Molecular pathology selected abstracts

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Personalized oligonucleotide therapy for treatment of a rare genetic disease

December 2019—A variety of molecular diagnostic laboratory tools are available to diagnose diseases caused by mutations in the human genome. However, few treatments are available to correct the underlying pathophysiology driven by these mutations. This is due, in part, to pharmaceutical companies' inability to justify, from a business perspective, the expense and time necessary to develop and obtain FDA approval for novel therapies that benefit only a small number of patients. In a recent study, investigators at Boston Children's Hospital showed that the conventional drug development and approval paradigm can be significantly disrupted and accelerated, given the right set of circumstances, without involving pharmaceutical companies. According to their findings, within 12 months of the initial molecular diagnosis of a disease-causing mutation, an effective targeted therapy applicable to a single patient can be designed; tested in vitro, in vivo, and for safety; approved by the FDA; administered; and demonstrate therapeutic efficacy. The authors reported on a six-year-old girl for whom a drug was designed to treat ceroid lipofuscinosis, neuronal, 7 (CLN7), which is a form of Batten's disease, a rare and fatal autosomal recessive neurodegenerative disease. The child's disease was caused by compound heterozygous mutations in the *CLN7* gene. One of the child's two *CLN7* mutations was found to be an intronic insertion of a retrotransposon, a rogue section of DNA known to evolutionarily "jump" between genes. This intronic insertion, which had not been described previously, could cause the disease by generating an abnormally spliced mRNA and, ultimately, an abnormal downstream protein. Previous studies have shown that abnormally spliced RNA can be treated using antisense oligonucleotides that mask the pathogenic cryptic splice site and, therefore, promote use of the normal gene's functional splicing sites. The authors of the study on the six-year-old girl, reported herein, designed an antisense oligonucleotide that targeted the DNA sequence of the patient's unique pathogenic cryptic splice acceptor site. This antisense oligonucleotide drug (Milasen) was shown to partially correct the splicing defect and consequent lysosomal pathology in the patient's cells grown in culture. After the investigators conducted a rapid series of animal tests to confirm safety, the FDA granted them permission to test Milasen in a single-patient clinical trial. The first intrathecal injections began within eight months of the child's diagnosis. After one year of treatment, the patient's number and duration of seizures significantly decreased, with no serious adverse events, although she continued to lose brain volume. This landmark proof of concept N-of-1 clinical trial is the first instance of an FDA-approved drug being created and administered for a single patient. Although this study provides hope for patients with rare genetic diseases, there are many obstacles to applying this model to other syndromes. From a scientific perspective, antisense oligonucleotide therapeutics will only apply to a very small number of diseases with a splice-switching molecular pathogenesis. From a societal perspective, cost is an issue. This treatment model also raises the questions, How should individualized drugs be regulated by the FDA? Should safety and efficacy benchmarks be loosened? Even for a progressive fatal disease, how can patient safety be ensured? The ability to answer these important questions will impact whether N-of-1 clinical trials can be applied to larger groups of patients.

Kim J, Hu C, El Achkar CM, et al. Patient-customized oligonucleotide therapy for a rare genetic disease. *N Engl J Med.* 2019;
381:1644–1652.

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Detecting recurrent colon cancer using circulating tumor DNA analysis

Colon cancer typically is initially treated with surgery and, for a subset of patients, adjuvant chemotherapy. While treatment improves the overall survival for patients with advanced colon cancer, no reliable post-treatment biomarker can predict which patients have minimal residual disease, which can lead to disease recurrence. Reliable identification of this high-risk patient population could provide an opportunity to treat these patients with additional therapy to decrease disease burden or delay or prevent disease recurrence. Identification of tumor-specific mutations in circulating tumor DNA (ctDNA) that has leaked into the blood may allow early and accurate detection of minimal residual disease. In this study, the authors measured patient-specific ctDNA mutations after surgery for advanced colon cancer and, again, after adjuvant chemotherapy. They showed that the detectable presence of these mutations in post-treatment plasma-derived DNA was a strong and significant prognostic biomarker for future disease recurrence. The patients enrolled in the study had at least one tumor-specific somatic mutation identified by next-generation sequencing of their pretreatment tumor. After therapy, the somatic mutation with the highest variant allele fraction was targeted for ultra-sensitive plasma-based ctDNA detection by a patient-specific assay that used an error-corrected polymerase chain reaction–based sequencing method. Circulating tumor DNA was identified in 20 of 96 (21 percent) patients after surgery and 15 of 88 (17 percent) patients after adjuvant chemotherapy. After a median follow-up of 29 months, 24 patients experienced disease recurrence. Circulating tumor DNA positivity at both post-treatment time points was strongly, significantly, and independently prognostic for subsequent disease recurrence. However, the overall diagnostic sensitivity of this ctDNA assay was suboptimal, with only 42 percent of disease recurrences having been preceded by a positive ctDNA result after surgery. In comparison, traditional carcinoembryonic antigen (CEA) tumor marker monitoring, which is traditionally used in invasive colon cancer follow-up, was positive in only seven of the 96 patients, compared to 20 of 96 for ctDNA. This suggests that ctDNA is likely a more analytically sensitive method for detecting residual tumor cells and possibly informing the need for additional therapy. The ability of this ctDNA assay to detect minimal residual disease after treatment has obvious utility for the clinical management of colon cancer patients. The availability of sensitive, prognostic, blood-based assays for minimal residual disease may provide solid tumor oncologists with a laboratory tool akin to those in the standard diagnostic toolkit of leukemia-focused hematological oncologists who have been monitoring minimal residual disease-based leukemic disease burdens via a variety of methods for many years.

Tie J, Cohen JD, Wang Y, et al. Circulating tumor DNA analyses as markers of recurrence risk and benefit of adjuvant therapy for stage III colon cancer. *JAMA Oncol.* 2019. doi:10.1001/jamaoncol.2019.3616.

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