**Poster 17**

# Formulation of CRISPR Cas-9 loaded polymer mesoscale nanoparticles

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## Purpose

The CRISPR/Cas9 system is a molecular mechanism of gene editing, and it can target specific genes for insertion, deletion, or modification. Earlier studies have shown knockouts in metastatic renal carcinoma as well as knockdown in tumor suppressing gene associated to kidney cancer. Knocking out the oncogenes with this technology can act as a therapy to inhibit growth of cancer cell and tumors. Different vectors can be used for the targeted delivery of CRISPR Cas-9 because intracellular delivery to the mutated gene can be difficult. Previously designed polymeric mesoscale nanoparticles (MNPs) can specifically target renal tubules, has controlled release and are highly biocompatible. The use of nanomaterials for drug delivery can overcome multiple barriers and limitations of viral delivery system.

## Methods

In this work, we synthesized MNPs with EGFP single guide RNA (sgRNA) and Cas9 protein to target green fluorescent protein (GFP) transfected in renal carcinoma cells. We performed in vitro studies using a GFP-transfected renal cell carcinoma cell line (RCC-4). Fluorescence imaging, PCR, and flow cytometry were used to validate knockout of GFP in these cells.

## Results

In vitro studies by fluorescence imaging and flow cytometry revealed significant knockouts with these CRISPR Cas9 loaded nanoparticles. Our work demonstrated that dual-loaded MNPs had somewhat better performance than separate MNP formulations each containing gRNA or Cas9. We found that control MNPs with no CRISPR contrast or with nonsense gRNA demonstrated no reduction in GFP expression.

## Conclusion

This work demonstrates successful loading and in vitro delivery of kidney targeted polymeric MNPs with CRISPR gene editing mechanisms. As many kidney diseases involve chromosomal alterations and gene mutations, in the future we aim to target these genes in the kidneys in vivo to deliver CRISPR/Cas9. We anticipate that this may lead to a ‘dual-targeted’ therapy with high precision and thus reduce the progression of kidney cancer and renal disease.

**Keywords:** Polymer nanoparticles, CRISPR, kidneys, cancer