In Vivo Nanoparticle Assessment of Neointimal Hyperplasia in Murine Arteriovenous Fistula

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Background

- AVF high maturation failure rate
- Stenosis and thrombosis are the most common cause of access failure
- The primary patency after percutaneous angioplasty is not satisfactory
- Annual cost of vascular access maintenance is around $2 billion in the U.S

Treatment of stenosis and thrombosis in hemodialysis fistulas and grafts by interventional radiology Luc Turmel-Rodrigues et al. NDT 2000;15:2029-2036
Background

Underlying pathology of AVF failure—neointimal hyperplasia (NH)

A: Day 0 of AVF placement: thin intimal layer
B: Day 115 of AVF placement: severe neointimal hyperplasia
Michael Allon et al. AJKD 2011; 58(3): 437-443

NH is alpha-smooth muscle actin (+)

Prabir Roy-Chaudhury et al. Neointimal hyperplasia in early arteriovenous fistula failure. AJKD 50: 782-790
Prior Lab Work

1. Assessed pathological endothelial function *in vivo* in AVF using endothelial targeted nanoparticles

2. Study whether there is a relationship between pathological endothelial function and subsequent neointimal hyperplasia development in AVF

Animal Model

AVF was created in 12-16 weeks old C57BL/6 mice. End to side carotid artery to jugular vein anastomosis.

AVF pathology at day 42 post-AVF surgery

Scale bar: 100 µm

Distance away from the anastomosis (µm)

Day42 NH area (µm²)

Sham
Cross-linked iron oxide (CLIO)

Cross-linked iron oxide: stable under harsh conditions (do not change in size, blood half-life, or loss of its dextran coat)

Pathological endothelial response was illuminated by CLIO-VT680

On day 14 post AVF surgery, confocal microscopy demonstrated CLIO-VT680 colocalized with CD31 positive endothelial cells near the anastomosis.
The intensity of CLIO signals on day 14 predicts neointimal hyperplasia on day 42.
Conclusion

- Pathological endothelium in AVF can be imaged in vivo using dextranated magnetofluorescent nanoparticles (CLIO-VT680)

- The day 14 IVM CLIO-VT680 signal predicted the subsequent site and magnitude of AVF neointimal hyperplasia at day 42 (r=0.58 p<0.05)
Limitations

• The AVF model mobilized the artery, does not mimic human AVF

• Could not image venous side due to scar tissue

• Confocal fluorescence IVM has limited depth of 200μm

• Lack of uremic environment
New AVF model

- New AVF model: end to side internal jugular vein-carotid artery anastomosis
- Evaluate venous stenosis and thrombus formation
- Future clinical translation
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Scale bar: 100µm
Figure A. Neointimal hyperplasia (NH) at week 1 and week 2 post AVF creation. The area of NH at week 2 is significantly higher than those at week 2.

Figure B. The area of thrombus at week 1 and week 2 post AVF creation.
Blood flow in AVFs

![Chart showing blood flow (ml/min) over duration post surgery (days). The chart includes data points for during surgery, week 1, week 2, and week 3, with varying blood flow levels across these time periods.]
Macrophages accumulated in the thrombus and adventitia of the AVFs
CLIO-VT680 was not colocalized with macrophages (CD68) and alpha-smooth muscle cells (aSMA).
Statin

- Statin—anti-inflammation
- Animal study: decrease neointimal hyperplasia
- Human study: Controversial*
- Higher dose may be required to achieve the clinical effects

* Roberto Pisoni et al. Statin therapy is not associated with improved vascular access outcomes. CJASN Aug 2010. vol 5. no. 8 1447-1450;
Nanostatin

- Size: 25 to 30nm
- Vehicle: reconstituted high density lipoprotein rHDL

- [s]-rHDL in vivo showed decreased aSMA and macrophages activities

Dio-rHDL colocalized with CD68+ macrophages

Day 7

Day 14

Scale bar: 250μm
Study protocol

Nanostatin (40mg/kg)
- Day 0 AVF
- Day 3 Nanostatin
- Day 6 Nanostatin
- Day 9 Nanostatin
- Day 12 Nanostatin
- Day 14 Sac

Oral atrovastatin (1.14mg/kg)
- Day 0 AVF
- Atrovastatin oral gavage daily
- Day 14 Sac

Control
- Day 0 AVF
- Day 14 Sac
NH area on day 14 post AVF  Thrombus area on day 14 post AVF

![Graph showing NH area and Thrombus area over distance from the anastomosis](image-url)
Blood flow at 2 weeks

- Control
- Statin
- Nanostatin
Conclusion

• Nanostatin binds to the macrophages after AVF creation

• Animals treated with nanostatin showed a trend of decreasing NH and increasing blood flow
Future Direction

- Identify the subset of macrophages that nanostatin binds in AVF
- Evaluate the role of nanostatin in maintaining AVF patency
- Clinical translation
Acknowledgements

MGH CSB
Jason R. McCarthy, PhD
Charles P. Lin, PhD
Matthias Narrender, PhD
Ralph Weissleder, MD

MGH CVRC/Cardiology
Farouc Jaffer, MD, PhD
Chase Kessinger, PhD
Harkamal Jhajj, BS
Adam Mauskapf, BS
Victory Qi, MD, PhD

MGH Radiology
Zubin Irani, MD

BWH Cardiology
Peter Libby, MD

BWH Nephrology

MGH Nephrology
Amin Arnaout, MD
Ravi Thadhani, MD

Mount Sinai Medical School
Willem Mulder, PhD

Funding
American Society of Nephrology Ben. J Lipps Research Fellowship Grant
American Society of Diagnostic and Interventional Nephrology Fellowship Grant