Molecular pathology selected abstracts

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In vivo gene editing as a therapeutic strategy for transthyretin amyloidosis

October 2021—Transthyretin amyloidosis, also known as ATTR amyloidosis, occurs secondary to accumulation of misfolded transthyretin (TTR) protein in the form of amyloid fibrils. The acquired form of ATTR amyloidosis is referred to as wild-type ATTR amyloidosis. In rare cases, ATTR amyloidosis manifests as hereditary ATTR (hATTR), which is inherited in an autosomal-dominant pattern due to mutations of the *TTR* gene. Most patients with ATTR amyloidosis develop polyneuropathy or cardiomyopathy, or both. The natural history of the disease involves progressive illness with death within two to six years of diagnosis in those with amyloid cardiomyopathy and four to 17 years after symptom onset in those with amyloid polyneuropathy in the absence of cardiomyopathy. Therapeutic strategies for treating ATTR amyloidosis involve long-term use of TTR-stabilizing agents, yet most of these patients experience disease progression. Because this is a monogenic disease, the authors evaluated the safety and efficacy of the in vivo gene-editing technique CRISPR Cas9-mediated gene editing, which is targeted toward reducing serum TTR protein concentrations. The protein is almost exclusively produced by the liver. Clustered regularly interspaced short palindromic repeats (CRISPR) and associated Cas9 endonuclease (Cas9) were directed at the *TTR* gene. NTLA-2001 is a CRISPR Cas9-based in vivo gene-editing therapy intended to edit TTR in hepatocytes, thereby decreasing the production of wild-type and mutant TTR after a single administration. NTLA-2001 consists of a proprietary lipid nanoparticle delivery system with liver tropism, carrying an mRNA-encoding Cas9 protein and a single-guide RNA (sgRNA) targeted toward knockdown of mutant and wild-type human TTR. NTLA-2001 was administered as an intravenous infusion and predominantly taken up by hepatocytes with the help of low-density lipoprotein receptors on the surface of the hepatocytes. Following intracellular assembly of the CRISPR-Cas9 complex, the sgRNA targeted the *TTR* gene sequence, which was followed by Cas9-mediated cleavage at these sites. Errors in endogenous DNA-repair mechanisms lead to insertions and deletions (indels) within the *TTR* gene sequence, resulting in deleterious mutations, decreased expression of full-length mRNA, and, ultimately, decreased TTR protein expression. A concern with the use of in vivo gene-editing techniques is off-target effects. To address this, the authors performed preclinical in vitro studies in primary cultures of human hepatocytes. They used multiple computational tools to identify potential off-target loci of interest. Seven such loci were identified, all within noncoding regions. No evidence of off-target gene editing was identified with extremely high doses of NTLA-2001. In addition, in vivo preclinical studies were conducted in transgenic mice and cynomolgus monkeys. A single dose of NTLA-2001 was found to reduce serum levels of mutant and wild-type TTR in the transgenic mice, with a nadir at four weeks. This effect was maintained at 12 months, suggesting permanent gene editing. Similarly, a single dose of the nonhuman primate surrogate of NTLA-2001 achieved a more than 94 percent reduction of serum TTR in the cynomolgus monkeys, and this effect was sustained over a 12-month period. In this proof-of-concept study, a single dose of intravenous NTLA-2001 was administered to six patients with hATTR amyloidosis with polyneuropathy. Mild (grade one) adverse effects were reported in three of six patients. NTLA-2001 doses of 0.1 mg/kg and 0.3 mg/kg reduced serum levels of mutant and wild-type TTR by 52 percent and 87 percent, respectively, at four weeks of follow-up. Limitations of this study included the four-week follow-up and a need for participants who received NTLA-2001 to undergo long-term safety monitoring. In addition, these patients need to receive vitamin A supplementation, as TTR has a normal physiologic role in vitamin A transport. In summary, these results provide clinical proof-of-concept for in vivo gene editing as a strategy for treating hATTR amyloidosis. In vivo studies in transgenic mice and cynomolgus monkeys suggest the possibility of permanently reduced TTR expression.

Gillmore JD, Gane E, Taubel J, et al. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. *N Engl J Med*. 2021;385(6):493–502.

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Evolution of the actionable metastatic cancer genome under therapeutic pressure

In this pan-cancer study, the authors sought to determine whether systemic therapy for metastatic cancer imposes evolutionary pressures that contribute to genomic diversification. This has important clinical implications pertaining to whether repeated molecular analysis is warranted to guide clinical treatment decisions. The authors used whole genome sequencing (WGS) to analyze multiple samples from 231 patients with metastatic cancer. Ninety-two percent (213 of 231) of patients had two biopsies analyzed. Multiple biopsies of metastatic lesions were sampled in 90 percent of cases, and a limited number of patients had a primary tumor sample and a paired metastatic sample available for analysis. The median time between biopsies was 6.4 months. Information regarding treatment administered between biopsies was available for 89 percent of patients. Although this was a pan-cancer study, most specimens belonged to five tumor types—breast, prostate, colorectal, lung, and skin. The authors noted that tumor purity, an important confounder, was not significantly different between the first and second biopsies. With regard to metrics of alterations across the genome, the rank correlation of tumor mutational burden between paired biopsies was high, with only a modest increase in the second biopsy (median increase from the first to the second biopsy, 0.35 mutations per megabase). The authors did not find any differences in the metrics for microsatellite instability between any of the paired samples. When comparing different categories of genomic alterations, such as single-nucleotide variants, insertions/deletions, structural variants, and copy number changes, the highest discordance between paired samples was for structural variants. However, the authors reported that this may be due to the lower confidence for identifying such events using WGS since, in many cases, a low number of supporting reads suggested the presence of a structural variant. When looking at specific genomic alterations, at least one driver event was identified for 97 percent of samples. It is noteworthy that the median number of drivers unique to the second biopsy was zero (interquartile range, 0–1). Therefore, although the metastatic cancer genome showed increased complexity over time, the number of functional driver events remained constant and the standard-of-care genomic indications, such as actionable *EGFR* gene alterations in lung cancer, remained unchanged over time in 99 percent of cases. However, this study highlighted two clinical scenarios in which a single genomic analysis likely would be insufficient for providing optimal patient care. The first scenario involved using small-molecule inhibitors, primarily for lung cancers, with standard-of-care genomic targets. The authors identified a much higher frequency of therapy-induced genomic alterations when the therapy targeted a mutant rather than wild-type gene (46 percent versus five percent, respectively). The second scenario involved using hormonal therapies, for which on-target evolution was identified in 22 percent of cases. This included gain of *ESR1* hotspot mutations in breast cancer and amplification of *AR* in prostate cancer, both of which are known mechanisms of acquired resistance. Other findings of interest included driver alterations (gain/loss) in rare cases in such genes as *SMAD2, MYC, ZBTB10*, and *STK11*. Some limitations of this study include a limited time interval between biopsies (median interval, 6.4 months), limited subgroup analysis for rarer cancer types, and infrequent detection of subclonal alterations due to a combination of lower tumor purity and a median depth of sequencing of approximately 100 times. However, these results provide insight into resource utilization and clinical scenarios in which repeated molecular analysis may be warranted to guide clinical treatment decisions.

Van de Haar J, Hoes LR, Roepman P, et al. Limited evolution of the actionable metastatic cancer genome under therapeutic pressure. *Nat Med*. 2021. https://doi.org/10.1038/s41591-021-01448-w

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