


# Nucleic Acid Aptamer: A Novel Potential Diagnostic and Therapeutic Tool for Leukemia

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Yuan Tan  
Yuejin Li  
Faqing Tang 

Department of Clinical Laboratory,  
Hunan Cancer Hospital and The  
Affiliated Cancer Hospital of Xiangya  
School of Medicine, Central South  
University, Changsha 410013, People's  
Republic of China

**Abstract:** Leukemia immunotherapy has been dominant via using synthetic antibodies to target cluster of differentiation (CD) molecules, nevertheless inevitable cytotoxicity and immunogenicity would limit its development. Recently, increasing reports have focused on nucleic acid aptamers, a class of high-affinity nucleic acid ligands. Aptamers purportedly serve as “chemical antibodies”, have negligible cytotoxicity and low immunogenicity, and would be widely applied for the therapy and diagnosis of various diseases, especially leukemia. In the preclinical applications, nucleic acid aptamers have displayed the augmented specificity and selectivity via recognizing targets on leukemia cells based on unique three-dimensional conformations. As small molecules with nucleic acid characteristics, aptamers need to be chemically modified to resist nuclease degradation, renal clearance and improve binding affinities. Moreover, aptamers can be linked with neoteric detection techniques to enhance sensitivity and selectivity of diagnosis and therapy. In this review, we summarized aptamers’ preparation, chemical modification and conjugation, and discussed the application of aptamers in diagnosis and treatment of leukemia through highly specifically recognizing target molecules. Significantly, the application prospect of aptamers in fusion genes would be introduced.

**Keywords:** nucleic acid aptamer, diagnosis, therapy, leukemia

## Introduction

Leukemia is a hematological malignancy that arises from bone marrow (BM), characterized by the abnormal proliferation of BM precursor cells, contributing to a series of symptoms including anaemia, bleeding, fever and life-threatening infections. Leukemia is composed of four types, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphoblastic leukemia (CLL), and chronic myeloid leukemia (CML). Routine methods to diagnose leukemia contain morphological examination, cytochemical immunophenotyping, cytogenetics and molecular analysis of BM samples, according to the 2016 revision to WHO classification of myeloid neoplasms and acute leukemia.<sup>1</sup> However, the drawbacks of traditional methods like invasion, procedural complications and impractical testing equipment limit the further development of clinical medicine,<sup>2</sup> demonstrating the importance of the development of new amplification strategies or diagnostic technologies. The amplification strategies simultaneously detect DNA mutations and copy number variation via amplifying response signal, such as multiplex ligation-dependent probe amplification (MLPA) for monitoring gene aberrations.<sup>3,4</sup> As previous researches reveal, conventional therapies for leukemia are composed of immunotherapy, stem cell

Correspondence: Faqing Tang  
Hunan Cancer Hospital and The Affiliated  
Cancer Hospital of Xiangya School of  
Medicine, Central South University,  
Changsha, People's Republic of China  
Tel/Fax +86-731-89762688  
Email tangfaqing33@hotmail.com

therapy, chemotherapy, traditional Chinese medicine treatment, targeted therapy and BM transplantation, which markedly improve anti-leukemic efficiency, but still have a poor prognosis and a high fatality rate.<sup>5</sup> Among these, the conjugation of chemotherapeutics with antibodies (mostly monoclonal antibodies, mAbs) formulates antibody–drug conjugates to directly deliver targeted drugs to cluster of differentiation (CD) antigens and other external targets, which is a promising strategy for clinical application.<sup>6–8</sup> Nevertheless, there is still an inevitable drawback that targets possibly escape from the attack of monoclonal antibodies in the unstable environment of progressive leukemia,<sup>9</sup> suggesting that a therapeutic platform with improved therapeutic efficacy and reduced non-specific toxicity is urgently in demand. Fortunately, with the establishment of new therapies, like aptamers-mediated methods, the curative effect of leukemia has been greatly improved.<sup>10</sup>

Recently, multifunctional nucleic acid aptamers have shown the superiority over monoclonal antibodies and might be excellent alternatives or supplements to monoclonal antibodies in theranostics (therapy and diagnosis) of leukemia.<sup>10</sup> Remarkably, the application of aptamers in other hematologic malignancies also shows dramatic prospect. For example, TD05 aptamers were developed against Ramos cells to detect Burkitt's lymphoma, which were also engineered as drug carriers to therapy diseases.<sup>11</sup> Aptamers purportedly serve as “unique antibodies”, consisting of short single strands of DNA or RNA, which are selected by “systemic evolution of ligands by exponential enrichment (SELEX)” including cell-SELEX and protein-SELEX,<sup>12</sup> then selectively bind to a wide range of targets including small organic molecules, peptides, proteins, viruses, bacteria, whole cells and even living animals. The interaction of aptamers with target molecules has low immunogenicity, superior stability, high affinity and specificity.<sup>12</sup> Therefore, aptamers have been widely applied for the detection and treatment of various diseases, including inflammatory, infections,<sup>13</sup> cardiovascular,<sup>14</sup> neurodegenerative,<sup>15</sup> autoimmune diseases,<sup>16</sup> and cancer.<sup>17,18</sup> Especially, aptamers-based methods for leukemia therapy and diagnosis have shown the preferable potential when conjugated with drugs, imaging technologies and other detection platforms.<sup>19,20</sup> Unfortunately, aptamers need to be chemically modified to significantly overcome nuclease degradation, rapid renal excretion and deficient binding affinity due to the nucleic acid characteristics.<sup>21</sup>

Herein, we summarized aptamers' preparation, chemical modification and conjugation, and discussed the application of aptamers in diagnosis and treatment of leukemia through highly specifically recognizing target molecules. Significantly, the application prospect of aptamers in fusion genes would be introduced.

## Aptamers' Generation and Optimization

### Aptamers' Isolation and Constitution

The nucleic acid aptamers are isolated by “SELEX” including protein-SELEX and cell-SELEX. In protein-SELEX, the purified or recombinant protein functions as a target for SELEX, and the procedure is simpler compared to cell-SELEX, but some selected aptamers binding to purified membrane proteins fail to recognize targets in whole cells,<sup>22</sup> and the instability of protein structure also affects its recognition function, which limit aptamers' application in medical fields.<sup>23</sup> In cell-based SELEX, the selected targets are embedded on viable cells, therefore the used cells must be available, cultivable and stable. Moreover, the cell-based SELEX does not need complicated purification steps.<sup>24</sup> The whole cell-SELEX process usually contains the following steps: Firstly, designing an initial single-stranded DNA oligonucleotide pool composed of  $10^{14}$ – $10^{15}$  random sequences. Next, random sequences fold into different secondary and tertiary structures and are incubated with immobilized targets to form aptamers-target complexes, which are used for positive selection. Then, the unbound sequences are washed off during the partitioning step. Next, the target-bound sequences are amplified by PCR in DNA aptamers or RT-PCR in RNA aptamers to create an enriched library for the next round of selection. Lastly, the enriched aptamers' sequences are frequently identified by high-throughput sequencing methods. This selection process is repeated 10–15 cycles, until the specific sequences of the target are enriched in the pool.<sup>25</sup> Remarkably, the more advanced cell-ExSELEX (genetic alphabet Expansion for SELEX) technique has been developed to generate unnatural base (UB)-containing DNA aptamers with higher affinity and specificity, which is based on hydrophobic UB pairs (UBPs) between 7-(2-thienyl)imidazo[4,5-*b*]pyridine (Ds) and 2-nitro-4-propynylpyrrole (Px).<sup>26</sup>

### Aptamers' Chemical Modification

The nucleic acid aptamers consisting of ssDNA or RNA need to be chemically modified to prevent degradation and

enhance aptamers' biological and physicochemical stability. In current, there are the following modifications: 1) 2'-substitutions on the sugar ring: 2'-OH moieties of RNA aptamers are heavily modified with fluorine (2'-F), Methoxy (2'-OMe), amine (2'-NH<sub>2</sub>) and locked nucleic acids (LNAs).<sup>27-29</sup> Unlocked nucleic acid (UNA), 2'-deoxy-2'-fluoro-D-arabinonucleic acid (2'-F ANA) and 2'-O-methyl RNA analogues are used to modify 2'-position of the DNA aptamers' sugar ring.<sup>30-32</sup> 2) Terminal 3'-3' and 5'-5' internucleotide linkage: Aptamers are modified by 3'-inverted dT and then extend chain in the standard 3'→5' pattern, which needs controlled pore glass (CPG) modified with 5'-hydroxyl of the first nucleoside.<sup>33-35</sup> 3) 3'-biotin conjugates: 3'-biotin is similar to 3'-inverted dT modification.<sup>35</sup> 4) Modifications on phosphodiester linkage: Replacing DNA phosphodiester linkage with triazole linkages,<sup>36</sup> methylphosphonate or phosphorothioate dramatically enhances resistance to nuclease hydrolysis.<sup>37-39</sup> 5) Mirror Image L-DNA: Based on natural D-form DNA aptamers, L-enantiomeric oligonucleotide aptamers (Spiegelmers) are chemically synthesized. L-DNA aptamers, like NOX-A12 and NOX-H94, have been proven to be more stable.<sup>40,41</sup>

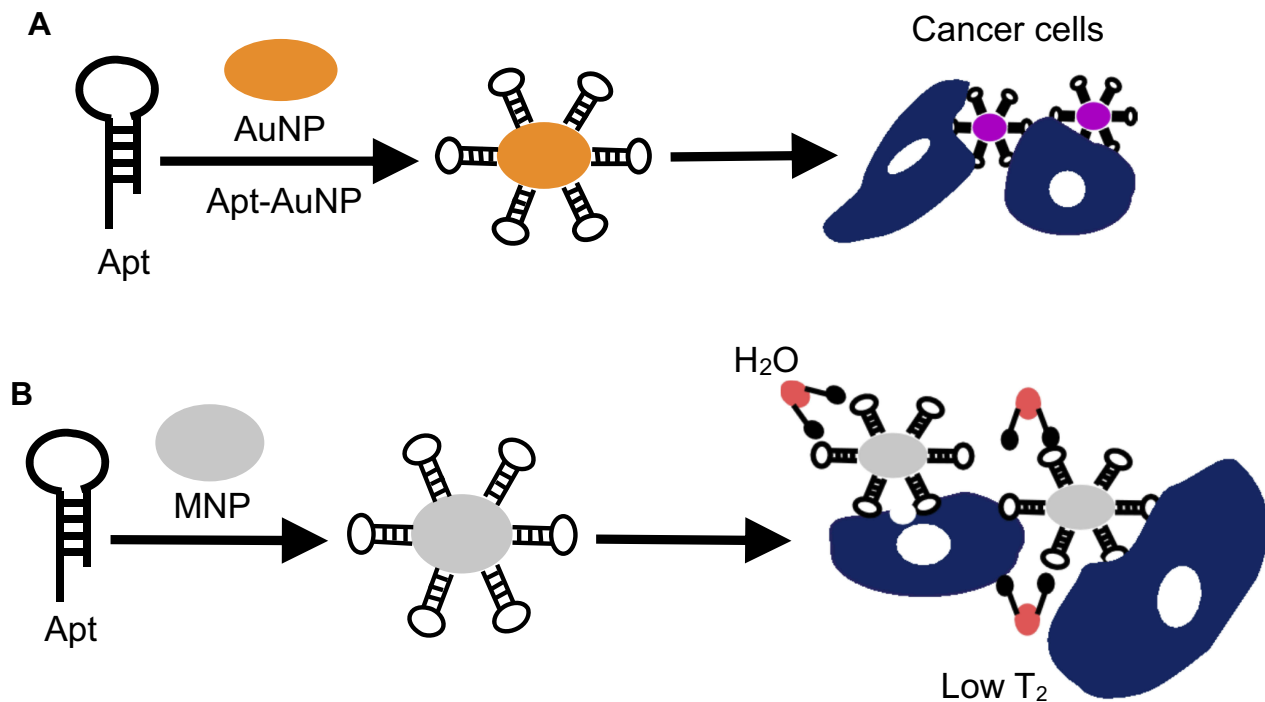
Aptamers are easy to be excreted rapidly through renal clearance due to the unique characteristics of small nucleic acid molecules. Some methods have been designed to extend half-life: 1) 5'-End with cholesterol: Cholesterol is added to the 5'-end of aptamer to form a cholesterol-oligonucleotide (cholODN) conjugate.<sup>42</sup> 2) 5'-End with Dialkyl Lipids: Dialkylglycerol (DAG) is linked to the 5'-end of aptamer.<sup>43</sup> 3) 5'-End PEGylation: Clinical trials have demonstrated that PEGylated aptamers have not only increased blood residence but also enhanced targeted delivery of drugs.<sup>44,45</sup> 4) Gold nanoparticles: The bioavailable nanomaterial is used to modify aptamers for reducing renal clearance.<sup>46</sup>

Considerably, there are several kinds of aptamer-modification strategies to augment binding affinity and target selectivity: 1) Modifications on bases slow off-rate modified aptamers (SOMAmers): 5-BzdU [5-(N-benzylcarboxamide)-2-deoxyuridine]-modified aptamer and SOMAmers containing 5-(N-benzylcarboxamide)-2-deoxyuridine (BndU) or 5-[N-(1-naphthylmethyl) carboxamide]-2-deoxyuridine (Nap-dU) have a strong affinity and stability.<sup>47</sup> 2) Based on crystal structure modifications: Crystal-modified aptamer prominently inhibits ATX activity that is a recognized therapeutic target for a number of diseases.<sup>48</sup> 3) Nuclear magnetic resonance (NMR) spectroscopy-guided aptamer optimization: Aptamer complexes formed by NMR-guided

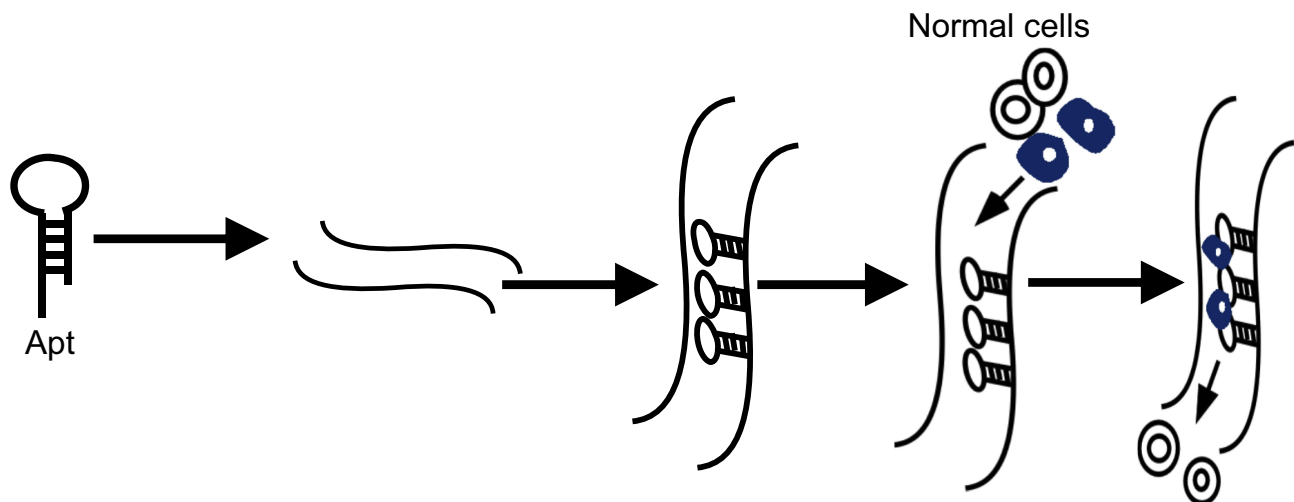
optimization could actually improve binding affinity.<sup>49,50</sup> 4) Phosphorodithioate (PS2) modification: PS2 substitution on RNA aptamers dramatically improves target binding affinity by ~ 1000-fold by inducing the fit rearrangement of the PS2-containing nucleotide.<sup>38,51</sup> 5) Deoxyuridines modification: 5'-position modified deoxyuridines also improve aptamer-binding affinity by promoting additional hydrophobic interactions between aptamers and their cognate targets.<sup>52</sup> 6) The hydrophobic base Ds modification: The data have indicated that the incorporation of unnatural base Ds can yield aptamers with greatly augmented affinities.<sup>53</sup>

## Aptamers' Conjugations

The sensitivity and selectivity of aptamers for the diagnosis and treatment of leukemia have been significantly improved after being combined with novel detection techniques. 1) Aptamer-nanoparticle: Aptamer-gold nanoparticle (Apt-AuNP) complexes selectively bind to target cells, generating discernible color changes from red to violet (Figure 1A). Moreover, aptamer-nanoparticle bioconjugates could enhance the intracellular delivery of drugs to target cells.<sup>54</sup> By contrast, aptamer-magnetic nanoparticle (Apt-MNP) has a peculiar character, which leads to a decrease in adjacent water T<sub>2</sub> by binding to target cells (Figure 1B), then detected by magnetic resonance imaging (MRI) or relaxometry.<sup>19</sup> 2) Microfluidic devices: Aptamers are immobilized on the surface of a microfluidic channel. The specific immobilized aptamers effectively capture target cells (Figure 2).<sup>55</sup> When combined with nanochannel-ion channel hybrid, the captured cells dramatically block the ionic flow, resulting in the obvious variation of I-V property detected by electrochemical linear sweep voltammetry technique with greatly enhanced sensitivity.<sup>56</sup> 3) Quantum dots (QDs) probes: QDs-aptamer complexes could not only emit a visible fluorescence spectrum in the presence of target cells but also differentiate different types of leukemic cells, which is suitable for efficient cancer detection and targeted imaging (Figure 3).<sup>57</sup> 4) Molecular Beacon (MB): MB is a dual-labeled single-stranded DNA with a fluorophore at 5' end and a quencher at the 3' end (Figure 4). The fluorescent imaging of MB probes can be extended to the rapid detection of circulating tumor cell, ATP, proteins and small molecules.<sup>58,59</sup> 5) Fluorescent probes: The fluorescence probes can greatly augment the sensitivity of detection via cell imaging (Figure 5).<sup>60</sup> and 6) MRI: In order to improve the sensitivity of MRI, gadolinium [Gd(III)], manganese [Mn(II)] or superparamagnetic iron oxide



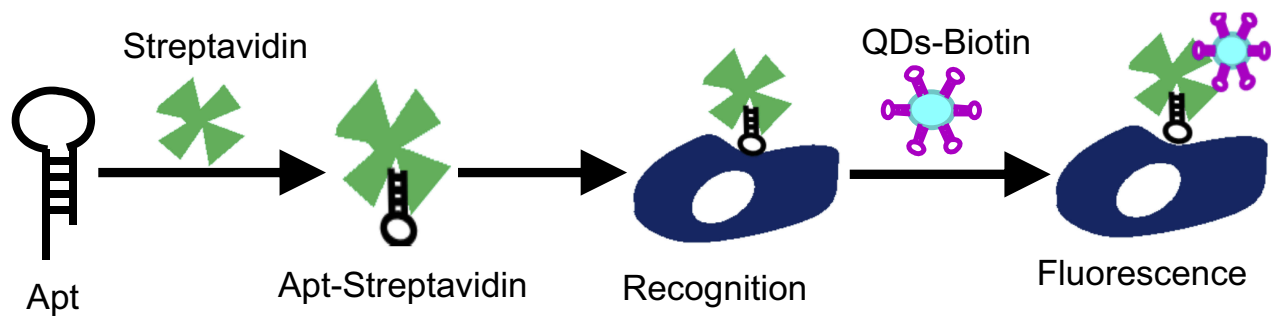
**Figure 1** A model of Apt-AuNP and Apt-MNP generation. **(A)** Aptamer is conjugated with AuNP. **(B)** Aptamer is conjugated with MNP: Apt-AuNP targeted cancer cells, which generated discernible color changes from red to violet. Apt-MNP targeted cancer cells, leading to a decrease in adjacent water T2 detected by MRI or relaxometry. **Abbreviations:** Apt, aptamer; Apt-AuNP, aptamer-gold nanoparticle; Apt-MNP, aptamer-magnetic nanoparticle; MRI, magnetic resonance imaging.



**Figure 2** A model of microfluidic devices. A model of microfluidic devices: Aptamers were immobilized on the surface of a microfluidic channel. The specific immobilized aptamers effectively captured target cells for diagnosis of leukemia.

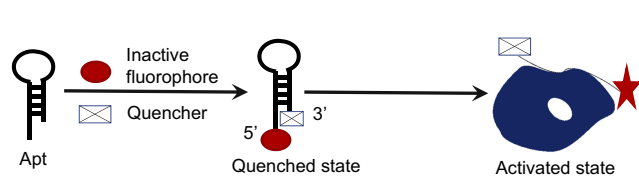
nanoparticles (SPIONs) complexes could be used as contrast agents (Figure 6).<sup>61</sup> In another report, monodispersed carboxylated magnetic nanocrystal (MNC, Fe<sub>3</sub>O<sub>4</sub>) with high crystallinity was conjugated with vascular endothelial growth factor receptor 2 (VEGFR2)-specific aptamers to increase the specific imaging of VEGFR2, displaying

a high magnetic resonance signal and efficient VEGFR2-detecting ability.<sup>62</sup> 7) Mass cytometry: Mass cytometry is a novel technique that quantitatively analyzes cell number one by one through using biotinylated aptamers in conjunction with metal-labeled neutravidin to characterize target cells, the characteristic cells undergo rapid



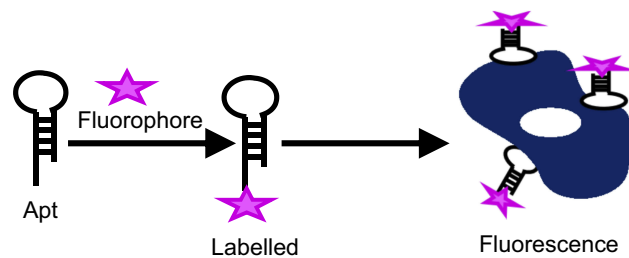
**Figure 3** A model of QDs-aptamer generation. Aptamer was conjugated with streptavidin. Apt-Streptavidin recognized leukemia cells, which was linked with QDs-Biotin, and then fluorescence was excited rapidly.

**Abbreviation:** QDs, quantum dots.



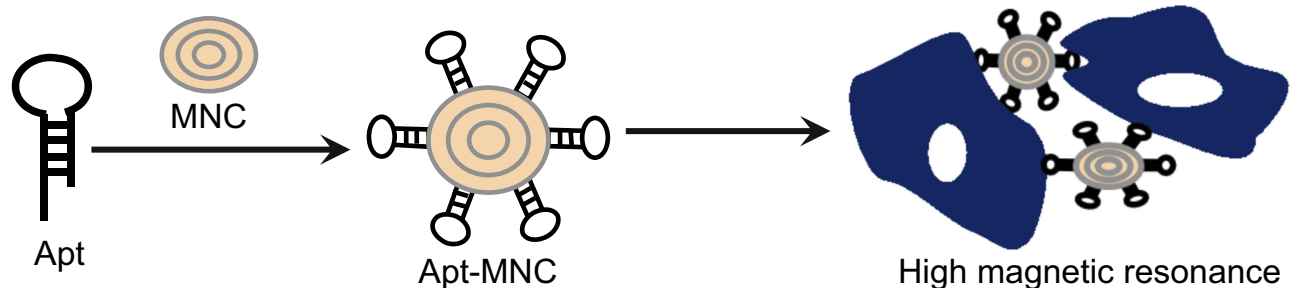
**Figure 4** Aptamer conjugated with MB. Aptamer was conjugated with a fluorophore at 5' end and a quencher at the 3' end. The labeled aptamer-targeted leukemia cells, and the inactive fluorophore was activated.

**Abbreviation:** MB, molecular beacon.



**Figure 5** Aptamer labeled with fluorescent probe. Aptamer was labeled with fluorescent probe. The labeled aptamer-targeted leukemia cells and then excited fluorescence.

ionization and the ionized elemental tags are analyzed by means of time-of-flight mass spectrometry (Figure 7).<sup>63</sup>



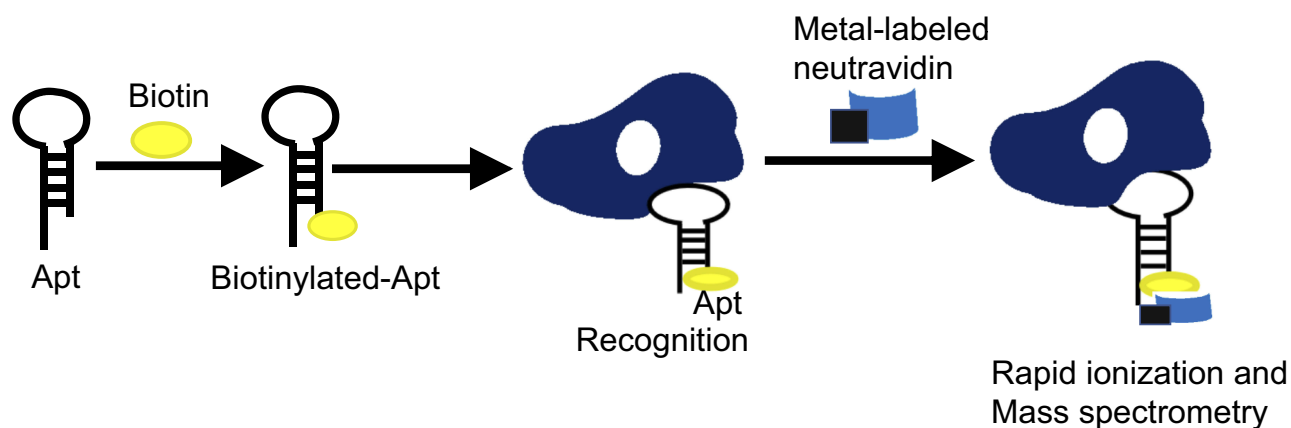
**Figure 6** MRI techniques using aptamer. MRI technique linked with aptamer: Aptamer was coupled with monodispersed MNC (Fe<sub>3</sub>O<sub>4</sub>) to recognize leukemia cells, then displaying a high magnetic resonance signal. The labeled MNC with aptamers increased the specific imaging of leukemia cells.

**Abbreviations:** MRI, magnetic resonance imaging; MNC, monodispersed carboxylated magnetic nanocrystal.

## Biomarkers Used for Aptamers-Mediated Diagnosis and Therapy in Leukemia

In patients with leukemia, a variety of biomarkers provide specific targets for target therapy. Such as, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) expressed on CLL cells has been used in leukemia therapy.<sup>64</sup> FcαRI (CD89) is a new target antigen expressed on different myeloid leukemia cell populations and mutated protein of nucleophosmin 1 (NPM1) has become an important prognostic and predictive maker.<sup>65</sup> Collectively, accumulated findings indicate that lords of markers of leukemia are frequently applied for aptamers-mediated detection and therapy, like AP-1 aptamer targeting CD20, Sgc8 aptamer targeting protein tyrosine kinase-7 (PTK-7), anti-CD33(+)/CD34(+) aptamer targeting CD33 and CD34, K19 aptamer targeting Siglec-5 protein, CD38-specific aptamer-drug conjugates (ApDCs), CD117 aptamer-methotrexate (MTX) complexes, CD123-aptamer-mediated targeted drug train (TDT), and human OX40 (hOX40) aptamer (Table 1).





**Figure 7** A model of mass cytometry. Biotinylated aptamer-captured target cells, which was recognized by metal-labeled neutravidin, then resulting in rapid ionization and the ionized elemental tags were analyzed by means of time-of-flight mass spectrometry.

CD20 is a B-cell differentiation antigen, located only in pre-B cells and mature B cells. It can act as the diagnostic target in CLL and ALL. Haghghi M et al has selected and characterized ssDNA aptamer against CD20

**Table 1** The Potential Application of Leukemic Biomarkers

Biomarkers	Expression Cell	Potential Applications	References
CD20	Pre-B cells and mature B cells	AP-1 ssDNA aptamer takes place of antibodies for diagnosis and therapy of ALL and CLL.	[66]
PTK-7	Molt-4 ALL cells	Sgc8 aptamer has shown great application for ALL detection and therapy.	[67]
CD33	Hematopoietic stem cells	Anti-CD33 antibody and aptamer are widely used for AML treatment.	[68]
CD34	Human and other mammalian hematopoietic stem/progenitor cells	CD34 is used to detect MRD and functions as an adverse prognostic factor in AML. AML-M <sub>2</sub> CD33(+)/CD34(+) cells have been used as targeted cells to select specific aptamers.	[69]
Siglec-5	AML cells	Siglec-5 protein-specific K19 aptamer holds the significant potency to detect and therapy AML.	[81]
CD38	Lymphocytes	CD38 is a predictor of B-CLL. Anti-CD38 antibody-drug conjugates and CD38-specific aptamer-drug conjugates have been used for targeted MM therapy.	[70]
CD44	Mainly in AML and B-CLL cell types	Anti-CD44 therapy inhibits AML and B-CLL progress, and anti-CD44 aptamer is an ideal tool for AML and B-CLL treatment.	[73]
CD47	Leukemia stem cells, AML, bcCML and T-ALL.	The combination treatment of cytosine Ara-C with anti-CD47 mAb represents an attractive option for AML targeted therapy, and anti-CD47 aptamer would become a powerful therapeutic strategy for AML.	[77]
CD117	Normal hematopoietic stem/progenitor cells	CD117 is a myeloid immunological marker, and CD117 aptamer (Apt)-methotrexate (MTX) complex inhibits AML cell growth.	[78]
CD123	High expression on LSCs	CD123-aptamer-mediated targeted drug train (TDT) is excellent therapeutic options for AML patients.	[74]
OX40	Leukemic blasts of AML	OX40-OX40L interaction promotes NK cells, and OX40 (hOX40) aptamer stimulates effector, memory T cell functions for leukemia treatment, and enhances the potency of dendritic cell-based tumor vaccines.	[75]

**Abbreviations:** ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; Ara-C, arabinoside; CD, cluster of differentiation; CLL, chronic lymphoblastic leukemia; CML, chronic myeloid leukemia; MRD, minimal residual disease; PTK-7, protein tyrosine kinase-7; Apt, aptamer; LSCs, leukemia stem cells; bcCML, blast crisis stages of CML; TDT, aptamer-mediated targeted drug train.

via cell-SELEX, termed as AP-1, which could take place of antibodies for diagnosis and therapy of leukemia.<sup>66</sup> PTK-7 is a significant biomarker expressed on the surface of Molt-4 cells, the specific interaction of DNA Sgc8 aptamer and PTK-7 has shown the significant potential for ALL detection.<sup>67</sup> CD33 is expressed in more than 90% of patients with AML and is a good target for AML treatment. The functionalized gold nanoparticles (FGNs) were designed through attaching five selected antisense oligonucleotides (AOs) and one anti-CD33(+)/CD34(+) aptamer to naked gold nanoparticles (NGNs), which blocked five oncogenes expression in AML subtype 2 (AML M2) containing BAG1, MDM2, Bcl-2, BIRC5 and XIAP.<sup>68</sup> Interestingly, AML-M2 CD33(+)/CD34(+) cells have been classified and used as targeted cells to select specific aptamers, which laid the basis for further looking for the more significant markers for diagnosis of AML-M2 leukemia.<sup>69</sup> CD38 is widely accepted as a marker for unfavorable prognosis in leukemia. CD38-specific ApDCs provide a new avenue for targeted MM therapy with higher tissue-penetrating potential and negligible immunogenicity.<sup>70</sup> CD44, mainly expressed in AML and B-CLL cell types, plays a key role in the proliferation, differentiation and migration of cells.<sup>71,72</sup> The anti-CD44 aptamer has functioned as ideal tools for selective target-specific therapy when conjugated to the surface of liposome in breast cancer and lung cancer,<sup>73</sup> which would be used in AML and B-CLL treatment by binding to CD44 on the surface of leukemic cells. CD123 (the interleukin-3 receptor  $\alpha$ -chain) is a leukemia-associated antigen expressed on leukemia stem cells (LSCs) at high levels. CD123-aptamer-mediated TDT was synthesized to selectively deliver cytotoxic agents to CD123-expressed cells in AML.<sup>74</sup> OX40, a TNF receptor family member, is expressed on leukemic blasts. The agonistic hOX40 aptamer has been designed to stimulate effector and memory T cell functions for leukemia treatment and even enhance the potency of dendritic cell-based tumor vaccines.<sup>75,76</sup>

Significantly, there are two important targets in AML, including CD47 and CD117. CD47 is identified as a negative prognostic marker for AML patients, anti-CD47 aptamer might also become a powerful therapeutic strategy for AML.<sup>77</sup> CD117 is also highly expressed in AML and can be used as a myeloid immunological marker to differentially diagnose AML. The chemosynthetic CD117 aptamer was conjugated with MTX to construct Apt-MTX complex, which inhibited AML cells' growth

and triggered cells' apoptosis, indicating the potential clinical value of Apt-MTX for AML targeted therapy.<sup>78</sup>

## Aptamers as Diagnostic Tools in Leukemia

It is acknowledged that increasing number of preclinical applications of aptamers for leukemia diagnosis have been reported, such as Siglec-5 aptamer binding to AML, Sgc8 aptamer binding to ALL, AP-1 aptamer binding to CLL. Noteworthy, the translational application of aptamers remains to be explored in clinics (Table 2).

Profoundly, aptamer-mediated avenues can be combined with other conventional tools to achieve early diagnosis. For instance, aptamer-based network hydrogel nanostructure in combination with molecular analysis to identify the PCR products of PML/RAR $\alpha$  presents enormous potential for early diagnosing disease.<sup>79</sup> It is approved that leukemia-derived exosomes are composed of CD63 and nucleolin (NCL). Conjunction of anti-CD63 antibody modified magnetic beads (CD63-MBs) with NCL-specific aptamer (AS1411) is applied to sensitively diagnose leukemia through binding the exosomes.<sup>79</sup> Furthermore, combination of novel artificial antibody with the site-enhanced multivalent aptamers (APT-CIH) also shows high capture efficiency and selectivity to leukemia cells, providing a rational basis for diagnosing leukemia and discovering more therapeutic targets.<sup>80</sup>

Some reports have shown aptamers' potential for AML diagnosis such as Siglec-5 protein-specific aptamers JH6, JH19, and K19 selected from aptamers pools. Among them, K19 aptamer represented the highest affinity and was labeled with biotin to construct a biotin-labeled K19 aptamer, namely Siglec-5 aptamer, which effectively identified and enriched Siglec-5 protein on the surface of AML cells. Thence, Siglec-5 aptamer provided an amazing approach for the detection of AML cells.<sup>81</sup> Importantly, the specific combination of Siglec-5 with the K19 aptamer sequence resulted in a conformational change that generated a split G-quadruplex structure recognized by the G-quadruplex-specific iridium(III) complex, which displayed an enhanced luminescent response and ultrasensitive detection.<sup>82</sup> Another aptamer (KH1C12) also showed augmented affinity and selectivity to the target AML cell line (HL60). Moreover, KH1C12 aptamer/gold nanoparticles-poly (3,4-ethylene dioxythiophene) (PEDOT) modified GC electrode (GC/PEDOT-Au/aptamer) could precisely count concentration of HL60 cells.<sup>83</sup> In theory,

**Table 2** The Application of Aptamers in Leukemia Diagnosis

Types	Aptamer Name	Target	Affinity (nM)	Combined with	References
AML	K19/Siglec-5 aptamer KHIC12 aptamer	Siglec-5 HL60 cell	12.37 nM	Biotin Au <sub>nano</sub> -PEDOT/GC	[74] [76]
ALL	Sgc8 aptamer Sgc8c and ATP aptamers Sgc8 aptamer Sgc8 aptamer Sgc8 aptamer Sgc8 aptamer Sgc8 aptamer Sgc8 aptamer Sgc8 dual aptamer Terbium(iii)-aptamer Ds-DNA aptamer, 05-MB231	PTK-7 PTK-7 PTK-7 PTK-7 PTK-7 PTK-7 PTK-7 PTK-7 PTK-7 PTK-7 PTK-7		QDs GO Carboxyfluorescein and GO FSNPs Cu-Au NPs APBA-AuNPs GMNPs Magnetic beads Magnetic and RCA probe Terbium(iii) Cell-ExSELEX	[63] [79] [80] [82] [81,83] [84] [85] [86] [87] [88] [22]
CML	Aptamer Aptamer	Glycoprotein Glycoprotein		H-RGO-Au NFs ZnO@CQDs	[89] [90]
CLL	AP-1 aptamer R 1.2 and R1.3 aptamer	CD20 mIgM	96.91 ± 4.5 nM	FITC	[62] [91]

**Abbreviations:** ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; CLL, chronic lymphoblastic leukemia; APBA-AuNPs, aminophenylboronic acid-modified gold nanoparticles; CD, cluster of differentiation; FITC, fluorescein isothiocyanate; GO, graphene oxide; Au<sub>nano</sub>-PEDOT/GC, gold nanoparticles-poly(3,4-ethylene dioxathiophene) modified GC electrode; RCA, rolling cycle amplification; GMNPs, gold nanoparticles-coated magnetic FeO nanoparticles; QDs, quantum dots; FSNPs, fluorescent silica nanoparticles; Cu-Au NPs, Cu-Au nanoparticles; H-RGO-Au NFs, graphene-hemin-composite coupled with Au nanoflowers; ZnO@CQDs, carbon quantum dots (CQDs) coated ZnO nanosphere.

CD33 functions as the biomarker of AML, and CD33 specific aptamers have been selected and characterized to bind to CD33+/CD34-cells in patients with AML M2, which might be used for the diagnosis of leukemia.<sup>84</sup>

ALL is the most common leukemia in children. Patient's improvement completely depends on the early of ALL. Thus, rapid, selective and specific methods are urgently in demand. Based on the specific interaction of DNA Sgc8c aptamer and PTK-7 on ALL cells,<sup>85</sup> a growing number of sophisticated diagnostic methods have been created. In one report, biotin-modified semiconductor QDs were labeled with the Sgc8 aptamer via streptavidin, then a stronger fluorescent signal would be generated by biotin amplification interactions when Sgc8 aptamer identified CCRF-CEM cells in T-ALL.<sup>67</sup> In another report, dual-aptamer (Sgc8c and ATP aptamers)-functionalized graphene oxide (DAFGO) complex, consisting of bispecific aptamers (Sgc8c and ATP aptamers) and graphene oxide (GO), was designed for internalization into Molt-4 T-ALL cells,<sup>86</sup> Sgc8c aptamer was selected and characterized to target to PTK-7. FAM (fluorophore)-labeled ATP aptamer was synthesized to detect the concentration of ATP. Theoretically, in the presence of target cells, DAFGO complex internalized into Molt-4 cells through interaction of

Sgc8c aptamer with PTK-7, subsequently, FAM-labeled ATP aptamer released from GO surface and then linked to ATP, leading to a strong fluorescence emission.<sup>86</sup> Significantly, Shan et al directly connected carboxyfluorescein-labeled Sgc8 aptamer (FAM-apt) to GO to target CCRF-CEM cells, when CCRF-CEM cells were captured, Sgc8 aptamer released from GO, and quenched fluorescence was recovered rapidly and significantly.<sup>87</sup> In addition, aptamer-modified fluorescent silica nanoparticles (FSNPs) and Cu-Au nanoparticle (Cu-Au NP)-based colorimetric or theranostic platform could also be a highly sensitive and selective method to diagnose ALL.<sup>88-90</sup>

In the further study, the combination of amplification strategy with nanoparticles has attracted immense attention. For instance, aptamer-based quartz crystal microbalance (QCM) biosensor was developed, which was based on the interaction of aminophenylboronic acid-modified gold nanoparticles (APBA-AuNPs) with silver enhancement.<sup>91</sup> In the system, Sgc8c aptamer was immobilized on QCM to capture CCRF-CEM cells by interacting with PTK7, APBA-AuNPs were used to label the captured cells. The frequency response was amplified by silver enhancement within a short time. Lastly, the captured cells were stained and quantitatively analyzed with



the fluorescence microscope.<sup>91</sup> Another new amplification strategy also immobilized Sgc8c aptamer onto gold nanoparticles-coated magnetic FeO nanoparticles (GMNPs) to constitute Apt-GMNPs complex, the process required a nitrogen-doped graphene modified electrode. Subsequently, the hairpin structure of the aptamer would be disrupted when Sgc8c aptamer captured CCRF-CEM cells, resulting in the decrease of the electrochemical signal.<sup>92</sup> On the other side, the combination of aptamer-conjugated magnetic beads (apt-MBs) and the magnet-QCM system was also applicable to detect and diagnose ALL. Sgc8c aptamer-conjugated MBs were used for extracting CCRF-CEM cells, and the magnet-QCM system was successfully applied for the quantitative analysis of cell. When MBs-captured CCRF-CEM cells accumulated on a quartz crystal gold electrode surface under a magnetic field, the resonant frequency would decrease.<sup>93</sup> For minimal residual diseases (MRD) of T-ALL, a smart dual-specific detection system containing magnetic aptamer Sgc8 probe (M-sgc8 probe) and rolling cycle amplification probe (RCA-sgc8 probe) has been developed, which would be successfully utilized for diagnosing and monitoring ALL progress.<sup>94</sup>

Lately, a label-free signal-on fluorescence aptasensor based on terbium(iii)-aptamer (Tb-apt) has been designed. The aptamer sensitized the fluorescence of Tb and formed a strong fluorescent Tb-apt probe. When the probe captured the CCRF-CEM cells, the released fluorescence signal was positively associated with cell concentration. Therefore, the aptamer-sensitized terbium(iii) luminescence method was rapid, ultrasensitive, economical and highly specific, which also held great potential to diagnose other types of leukemia at the early stage.<sup>95</sup> Excellently, the Ds-containing DNA (Ds-DNA) aptamer, 05-MB231 generated by cell-ExSELEX, could bind to CCRF-CEM cells with higher affinity, extensive specificities and biological activities, indicating that the Ds-DNA aptamers and the novel cell-ExSELEX would be broadly applied for leukemia cell imaging, biomarker discovery, and detection.<sup>26</sup>

CML is a clonal myeloproliferative disease characterized by increased neutrophils in peripheral blood and myeloid proliferation in BM. Interestingly, graphene-hemin-composite coupled with Au nanoflowers (H-RGO-Au NFs) has been demonstrated as an electrochemical aptamer biosensor, which possessed enhanced peroxidase-like properties and sensitively realized glycoprotein on the surface of K562 CML cells.<sup>96</sup> Consistently, a new electrochemiluminescence (ECL) platform based on the complex

of aptamers and carbon quantum dots (CQDs) coated ZnO nanosphere (ZnO@CQDs) could offer great promise for ultrasensitive and selective detection of K562 CML cells.<sup>97</sup> It is accepted that the progression of CML is characterized by the generation of BCR/ABL1 fusion gene. Based upon the unique expression of BCR/ABL1 gene in CML cells, the application of BCR/ABL1-based aptamer has a great application prospect in the diagnosis, treatment and prognosis of CML.

In consideration of CLL, Haghghi M et al have selected and characterized ssDNA aptamers against CD20 via cell-SELEX, namely AP-1, AP-2 and AP-3, which could conjugate with other imaging technologies to improve diagnostic sensitivity and have the potency to take place of antibodies. AP-1 aptamer would be extensively used for diagnosis and therapy of CLL with the most stable thermodynamic property and the highest binding affinity.<sup>66</sup> Recently, two novel truncated G-rich aptamers, R1.2 and R1.3, have been generated by “Ligand-Guided Selection” (LI-GS), both of them folded into G-quadruplex structures in the bioactive conformations, which was essential for specific and effective recognition of mIgM expressed in B cells and provided the fundamental basis for designing effective aptamer-based biosensors potentially applied for the highly sensitive and selective diagnosis of B cell lymphoma and CLL.<sup>98</sup>

## Aptamers as Therapeutic Tools in Leukemia

Collectively, there are numerous preclinical applications of aptamers for leukemia treatment, for instance, CD117-specific ssDNA aptamer, ZW25 aptamer, anti-CD33 (+)/CD34(+) aptamer, S30-T1 aptamer, AS-1411 aptamer, K19/Siglec-5 aptamer, RNA aptamers and peptide aptamers targeting AML, Sgc8 aptamer targeting ALL,  $\beta$ -arrestin 2 aptamer, NOX-A12 aptamer and F79 aptamer targeting CML. Nevertheless, only AS1411 aptamer combined with cytarabine has entered clinical trials in the treatment of patients with primary refractory or relapsed AML (Table 3).

It is worth noting that the conjunction of aptamers with routine therapies will provide more precise and higher-quality therapeutic efficacy. Recently, increasing researches have approved aptamer-based targeted drug delivery systems prominently alleviated side effects and improved therapeutic efficacy of chemotherapy, such as CD117 ssDNA aptamer delivering MTX, S30-T1 aptamer

**Table 3** The Application of Aptamers in Leukemia Therapy

Types	Aptamer Name	Target	Affinity (nM)	Combined with	References
AML	CD117-specific ssDNA aptamer	CD117	4.24 nM	MTX	[78]
	ZW25	CD123	29.41 nM	Dox	[74]
	Anti-CD33(+)/CD34(+) aptamer	CD33/CD34		NGNs	[68]
	S30-T1 aptamer	CD33		DOX	[103]
	AS-1411 aptamer	NCL		Cytarabine	[104]
	K19/Siglec-5 aptamer	Siglec-5	12.37 nM	Biotin/saporin	[81]
	RNA aptamers Peptide aptamers	RD TCTP			[105] [108]
ALL	Sgc8 aptamer	PTK7		Dau	[109,110]
	Sgc8 aptamer	PTK7		AuNPs/Dau	[111]
	AS1411 aptamer	NCL			[115]
	Sgc8 aptamer	PTK7		VCR/liposome	[112]
CML	$\beta$ -arrestin 2 aptamer	$\beta$ -arrestin 2	22.03 nM	NCL aptamer	[116]
	NOX-A12	SDF-1 or CXCL-12		Nilotinib	[118]
	F79 aptamer	RAD52			[119]

**Abbreviations:** ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; CD, cluster of differentiation; Dox, doxorubicin; MTX, methotrexate; NGNs, naked gold nanoparticles; Dau, daunorubicin; AuNPs, gold nanoparticles; RD, runt domain; NCL, nucleolin; VCR, vincristine; SDF-1, stromal-cell derived factor-1; CXCL-12, chemokine (C-X-C motif) ligand-12; PTK-7, protein tyrosine kinase-7; TCTP, translationally controlled tumor protein.

delivering doxorubicin (Dox), Sgc8 aptamer delivering Daunorubicin (Dau) and vincristine (VCR) sulphate.

Of note, the polyvalent aptamer system composed of multiple aptamer units and physically inserted chemotherapy agents, namely “Poly-Aptamer-Drug”, naturally presents enhanced binding affinity ( $\sim 40$  fold greater) and cellular internalization efficiency than monovalent counterpart in targeting and killing leukemia cells due to multivalent effects.<sup>99,100</sup> For further monitoring anti-leukemia drug effect, the complex of localized surface plasmon resonance (LSPR) and gold nanorods (AuNR) functionalized with aptamers has been effectively used in preclinical stage through sensing the cytochrome-c released from apoptotic leukemia cells.<sup>101</sup>

The current treatment strategies for AML might induce relapse and immunological rejection. Thus, promising therapeutic approaches for AML with fewer side effects are urgently needed. CD117, a transmembrane receptor highly expressed on AML cells, is beneficial for detecting MRD, which indicates that the CD117 may be a potential therapeutic biomarker for AML.<sup>102</sup> Recently, CD117-specific ssDNA aptamer was produced, and the Apt-MTX complex was formulated by conjugating aptamer with MTX. Importantly, Apt-MTX efficiently inhibited the growth of primary AML cells and had no side effects on off-target normal marrow cells.<sup>78</sup> Another AML marker, CD123-aptamer (ZW25)-mediated TDT was created to selectively deliver drugs to target cells via binding to

CD123, which demonstrated potential applications for effectively delivering therapeutic drugs to CD123-expressed cells.<sup>74</sup> Similarly, CD33 is also the significant marker of AML, in the further study, FGNs were designed by attaching five selected antisense AOs and one anti-CD33(+)/CD34(+) aptamer to NGNs, which hindered AML-M2 development through blocking five important oncogenes expression, including BAG1, MDM2, Bcl-2, BIRC5 and XIAP.<sup>68</sup> Moreover, the optimized CD33-targeting aptamer S30-T1 could highly recognize the C2 domain of the CD33 antigen in vitro and in vivo and specifically arrest the cell cycle at the G2 phase to inhibit CD33 positive AML HL-60 cell proliferation via delivering Dox to target cells.<sup>103</sup> In consideration of Siglec-5 protein, three new aptamers (JH6, JH19, and K19) were selected and characterized, and K19 had the highest affinity and specificity. The biotin-labeled K19 aptamer was used to enrich and identify Siglec-5 protein, which acted as a prospective strategy for AML targeted therapy.<sup>81</sup> Additionally, we have found AS-1411, a guanosine-rich oligonucleotide aptamer, was designed for specifically binding to NCL, demonstrating obvious inhibitive activity against AML cell lines. Combination of AS-1411 with cytarabine showed the superiority to cytarabine alone.<sup>104</sup>

Further, a recent research has reported RNA aptamers were created to link with the Runt domain (RD) of the AML1 protein with high affinity. AML1 (RUNX1) is a transcription factor isolated from a chromosomal

breakpoint and its genetic variations are frequently observed in human leukemia, leading to abnormal hematopoiesis and immunodeficiency.<sup>105</sup> The repair of AML1 function is dependent on RNA aptamers targeting to RD, providing new insights into treatment of AML1-related diseases.<sup>106,107</sup> Besides, some aptamers were designed for treating AML via controlling cell cycle and inhibiting malignant cell proliferation. For instance, the peptide aptamers were selected to prohibit translationally controlled tumor protein (TCTP) expression in Molt-4 cells in AML, which down-regulated the expressions of cell-cycle-related downstream proteins, suggesting potential promise for AML therapy.<sup>108</sup>

Clinically, the treatment of ALL is also confronted with great challenges. Dau has been attractive in the treatment of ALL but with limitations due to its cardiotoxicity. To reduce the cardiotoxicity, Sgc8 Apt-Dau conjugate, a simple and efficient complex was constructed to specifically deliver Dau to ALL T cells through the interaction between Sgc8 and PTK7 with negligible toxicity.<sup>109,110</sup> In order to further enhance the specificity and sensitivity of treatment. The Apt-Dau complex was connected to AuNPs which has widely been used to deliver therapeutic drugs with a large surface to volume ratio, simple synthesis and low toxicity.<sup>111</sup> The release of Dau from the complex depended on the pH levels. In pH 7.4, about 18% of Dau was released from Apt-Dau-AuNPs complex, while switching to pH 5.5, the release of drug from the complex was up to 75% under the same condition. Subsequently, flow cytometry analysis indicated the internalization efficiency of Dau was dramatically increased in comparison with Dau- and Apt-Dau conjugate-treated Molt-4 cells. Hence, Apt-Dau-AuNPs complex has been explored as a specific delivery system to internalize agents into target cells with reduced cytotoxic effects.<sup>110</sup>

In addition to Dau and DOX, VCR is widely used in hemato-oncology, especially ALL. The VCR-loaded and Sgc8 aptamer-conjugated liposomal drug delivery system (Sgc8/VCR-Lipo) significantly enhanced the therapeutic effects of VCR against ALL.<sup>112</sup> Amazingly, the novel N-heterocyclic carbene (NHC)-gold(I) complexes and aptamer-guided DNA tetrahedral nanostructure (s-TDN) could also be utilized to transport drugs when conjugated with Sgc8c aptamer.<sup>113,114</sup> As we know, NCL is a 9-O-acetylated sialoglycoprotein presenting on the surface of pre-B ALL, NCL-recognition aptamer AS1411 has shown evident therapeutic effects.<sup>115</sup> Strikingly, the

conjugation of 5-MB231 Ds-DNA aptamer with personalized medicine held the potency to augment anti-ALL activity.<sup>26</sup>

In terms of CML, regulators of signaling pathways and oncogenes might be ideal therapeutic targets. It has been reported that  $\beta$ -arrestin 2 was critical for the onset and maintenance of both the chronic and blast crisis stages of CML (bcCML). The specific  $\beta$ -arrestin 2-binding aptamers were constructed, including  $\beta$ -arr2A1,  $\beta$ -arr2A2 and  $\beta$ -arr2A3.  $\beta$ -arr2A3 had the highest binding affinity. In order to selectively deliver  $\beta$ -arrestin 2 aptamer to leukemia cells,  $\beta$ -arr2A3 was linked to the NCL aptamer through complementary base-pair annealing. The NCL aptamer- $\beta$ -arr2A3 chimera internalized into cells and delivered the  $\beta$ -arr2A3 aptamer to its intracellular target, which effectively inhibited the activation of the Hh/Smo and Wnt/Fz (Wingless/Frizzled) signaling pathways, and thereby suppressed CML progression.<sup>116</sup> Besides, there is an RNA aptamer targeting oncogene-induced Leukemia. NOX-A12 is an RNA oligonucleotide in L-configuration spiegelmer aptamer, which could block stromal-cell derived factor-1 (SDF-1) or chemokine (C-X-C motif) ligand-12 (CXCL-12)-induced BCR-ABL1 expression through preventing SDF-1 from reacting with its receptors, chemokine (C-X-C motif) receptor 4 (CXCR4) and CXCR7.<sup>117,118</sup> Moreover, the cooperation of NOX-A12 with nilotinib (ABL-kinase inhibitor) could suppress CML development, indicating a cooperative effect of two inhibitors for diminishing or eradicating residual CML cells. The finding also suggested that NOX-A12 had the ability to improve the efficacy of tyrosine kinase inhibitors (TKI) against oncogene-driven leukemia.<sup>118</sup>

It is identified that RAD52 has 2 DNA binding domains I and II (DNA I and DNA II) that bind to amino acid residues F79 and K102, respectively, which plays a critical role in BCR-ABL1-mediated CML cells growth. Of note, F79 aptamer could abrogate DNA binding activity of RAD52, leading to homologous recombination repair (HRR) inhibition and CML elimination through inducing synthetic lethality in BRCA-deficient CML cells. Theoretically, targeting RAD52 by F79 aptamer might enhance the therapeutic effect of oncogenes-induced leukemia including B-ALL, T-ALL and PML-RAR-positive acute promyelocytic leukemia (APL).<sup>119,120</sup>

## Prospective

Recently, rapid, ultrasensitive, and highly specific diagnostic methods for leukemia have been emerging. Based on

aptamer–target interaction, a functional DNA nanodevice has been built for recognizing target cells by aptamer-integrated DNA nanoassembly, which presented the potential applications for point-of-care leukemia diagnosis with excellent sensitivity and selectivity.<sup>121</sup> Besides conventional biomarkers, some promising markers have already emerged, like miRNA, exosomes and so on. Herein, leukemia-derived exosomes containing CD63 and NCL have also been identified as nanosized biomarkers highly sensitively detected by a fluorescent biosensing platform, namely a dual-signal amplification. The process required anti-CD63 antibody modified magnetic bead conjugates (MB-CD63) and a DNA primer comprising an NCL-recognition AS1411 aptamer.<sup>122</sup> Meanwhile, an aptamer biosensor provided a novel strategy for label-free detection of leukemia-derived miR-16 with high sensitivity due to the polymerase cyclic amplification and the aggregation of the illuminator. Significantly, the neoteric technique would offer a promising strategy for early diagnosis of fusion genes.<sup>123</sup>

Generally, aptamers or aptamer/antibody bispecific system (AAbs) are often designed to target to biomarkers on the surface of leukemia cells or to influence the intracellular targets. However, there are only a few reports about aptamers directly against fusion genes. A novel research has developed an enzyme-free and label-free surface plasmon resonance (SPR) biosensor applied for ultrasensitive detection of fusion genes, which required the DNA self-assembly aptamer-based network hydrogel nanostructure via streptavidin (SA) encapsulation, and the SA aptamer, as a capture aptamer, dramatically amplified the SPR signal by strongly linking to SA. Furthermore, the sophisticated biosensing method has been applied to capture the PML/RAR $\alpha$ , a gene marker of M<sub>4E0</sub>, displaying excellent feasible potency for detecting fusion genes and monitoring leukemia progress.<sup>79</sup> Besides, there are other traditional fusion genes such as AML1-MTG8 and CBF $\beta$ -MYH11, typical symbols of M<sub>2b</sub> and M<sub>3</sub>, respectively, based on which we speculate that it is available to extract fusion genes from leukemia samples to directly construct ssDNA library or indirectly formulate RNA library, which provides qualifications for selecting and characterizing fusion genes-specific nucleic acid aptamers. Nevertheless, we may face inevitable challenges due to the insufficient sample and uncertain length of the randomized sequence. In the future, efforts should be focused on how to design and optimize nucleic acid aptamers to directly target and prohibit fusion genes' expressions and further control

leukemia development. Interestingly, aptamers can also be utilized to discover more gene targets related to leukemia.

## Conclusion

Even though monoclonal antibody-based complexes still dominated in leukemia diagnosis and therapy, aptamers and SELEX technology have brought a revolution to biomedical fields. Compared with monoclonal antibody therapy, aptamers can be used to directly kill leukemia cells or deliver agents to targets with faster internalization, higher selectivity and affinity, lower toxicity, lower immunogenicity, longer half-life and more stable properties at a vast range of variable environment. What's more, after being conjugated with imaging and other advanced technologies, such as nanoparticles, microfluidic devices, QBs probes, MB, fluorescent probes, MRI and mass cytometry, the sensitivity and specificity of aptamers are significantly improved in diagnosis and treatment. However, aptamers require chemical modifications to significantly overcome nuclease degradation, rapid renal excretion and deficient binding affinity due to the nucleic acid characteristics, which result in increased cost and time procedure. In addition, SELEX is an expensive and tedious process. All of these problems bring great challenges to the clinical application of aptamers. Here, future researches should try to make aptamers' selection and modifications procedures faster, cheaper and simpler.

In summary, nucleic acid aptamers have attracted great attention and achieved tremendous progress in increasingly diverse fields. It is recognized that nucleic acid aptamers are powerful diagnostic and therapeutic tools for leukemia, which also can conjunct with conventional strategies. But numerous clinical applications of aptamers are still confronted with immense challenges including the automation of SELEX process, the increase of biostability and bioavailability and so on. Herein, the aptamer-based strategies actually hold the potential to be excellent alternatives to traditional approaches for diagnosis and treatment of leukemia, and the application of aptamers in detecting fusion genes is urgent to be explored.

## Abbreviations

AAbs, aptamer/antibody bispecific system; BM, bone marrow; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CPG, controlled pore glass; APBA-AuNPs, aminophenylboronic acid-modified gold nanoparticles; Ara-C, arabinoside; Apt, aptamer; Apt-AuNP, aptamer–gold nanoparticle; Apt-MNP, aptamer–magnetic nanoparticle;



ATX, autotoxin; AuNPs, gold nanoparticles; Bn-Du, 5-(N-benzylcarboxamide)-2-deoxyuridine; CD, cluster of differentiation; CLL, chronic lymphoblastic leukemia; CML, chronic myeloid leukemia; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; DAFGO, dual-aptamer-functionalized graphene oxide; Dau, daunorubicin; Dox, doxorubicin; FGNs, functionalized gold nanoparticles; GO, graphene oxide; MNC, monodispersed carboxylated magnetic nanocrystal; NMR, nuclear magnetic resonance; VEGFR2, vascular endothelial growth factor receptor 2; MNPs, magnetic nanoparticles; MRD, minimal residual disease; ApDCs, aptamer-drug conjugates; MTX, methotrexate; NGNs, naked gold nanoparticles; NPM1, nucleophosmin 1; ECL, electrochemiluminescence; QCM, quartz crystal microbalance; QDs, quantum dots; RD, runt domain; SELEX, systemic evolution of ligands by exponential enrichment; ExSELEX, genetic alphabet Expansion for SELEX; SOMAmers, slow off-rate modified aptamers; SPIONs, super paramagnetic iron oxide nanoparticles; TCR, T cell receptor; NCL, nucleolin; PTK-7, protein tyrosine kinase-7; AML M2, AML subtype 2; AOs, antisense oligonucleotides; siRNA, small interfering RNA; RNAi, RNA interference; DAG, dialkylglycerol; MB, molecular beacon; MRI, magnetic resonance imaging; SPR, surface plasmon resonance; SA, streptavidin; APL, acute promyelocytic leukemia; VCR, vincristine sulphate; TCTP, translationally controlled tumor protein; Au<sub>nano</sub>-PEDOT/GC, gold nanoparticles-poly(3,4-ethylene dioxithiophene) modified GC electrode; LSCs, leukemia stem cells; UB, unnatural base; UBPs, UB pairs; Px, 2-nitro-4-propynylpyrrole; Ds, 7-(2-thienyl)imidazo[4,5-*b*]pyridine; Ds-DNA, Ds-containing DNA; GMNPs, gold nanoparticles-coated magnetic FeO nanoparticles; FSNPs, fluorescent silica nanoparticles; Cu-Au NP, Cu-Au nanoparticle; FAM, fluorophore; LI-GS, Ligand-Guided Selection; LSPR, localized surface plasmon resonance; NHC, N-heterocyclic carbene; TKI, tyrosine kinase inhibitors; HRR, homologous recombination repair; H-RGO-Au NFs, graphene-hemin-composite coupled with Au nanoflower; ZnO@CQDs, carbon quantum dots (CQDs) coated ZnO nanosphere; AuNR, gold nanorods; bcCML, blast crisis stages of CML; SDF-1, stromal-cell derived factor-1; CXCL-12, chemokine (C-X-C motif) ligand-12; CXCR4, chemokine (C-X-C motif) receptor 4.

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## Author Contributions

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