Future of Cancer Immunotherapy: Advancing CAR-Ma with Innovative RNA Vector Multi-Modality

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1 Introduction

Cancer remains a leading global health challenge, with 9.7 million deaths in 2022 and nearly 30 million new cases projected by 2040 (1,2). In Hong Kong, cancer accounted for ~30% of deaths in 2020 (3).

Current therapies, including radiotherapy, chemotherapy, and immunotherapy, face significant challenges such as side effects, high costs, limited accessibility, and ineffective targeting of solid tumors, which comprise over 90% of cancers (4,5,6). These tumors often resist treatment due to the tumor microenvironment (TME), which obstructs drug penetration and hampers the persistence of Chimeric Antigen Receptor (CAR)-T cells (7,8). Advances in genomic technologies have revealed the complexities of cancer, including tumor heterogeneity and acquired resistance, complicating precision in treatment (9).

While existing therapies are powerful, they lack the sophistication needed to effectively address the multifaceted nature of cancer.

2 Objective

This project, under the HKU International Genetically Engineered Machine (iGEM) 2024 Team, aims to synthesize CAR-Ma—CAR on macrophages—powered by the world's first multi-modal RNA.

3 Methodology

The simplified procedural overview of this research is as follows:

1. DNA fragments amplification and plasmid assembly
2. Linearization of plasmids
3. In-vitro transcription (IVT) of DNA plasmids to RNA
4. Electroporation of the RNA product into target cell lines
5. Isolation of macrophage yield

Cloning, minipreparation, and transformation of bacterial cultures (DH5α and DH10B) were also conducted, alongside cell culturing of THP-1 (human monocyte) and HEPG2 (human liver cancer) cell lines.

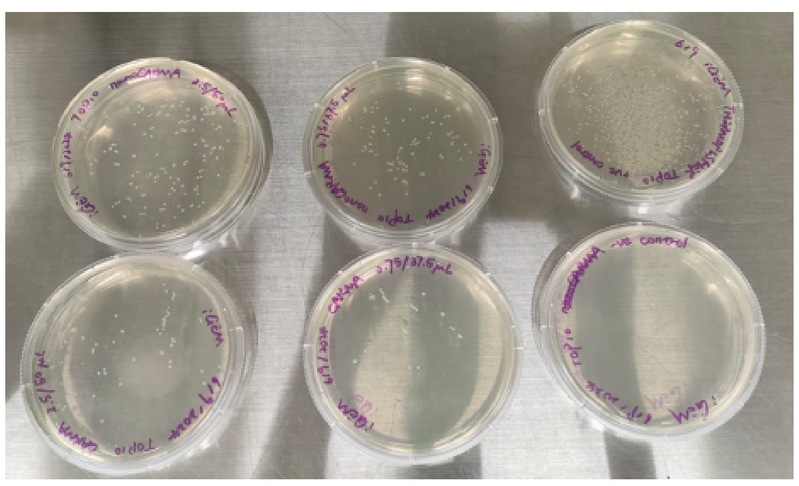

Fig. 1. Image illustrating the successful cloning process of nanoCAR-Ma and CAR-Ma.

Primary Engineering Components

Multimodal RNA

Designed, with the addition of 26S subgenomic promoters and internal ribosomal entry sites (IRES), to perform three key functions (10):

1. **Replicative**: Uses a code from the Venezuelan Equine Encephalitis Virus (VEEV) to maintain regulated expression and replenish CAR during phagocytosis, enhanced by high-fidelity RNA-dependent RNA polymerase and a self-replicating RNA (srRNA) to minimize mutations.
2. **Transience**: Temporarily expresses genes to disable tumor immune evasion pathways, particularly the SIRPa-CD47 and CRISPR-Cas12a pathways, enhancing safety and specificity in targeted cancer therapies.
3. **Release**: Delivers functional non-coding RNAs, including gRNAs and small interfering RNAs (siRNAs) to target and degrade complementary mRNA, regulating gene expression through RNA interference (RNAi) (11).

Being non-integrative, this RNA system mitigates risks associated with insertional mutagenesis and cytotoxicity, allowing cells to revert to their native state post-therapy (12).

The Heart of CAR-Ma: 5th Generation CAR Design

CAR-Ma is powered by a humanized nanobody from camelid antibodies, targeting glypican-3 (GPC3), a glycoprotein prevalent in cancers, including hepatocellular carcinoma (13). Its modularity enables simultaneous targeting of multiple antigens and includes co-stimulatory domains to enhance phagocytosis and immune coordination. The CAR also expresses IFN-γ to polarize macrophages toward an anti-cancer state and features a small-molecule control system for precise CAR expression regulation.

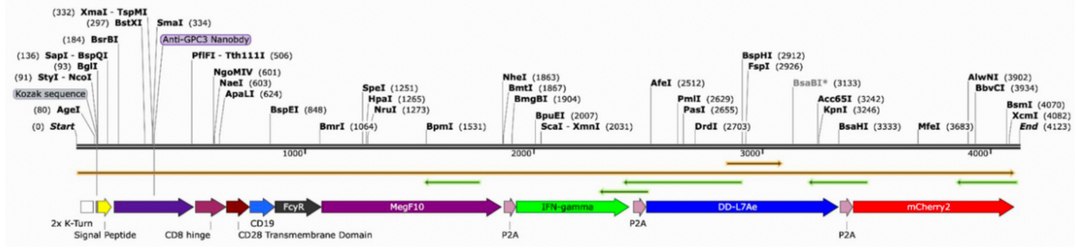

Fig. 2. Plasmid design for the 5th Generation CAR (4123 base pairs).

Molecular Targeting of Immune Evasion Checkpoints in Cancer

Solid tumors evade immune detection by utilizing checkpoints like CD47 and PD-L1. CAR-Ma knocks out these checkpoints and proteins, using Cas9-GFP or Cas12, that inhibit macrophage and T-cell activity. A novel shRNA pipeline, with CRISPR, allows for the transient silencing of SIRPa, mimicking the effects of permanent knockouts while enabling cells to revert to their native state.

4 Results

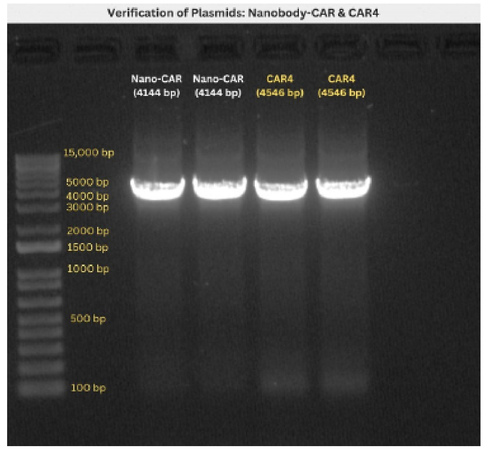

Fig. 3. UV-visualized electrophoresis image verifying CAR plasmids.

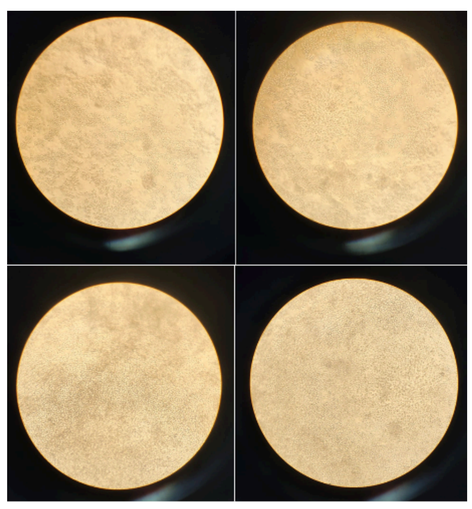

Fig. 4. Microscope image of THP-1 cells treated with puromycin (0.2 to 5 μg/mL) for visualizing cell culture and selecting transfected cells.

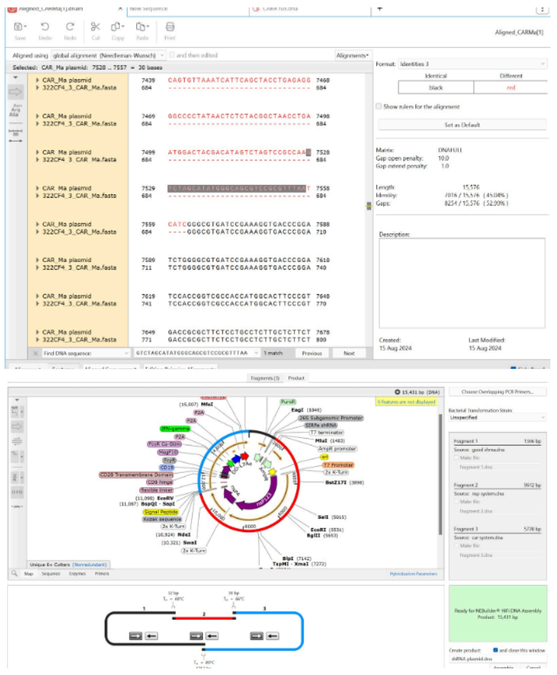

Fig. 5. Screen captures of plasmid sequence verification, confirming the presence of specific fragments to assess wet lab results.

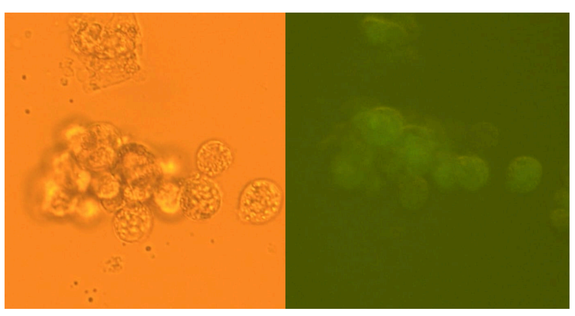

Fig. 6. Microscope images of transfected THP-1: left without green fluorescent protein (GFP), right with GFP phagocytosing HEPG2 cells.

5 Discussion

Challenges included non-specific amplification in PCR, low transformation efficiency in cloning, and inhibitors. To improve outcomes, we can optimize primer sequences and conditions, use advanced primer design software, and incorporate in silico validation tools, enhancing the reliability and success of results.

6 Conclusion

CAR-Ma enhances cancer immunotherapy through a humanized nanobody and multi-modal RNA, improving immune coordination while enabling precise genetic regulation. By targeting immune evasion, CAR-Ma boosts therapeutic efficacy and safety.

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