Escort aptamers: a delivery service for diagnosis and therapy

Brian J. Hicke and Andrew W. Stephens

Gilead Sciences, Boulder, Colorado, USA

Address correspondence to: Brian J. Hicke, Gilead Sciences, 2860 Wilderness Place, Boulder, Colorado 80301, USA.
Phone: (303) 546-7748; Fax: (303) 444-0672; E-mail: bhicke@gilead.com.

In 1990, the discovery of aptamers by Tuerk and Gold (1) and subsequently by Ellington and Szostak (2) spawned significant interest within academia and industry. Aptamers have quickly become valuable research tools (3–5). More than that, a therapeutic aptamer (6) has entered clinical evaluation just eight years after the inception of the technology. While aptamers have built inroads into therapeutic applications, they may also become important in vitro diagnostic tools (7). Here, we discuss a new development: the use of "escort aptamers" as targeting agents for in vivo diagnosis and therapy. Instead of directly interrupting a disease process, as would a function-blocking aptamer, escort aptamers are designed to deliver radionuclides, toxins, or cytotoxic agents to diseased tissue. Specifically, we focus on the delivery of benign radionuclides for in vivo diagnosis of disease. For discussion of function-blocking aptamers, see White et al., this Perspective series (8).

Aptamers and antibodies

The advent of monoclonal antibodies in 1974 brought to mind Paul Ehrlich's turn-of-the-century insight that molecules could serve as "magic bullets" that home to pathological organisms with precision (http://nobel.sdsc.edu/medicine/laureates/1908/ehrlich-bio.html). Indeed, the high affinity and specificity of antibodies provide some of the key properties in Ehrlich's concept. Despite some successes in tissue targeting, antibodies are saddled with a fundamental disadvantage: their large size (~155 kDa) results in slow tissue penetration and long blood residence. For example, in clinical settings where an antibody is coupled to a cell-killing radionuclide, this long circulation half-life leads to bone marrow toxicity that limits the permissible dose (9). To decrease blood half-life while maintaining target specificity, a second generation of smaller antibody fragments has been designed (10, 11). Antibody pretargeting strategies also show promise (12–14), and small peptides can have excellent pharmacokinetic profiles (15). However, many approaches are limited by complexity of clinical protocols, paucity of available targeting molecules, low-affinity binding, or immune responses by patients that prevent repetitive treatment cycles.

Because aptamers may provide solutions to many of these problems, they represent a promising new class of targeting agents. Having high affinity and specificity, and being synthetic polymers, aptamers combine the advantages of antibodies and small peptides in tissue targeting. To date, aptamers have not shown toxicity or immunogenicity following testing in several mammalian species (D. Drolet and R. Bendele, personal communication), suggesting that repeat dosing is possible in clinical settings. Finally, during the genomic/proteomic age, rapid discovery and development of high-affinity binding agents, as is possible with aptamer technology, will likely be advantageous in keeping pace with discoveries (16).

What is an aptamer?

Aptamers are modified oligonucleotides that are isolated by the systematic evolution of ligands by exponential enrichment (SELEX) process. Formally, aptamers are similar in composition to natural nucleic acids but are built with 2′-modified sugars to enhance resistance to blood and tissue nucleases. Aptamers are not linear molecules that carry genetic information. Rather, they are globular molecules, as exemplified by the shape of tRNA. Like antibodies, aptamers most frequently function through high-affinity binding to a target protein. This distinguishes aptamers from antisense oligonucleotides and ribozymes, which are designed to interrupt the translation of genetic information from mRNAs into proteins. At 8–15 kDa, escort aptamers are intermediate in size between small peptides (~1 kDa) and single-chain antibody fragments (scFv's; ~25 kDa).

Chemical synthesis, an advantage over proteins that aptamers share with small peptides (15), enables a wide range of site-specific modifications. This allows for engineering of an escort aptamer toward a specific purpose. For research, aptamers are readily tagged with fluorescent dyes, radionuclides, or biotin. For clinical purposes, escort aptamers can be conjugated to a variety of molecules, such as radionuclides or cytotoxic agents.

A notable example of aptamer plasticity was reported by Smith and colleagues (17), who used a modified SELEX process to blend high-affinity binding with covalent inhibition of an enzyme. To achieve enzyme inactivation, Smith and colleagues linked a weakly reactive valyl phosphonate moiety to a random aptamer pool, and selected for aptamers capable of rapid covalent linkage to human neutrophil elastase. The result is a combination of high-affinity binding with specific active-site inhibition. This pairing inactivates elastase nearly 100-fold more rapidly than do peptide-based phosphonate inhibitors. This aptamer has been further modified to incorporate a radio-metal chelation moiety and has been used to target neutrophil-bound elastase in an in vivo inflammation model (17).

Many aptamer adaptations use simple succinimidyl ester chemistry, which is accessible even to the most faint-of-heart among us. Importantly, modification can be directed to a single site away from the aptamer's active surface, preventing loss of function. Radiolabeling and conjugations can be performed using high temperatures (95°C), organic solvents, and pH ranging from 4 to 8.5. Thus, escort aptamers can attend a variety of functions through their chemical adaptability.

Aptamer isolation: the SELEX process

The SELEX process at the heart of aptamer isolation consists of iterative steps of binding and amplification using a combinatorial library of oligonucleotides (see White et al., this Perspective series, ref. 8; and ref. 18). In this respect, it is fundamentally similar to phage display (19) and ribosome display (20) technologies that are used for generation of antibody fragments and peptides. Before beginning the SELEX process, a single-stranded DNA oligonucleotide pool must be chemically synthesized with fixed sequences at either end, flanking a region of (typically) 40 randomized nucleotides. From this theoretical maximum "sequence space" of 10^{24} distinct nucleotide sequences, about 1 nmol, corresponding to about 10^{15} sequences, is used as a template for generating a 70-nucleotide transcript with RNA polymerase. To initiate the SELEX process, the oligonucleotide pool is incubated with the target protein. Aptamers bound to the protein are partitioned away from unbound oligonucleotides and then amplified and transcribed to close one round of the process. Subsequent selection rounds further cull the pool, as pressure is applied to yield only high-affinity interactions. Typically, five to eight rounds are required for completion, which is usually defined by a plateau in affinity for the target protein. These steps are automatable (21, 22), which implies that the SELEX process can keep pace with accelerating target discovery rates.

To diversify aptamer libraries, 5-position adducts on pyrimidines can be blended into the SELEX process. As an example, introduction of benzyl and pyridyl moieties allows for presentation of increased hydrophobicity to target proteins that are less likely to find polyanionic oligonucleotides attractive. Protein-like functionality can also be incorporated: primary amine, carboxylic acid, and imidazole side chains increase the chemical diversity of aptamer libraries (23). In addition, the SELEX process can be adapted to identify aptamers that interact with their targets in a covalent manner. For example, the pyrimidine 5-position is available for attachment of chemically and photochemically reactive moieties. The adducts chosen for this purpose are generally weakly reactive except in the context of an appropriate aptamer, so undesirable crosslinking to nontarget serum proteins is rare, and covalent linkage is strictly dependent on formation of a specific aptamer–protein complex.

At completion of the SELEX experiment, an aptamer pool is cloned and sequenced, and aptamers are screened for affinity. For the most efficient chemical synthesis, clones are truncated to the smallest size possible while retaining high affinity (Figure 1). Typically, such size-minimized aptamers range from 25 to 45 nucleotides in length.

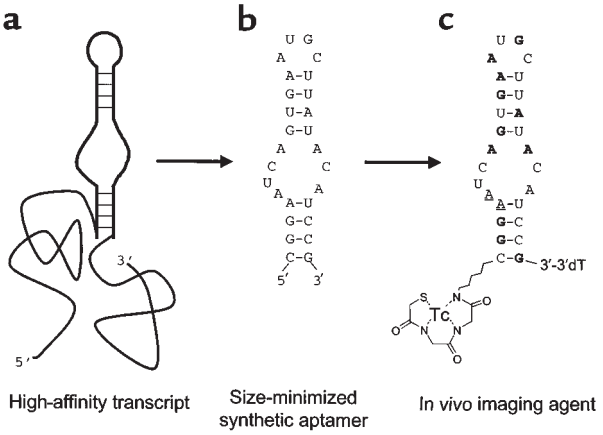

Figure 1
An archetypal escort aptamer. (**a**) A high-affinity aptamer is identified by the SELEX process. 2′-F pyrimidines are incorporated during selections. (**b**) The aptamer is truncated to minimal size and is now a synthetic molecule. (**c**) The escort aptamer as an in vivo diagnostic agent. Further nuclease stabilization is achieved: only two positions remain 2′-OH (underlined); the remainder are 2′-F pyrimidine and 2′-OCH_3 purine (bold). Chemical synthesis adds a 3′-3′ exonuclease cap and a primary amine (or thiol, etc.) for desired modifications. For in vivo imaging, a radiometal chelator is conjugated to the 5′ amine and ^{99m}Tc is incorporated.

Engineering chemical stability

Early in the development of function-blocking aptamers, it was appreciated that the inherent instability of RNA and DNA in blood is a fundamental limitation to therapeutic utility. This observation led the antisense research community to develop nucle-

ase-resistant oligonucleotides with modified phosphate backbones, including phosphorothioate and methyl phosphonate linkages. However, such backbone modifications are not compatible with the enzymatic steps of the SELEX process, and this forces the development of alternative solutions to the nuclease cleavage problem.

During nuclease cleavage, the ribose 2′-OH engages in nucleophilic attack on the neighboring 3′ phosphodiester bond. Therefore, 2′ modifications that diminish reactivity can effect significant nuclease resistance in plasma (24). In contrast to backbone substitutions, many 2′ ribose modifications are compatible with the SELEX process enzymes. Further, unlike phosphorothioate oligonucleotides, 2′-modified aptamers maintain low binding to serum proteins, a feature that is critical in permitting escort aptamers to be targeted specifically to the tissue of interest. As a result of the modifications, RNAs containing 2′-F and 2′-NH_2 pyrimidines are at least 1,000-fold more resistant to degradation in plasma than their unmodified RNA counterparts (24).

Final steps in escort aptamer preparation involve synthetic modifications of the truncated aptamer. Further nuclease stabilization is achieved by substitution of 2′-OCH_3 for 2′-OH at purine positions. As the 2′-OCH_3 moiety is not compatible with current SELEX process enzymes, this alteration must occur during chemical synthesis following evolution of a specific aptamer sequence. Generally, most of the 2′-OH purines can be substituted without loss of binding activity. At some locations, purines cannot be substituted without loss of affinity. In addition to protection against endonucleases, it is useful to protect against 3′ exonuclease activity. Therefore the 3′ nucleotide is inverted to form a new 5′-OH, with a 3′-3′ linkage to the penultimate base. Finally, synthesis incorporates nucleophilic amines or thiols, lending flexibility for attachment of the escorted species or other desirable modifications.

An archetypal escort aptamer structure is shown in Figure 1c. It is a size-minimized oligonucleotide that exits the SELEX process and is truncated, further protected against nucleases, and conjugated to its payload. For in vivo imaging, we typically couple a radiometal chelator to the 5′ terminus. In the example shown, the chelator (25) incorporates the metastable isotope technetium-99m (^{99m}Tc), an isotope commonly used in clinical settings.

Aptamers as targeting agents

At least six properties can be ascribed to the ideal targeting agent: high affinity and specificity for the target molecule, rapid uptake in the target tissue, rapid blood clearance, urinary excretion, durable tissue retention, and accumulation of high concentrations in the target tissue. The efficacy of a targeting agent is the aggregate of performance in each area. To interpret the strengths and weaknesses of escort aptamers in each category, we have initially focused on delivery of a benign radionuclide, ^{99m}Tc. Unless otherwise stated, pharmacokinetic parameters discussed here refer to the escorted ^{99m}Tc radiolabel, not to the aptamer per se.

Affinity and specificity are crucial for retention in the target tissue and low uptake in nontarget tissues. For antibodies, the relationship of affinity with uptake level in the target tissue is clear: within limits, increased affinity leads to greater uptake (26). Aptamers typically have high affinity for their target proteins, ranging from 0.05–10 nM, equilibrium dissociation constants that compare favorably with those of scFv's and are considerably better than those generally reported for peptides derived from phage display experiments. Tumor-targeting agents need to differentiate between normal and malignant forms of the same tissue, again requiring the specificity that aptamers generate (6). Affinity and specificity are not sufficient, however. Pharmacokinetic and tissue distribution characteristics are also critical and can doom an otherwise promising agent.

In comparing antibody fragments to antibodies (10), the transition from monomeric to multimeric forms leads to improved tumor uptake, but generally decreases tumor/blood ratios. At 15–30 kDa, a dimeric aptamer would have roughly equivalent mass to a monomeric scFv, ~25 kDa. In one case, dimerization of a function-blocking aptamer that binds to a cell surface protein results in a tenfold decrease in aptamer dissociation rate from lymphocytes (27). For escort aptamers, the effects of dimerization on tissue target-

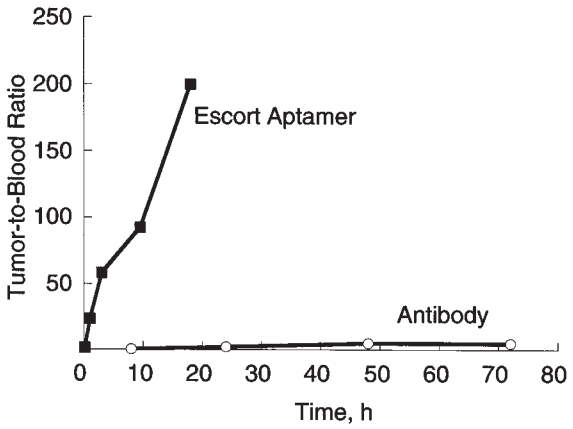

Figure 2
Tumor/blood ratios of escort aptamer and antibody. Radiolabeled aptamer and antibody against the same target protein were administered by intravenous bolus injection into tumor-bearing mice. Concentrations in the target tissue and blood were determined, and the target/blood concentration ratio was plotted against time. Because of differing distribution and clearance kinetics, different time points were chosen for aptamer and antibody.

ing are not yet known. Regardless of any changes made by increasing avidity, current escort aptamers have very high affinity and specificity.

Rapid image development is an increasingly important clinical parameter in targeting, in part because of the managed health care environment, which demands that diagnostic studies be performed quickly in an outpatient setting. Aptamers display rapid image development, as depicted graphically in Figure 2, which compares tumor targeting by an aptamer and an antibody that bind to the same protein. The antibody requires days to achieve an appreciable signal/noise ratio. In contrast, the aptamer rapidly develops a far higher signal/noise ratio that results in high-quality tumor images (B.J. Hicke, unpublished observation). The slow uptake and clearance of antibodies is due in part to their large size. While smaller antibody fragments show improvements relative to intact antibodies, peptides (15) and now escort aptamers have still more favorable kinetics, which fit well with anticipated clinical needs for in vivo imaging.

For in vivo imaging using ^{99m}Tc, signal/noise ratios are important parameters, and here we focus on a critical parameter, the target/blood ratio. After intravenous injection of two different ^{99m}Tc-radiolabeled aptamers, maximal levels are observed in either clots or tumors within 10–30 minutes. In addition to rapid tissue uptake, these escort aptamers display rapid clearance, as defined here by ^{99m}Tc removal from blood. For tumor- and clot-targeting escort aptamers, 99% of the blood ^{99m}Tc is cleared with a half-life of less than 5 minutes in the mouse. This desirable clearance rate is more comparable to small peptides than to scFv's or antibodies. At 8–15 kDa, escort aptamers are small enough to permit rapid renal filtration and rapid access to extravascular tissues (for function-blocking aptamers against intravascular proteins, longer blood residence is desirable and is obtained by conjugation to 40 kDa polyethylene glycol [28]). As nuclease cleavage occurs during the time scale of these measurements, ^{99m}Tc-labeled aptamer metabolites will also contribute to clearance. For in vivo imaging, current escort aptamer forms develop favorable target/blood ratios in animal models, primarily driven by rapid blood clearance rates.

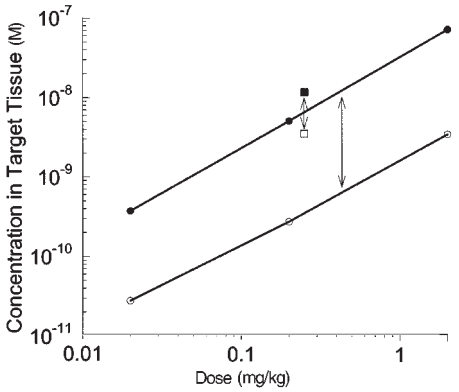

Figure 3
Comparison of tumor targeting by escort aptamer and antibody. ^{99m}Tc-radiolabeled aptamer and ^{125}I-radiolabeled antibody against the same target protein were administered by intravenous bolus injection into tumor-bearing mice. Concentration of aptamer was measured at 1 hour in tumor (filled circles) and blood (open circles), and concentration of antibody was measured at 24 hours in tumor (filled square) and blood (open square). Three doses of aptamer are compared with each other and with a single dose of antibody. Signal/noise ratios of antibody and aptamer at equivalent tumor loading are represented by the lengths of the double-headed arrows. Note: for both aptamer and antibody, the time of measurement was before optimal tumor/blood ratios were achieved (see Figure 2).

Another desired feature of the ideal targeting agent is urinary excretion, which tends to be much more rapid than hepatobiliary excretion. In mice, current escort aptamers produce approximately 60% urinary clearance of the injected ^{99m}Tc within 3 hours (in rabbits, urinary excretion proceeds to a greater extent). The remaining radioactivity (40%) is cleared through the hepatobiliary system. This is a disadvantage to current escort aptamer formulations, as radioactivity passing through the liver and intestines can obscure in vivo images of abdominal targets. It remains to be seen whether hepatobiliary clearance is a clinically significant hurdle. For peptide targeting agents, the balance of urinary and hepatobiliary excretion can be readily altered by modification of the ^{99m}Tc chelating moiety (15), and this is also the case for escort aptamers in rodent studies.

An unexpected feature of aptamers is durable retention in the preferred tissue, with a half-life of more than 12 hours for a tumor-targeting aptamer in the mouse. While the reason for long retention has not been addressed, we were surprised to discover that tumor- and clot-associated escort aptamers remain mostly intact for hours, with some variation depending on the aptamer and the animal species. In contrast to target-bound aptamers, nuclease degradation of blood-borne aptamers occurs rapidly, with greater than 95% destruction within 30 minutes in blood. Currently, we feel that targeted, as opposed to blood-borne, escort aptamers are stable because the bulk of degradation occurs at sites (perhaps the liver and kidney) that are anatomically separate from the target tissue. Consistent with this model, we have noted that aptamer clearance from the blood is dramatically slower in hepatectomized rats (A.W. Stephens, unpublished observation). As an aside, the observed protection of target-bound aptamer from nucleases has implications for the development of function-blocking nucleic acids, including aptamers, antisense, and ribozymes, that operate in extravascular tissues: in optimizing pharmacokinetics, it may

become important to measure target tissue levels as well as blood residence time. Independent of the mechanism, durable target tissue residence is a primary factor in the high signal/noise ratios (as high as 50 within 3 hours) achieved with escort aptamers.

Finally, an ideal escort displays high uptake in the target tissue. High uptake increases the sensitivity of an imaging agent. Often, uptake of imaging agents is measured as percent of the injected dose per gram (% ID/g) of tissue. We have compared an escort aptamer with an antibody for the same target protein. When quantitated using the % ID/g method, the aptamer has 15-fold lower uptake than the antibody at an equivalent dose (data not shown). An alternative analysis measures molar concentration in the target tissue. Figure 3 compares antibody and aptamer in this way and reveals three principles. First, at an equivalent dose, molar uptake of the aptamer is reduced only twofold compared with the antibody. Second, to achieve equivalent tumor loading, one can simply increase the aptamer dose twofold. Third, at a comparable tumor loading level, denoted by the double-headed arrows in the figure, the aptamer displays much higher signal/noise ratios (20 at 1 hour after injection) than does the antibody (3.5 at 24 hours after injection). Like the escort aptamer, scFv's and high-affinity peptides have improved clearance rates but lower % ID/g than antibodies. The desired comparison between aptamer, scFv, and peptide has not yet been possible. To summarize, small increases in escort aptamer dose compensate for lower % ID/g tissue uptake compared with antibodies and fragments thereof, with retention of high signal/noise ratios.

Since all targeting molecules fall short in one or more of these six categories, there is as yet no magic bullet. As targeting agents, aptamers are currently described as having high-affinity binding and durable retention in target tissue, rapid tissue penetration and blood clearance, and both urinary and hepatobiliary clearance pathways. Experimental approaches to improvement include testing dimeric aptamer forms with increased avidity, exploring the effect of increases in nuclease stability, and chemical alterations to increase urinary clearance and decrease hepatobiliary clearance. In optimizing the properties of escort aptamers, it will be necessary to more thoroughly define clearance pathways and the effect of aptamer metabolism on tissue targeting. The ease of chemical synthesis and modification allows one to rapidly screen aptamer formulations for increased performance.

Radiotherapeutic applications of escort aptamers

While improved in vivo imaging would be valuable, escort aptamer characteristics also suggest utility in cancer therapy. The transition to therapy awaits clinical assessment of escort aptamers as imaging agents. Nevertheless, current preclinical work identifies some issues to be addressed for this transition. Hepatobiliary clearance, if observed clinically, is not favorable due to increased exposure of radiosensitive intestinal epithelia. Radioisotope choice can help alleviate concerns caused by hepatobiliary clearance. For example, α-particle radiotherapy is attractive for two reasons. First, a short half-life (46 minutes in the case of ^{213}Bi) decreases intestinal exposure because of decay during transit to the intestines. Second and more important, the α particle has a very short path length (<100 μM) relative to the intestinal lumen diameter, suggesting that a minute fraction of intestinal ^{213}Bi decay events will reach the radiosensitive epithelium. In terms of efficacy, the escort aptamer's tumor penetration rate exceeds the ^{213}Bi decay rate. Because escort aptamer pharmacokinetics match ^{213}Bi decay kinetics, a large increase in therapeutic index may be possible with an α particle–emitting aptamer as compared with an aptamer conjugated to the β-emitting isotope ^{90}Y. In fact, a preclinical radiotherapy comparison using a 50-kDa antibody fragment indicates that the α particle–emitting fragment has a far higher cure rate than the corresponding β particle–emitting antibody fragment (29).

Escort aptamers as a new class of targeting molecules

As oligonucleotide analogs of antibodies, escort aptamers are well tailored for delivering radionuclides to sites of diseased tissue. Significant work is needed to fully assess escort aptamer potential, but it appears that aptamers will be important targeting agents due to their high affinity, rapid blood clearance, and adaptability through organic synthesis. An aptamer can be rapidly identified and carried through discovery, optimization, and application to research and clinical problems.

In the near future, escort aptamers will need to be tested to determine how animal models translate into the clinic. Clinical behavior will provide important feedback to preclinical design in both imaging and therapy. While no single magic bullet will likely be found, the emergence of scFv's, antibody pretargeting strategies, and small peptides suggests that over the horizon lies an array of specific targeting agents. What will escort aptamers bring to the party?

Acknowledgments

Critical contributions came from many, including Philippe Bridonneau, Gary Cook, David Parma, Paul Schmidt, Drew Smith, and Steve Warren. Colleagues Ulrich Speck, Ludger Dinkelborg, and Stephan Hilger have supplied additional expertise. Martin Brechbiel and Brian Moyer provided helpful input on α-particle radiotherapy. Dan Drolet and Ray Bendele

made helpful additions to this manuscript. Finally, Larry Gold's commitment to aptamer research has made this work possible.

1. Tuerk, C., and Gold, L. 1990. Systematic Evolution of Ligands by EXponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science*. **249**:505–510.
2. Ellington, A.D., and Szostak, J.W. 1990. In vitro selection of RNA molecules that bind specific ligands. *Nature.* **346**:818–822.
3. Gold, L. 1995. Oligonucleotides as research, diagnostic, and therapeutic agents. *J. Biol. Chem*. **270**:13581–13584.
4. Famulok, M., and Mayer, G. 1999. Aptamers as tools in molecular biology and immunology. *Curr. Top. Microbiol. Immunol*. **243**:123–136.
5. Gold, L., et al. 1995. Diversity of oligonucleotide functions. *Annu. Rev. Biochem*. **64**:763–797.
6. Ruckman, J., et al. 1998. 2'-Fluoropyrimidine RNA-based aptamers to the 165-amino acid form of vascular endothelial growth factor (VEGF165). Inhibition of receptor binding and VEGF-induced vascular permeability through interactions requiring the exon 7-encoded domain. *J. Biol. Chem.* **273**:20556–20567.
7. Jayasena, S.D. 1999. Aptamers: an emerging class of molecules that rival antibodies in diagnostics. *Clin. Chem*. **45**:1628–1650.
8. White, R.R., Sullenger, B.A., and Rusconi, C.P. 2000. Developing aptamers into therapeutics. *J. Clin. Invest.* **106**:929–934.
9. Knox, S.J., and Meredith, R.F. 2000. Clinical radioimmunotherapy. *Semin. Radiat. Oncol*. **10**:73–93.
10. Adams, G.P., and Schier, R. 1999. Generating improved single-chain Fv molecules for tumor targeting. *J. Immunol. Methods*. **231**:249–260.
11. Wu, A.M., et al. 1996. Tumor localization of anti-CEA single-chain Fvs: improved targeting by non-covalent dimers. *Immunotechnology.* **2**:21–36.
12. Boerman, O.C., et al. 1999. Pretargeting of renal cell carcinoma: improved tumor targeting with a bivalent chelate. *Cancer Res*. **59**:4400–4405.
13. Paganelli, G., et al. 1999. Antibody-guided three-step therapy for high grade glioma with yttrium-90 biotin. *Eur. J. Nucl. Med*. **26**:348–357.
14. Axworthy, D.B., et al. 2000. Cure of human carcinoma xenografts by a single dose of pretargeted yttrium-90 with negligible toxicity. *Proc. Natl. Acad. Sci. USA*. **97**:1802–1807.
15. Lister-James, J., Moyer, B.R., and Dean, T. 1996. Small peptides radiolabeled with 99mTc. *Q. J. Nucl. Med*. **40**:221–233.
16. Gold, L., and Alper, J. 1997. Keeping pace with genomics through combinatorial chemistry. *Nat. Biotechnol.* **15**:297.
17. Charlton, J., Sennello, J., and Smith, D. 1997. In vivo imaging of inflammation using an aptamer inhibitor of human neutrophil elastase. *Chem. Biol*. **4**:809–816.
18. Fitzwater, T., and Polisky, B. 1996. A SELEX primer. *Methods Enzymol.* **267**:275–301.
19. Winter, G., et al. 1994. Making antibodies by phage display technology. *Annu. Rev. Immunol.* **12**:433–455.
20. Hanes, J., and Pluckthun, A. 1997. In vitro selection and evolution of functional proteins by using ribosome display. *Proc. Natl. Acad. Sci. USA*. **94**:4937–4942.
21. Drolet, D.W., et al. 1999. A high throughput platform for systematic evolution of ligands by exponential enrichment (SELEX). *Comb. Chem. High Throughput Screen.* **2**:271–278.
22. Cox, J.C., Rudolph, P., and Ellington, A.D. 1998. Automated RNA selection. *Biotechnol. Prog.* **14**:845–850.
23. Eaton, B.E. 1997. The joys of in vitro selection: chemically dressing oligonucleotides to satiate protein targets. *Curr. Opin. Chem. Biol.* **1**:10–16.
24. Pieken, W.A., et al. 1991. Kinetic characterization of ribonuclease-resistant 2'-modified hammerhead ribozymes. *Science*. **253**:314–317.
25. Hilger, C.S., et al. 1999. Tc-99m-labeling of modified RNA. *Nucleosides Nucleotides*. **18**:1479–1481.
26. Adams, G.P., et al. 1998. Increased affinity leads to improved selective tumor delivery of single-chain Fv antibodies. *Cancer Res*. **58**:485–490.
27. Ringquist, S., and Parma, D. 1998. Anti-L-selectin oligonucleotide ligands recognize CD62L-positive leukocytes: binding affinity and specificity of univalent and bivalent ligands. *Cytometry*. **33**:394–405.
28. Watson, S.R., et al. 2000. Anti-L-selectin aptamers: binding characteristics, pharmacokinetic parameters, and activity against an intravascular target in vivo. *Antisense Nucleic Acid Drug Dev*. **10**:63–75.
29. Behr, T.M., et al. 1999. High-linear energy transfer (LET) alpha versus low-LET beta emitters in radioimmunotherapy of solid tumors: therapeutic efficacy and dose-limiting toxicity of 213Bi- versus 90Y-labeled CO17-1A Fab' fragments in a human colonic cancer model. *Cancer Res*. **59**:2635–2643.