Chapter 7

Aptamers as a Promising Therapeutic Tool for Cancer Immunotherapy

Mario Martínez Soldevilla, Helena Villanueva and Fernando Pastor

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66964

Abstract

Aptamers are single-chained RNA or DNA oligonucleotides (ODNs) with a three-dimensional conformation that provides the ability to fit their targets with high affinity and specificity obtained by a method called SELEX. Cancer immunotherapy has nowadays come back to prominence due to its encouraging results in the clinic with monoclonal antibodies. Aptamers display some important advantages over antibodies at the time of translation into the clinic. They are very suitable for targeting and delivery, reducing off-target side effects, and increasing the therapeutic index of a given strategy. Hundreds of aptamers have been described for very different purposes within biomedical research. Some of the aptamers described recently have been isolated with immunotherapeutic applications to overcome current challenges in cancer immunotherapy. To elicit a specific antitumor immune response, some of these aptamers are engineered to activate co-stimulatory receptors or blocking immunosuppressive signals. Aptamers would hopefully gain an important niche in cancer immunotherapy due to their specific properties.

Keywords: aptamer, oligonucleotide, receptor, cancer, immunotherapy, immune system

1. Introduction

Oligonucleotides (ODNs) are short DNA or RNA oligomers presented as single- or double-stranded molecules containing a specified sequence. This kind of molecules can be generated to be used for a large variety of purposes, such as artificial gene synthesis, DNA sequencing, library construction, molecular probes, and regulation of gene expression, among others. The technical support in terms of detection and analysis that ODNs provide in daily laboratory work is not but a small part of their current use. Nowadays, ODN molecules such as small

interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), CpGs, and aptamers are being currently used as therapeutic agents for the treatment of diseases and malignancies of very different nature.

Aptamers are single-stranded DNA or RNA (ssDNA or ssRNA) oligonucleotides; their three-dimensional conformation provides them with the capability to fit in their targets with high affinity and specificity. The word "aptamer" was coined by Jack Szostak and results from the junction of two words, "aptus" which comes from Latin and means "to fit" and "meros" which comes from Greek and means "particle." The first aptamer was isolated by Andy Ellington and Jack Szostak in 1990 toward organic dyes and paralleled by Craig Tuerk and Larry Gold against the T4-bacteriophage DNA polymerase [1, 2]. Aptamers are isolated through a combinatorial chemical method named SELEX, meaning **s**ystematic **e**volution of **l**igands by **ex**ponential enrichment. The SELEX method (schematically represented in **Figure 1**) is an iterative process that consists in rounds of selection. Each round comprises three main steps: "binding," "partition," and "amplification" [1, 2]. The first step is called "binding" and begins with a complex randomized library of 10^{12} to 10^{15} different sequences to ensure the majority of potential structures. Each sequence comprises two constant regions at

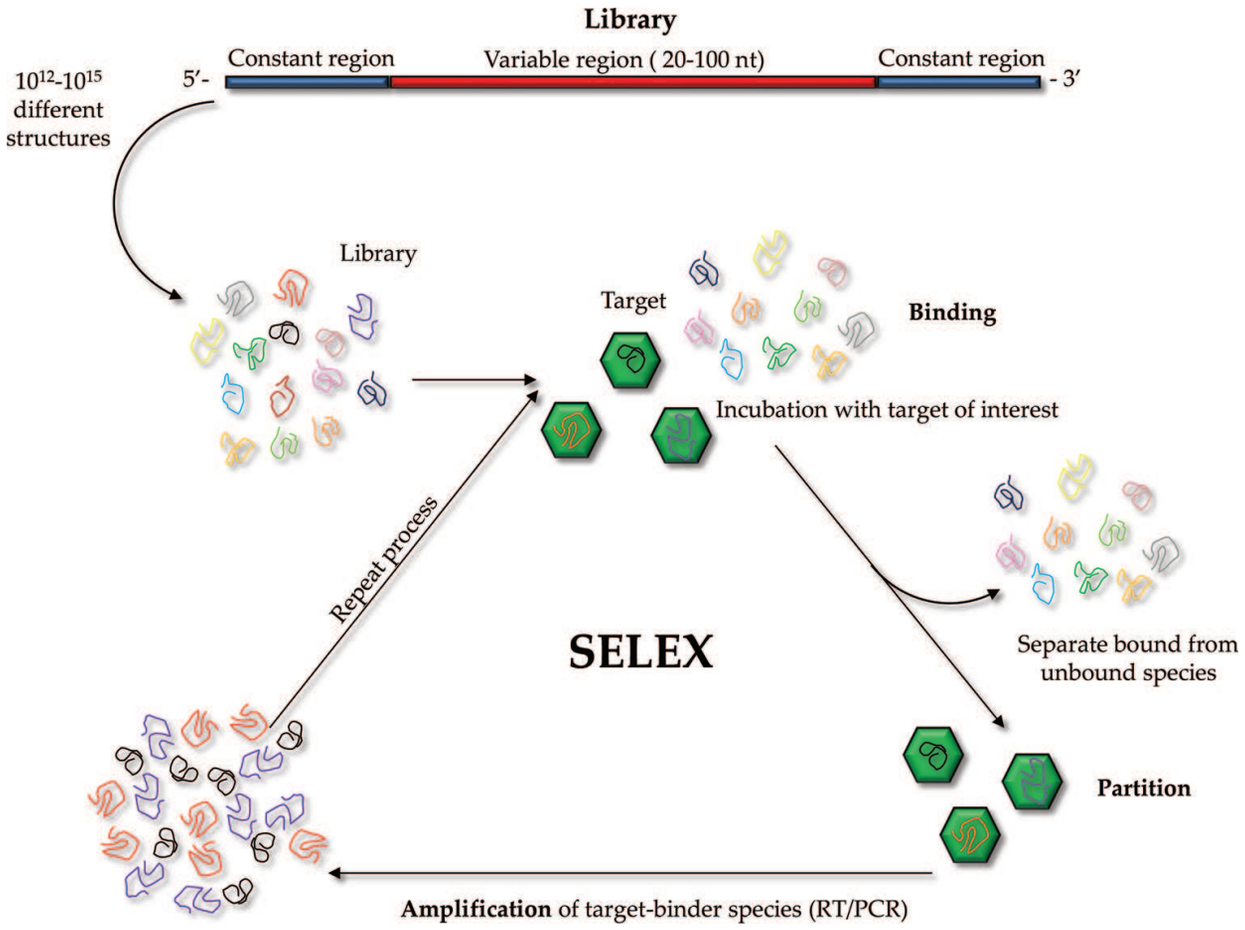

Figure 1. The SELEX procedure. The systematic evolution of ligands by exponential enrichment consists in three main steps: binding, partition, and amplification.

3′ and 5′ ends allowing the primers to anneal flanking a variable region that can vary from 20 to 100 nucleotides (nt). Throughout this step, the library is mixed with the target of interest to allow for some of the sequences to join the target. The following step is known as "partition" and consists in separating the target-binding species from non-binders. Finally, the binder species are amplified by polymerase chain reaction (PCR) in the "amplification" step to serve as library for the next round of selection. It is to note that if the aptamer of interest is an RNA aptamer, in vitro transcription shall be performed before starting each round. The SELEX process usually takes from 9 to 15 rounds, which implies months of work, but new tools such as high-throughput sequencing enable the researchers to identify already enriched sequences at early selection stages, thereby reducing the number of rounds and hence the amount of work to be done [3, 4]. Since the first aptamers isolated by the conventional selection procedure, the SELEX method has evolved and varied through time with the objective of isolating aptamers against targets from every possible nature, including sugars, vitamins, proteins, or even small molecules [5, 6]. Some of the variations are CE-SELEX, which comes from capillary electrophoretic SELEX [7]; cell-SELEX, in which selection is carried out with cells [8]; toggle-SELEX, which on the other hand is used to obtain cross-reactive aptamers [4, 9]; and tailored-SELEX [10], which is used to identify 10-fixed nucleotide aptamers without primer-binding sites. Tailored-SELEX was validated when a Spiegelmer against the migraine-associated target calcitonin gene-related peptide 1 (alpha-CGRP1) was isolated [10]. Spiegelmers are a recently described new class of aptamers, which are "mirror-image" L-conformed enantiomer aptamers [11]. Moreover, in vivo SELEX performs the rounds of selection in animals [12], and genomic SELEX is otherwise used to achieve what are called genomic aptamers directed to bind genomic-encoded functional domains [13].

As mentioned above, aptamers can be isolated against molecule from almost every nature with high affinity and specificity which, in the majority of cases, is comparable or even superior to that of their corresponding monoclonal antibodies (mAbs). Indeed, a DNA aptamer against IL-6 that recognizes this interleukin with a dissociation constant (K_d) of 0.2 nM has been recently described [14]. Following this, line aptamers show several advantages over cell-based products such as antibodies (Abs) or recombinant proteins, as summarized in **Table 1**. Aptamers are smaller than cell-based products, which provide them with an ease to penetrate tissues and therefore make them very suitable for targeting. Thanks to their chemical nature aptamers can be modified to optimize yield and easily customized to add tailored properties to carry cargoes from very diverse nature such as drugs, radioisotopes, proteins, enzymes, RNAs, or even nanostructures, greatly favoring their use for delivery [15–17]. Moreover, aptamers can be easily multimerized to modulate the immune system [18–22]. Throughout the SELEX technique, the process is not interfered with by the toxicity or low immunogenicity of specific antigens as might befall, for example, with Abs [22]. Cell-based products such antibodies or recombinant proteins usually show T-cell-dependent immunity, meaning that an immune response can be directed against these compounds unlike what happens with short ODNs such as aptamers. At the time of translating, the approaches to the clinic aptamers possess a great advantage over other kinds of molecules since they inherently present an antidote [23, 24]. Aptamers are chemical products that can be synthetically manufactured what facilitated their exportation to GMP grade (good manufacturing practices or the practices required

Feature	Aptamer	Cell-based product	Advantage
Nature	Chemically synthesized	Produced by cells	Easy to multimerize for activation of immune receptors
Immunogenicity	Not or very low immunogenic	T-cell-dependent immunity	Do not trigger immune response against them
Size	Small (5–90 KDa)	Big (50–200 KDa)	Easy tissue penetrating, very suitable for targeting
Customization	Easy procedure	Requires specific skills	Tailored properties easy to add, very suitable for delivery
Antidote	Yes	No	Possibility of reversion any undesired effect
GMP grade	Lower cost of manufacturing	Higher complexity and cost of manufacturing	Easier regulatory approval process

Table 1. Advantages of aptamers over cell-based products.

to manufacture and sell any pharmaceutical product). This feature privileges aptamers over antibodies since regulatory approval processes are tougher on cell-based products due to their high complexity and cost of manufacturing.

Aptamers present one disadvantage over other agents currently used in translational medicine—their low plasma stability. Nonetheless, their half-life in plasma can be significantly enhanced by using different approaches, such as selective substitution of HO residues by *O*-methyl or F analogs at the 2′ position of the pyrimidine interactions, thus increasing their resistance to RNA-degrading enzymes [5, 25]. Aptamers can be conjugated to cholesterol to enhance their half-life at the same time that improves their biological activity [26–28]. Another alternative clinically compatible carrier is polyethylene glycol (PEG) [29], which prevents its renal exclusion [5]. PEG conjugation highly increases aptamer survival as exemplified by a PEGylated anti-MUC1 aptamer-doxorubicin conjugate [30]. The PEGylated form of this MUC1-doxorubicin conjugate increased its survival rate to a maximum of sixfold [30]. Furthermore, the addition of nonnatural analogous bases can widen the aptamer-target interactions [5, 25]. This is the case of slow off-rate modified aptamers (SOMAmers), in which aromatic hydrophobic modified nucleotides such as benzyl-dU (Bn-dU) and naphthyl-dU (Nap-dU) are added [31]. Slow off-rate modified aptamers (SOMAmers) show protein-like modified side chains. These substitutions advantage them over conventional aptamers in decreasing the number of exposed polar groups and augmenting their affinity [31].

A tremendous amount of new selected aptamers has been published since the first aptamer isolated in the early 1990s [5, 32, 33]. Some of them are currently undergoing clinical trials for the treatment of several diseases, such as macular edema and age-related macular degeneration as in the case of the antiplatelet-derived growth factor (PDGF) and the anticomplement component (C5) RNA aptamers [34, 35]. Some RNA aptamers including the antifactor IXa of coagulation and the anti-A1 domain for activated von Willebrand factor (vWf) are directed

http://dx.doi.org/10.5772/66964

to control hemostasis [35–37]. For the treatment of diabetes mellitus, one Spiegelmer is being used to target the monocyte chemoattractant protein 1 (MCP-1 also called CCL2) [35]. The two most advanced aptamers for cancer treatment are the anti-nucleolin aptamer AS1411 and the anti-stroma cell-derived factor-1 (SDF-1 also called CXCL12) NOX-A12 [35]. Among every aptamer tested in clinical trials, the first in class was the anti-VEGF RNA aptamer approved in 2004 by the Food and Drug Administration (FDA) which is used for the treatment of age-related macular degeneration and is called MACUGEN.

2. Aptamers in cancer immunotherapy

The cancer burden around the world is extremely growing, to the extent that estimates calculate 21 million new cancer cases and 13 million cancer deaths from now until 2030 [38]. There exist nowadays three main strategies to tackle cancer, namely, chemotherapy, radiotherapy, and surgery in cases of resettable tumors. The elevated relapsing rates and the high toxicity associated with current treatments due to their lack of specificity usually make these conventional treatments not powerful enough in many kinds of tumors. Surgery has the inherent problem of not removing every single malignant cell, causing tumor relapses in the majority of cases. On the other hand, radiotherapy and chemotherapy often show serious side effects, which is not only very harmful but also very uncomfortable for the patient. Immunotherapy is now given prominence thanks to the encouraging results obtained in some clinical trials over the last few years [39] and the use as monotherapy or in combination of several FDA-approved immune-checkpoint blocking Abs such as ipilimumab, nivolumab, or pembrolizumab [40–46]. Nonetheless, due to the severe toxicity associated with the use of agonistic mAbs such 4-1BB Ab or super agonistic CD28 Ab (TGN1412) causing hepatic toxicity and cytokine storm, respectively [47–50], new immunomodulatory ligands with lower associated side effects are strongly needed.

As mentioned above, aptamers have been used in different research fields such as metabolic and cardiovascular diseases or cancer [5]. Among the number of aptamers used in cancer research, some of them have been used to treat cancer within an immunotherapeutic context [51]. This chapter will be focused on aptamers used to date for cancer immunotherapy, which in turn will be subdivided in four main parts: (I) aptamers developed to block immunosuppressive signals, (II) agonistic aptamers directed to trigger activating signals, (III) bi-specific aptamers to target the immune response to the tumor site, and (IV) aptamer-based approaches to enhance tumor immunogenicity.

2.1. Aptamers developed to block immunosuppressive signals

In order to find the first aptamer developed with immunotherapeutic intention, we must go back to 2003. It was then when a CTLA-4 RNA aptamer was isolated and multimerized to block CTLA-4 signaling [18]. Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is a member of the immunoglobulin superfamily expressed by activated T cells, and its engagement with its natural ligand B7 (B7.1=CD80 and B7.2=CD86) leads to T-cell exhaustion [52]. The work published by Gilboa's group laid down the foundations for aptamers as new immunotherapeutic agents. In this work, the multimerized blocking anti-CTLA-4 RNA aptamer showed to bind its target with high affin-

ity and inhibited CTLA-4 action in vitro. This tetrameric form (schematically represented in **Figure 2a**) enhanced its in vitro and in vivo effects similar indeed to that of the mAb [18].

Programmed cell death protein 1 (PD-1) is a protein expressed in several cell types. It is expressed on the surface of CD8+ T lymphocytes especially in tumor-infiltrating lymphocytes (TILs). Its engagement with its ligand PDL-1 expressed on tumor cells induces T-cell exhaustion leading to their dysfunction and therefore tumor progression [53]. An anti-PD-1 DNA aptamer (represented in **Figure 2b**) has been published, which is able to block the PD-1-PDL-1 axis, thereby decreasing tumor burden and increasing survival in murine tumor models [54].

TIM3 is another T-cell exhaustion maker expressed in CD4+ interferon-γ expressing cells and cytotoxic CD8+ T lymphocytes [55]. It is usually expressed on T lymphocytes together with PD-1 [55]. Moreover, the upregulation of TIM3 on a subpopulation of infiltrating Tregs has been correlated with bad prognosis in patients [56]. Our group has recently described a TIM3 RNA aptamer with antagonistic capacity [57]. This aptamer (represented in **Figure 2c**) was able to counteract TIM3 inhibitory signal on T lymphocytes in vitro and reduce tumor burden in a mouse colorectal tumor model in combination with PDL-1 blockade [57] Moreover, we have published at the present time a work that describes the use of in silico and docking studies to predict the mode of action and potential binding site of novel and the already published murine TIM3 RNA aptamer [58].

We have recently selected a murine CD40 RNA aptamer with high affinity for its target (represented in **Figure 2d**). Since CD40 is expressed in several B-cell malignancies, we used it

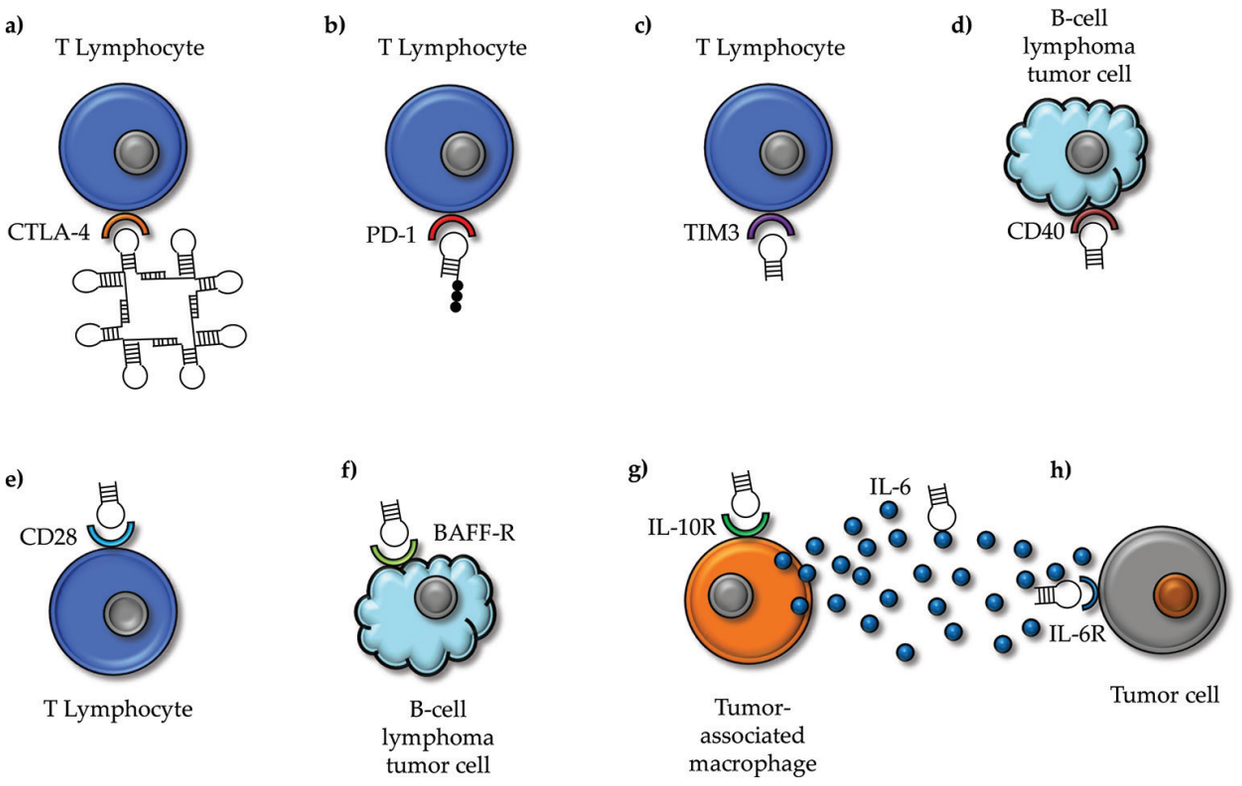

Figure 2. Antagonistic aptamers. (a) CTLA-4, (b) PD-1, (c) TIM3, (d) CD40, (e) CD28, (f) BAFF-R, (g) IL-10R, and (h) IL-6 and IL-6R.

to block the CD40 receptor in vitro and in vivo. The experiments resulted in tumor growth reduction as we increased mice survival by 30% [21]. We had previously described a CD28 antagonistic RNA aptamers which in its monomeric form was able to compete for CD28 ligand B7 and to revert in vitro the co-stimulation induced by B7 in CD4+ T lymphocytes (represented in **Figure 2e**) [20]. This antagonistic aptamer could serve as immunosuppressant in several autoimmune diseases or transplants. For example, in the host-versus-donor immune responses triggered upon transplant engraftment, drug administration is needed to suppress the acute response. Thus, the use of this antagonistic CD28 aptamer might serve as suppressor of the T-cell-guided immune responses against the graft, easing donor engraftment.

The B-cell-activating factor (BAFF) is produced by dendritic cells, monocytes, macrophages, and B cells [59]. The engagement with its receptor has been described to induce B-cell proliferation and survival, and its overexpression has been identified in different B-cell malignancies [60–64]. Aptamers that block BAFF-BAFF-R axis have been selected (represented in **figure 2f**). These aptamers were able to block BAFF-dependent proliferation and survival as well as B-cell malignant proliferation [65].

Several aptamers have been described in the last few years aimed to block cytokine signaling. IL-10 is an immunosuppressive cytokine that promotes immunomodulatory responses favoring tumor growth. It has been shown to be elevated in plasma of cancer patients, which can be used as a prognostic factor in cancer progression [66]. The blockade of its receptor IL-10R has been described to inhibit tumor growth in murine tumor models [67, 68]. An IL-10R-blocking aptamer has been isolated (represented in **figure 2g**). This aptamer was optimized by truncation by removing stearic domains, resulting in an increased affinity for its target [69]. The in vivo experiments revealed that it was able in its truncated monomeric form to inhibit tumor growth at comparable levels to those of the mAb. It was further tetramerized, and this multimeric form was able to block IL-10-IL-10R axis in vitro [69]. Further, a human and murine cross-reactive aptamer against IL-10RA has been recently isolated [4].

A very interesting immunotherapeutic strategy is IL-6-IL-6R axis interruption. IL-6 is a proinflammatory cytokine expressed by B and T cells, monocytes, and fibroblasts [70]. Its presence within the tumor microenvironment leads to immunoregulatory responses favoring tumor growth. Two SOMAmers (represented in **Figure 2h**) have been selected that bind IL-6 with high affinity and inhibit IL-6-mediated signaling by blocking its interaction with IL-6R [71]. The in vitro experiments revealed a similar effect to that obtained by the corresponding IL-6 mAb tocilizumab [71]. Furthermore, an RNA aptamer toward IL-6 (represented in **Figure 2h**) has been selected which showed no blocking activity but was able to effectively deliver cargoes to IL-6 expressing cells [14].

Aptamers toward other cytokines, such as IL-4R or tumor necrosis factor alpha (TNF-α), have been selected. A human and murine RNA cross-reactive aptamer was isolated toward IL-4R able to induce apoptosis in myeloid-derived suppressor cells (MDSCs). The IL-4R-mediated signaling in MDSCs and tumor-associated macrophages (TAMs) resulted in an increased number of TILs and reduction of tumor burden in a mammary carcinoma tumor model [72]. Finally, a TNF-α DNA-blocking aptamer has been isolated that is able to inhibit its activity in vitro [73]. These antagonistic and the remaining aptamers used to date in cancer immunotherapy are summarized in **Table 2**.

Type	Function	Target/s	Nature	Specie/s	Treatment/s	Reference
T-cell exhaustion markers	Antagonists	CTLA-4	RNA	Murine	Melanoma	Santulli-Marotto [18]
		PD-1	DNA	Murine	Colon carcinoma	Prodeus [54]
		TIM3	RNA	Murine	Colon carcinoma in combination with PD-1 blockade	Hervas-Stubbs [57]
Cytokines		IL-10R	RNA	Murine	Colon carcinoma	Vicari [68]
			RNA	Human and murine	Not described (*)	Levay [4]
		IL-6	DNA	Human	Glioma and hepatoma (in vitro)	Gupta [14]
		IL-6R	RNA	Human	Not described (*)	Scheller [70]
		IL-4R	RNA	Human and murine	Mammary carcinoma	Meyer [71]
		TNF-α	DNA	Human	Prevention of TNF-α-induced apoptosis (in vitro)	Roth [72]
Immune receptors		CD28	RNA	Murine	Reversion of CD4 T-cell proliferation (in vitro)	Pastor [20]
		CD40	RNA	Murine	B-cell lymphoma	Soldevilla [21]
		BAFF-R	RNA	Human	Mantle cell lymphoma	Kern [64]
	Agonists	CD28	RNA	Murine	B-cell lymphoma	Pastor [20]
		4-1BB	RNA	Murine	Mastocytoma	McNamara [19]
			RNA	Human and murine	Not described (*)	Levay [4]
		OX-40	RNA	Human	CD4 proliferation (in vitro)	Vinay [74]
			RNA	Murine	Melanoma	Dollins [75]
		CD40	RNA	Murine	Aplasia recovery	Soldevilla [21]

Type	Function	Target/s	Nature	Specie/s	Treatment/s	Reference
Bi-specific immune receptor-tumor marker	Tumor-targeted co-stimulation	4-1BB-PSMA	RNA-RNA	Murine	Colon carcinoma	Niu [76]
		4-1BB-VEGF	RNA-RNA	Murine	Breast carcinoma	Pastor [77]
		CD28-MRP1	RNA-RNA	Murine	Chemotherapy-resistant melanoma	Gilboa [78]
	Tumor-targeted ADCC	CD16α-c-Met	RNA-RNA	Human	ADCC of gastric carcinoma (in vitro)	Dean [79]
Increase tumor antigenicity	Immunomodulating aptamer-based approaches	PSMA-NMD	RNA-siRNA	Murine	Colon carcinoma	Pastor [20]
		CD40-NMD	RNA-shRNA	Murine	B-cell lymphoma	Soldevilla [21]
Increase tumor immunogenicity		CTLA-4-STAT-3	RNA-siRNA	Murine	T-cell lymphoma	Kortylewski [80]
		4-1BB-mTORC1	RNA-siRNA	Murine	Melanoma	Herrmann [81]
		CD28-FOXP3	RNA-peptide	Murine	Colon carcinoma	Casares [82]
		DEC205	RNA	Murine	Melanoma	Berezhnoy [3]

Aptamers are divided into categories depending on type and function. Types: T-cell exhaustion markers, cytokines, immune receptors, bi-specific immune receptor-tumor marker, increase tumor antigenicity, and increase of tumor immunogenicity. Functions: antagonists, agonists, tumor-targeted co-stimulation, tumor-targeted ADCC, and immunomodulating aptamer-based approaches.
Are not described as antagonists.

Table 2. Summary of aptamers used in cancer immunotherapy.

http://dx.doi.org/10.5772/66964

2.2. Agonistic aptamers directed to trigger activating signals

T lymphocytes need at least two signals to be properly activated. The first one comes from the engagement of major histocompatibility complex (MHC) class I in the case of CD8+ and MHC class II in CD4+ cells with the T-cell receptor (TCR) along with CD3. The second signal, well known as co-stimulatory signal, comes mainly from the engagement of CD28 expressed on the surface of T lymphocytes and its ligand B7 expressed on antigen-presenting cells (APCs). Within the tumor microenvironment, lack of co-stimulatory ligands leads to T-cell exhaustion, what turns them into anergic cells unable to trigger an immune response. Thus, the search for agonistic agent has always been of great interest in cancer immunotherapy. The first agonistic aptamer was not described until 2008. It was isolated by conventional SELEX toward murine 4-1BB, which is one of the major co-stimulatory receptors expressed in T lymphocytes. Its ligand 4-1BBL is expressed on the surface of APCs, and their engagement leads to T-cell proliferation [74, 83]. This aptamer was dimerized with the intention of displaying agonistic functions. To that end the agonistic aptamer was generated by adding a complementary 21 nt length to the 3′ end of each monomer using polymerase chain reaction (PCR). After in vitro transcription, monomers were hybridized by pair-wise fashion generating a dimer with a double-stranded linker, which provides a more rigid structure and mirrors the average distance of the two Fv of an IgG (represented on **Figure 3a**). The use of this 4-1BB agonistic RNA aptamer in murine tumor models resulted in inhibition of tumor growth [19]. This work strengthened the idea of using aptamers as novel agents for cancer immunotherapy. Moreover, a human and murine cross-reactive aptamer has been recently published [4]. In this work, they describe a parallel both human- and murine-specific target selection against IL-10RA and 4-1BB followed by identification of common sequences by HTS. This is a "toggle-type" SELEX, which shows a very feasible manner to isolate cross-reactive species aptamers [4].

CD28 is one of the main co-stimulatory receptors with a very important role in immunotherapy. Our research team has generated multimerized CD28 agonistic RNA aptamers able to provide proper CD28 what lead CD4+ and CD8+ proliferation in vitro [20]. Two different dimeric structures were generated to evaluate their effect on T-cell co-stimulation and therefore optimize the strategy. The first dimer was generated as previously described [19] by pair-wise annealing fashion. However, the dimeric structure generated by in vitro transcribing two contiguous monomer units exerted in this case the highest co-stimulatory capacity in CD4+ and CD8+ T lymphocytes [20]. This dimer provides a shorter linker reducing the distance to the minimum and a more flexible structure (represented in **Figure 3b**). This agonistic CD28 aptamer was able to induce both cellular and humoral response in mice in a context of idiotypic vaccination. The use of this agonistic aptamer as adjuvant in an already established idiotypic vaccination protocol to treat B-cell lymphoma resulted in decreased tumor growth and increased survival rate. This aptamer showed a similar effect to that of the mAb [20].

OX-40 is another co-stimulatory receptor upregulated on the surface of CD4+ T cells upon activation, and the engagement with its natural ligand OX-40L expressed on APCs promotes T-cell proliferation, increased cytokine release, and long-term survival [75]. An RNA aptamer toward murine OX-40 was isolated and engineered to exert agonistic functions (represented

http://dx.doi.org/10.5772/66964

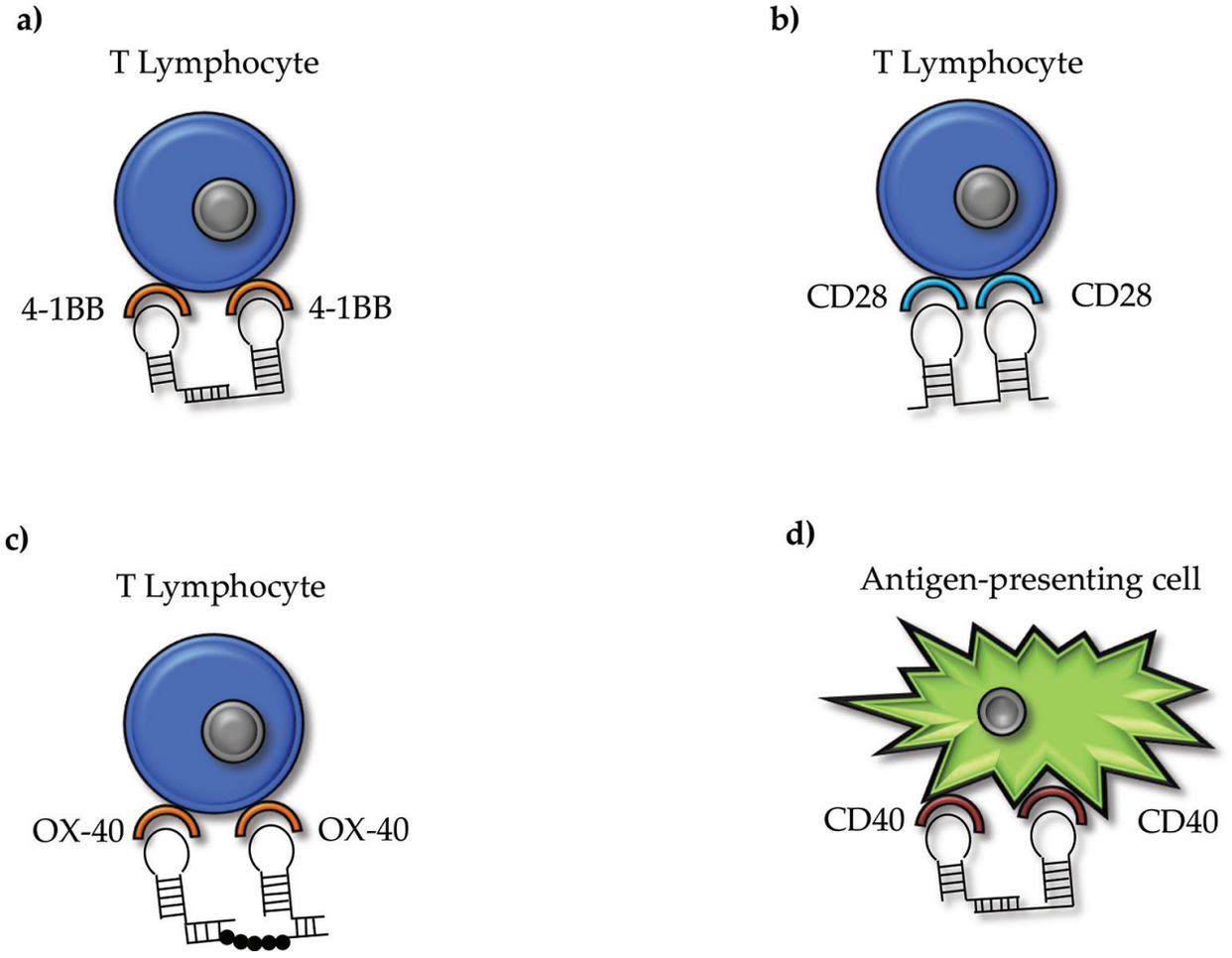

Figure 3. Agonistic aptamers. (a) 4-1BB, (b) CD28, (c) OX-40, and (d) CD40.

in **Figure 3c**) [75]. It was generated as a two-copy scaffold with 18 nt length polycarbon units between the two 3′ aptamer end of the complementary sequences that will anneal by pair-wise fashion [75, 84]. Further, a human OX-40 aptamer has been described which in its dimeric form provided a co-stimulatory effect as demonstrated by cellular proliferation and increased INF-γ production [84].

CD40 is a receptor expressed on the surface of APCs, and its ligand is expressed on T lymphocytes. Their engagement promotes clonal expansion, isotype switching, maturation, proliferation, generation of plasma cells in the case of B cells, and increased antigen presentation on dendritic cells [85, 86]. We have recently published two different agonistic CD40 RNA aptamer-based constructs able to recover bone marrow aplasia while increasing antigen presentation [21]. As mentioned above, in this work, one of the isolated aptamers acted as an antagonist which by simple dimerization was turned into agonist (represented in **Figure 3d**).

2.3. Bi-specific aptamers to target the immune response to the tumor site

The use of agonistic mAbs has been demonstrated to exert severe toxicities as happened with the CD28 superagonistic mAb TGN1412, which resulted in cytokine storm leading a multi-

organ failure forcing the clinical trial to be concluded [47]. Another example is the elevated liver toxicity displayed by the 4-1BB mAb [48]. Moreover, the use of immune-checkpoint blockade mAbs raises the major concern of the appearance of several side effects such as hepatotoxicity, lymphopenia, and thrombocytopenia [76]. Thus, targeting the immune response to the tumor site would reduce the toxicity owed to the off-target effects while increasing the therapeutic index. This approach was published for the first time in 2011 by generating the first bi-specific aptamer, which consisted of both the PSMA and the agonistic 4-1BB aptamers (represented in **Figure 4a**) [77]. This new approach displayed a more potent antitumor immunity at lower doses than that of the corresponding mAbs or the nontargeted co-stimulation. Targeting the immune response to the tumor site is a strategy that requires less amount of reagent as demonstrated in this work, which shows that targeted co-stimulation works as effectively as 10-fold levels of the corresponding controls [77]. A new bi-specific aptamer has been published with the intention of targeting 4-1BB co-stimulation to the vascular endothelial growth factor (VEGF). This new construct consisting of both the VEGF and the agonistic 4-1BB aptamers (represented in **Figure 4b**) showed less toxicity than the rest of the controls while obtaining the same therapeutic effect [87]. In fact, this therapeutic index widening was mirrored in a similar antitumor effect while reducing CD8+ T-cell hyperplasia as well as spleen, lymph node, lung, and liver weights [78, 88]. These described works demonstrate the feasibility of the strategies based on targeting co-stimulation to the tumor site using aptamers.

We have recently published a bi-specific CD28-MRP1 aptamer to target CD28 co-stimulation to cancer stem cells (represented in **Figure 4c**) [89]. The targeted co-stimulation to cancer stem cells which imply chemotherapy resistance would exert a selection pressure on these cells usually responsible for tumor metastasis and tumor relapses [79, 90]. We isolated an aptamer that recognizes multidrug-resistant protein 1 (MRP1) with high affinity, and we used it to generate the

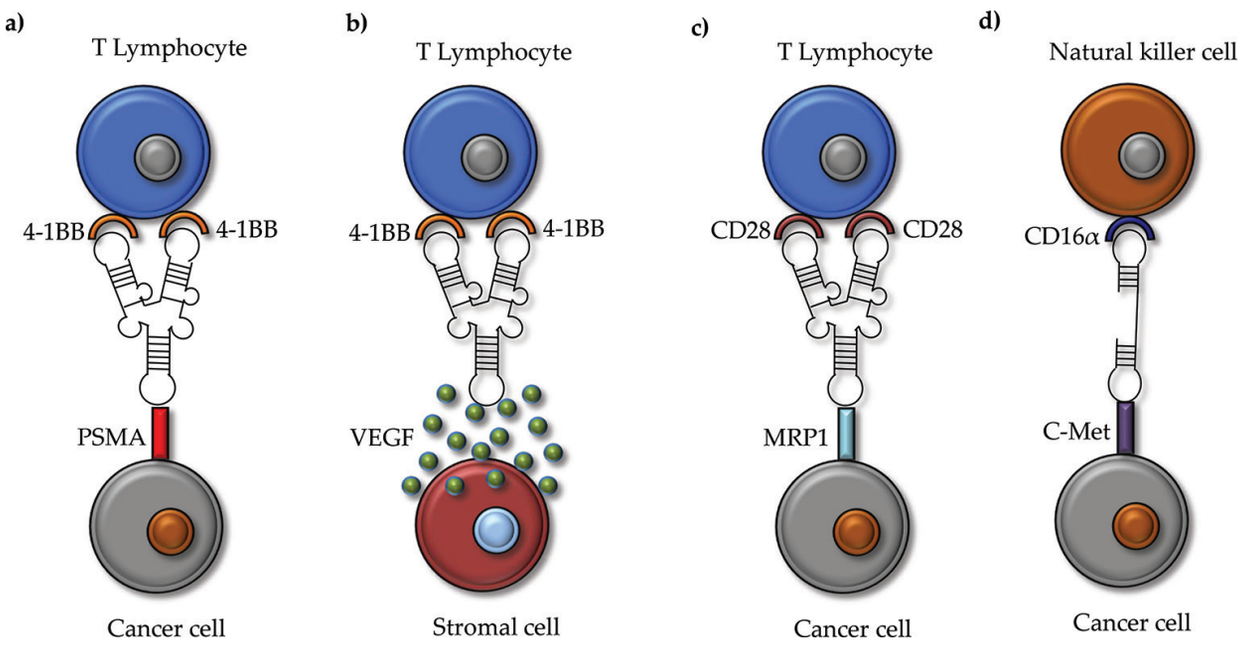

Figure 4. Bi-specific aptamers. (a) 4-1BB-PSMA, (b) 4-1BB-VEGF, (c) CD28-MRP1, and (d) CD16α-c-Met.

CD28-MRP1 bi-specific aptamer together with the already published agonistic CD28 aptamer. This bi-specific aptamer was able to target and properly provide co-stimulation signal to MRP1-overexpressing cells in vitro and in vivo. It was tested in a murine tumor model in the presence of a vaccine (Gvax) and a transient inhibitor peptide of FOXP3 resulting in higher T-lymphocyte tumor infiltration, slower tumor growth, and longer survival [89]. Further, we developed a new vaccination strategy consisting in irradiated MRP1-overexpressing cells coated ex vivo with the CD28-MRP1 bi-specific aptamer termed CD28 Aptvax. CD28 Aptvax exerted delay in MRP1-expressing tumors as well as 50% survival after 50 days of follow-up [89].

Finally, targeted antibody-dependent cell-mediated cytotoxicity (ADCC) can be achieved as well. To that purpose, a DNA aptamer against the Fcγ receptor III (CD16α) was developed to generate a bi-specific aptamer to target ADCC to c-Met-overexpressing tumor cells (represented in **Figure 4d**) [91]. This bi-specific aptamer was tested in both human gastric and lung cancer cell lines resulting in specific c-Met-targeted ADCC [91].

2.4. Aptamer-based approaches to enhance tumor immunogenicity

Despite efforts invested in blocking immunosuppressive signals and activating positive signals, tumor antigenicity is a challenge that remains mostly unsolved. In 2010, a feasible approach was described to increase tumor antigenicity by expressing new tumor antigens. In this work published by Pastor et al. [92], new and therefore more potent antigens are expressed by the tumor triggering a powerful immune response [92, 93]. This approach was based on generating an aptamer-based chimera consisting of the PSMA aptamer and an siRNA for one of the NMD factors. Nonsense-mediated RNA decay (NMD) is a mechanism that controls abnormal transcripts in charge of deleting mRNAs that encode premature termination codons (PTCs). This targeted NMD inhibition resulted in triggering an increased tumor antigenicity leading to a potent immune response in vivo, thereby reducing tumor growth [92]. Moreover, it has been demonstrated that higher lymphocyte infiltration in the tumor is correlated with lower NMD expression, as was shown by the inverse correlation between the accumulation of CD3+ and the expression of the NMD in colorectal cancer with microsatellite instability [94]. Since tumor regression has been demonstrated by the expression of new antigens in the tumor by NMD inhibition [92–94], we decided to apply this strategy to B-cell lymphoma. In this work recently published by our research team, we generated a chimera with the CD40 agonistic aptamer coupled with an shRNA aimed at inhibiting the NMD [21]. In this work, the optimized chimera led to the expression of new powerful antigens, thus triggering an immune response against the tumor. This chimera was able to generate higher lymphocyte infiltration, decreasing tumor growth and increasing mice survival in a B-cell lymphoma tumor model [21].

The expression of new antigens is in some cases insufficient due to the immunosuppressive microenvironment. In fact, the expression of new antigens induces regulatory T-cell (Treg) infiltration indicating that the combination with other aptamer-based strategies would serve to optimize the antitumor immune responses. Signal transducer and activator of transcription 3 (STAT-3)-targeted inhibition can be achieved using Toll-like receptor 9 (TLR9) natural ligands such as CpG. It has been demonstrated that this targeted inhibition triggers a strong antitumor immune response mediated by the activation of tumor-associated immune cells

[80, 95]. In addition, STAT-3 is upregulated in immunosuppressive cells and favors $CD4^+$ Treg expansion. Aptamer-based CTLA-4 delivery strategies have been demonstrated to target both $CD4^+$ Tregs and $CD8^+$ infiltrated lymphocytes [81]. CTLA-4 aptamer-based-targeting delivery of STAT-3 siRNA to T lymphocytes results in inhibition of tumor growth and of metastasis [81]. STAT-3 promotes tumor cell survival and proliferation in tumor cells, as well as invasion and immunosuppression [81]. This work shows an increase of CD8+ T-effector response in vivo thanks to the blockade of CTLA-4 in the first place and subsequently to STAT-3 silencing. STAT-3 inhibition provided a systemic antitumor response leading to inhibition of tumor growth in various cancer cell lines as well as metastasis [81].

mTOR is an intracellular mediator associated with the presence of immune-system shod-living cells [3]. A strategy that demonstrated the agonistic 4-1BB optima coupled with an siRNA for a key factor of the mTOR complex 1(mTORC1) was called raptor [3]. This strategy resulted in mTORC1 downregulation in vitro, and its combination with an already established vaccination protocol promoted a protective immunity in a murine tumor model. This achieved antitumor response showed memory features with cytotoxic effect function [3].

Moreover, an RNA aptamer toward DEC205 has been recently published able to delivery in vitro deliver-specific cargoes for cross-presentation. DEC205 is a surface receptor expressed on CD8+α dendritic cells, which promotes antigen cross-presentation and the subsequent CD8+ activation. The use of this aptamer in vivo displayed strong T-cell-mediated tumor immunity [96].

Our research team has recently shown a new strategy to increase tumor immunogenicity by targeting the inhibition of FOXP3. We generated a CD28 aptamer chimera coupled with the already published FOXP3 transient inhibitory peptide P60 [82, 97]. This peptide is able to penetrate into Tregs and inhibits its function [82]. Due to the absence of specificity of the P60 peptide, we decided to couple it with one of our CD28 described aptamers and therefore target FOXP3 inhibition to CD28-expressing cells. This targeted inhibition counteracted Treg immunosuppression activity while reducing the concentration hundreds of times up to 0.5 mictoM [82, 97]. A very similar antitumor effect in a colon carcinoma tumor model was achieved using 625 pmol of the CD28-P60 chimera compared with 500 nmol of the P60 control [97].

3. Conclusion

Aptamers have gained a large spot among therapeutic agents [5] in several research fields. They have colonized experimental approaches for the treatment of several metabolic and vascular diseases, and their preclinical use in cancer treatments has been widely used. Aptamers can be used to face the three major challenges that immunotherapy poses today [51]. To address the blockade of immunosuppressing signals, aptamers toward CTLA-4, PD1, TIM3, IL-10R, or IL-6 can be used. With the purpose of activating positive signals, agonistic aptamers directed to trigger CD28, 4-1BB, OX40, or CD40 receptors can be utilized. In order to increase tumor immunogenicity, several aptamer-based strategies can be used, such as targeting the NMD inhibition to the tumor, STAT-3-targeted inhibition in TILs, or FOXP3-targeted inhibition in Tregs. Finally, it is to be noted that bi-specific aptamers such as 4-1BB-PSMA, 4-1BB-VEGF, CD28-MRP1, or CD16α-c-MET can be utilized to direct the immune response

to the tumor site. To conclude, despite the youth of this emerging platform, aptamers might feasibly serve as a promising therapeutic tool for cancer immunotherapy.

Acknowledgements

This chapter is supported by Instituto de salud Carlos II FIS I+D [(PI14/01611) and (PI11702854)] Ramón y cajal (RYC-2012-10699) to Fernando Pastor.

Author details

Mario Martínez Soldevilla*, Helena Villanueva and Fernando Pastor

*Address all correspondence to: mmsoldevilla@unav.es

Molecular Therapy Program, Center for Applied Medical Research (CIMA), Foundation for Applied Medical Research (FIMA), Pamplona, Spain

References

[1] Ellington AD, Szostak JW. *In vitro* selection of RNA molecules that bind specific ligands. Nature. 1990;346(6287):818–822.

[2] Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990;249(4968):505–510.

[3] Berezhnoy A, Castro I, Levay A, Malek TR, Gilboa E. Aptamer-targeted inhibition of mTOR in T cells enhances antitumor immunity. J Clin Invest. 2014;124(1):188–197.

[4] Levay A, Brenneman R, Hoinka J, Sant D, Cardone M, Trinchieri G, et al. Identifying high-affinity aptamer ligands with defined cross-reactivity using high-throughput guided systematic evolution of ligands by exponential enrichment. Nucleic Acids Res. 2015;43(12):e82.

[5] Keefe AD, Pai S, Ellington A. Aptamers as therapeutics. Nat Rev Drug Discov. 2010;9(7):537–550.

[6] Cerchia L, de Franciscis V. Nucleic acid-based aptamers as promising therapeutics in neoplastic diseases. Methods Mol Biol. 2007;361:187–200.

[7] Mosing RK, Bowser MT. Isolating aptamers using capillary electrophoresis-SELEX (CE-SELEX). Methods Mol Biol. 2009;535:33–43.

[8] Daniels DA, Chen H, Hicke BJ, Swiderek KM, Gold L. A tenascin-C aptamer identified by tumor cell SELEX: systematic evolution of ligands by exponential enrichment. Proc Natl Acad Sci U S A. 2003;100(26):15416–15421.

[9] White R, Rusconi C, Scardino E, Wolberg A, Lawson J, Hoffman M, et al. Generation of species cross-reactive aptamers using "toggle" SELEX. Mol Ther. 2001;4(6):567–573.

[10] Vater A, Jarosch F, Buchner K, Klussmann S. Short bioactive spiegelmers to migraine-associated calcitonin gene-related peptide rapidly identified by a novel approach: tailored-SELEX. Nucleic Acids Res. 2003;31(21):e130.

[11] Oberthur D, Achenbach J, Gabdulkhakov A, Buchner K, Maasch C, Falke S, et al. Crystal structure of a mirror-image L-RNA aptamer (Spiegelmer) in complex with the natural L-protein target CCL2. Nat Commun. 2015;6:6923.

[12] Cooper TA. *In vivo* SELEX in vertebrate cells. Methods Mol Biol. 1999;118:405–417.

[13] Kim S, Shi H, Lee DK, Lis JT. Specific SR protein-dependent splicing substrates identified through genomic SELEX. Nucleic Acids Res. 2003;31(7):1955–1961.

[14] Gupta S, Hirota M, Waugh SM, Murakami I, Suzuki T, Muraguchi M, et al. Chemically modified DNA aptamers bind interleukin-6 with high affinity and inhibit signaling by blocking its interaction with interleukin-6 receptor. J Biol Chem. 2014;289(12):8706–8719.

[15] McNamara JO 2nd, Andrechek ER, Wang Y, Viles KD, Rempel RE, Gilboa E, et al. Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. Nat Biotechnol. 2006;24(8):1005–1015.

[16] Orava EW, Cicmil N, Gariepy J. Delivering cargoes into cancer cells using DNA aptamers targeting internalized surface portals. Biochim Biophys Acta. 2010;1798(12):2190–2200.

[17] Kanwar JR, Roy K, Kanwar RK. Chimeric aptamers in cancer cell-targeted drug delivery. Crit Rev Biochem Mol Biol. 2011;46(6):459–477.

[18] Santulli-Marotto S, Nair SK, Rusconi C, Sullenger B, Gilboa E. Multivalent RNA aptamers that inhibit CTLA-4 and enhance tumor immunity. Cancer Res. 2003;63(21):7483–7489.

[19] McNamara JO, Kolonias D, Pastor F, Mittler RS, Chen L, Giangrande PH, et al. Multivalent 4-1BB binding aptamers costimulate CD8+ T cells and inhibit tumor growth in mice. J Clin Invest. 2008;118(1):376–386.

[20] Pastor F, Soldevilla MM, Villanueva H, Kolonias D, Inoges S, de Cerio AL, et al. CD28 aptamers as powerful immune response modulators. Mol Ther Nucleic Acids. 2013;2:e98.

[21] Soldevilla MM, Villanueva H, Bendandi M, Inoges S, Lopez-Diaz de Cerio A, Pastor F. 2-fluoro-RNA oligonucleotide CD40 targeted aptamers for the control of B lymphoma and bone-marrow aplasia. Biomaterials. 2015;67:274–285.

[22] Gilboa E, McNamara J 2nd, Pastor F. Use of oligonucleotide aptamer ligands to modulate the function of immune receptors. Clin Cancer Res. 2013;19(5):1054–1062.

[23] Oney S, Lam RT, Bompiani KM, Blake CM, Quick G, Heidel JD, et al. Development of universal antidotes to control aptamer activity. Nat Med. 2009;15(10):1224–1228.

[24] Bompiani KM, Woodruff RS, Becker RC, Nimjee SM, Sullenger BA. Antidote control of aptamer therapeutics: the road to a safer class of drug agents. Curr Pharm Biotechnol. 2012;13(10):1924–1934.

[25] Schmidt KS, Borkowski S, Kurreck J, Stephens AW, Bald R, Hecht M, et al. Application of locked nucleic acids to improve aptamer *in vivo* stability and targeting function. Nucleic Acids Res. 2004;32(19):5757–5765.

[26] de Smidt PC, Le Doan T, de Falco S, van Berkel TJ. Association of antisense oligonucleotides with lipoproteins prolongs the plasma half-life and modifies the tissue distribution. Nucleic Acids Res. 1991;19(17):4695–4700.

[27] Lee CH, Lee YJ, Kim JH, Lim JH, Kim JH, Han W, et al. Inhibition of hepatitis C virus (HCV) replication by specific RNA aptamers against HCV NS5B RNA replicase. J Virol. 2013;87(12):7064–7074.

[28] Lee CH, Lee SH, Kim JH, Noh YH, Noh GJ, Lee SW. Pharmacokinetics of a cholesterol-conjugated aptamer against the hepatitis C virus (HCV) NS5B protein. Mol Ther Nucleic Acids. 2015;4:e254.

[29] Da Pieve C, Blackshaw E, Missailidis S, Perkins AC. PEGylation and biodistribution of an anti-MUC1 aptamer in MCF-7 tumor-bearing mice. Bioconjug Chem. 2012;23(7):1377–1381.

[30] Tan L, Neoh KG, Kang ET, Choe WS, Su X. PEGylated anti-MUC1 aptamer-doxorubicin complex for targeted drug delivery to MCF7 breast cancer cells. Macromol Biosci. 2011;11(10):1331–1335.

[31] Rohloff JC, Gelinas AD, Jarvis TC, Ochsner UA, Schneider DJ, Gold L, et al. Nucleic acid ligands with protein-like side chains: modified aptamers and their use as diagnostic and therapeutic agents. Mol Ther Nucleic Acids. 2014;3:e201.

[32] Bunka DH, Stockley PG. Aptamers come of age—at last. Nat Rev Microbiol. 2006;4(8):588–596.

[33] Zhou G, Wilson G, Hebbard L, Duan W, Liddle C, George J, et al. Aptamers: apromising chemical antibody for cancer therapy. Oncotarget. 2016; 7(12):13446–13463.

[34] Ricklin D, Lambris JD. Complement-targeted therapeutics. Nat Biotechnol. 2007;25(11):1265–1275.

[35] Sundaram P, Kurniawan H, Byrne ME, Wower J. Therapeutic RNA aptamers in clinical trials. Eur J Pharm Sci. 2013;48(1–2):259–271.

[36] Gilbert JC, DeFeo-Fraulini T, Hutabarat RM, Horvath CJ, Merlino PG, Marsh HN, et al. First-in-human evaluation of anti von willebrand factor therapeutic aptamer ARC1779 in healthy volunteers. Circulation. 2007;116(23):2678–2686.

[37] Jilma B, Paulinska P, Jilma-Stohlawetz P, Gilbert JC, Hutabarat R, Knobl P. A randomised pilot trial of the anti-von willebrand factor aptamer ARC1779 in patients with type 2b von willebrand disease. Thromb Haemost. 2010;104(3):563–570.

[38] Siegel RL, Miller KD, Jemal A. Cancer Facts & Figures 2015. Atlanta: American Cancer Society; 2015.

[39] Kyi C, Postow MA. Checkpoint blocking antibodies in cancer immunotherapy. FEBS Lett. 2014;588(2):368–376.

[40] No authors listed. Ipilimumab. Drugs R D. 2010;10(12):97–110

[41] Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–723.

[42] Lipson EJ, Drake CG. Ipilimumab: an anti-CTLA-4 antibody for metastatic melanoma. Clin Cancer Res. 2011;17(22):6958–6962.

[43] Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364(26):2517–2526.

[44] Raedler LA. Keytruda (pembrolizumab): first PD-1 inhibitor approved for previously treated unresectable or metastatic melanoma. Am Health Drug Benefits. 2015;8(Spec Feature):96–100.

[45] Raedler LA. Opdivo (nivolumab): second PD-1 inhibitor receives FDA approval for unresectable or metastatic melanoma. Am Health Drug Benefits. 2015;8(Spec Feature):180–183.

[46] Melero I, Berman DM, Aznar MA, Korman AJ, Perez Gracia JL, Haanen J. Evolving synergistic combinations of targeted immunotherapies to combat cancer. Nat Rev Cancer. 2015;15(8):457–472.

[47] Attarwala H. TGN1412: from discovery to disaster. J Young Pharm. 2010;2(3):332–336.

[48] Bartkowiak T, Curran MA. 4-1BB agonists: multi-potent potentiators of tumor immunity. Front Oncol. 2015;5:117.

[49] Naidoo J, Page DB, Li BT, Connell LC, Schindler K, Lacouture ME, et al. Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. Ann Oncol. 2015;26(12):2375–2391.

[50] Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. J Clin Oncol. 2015;33(17):1974–1982.

[51] Soldevilla MM, Villanueva H, Pastor F. Aptamers: a feasible technology in cancer immunotherapy. J Immunol Res. 2016;2016:1083738.

[52] Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med. 1995;182(2):459–465.

[53] Gros A, Robbins PF, Yao X, Li YF, Turcotte S, Tran E, et al. PD-1 identifies the patient-specific CD8(+) tumor-reactive repertoire infiltrating human tumors. J Clin Invest. 2014;124(5):2246–2259.

[54] Prodeus A, Abdul-Wahid A, Fischer NW, Huang EH, Cydzik M, Gariepy J. Targeting the PD-1/PD-L1 immune evasion axis with DNA aptamers as a novel therapeutic strategy for the treatment of disseminated cancers. Mol Ther Nucleic Acids. 2015;4:e237.

[55] Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, et al. Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. Nature. 2002;415(6871):536–541.

[56] Sakuishi K, Ngiow SF, Sullivan JM, Teng MW, Kuchroo VK, Smyth MJ, et al. TIM3+FOXP3+ regulatory T cells are tissue-specific promoters of T-cell dysfunction in cancer. Oncoimmunology. 2013;2(4):e23849.

[57] Hervas-Stubbs S, Soldevilla MM, Villanueva H, Manchero U, Bendandi M, Pastor F. Identification of TIM3 2′-fluoro oligonucleotide aptamer by HT-SELEX for cancer immunotherapy. Oncotarget. 2015;7(4):4522–4530.

[58] Rabal O, Pastor F, Villanueva H, Soldevilla MM, Hervas-Stubbs S, Oyarzabal J. In silico aptamer docking studies: from a retrospective validation to a prospective case study-TIM3aptamers binding. Mol Ther Nucleic Acids. 2016;5(10):e376. doi: 10.1038/mtna.2016.84.

[59] Craxton A, Magaletti D, Ryan EJ, Clark EA. Macrophage- and dendritic cell—dependent regulation of human B-cell proliferation requires the TNF family ligand BAFF. Blood. 2003;101(11):4464–4471.

[60] Shulga-Morskaya S, Dobles M, Walsh ME, Ng LG, MacKay F, Rao SP, et al. B cell-activating factor belonging to the TNF family acts through separate receptors to support B cell survival and T cell-independent antibody formation. J Immunol. 2004;173(4):2331–2341.

[61] Batten M, Groom J, Cachero TG, Qian F, Schneider P, Tschopp J, et al. BAFF mediates survival of peripheral immature B lymphocytes. J Exp Med. 2000;192(10):1453–1466.

[62] He B, Chadburn A, Jou E, Schattner EJ, Knowles DM, Cerutti A. Lymphoma B cells evade apoptosis through the TNF family members BAFF/BLyS and APRIL. J Immunol. 2004;172(5):3268–3279.

[63] Novak AJ, Grote DM, Stenson M, Ziesmer SC, Witzig TE, Habermann TM, et al. Expression of BLyS and its receptors in B-cell non-Hodgkin lymphoma: correlation with disease activity and patient outcome. Blood. 2004;104(8):2247–2253.

[64] Kern C, Cornuel JF, Billard C, Tang R, Rouillard D, Stenou V, et al. Involvement of BAFF and APRIL in the resistance to apoptosis of B-CLL through an autocrine pathway. Blood. 2004;103(2):679–688.

[65] Zhou J, Tiemann K, Chomchan P, Alluin J, Swiderski P, Burnett J, et al. Dual functional BAFF receptor aptamers inhibit ligand-induced proliferation and deliver siRNAs to NHL cells. Nucleic Acids Res. 2013;41(7):4266–4283.

[66] Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001;19:683–765.

[67] Guiducci C, Vicari AP, Sangaletti S, Trinchieri G, Colombo MP. Redirecting *in vivo* elicited tumor infiltrating macrophages and dendritic cells towards tumor rejection. Cancer Res. 2005;65(8):3437–3446.

[68] Vicari AP, Chiodoni C, Vaure C, Ait-Yahia S, Dercamp C, Matsos F, et al. Reversal of tumor-induced dendritic cell paralysis by CpG immunostimulatory oligonucleotide and anti-interleukin 10 receptor antibody. J Exp Med. 2002;196(4):541–549.

[69] Berezhnoy A, Stewart CA, Mcnamara JO 2nd, Thiel W, Giangrande P, Trinchieri G, et al. Isolation and optimization of murine IL-10 receptor blocking oligonucleotide aptamers using high-throughput sequencing. Mol Ther. 2012;20(6):1242–1250.

[70] Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta. 2011;1813(5):878–888.

[71] Meyer C, Eydeler K, Magbanua E, Zivkovic T, Piganeau N, Lorenzen I, et al. Interleukin-6 receptor specific RNA aptamers for cargo delivery into target cells. RNA Biol. 2012;9(1):67–80.

[72] Roth F, De La Fuente AC, Vella JL, Zoso A, Inverardi L, Serafini P. Aptamer-mediated blockade of IL4Ralpha triggers apoptosis of MDSCs and limits tumor progression. Cancer Res. 2012;72(6):1373–1383.

[73] Orava EW, Jarvik N, Shek YL, Sidhu SS, Gariepy J. A short DNA aptamer that recognizes TNF alpha and blocks its activity *in vitro*. ACS Chem Biol. 2013;8(1):170–178.

[74] Vinay DS, Kwon BS. Role of 4-1BB in immune responses. Semin Immunol. 1998;10(6):481–489.

[75] Dollins CM, Nair S, Boczkowski D, Lee J, Layzer JM, Gilboa E, et al. Assembling OX40 aptamers on a molecular scaffold to create a receptor-activating aptamer. Chem Biol. 2008;15(7):675–682.

[76] Niu L, Strahotin S, Hewes B, Zhang B, Zhang Y, Archer D, et al. Cytokine-mediated disruption of lymphocyte trafficking, hemopoiesis, and induction of lymphopenia, anemia, and thrombocytopenia in anti-CD137-treated mice. J Immunol. 2007;178(7):4194–4213.

[77] Pastor F, Kolonias D, McNamara JO 2nd, Gilboa E. Targeting 4-1BB costimulation to disseminated tumor lesions with bi-specific oligonucleotide aptamers. Mol Ther. 2011;19(10):1878–1886.

[78] Gilboa E, Berezhnoy A, Schrand B. Reducing toxicity of immune therapy using aptamer-targeted drug delivery. Cancer Immunol Res. 2015;3(11):1195–1200.

[79] Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer. 2005;5(4):275–284.

[80] Kortylewski M, Swiderski P, Herrmann A, Wang L, Kowolik C, Kujawski M, et al. *In vivo* delivery of siRNA to immune cells by conjugation to a TLR9 agonist enhances antitumor immune responses. Nat Biotechnol. 2009;27(10):925–932.

[81] Herrmann A, Priceman SJ, Swiderski P, Kujawski M, Xin H, Cherryholmes GA, et al. CTLA4 aptamer delivers STAT3 siRNA to tumor-associated and malignant T cells. J Clin Invest. 2014;124(7):2977–2987.

[82] Casares N, Rudilla F, Arribillaga L, Llopiz D, Riezu-Boj JI, Lozano T, et al. A peptide inhibitor of FOXP3 impairs regulatory T cell activity and improves vaccine efficacy in mice. J Immunol. 2010;185(9):5150–5159.

[83] Sica G, Chen L. Modulation of the immune response through 4-1BB. Adv Exp Med Biol. 2000;465:355–362.

[84] Pratico ED, Sullenger BA, Nair SK. Identification and characterization of an agonistic aptamer against the T cell costimulatory receptor, OX40. Nucleic Acid Ther. 2013;23(1):35–43.

[85] Foy TM, Laman JD, Ledbetter JA, Aruffo A, Claassen E, Noelle RJ. gp39-CD40 interactions are essential for germinal center formation and the development of B cell memory. J Exp Med. 1994;180(1):157–163.

[86] Arpin C, Dechanet J, Van Kooten C, Merville P, Grouard G, Briere F, et al. Generation of memory B cells and plasma cells *in vitro*. Science. 1995;268(5211):720–722.

[87] Schrand B, Berezhnoy A, Brenneman R, Williams A, Levay A, Kong LY, et al. Targeting 4-1BB costimulation to the tumor stroma with bispecific aptamer conjugates enhances the therapeutic index of tumor immunotherapy. Cancer Immunol Res. 2014;2(9):867–877.

[88] Schrand B, Berezhnoy A, Brenneman R, Williams A, Levay A, Gilboa E. Reducing toxicity of 4-1BB costimulation: targeting 4-1BB ligands to the tumor stroma with bi-specific aptamer conjugates. Oncoimmunology. 2015;4(3):e970918.

[89] Soldevilla MM, Villanueva H, Casares N, Lasarte JJ, Bendandi M, Inoges S, et al. MRP1-CD28 bi-specific oligonucleotide aptamers: target costimulation to drug-resistant melanoma cancer stem cells. Oncotarget 2016;7(17):23182–23196.

[90] Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer. 2002;2(6):442–454.

[91] Boltz A, Piater B, Toleikis L, Guenther R, Kolmar H, Hock B. Bi-specific aptamers mediating tumor cell lysis. J Biol Chem. 2011;286(24):21896–21905.

[92] Pastor F, Kolonias D, Giangrande PH, Gilboa E. Induction of tumour immunity by targeted inhibition of nonsense-mediated mRNA decay. Nature. 2010;465(7295):227–230.

[93] Holbrook JA, Neu-Yilik G, Hentze MW, Kulozik AE. Nonsense-mediated decay approaches the clinic. Nat Genet. 2004;36(8):801–808.

[94] El-Bchiri J, Guilloux A, Dartigues P, Loire E, Mercier D, Buhard O, et al. Nonsense-mediated mRNA decay impacts MSI-driven carcinogenesis and anti-tumor immunity in colorectal cancers. PLoS One. 2008;3(7):e2583.

[95] Kortylewski M, Kujawski M, Herrmann A, Yang C, Wang L, Liu Y, et al. Toll-like receptor 9 activation of signal transducer and activator of transcription 3 constrains its agonist-based immunotherapy. Cancer Res. 2009;69(6):2497–2505.

[96] Wengerter BC, Katakowski JA, Rosenberg JM, Park CG, Almo SC, Palliser D, et al. Aptamer-targeted antigen delivery. Mol Ther. 2014;22(7):1375–1387.

[97] Lozano T, Soldevilla MM, Casares N, Villanueva H, Bendandi M, Lasarte JJ, et al. Targeting inhibition of Foxp3 by a CD28 2′-Fluro oligonucleotide aptamer conjugated to P60-peptide enhances active cancer immunotherapy. Biomaterials. 2016;91:73–80.

IntechOpen