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RGD-targeted Nanoparticles Expressing Flt23k Inhibit CNV In a Murine CNV Model

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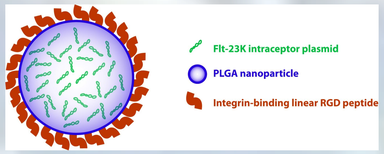

- **CNV models**
  - aav-shRNA.sFlt-1 CNV mice
  - Laser-CNV mice

Novel Delivery System:

Systemic Delivery Selectively and Safely Targeting Diseased Tissue.

RGD.flt23k.NR.NP homing to CNV lesion

- Immunohistology (Fig. 1, 2)

Quantification of CNV and fibrosis volumes

- Seg3D software in vivo (Fig. 3,4)
- Histology (Fig. 5)

Systemic toxicity evaluation

- Organ histology
- Life span

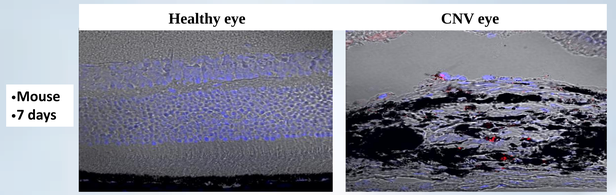

Fig. 1: RGD-targeted nanoparticles (red) home to CNV but not to normal tissue *(40×). Blue: DAPI staining.*

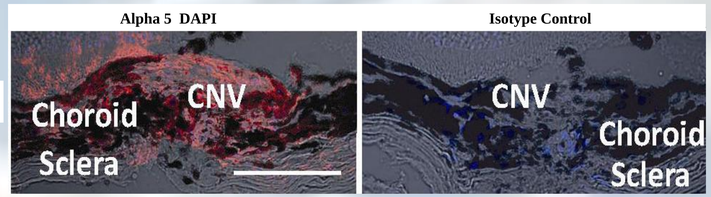

Fig. 2: Alpha 5 (red) selectively expressed in CNV *(40×).*

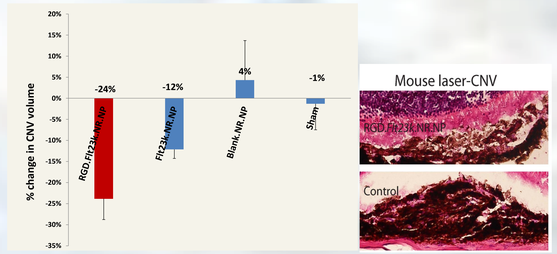

Fig. 3: RGD-targeted nanoparticles regress CNV. CNV decreased 24% with treatment (n=11, p<0.05, 2W).

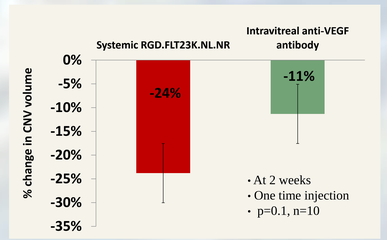

Fig. 4: Systemic RGD.Flt23k.NP is non-inferior in efficacy to intravitreal anti-VEGF Ab.

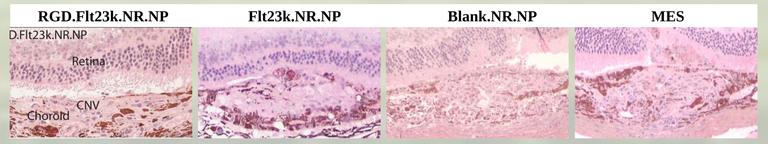

Fig. 5: H&E staining shows that the CNV lesion significantly regressed in RGD.Flt23k.NR.NP treated group compared with the controls in aav.shRNA.sFlt-1 induced CNV models (40×).

Purpose

To determine whether RGD.Flt23k PLGA nanoparticles can regress choroidal neovascularization (CNV) in murine and primate CNV models.

Methods

We prepared nile-red labeled nanoparticles which were blank, loaded with pCMV.Flt23k, or loaded with pCMV.Flt23k conjugated with RGD oligopeptides (which home to alpha-v-beta-3 integrin). All three nanoparticles were dissolved in MES buffer. A total volume of 4 µl (plasmid concentration is 0.1µg/µl) was delivered to each mouse, and similar volumes of MES buffer served as blank control. Murine CNV was induced by 532 nm laser or subretinal injection of adeno-associated virus mediated small hairpin ribonucleic acid (shRNA) sFlt-1. Tail vein injection was performed 2 weeks after induction of CNV. CNV regression was evaluated 2 weeks after tail vein injection in histological sections and CNV volume quantified using newly developed software, Seg3D in vivo image. RGD.Flt23k.NR.NP was detected by immunostaining in CNV.

Results

RGD.Flt23k.NR.NP only presents in CNV lesion. α5 expression in CNV area was confirmed by immunostaining. H&E stained sections show the CNV size was dramatically decreased in RGD.Flt23k.NR.NP injected mice (treatment group) as compared to the other three control groups (Flt23k.NR.NPs, blank NR.NPs or MES buffer). The treatment group mice CNV volume was decreased by 23%, which showed significantly more reduction than observed with unlabeled nanoparticles, blank nanoparticles, or MES control (all p value <0.05), As a positive control, CNV lesions treated with an intravitreal injection of an anti-mouse VEGF antibody were decreased by 11% (p=0.1). No systemic toxicity was detected. Life span of treated mice > 2 years.

Summary

One intravenous injection of targeted nanoparticles delivering Flt23k intraceptors regressed CNV murine models.

RGD oligopeptide enhanced selective localization.

No toxicity was observed.

RGD.Flt23k.NP could serve as an intravenous alternative or adjunct to monthly intravitreal injections.

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