Public Advisory:

Critical Risks from Overestimated sgRNA Purity in In Vivo CRISPR Therapies

Issued by: GeneLancet Biosciences Inc

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GeneLancet issues this public advisory to alert a profound humanity-level risk in the rapid scale-up of in vivo CRISPR gene editing trials using synthetic sgRNA products. Numerous scientific reports have repeatedly shown that the widely adopted FLP purities, determined by UV area in HPLC, dangerously overestimate actual purities. Analysis of the main FLP peak by mass spectrometry (MS) is needed to assure its safety, as this can mislead stakeholders on the true extent of impurity control. The public, regulators, and stakeholders must demand immediate reforms to prevent catastrophic outcomes.

Understanding sgRNA: The Key Component in CRISPR Therapies

Single guide RNA (sgRNA) is a synthetic RNA molecule central to CRISPR-Cas9 gene editing systems. It combines the functions of natural CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA) via a tetranucleotide loop into a single ~100-nucleotide strand, guiding the Cas9 enzyme to specific DNA targets for precise cuts. Chemically synthesized sgRNAs are favored in therapeutics for their customizable modifications (e.g., 2'-methoxy, phosphorothioate backbones for stability and reduced immunogenicity). However, synthesis flaws lead to impurities like truncated sequences (N-1), most of which misdirect Cas9 and cause off-target edits. Purity is critical because even low-level such impurities can amplify risks in in vivo applications, where billions of cells are exposed without screening. Commercial GMP sgRNAs often claim high purity via UV methods, but mass spectrometry (MS) reveals true levels around 50%, underscoring the need for better standards to ensure safe, effective therapies.

The Core Issue: Inadequate sgRNA Purity and Oversight in In Vivo Trials

In vivo CRISPR therapies, such as those developed by Intellia Therapeutics (e.g., nexiguran ziclumeran for ATTR-CM) and Verve Therapeutics (e.g., VERVE-101 for cholesterol reduction), involve direct delivery of CRISPR components—like single guide RNA (sgRNA) and Cas9—into the body via lipid nanoparticles (LNPs). Unlike ex vivo approaches (e.g., Casgevy for sickle cell disease, where cells can be screened before re-infusion), in vivo editing affects billions of cells without safeguards, making errors permanent and unscreenable.

A critical flaw lies in sgRNA quality: Vendors and sponsors claim >80% (often >90%) full-length product (FLP) purity using UV-based methods (e.g., ion-pair reversed-phase HPLC-UV), which overestimate true purity by failing to resolve co-eluting impurities like truncations or variants. Techniques like mass spectrometry (MS), which is a good-fit method for detecting molecular weights, often reveal actual purities below 74% even in the purest HPLC fractions, with data from a 2025 Organic Process Research & Development paper by Genentech showing that claimed >90% UV purities are actually around 50% for commercial GMP products. Claims of achieving single-nucleotide resolution for impurities (e.g., N-1 truncations) via optimized ion-pairing reversed-phase chromatography are shady because truncated species are highly diverse, the N-1 reference in method development is insufficient, they are demonstrated on specific reference sequences and do not generalize to other sgRNA sequences, and MS analysis of the main FLP peak is needed to assure its purity, potentially misleading stakeholders on the true extent of impurity control. This unsafely allows trials to proceed without formal risk-based justifications, as UV claims meet informal FDA benchmarks, potentially masking commercial motivations to expedite approvals and market entry.

Catastrophic Risks to Humanity

The negligence in handling sgRNA impurities exacerbates the inherent dangers of in vivo editing:

1. Off-Target Mutations and Genomic Instability: Impure sgRNAs can cause unintended DNA cuts, leading to large structural variations like chromosomal translocations, megabase-scale deletions, or chromothripsis. These may hit oncogenes and can also disrupt regulatory elements, causing delayed effects such as chronic inflammation, neurodegeneration, or metabolic disorders. In preclinical models, off-targets have persisted across generations, raising concerns for heritable changes if germline cells are inadvertently edited.
2. Long-Term and Transgenerational Effects: DNA changes are irreversible, with risks manifesting years later or in offspring. Unlike traditional drugs, there's no "off-switch," and current trials' short follow-ups so far (1-5 years) miss these latencies.
3. Batch Variability Issues: SgRNA impurities vary between batches due to chemical synthesis flaws, presenting serious challenges in cGMP productions and amplifying risks in unscreened in vivo applications.

Call to Action

To avert catastrophe:

- **Immediate Moratorium on In Vivo Trials**: Pause new enrollments until MS-based purity validation is mandated, ensuring true FLP ≥80% and completion of risk-based justifications according to FDA’s recommendation.
- Mandatory Risk Assessments: Require sponsors to disclose MS data of sgRNA drug substances in all trials.

- Public Transparency: Regulators must publish purity metrics and justifications; patients deserve full informed consent on permanence and unknowns.
- Ethical Reforms: Strengthen international guidelines (e.g., via WHO) to prioritize safety over speed.

This is not alarmism but a precautionary imperative. The promise of CRISPR is immense, but mishandling could undermine it forever. The public must demand accountability now.

About GeneLancet Biosciences

GeneLancet Biosciences is a leader in next-generation gene editing, focused on developing precise and safe CRISPR therapeutics. GeneLancet is committed to advancing guide RNA technologies like LgRNA to improve the potency, safety (specificity) and scalability of gene editing.

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