Modified Guide RNAs

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5. **Abstract: This disclosure relates to modified single and dual guide RNAs having improved in vitro and in vivo activity in gene editing methods.**
6. **Summary:**
    a. **This disclosure relates to the field of gene editing using CRISPR/Cas systems, a part of the prokaryotic immune system that recognizes and cuts exogenous genetic elements. The CRISPR/Cas system relies on a single nuclease, termed CRISPR-associated protein 9 (Cas9), which induces site-specific breaks in DNA. Cas9 is guided to specific DNA sequences by small RNA molecules termed guide RNA (gRNA). Guide RNA comprises trRNA (also known as tracrRNA) and crisprRNA (crRNA). The trRNA and crRNA may be contained within a single guide RNA (sgRNA) or in two separate RNA molecules of a dual guide RNA (dgRNA). Cas9 in combination with trRNA and crRNA or an sgRNA is termed the Cas9 ribonucleoprotein complex (RNP).**
    b. **Oligonucleotides, and in particular RNA, are sometimes degraded in cells and in serum by endonuclease or exonuclease cleavage. Improved methods and compositions for preventing such degradation, improving stability of gRNAs and enhancing gene editing efficiency is desired, especially for therapeutic applications.**
    c. **In some embodiments, therapeutic genome editing tools are provided comprising modified guide RNAs. The modified guide RNAs described herein may improve the stability of the guide RNA and the guide RNA/Cas9 complex and improve the activity of Cas9 (e.g., SpyCas9 and equivalents) to cleave target DNA. In some embodiments, the guide RNA is an sgRNA. In some embodiments, the guide RNA is a dgRNA. In some embodiments, the guide RNA is a tracrRNA. In some embodiments, the guide RNA is a crRNA.**