**Hierarchical Multi-Agent Reinforcement Learning For Optimizing CRISPR-Based Polygenic Therapeutic Design**

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**Background and aims.** Polygenic diseases require CRISPR therapies targeting multiple genes simultaneously while balancing editing efficiency, off-target effects, vector capacity, and immunogenicity. Current approaches optimize guides individually without considering gene interactions. We developed a hierarchical multi-agent reinforcement learning (MARL) framework to coordinate multi-gene CRISPR therapeutic design with explicit synergy modeling.

**Methods.** We implemented a three-tier MARL architecture: (1) Guide Design Agents (GDA) using PPO to optimize individual guide RNA sequences; (2) a Therapeutic Strategy Agent (TSA) using PPO with hierarchical feedback to select editing modes and maximize gene synergies; and (3) a Clinical Translation Agent (CTA) using DQN to enforce vector capacity and immunogenicity constraints. We incorporated a synergy matrix quantifying beneficial gene co-editing effects. The framework was validated on a polygenic retinal disease model involving 7 mutations across 5 genes (PDE6B, RHO, RP1, USH2A, PRPF8) using a 1Mb mini-genome for computational feasibility.

**Results.** The framework successfully designed a multi-gene therapy targeting 4/7 mutations with optimal editing strategies: prime editing for PDE6B (c.2071C>T) and RHO (c.541C>T), base editing for RP1 (c.2029C>T), and knockout for USH2A (c.11864G>A). The design achieved high editing efficiencies (0.48-0.76), remained within SaCas9 vector capacity (3,900/4,500 bp), minimized immunogenicity (1/2 epitopes), and maximized therapeutic synergy (score: 30.0). Processing completed in 10 minutes on standard hardware.

**Conclusion/Discussion.** Our hierarchical MARL framework demonstrates AI's capability to navigate complex multi-objective optimization in genetic medicine. By explicitly modeling gene synergies and clinical constraints, the system identifies personalized therapeutic strategies that would be computationally intractable using traditional approaches. This proof-of-concept establishes a foundation for AI-driven design of polygenic therapies, potentially accelerating development from years to days.