# Nanoparticle Enhancement of CCL21 Immunotherapy Treatment for Glioblastoma Demonstrates a Synergistic Anti-Tumor Effect

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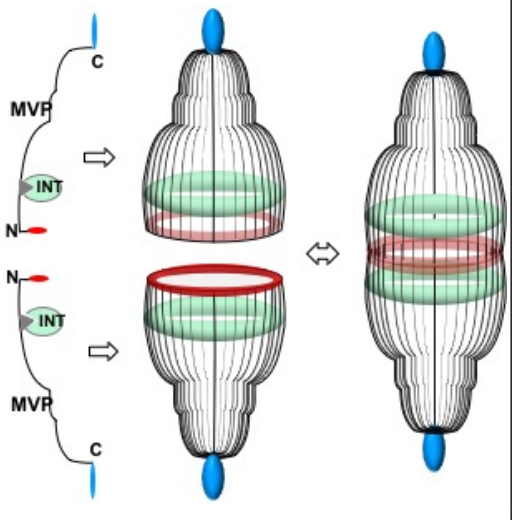
## Introduction

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults. Despite maximal therapy involving surgical resection, radiation and chemotherapy, median survival is approximately 15 months. Chemokine-based immunotherapy is an emerging treatment modality in GBM treatment. CCL21 is a lymphoid chemokine that enhances anti-tumor response by serving as a chemoattractant for immune cells. Nanotechnology has recently emerged as a viable treatment effector that can mediate antitumor activity. Vault nanoparticles are endogenous barrel-shaped ribonucleoproteins used as a novel drug delivery system. This study assesses the feasibility and potential of CCL21-packaged vault nanoparticles as an immunotherapeutic for GBM.

**Schematic diagram of engineered vault structures**

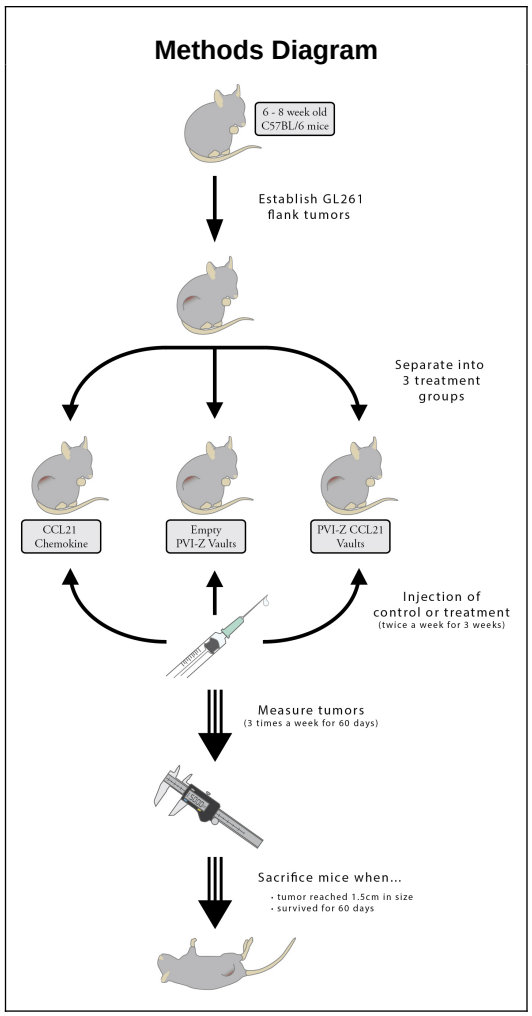



Han et al. ACS Nano 2011 23;5(8):6128-37

## Methods

In our pre-clinical model, GL261 cells (1.0 x 10^6) were implanted subcutaneously into the flank of 6-week-old C57BL/6 mice (n=9). The mice were treated with five intratumoral injections of CCL21-nanoparticle, vault nanoparticle alone, or CCL21 alone. Tumor size was measured at bisecting angles with calipers. At study conclusion, the presence and number of lymphocytes in tumors were analyzed using flow cytometry.

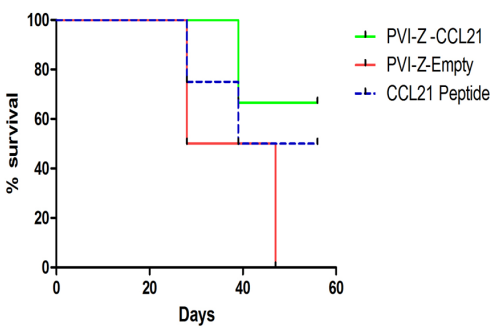
**Methods Diagram**




## Results

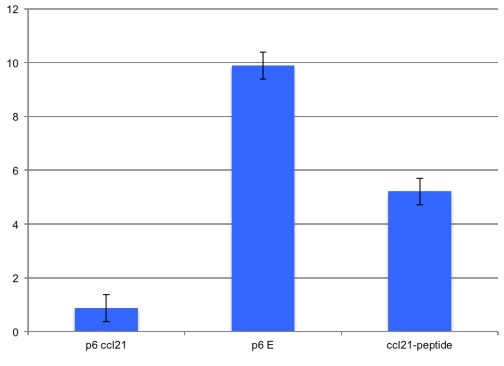
The CCL21-nanoparticle treatment group demonstrated a significant decrease in tumor growth rate when compared to CCL21 and nanoparticle alone. The fold change in tumor area in the CCL21-nanoparticle group was 4.3 compared to 5.2 and 6.4 for the nanoparticle group and CCL21 group, respectively. CCL21-nanoparticle treatment also increased CD3+ lymphocyte infiltrates in the tumor.

**CCL21 in vivo survival results**



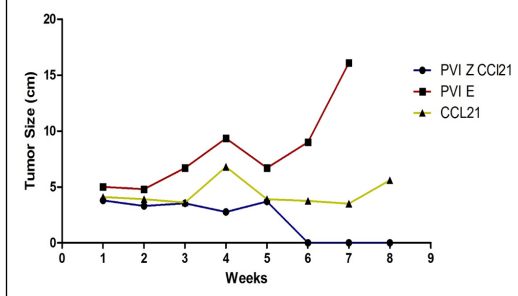

Survival of mice injected with empty vaults, CCL21, or vaults with CCL21.

**Tumors sizes at week 6**




Tumor size (cm) of mice injected with empty vaults, CCL21, or vaults with CCL21 after 6 weeks.

**CCL21 in vivo tumor size results**




Tumor size over time in mice injected with empty vaults, CCL21, or vaults with CCL21

**Table 1**
Tumor volumes by treatment group

| Treatment | Week 1 ($mm^3$) | Week 2 ($mm^3$) | Week 3 ($mm^3$) | Change (%) |
|---|---|---|---|---|
| pVI-E-CCL21 | 45.19 | 21.80 | 31.11 | -31.15 |
| rCCL21 | 46.55 | - | 94.46 | 102.9 |
| pVI-E-Z | 78.94 | 74.94 | 272.64 | 245.3 |

pVI-E-CCL21 = CCL21-vault, rCCL21 = recombinant CCL21, pVI-E-Z = empty vault, - = not recorded.

## Conclusions

This data suggests that CCL21-vaults can hinder tumor growth and promote lymphocyte antitumor activity in this pre-clinical model. Furthermore, our data demonstrates that nanoparticle delivery enhances the immunotherapeutic effect of CCL21. Further studies are needed to fully elucidate the synergy of combining chemokines with nanoparticles for targeted tumor therapy.

## Learning Objectives

By the conclusion of this session, participants should be able to: 1) Describe the importance of nanoparticle delivery of CCL21 to enhance antitumor effects, 2) Discuss, in small groups, the future potential for nanoparticle delivery of treatments, 3) Identify novel possible therapeutics for GBM.