, V., Kanwar, R.K., Eluchuri, S., Krishnakumar, S., 2015a. EpCAM aptamer mediated cancer cell specific delivery of EpCAM siRNA using polymeric nanocomplex. J. Biomed. Sci. 22, 4. doi:http://dx.doi.org/10.1186/s12929-014-0108-9.

Subramanian, N., Kanwar, J.R., Kanwar, R.K., Krishnakumar, S., 2015b. Targeting cancer cells using LNA-modified aptamer-siRNA chimeras. Nucleic Acid Ther. 25, 317–322. doi:http://dx.doi.org/10.1089/nat.2015.0550.

Sullenger, B.A., 2016. Aptamers coming of age at twenty-five. Nucleic Acid Ther. 26, 119. doi:http://dx.doi.org/10.1089/nat.2016.29001.sul.

Sun, H., Tan, W., Zu, Y., 2016. Aptamers: versatile molecular recognition probes for cancer detection. Analyst 141, 403–415. doi:http://dx.doi.org/10.1039/C5AN01995H.

Taghdisi, S.M., Abnous, K., Mosaffa, F., Behravan, J., 2010. Targeted delivery of daunorubicin to T-cell acute lymphoblastic leukemia by aptamer. J. Drug Target. 18, 277–281. doi:http://dx.doi.org/10.3109/10611860903434050.

Thiel, K.W., Giangrande, P.H., 2010. Intracellular delivery of RNA-based therapeutics using aptamers. Ther. Deliv. 1, 849–861. doi:http://dx.doi.org/10.4155/tde.10.61.

Thiel, K.W., Hernandez, L.I., Dassie, J.P., Thiel, W.H., Liu, X., Stockdale, K.R., Rothman, A.M., Hernandez, F.J., McNamara, J.O., Giangrande, P.H., 2012. Delivery of chemo-sensitizing siRNAs to HER2+-breast cancer cells using RNA aptamers. Nucleic Acids Res. 40 (13), 6319–6337. doi:http://dx.doi.org/10.1093/nar/gks294.

Trinh, T. Le., Zhu, G., Xiao, X., Puszyk, W., Sefah, K., Wu, Q., Tan, W., Liu, C., 2015. A synthetic aptamer-drug adduct for targeted liver cancer therapy. PLoS One 10, e0136673. doi:http://dx.doi.org/10.1371/journal.pone.0136673.

Tuerk, C., Gold, L., 1990. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 249, 505–510. doi:http://dx.doi.org/10.1126/science.2200121.

Varmira, K., Hosseinimehr, S.J., Noaparast, Z., Abedi, S.M., 2013. A HER2-targeted RNA aptamer molecule labeled with 99mTc for single-photon imaging in malignant tumors. Nucl. Med. Biol. 40, 980–986. doi:http://dx.doi.org/10.1016/j.nucmedbio.2013.07.004.

Varmira, K., Hosseinimehr, S.J., Noaparast, Z., Abedi, S.M., 2014. An improved radiolabelled RNA aptamer molecule for HER2 imaging in cancers. J. Drug Target. 22, 116–122. doi:http://dx.doi.org/10.3109/1061186X.2013.839688.

Wang, A.Z., Bagalkot, V., Vasilliou, C.C., Gu, F., Alexis, F., Zhang, L., Shaikh, M., Yuet, K., Cima, M.J., Langer, R., Kantoff, P.W., Bander, N.H., Jon, S., Farokhzad, O.C., 2008. Superparamagnetic iron oxide nanoparticle-aptamer bioconjugates for combined prostate cancer imaging and therapy. ChemMedChem 3 doi:http://dx.doi.org/10.1002/cmdc.200800091.

Wilner, S.E., Wengerter, B., Maier, K., de Lourdes Borba Magalhães, M., Soriano Del Amo, D., Pai, S., Opazo, F., Rizzoli, S.O., Yan, A., Levy, M., 2012. An RNA alternative to human transferrin: a new tool for targeting human cells. Mol. Ther. Acids 2, e79. doi:http://dx.doi.org/10.1038/mtna.2013.6.

Wu, X., Liang, H., Tan, Y., Yuan, C., Li, S., Li, X., Li, G., Shi, Y., Zhang, X., 2014. Cell-SELEX aptamer for highly specific radionuclide molecular imaging of glioblastoma in vivo. PLoS One 9, e90752. doi:http://dx.doi.org/10.1371/journal.pone.0090752.

Wu, X., Chen, J., Wu, M., Zhao, J.X., 2015. Aptamers: active targeting ligands for cancer diagnosis and therapy. Theranostics 5, 322–344. doi:http://dx.doi.org/10.7150/thno.10257.

Wullner, U., Neef, I., Eller, A., Kleines, M., Tur, M.K., Barth, S., 2008. Cell-specific induction of apoptosis by rationally designed bivalent aptamer-siRNA transcripts silencing eukaryotic elongation factor 2. Curr. Cancer Drug Targets 8 (7), 554–565. doi:http://dx.doi.org/10.2174/156800908786241078.

Xiang, D., Shigdar, S., Qiao, G., Zhou, S.-F., Li, Y., Wei, M.Q., Qiao, L., Shamaileh, H., Al Zhu, Y., Zheng, C., Pu, C., Duan, W., 2015. Aptamer-mediated cancer gene therapy. Curr. Gene Ther. 15, 109–119.

Yang, J., Xie, S.-X., Huang, Y., Ling, M., Liu, J., Ran, Y., Wang, Y., Thrasher, J.B., Berkland, C., Li, B., 2012. Prostate-targeted biodegradable nanoparticles loaded with androgen receptor silencing constructs eradicate xenograft tumors in mice. Nanomedicine (Lond). 7, 1297–1309. doi:http://dx.doi.org/10.2217/nnm.12.14.

You, X.-G., Tu, R., Peng, M.-L., Bai, Y.-J., Tan, M., Li, H.-J., Guan, J., Wen, L.-J., 2014. Molecular magnetic resonance probe targeting VEGF165: preparation and in vitro and in vivo evaluation. Contrast Media Mol. Imaging 9 doi:http://dx.doi.org/10.1002/cmmi.1584.

Yu, M.K., Kim, D., Lee, I.-H., So, J.-S., Jeong, Y.Y., Jon, S., 2011. Image-guided prostate cancer therapy using aptamer-functionalized thermally cross-linked superparamagnetic iron oxide nanoparticles. Small 7, 2241–2249. doi:http://dx.doi.org/10.1002/smll.201100472.

Zhang, K., Liu, M., Tong, X., Sun, N., Zhou, L., Cao, Y., Wang, J., Zhang, H., Pei, R., 2015. Aptamer-modified temperature-sensitive liposomal contrast agent for magnetic resonance imaging. Biomacromolecules 16, 2618–2623. doi:http://dx.doi.org/10.1021/acs.biomac.5b00250.

Zhou, J., Rossi, J.J., 2014. Cell-type-specific, aptamer-functionalized agents for targeted disease therapy. Mol. Ther. Nucleic Acids 3, e169. doi:http://dx.doi.org/10.1038/mtna.2014.21.

Zhou, J., Bobbin, M.L., Burnett, J.C., Rossi, J.J., 2012. Current progress of RNA aptamer-based therapeutics. Front. Genet. 3, 234. doi:http://dx.doi.org/10.3389/fgene.2012.00234.

Web references

[1] http://www.prnewswire.com/news-releases/aptamers-market--technology-trend-analysis-by-applications--therapeutics-diagnostics-biosensors-drug-discovery-biomarker-discovery-research-applications-with-market-landscape-analysis--global-forecasts-to-2018-261648911.html. In February, 24th, 2017.