DNA nanorobots

By Lauren Martz, Staff Writer

Researchers at the **Wyss Institute for Biologically Inspired Engineering at Harvard University** have designed drug delivery vehicles dubbed DNA nanorobots that allow for complex targeting and controlled drug release.¹ Although targeted delivery is hardly a new concept, the DNA nanorobots go one step beyond existing approaches by enabling conditional payload release based on the presence of combinations of markers on target cells, thus making delivery more specific and controlled.

The Wyss team's technology has the potential to improve the safety and efficacy of a variety of therapeutics, but several steps, including design changes to increase time in circulation and decrease immunogenicity, could be required before progressing to clinical trials.

Previous studies have shown that DNA constructs can be designed to perform robotic functions including computing and sensing.² Building on those studies, a Wyss team led by George Church used a computer-aided 'DNA origami' method, which folds single-stranded DNA into a customized shape, to design the nanorobot delivery system.

The nanorobots were designed with a two-part DNA barrel shape capable of holding 12 payload molecules inside. The two parts are held together by customized oligonucleotide aptamers that induce a conformational change upon binding specific peptide targets, which opens the barrel and exposes the payload.

Church is a founding core faculty member at the Wyss Institute, professor of genetics at **Harvard Medical School** and professor of health sciences at **Harvard University** and the **Massachusetts Institute of Technology**.

As proof of concept, the team loaded nanorobots with fluorescently labeled antibody fragments against human leukocyte antigen (HLA) and designed aptamers that specifically bound surface peptides found on cancer cells. Each nanorobot was held together by two aptamers designed to bind the same or different peptide targets. Nanorobots targeting two distinct cell surface peptides allowed better cell selectivity. The nanorobots exposed payload, as indicated by increased fluorescence signal, only when cultured in cell types expressing the target surface peptide or peptides.

To show that the nanorobots could bind to specific cell types, the team mixed large granular lymphocytic leukemia cells with a Burkitt's lymphoma cell line or whole-blood leukocytes and added a nanorobot with DNA aptamers that recognized a protein only found on the leukemia cells. In both cultures, the nanorobots specifically detected the leukemia cells.

The next step was determining the sensitivity of the nanorobots. The team

developed a culture of mixed lymphoma and leukemia cells with varying concentrations of each cell type. Nanorobots designed to target the leukemia cells were able to detect them at concentrations as low as the single-cell level.

Finally, the team tested the therapeutic potential of the structures *in vitro*. Nanorobots loaded with antibodies against the known cancer targets CD33 and sialic acid binding Ig-like lectin 7 (SIGLEC7; CDw328) and with aptamer locks targeting a leukemia surface protein produced dose-dependent arrest of leukemia cell growth.

The work was published in *Science*.

Rather than just steady release over time of a drug that can bind target and nontarget cells alike, Church noted that the nanorobot technology has programmable logic in a very tiny package—smaller than a cell.

"The idea is to develop the capability for controlled delivery or release of payloads in response to cell surface markers that may be present on one cell population while not responding to nontarget cells that do not bear the same combination of markers," added Shawn Douglas, technology development fellow at the Wyss institute and co-corresponding author on the paper.

"We can finally integrate sensing and logical computing functions via complex yet predictable nanostructures, which are some of the first hybrids of structural DNA, antibodies, aptamers and metal atomic clusters," added Douglas.

Nanorobot redesign

Church said the team's nanorobots could be applied to "any therapeutic or diagnostic that could benefit from sensing, logic, actuation and

targeted delivery." The indications his team are studying include cancer, immune modulation and autoimmunity.

Douglas told *SciBX*, "The first step is to scale up production of our devices so we have enough material to work in an animal model. We need about 1,000 times more material for these experiments than we did for the current study."

Church added that his team is "moving on to rodent efficacy and toxicity testing—then cost reduction protocols and clinical trials."

According to Douglas, the team hopes to publish follow-up studies showing function in animals in the next two years.

Both Church and Douglas said the nanorobots likely will need design alterations before the product is ready for the clinic.

"It is possible that the DNA sequence we used is immunogenic due to unmethylated CpG sites," noted Douglas. "We might address this by redesigning the sequence to not include those bases."

Additionally, he told *SciBX* that the team will probably need to redesign the nanorobot so that it can stably circulate in the bloodstream long enough for it to find its protein target and release its payload.

Harvard University has filed a patent application for the nanorobots, and the technology is available for licensing.

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—Shawn Douglas, Harvard University

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