Abstract MDP8

Blockade of CD47 using a novel anti-CD47 molecule, BRB-002, attenuates atherosclerosis in an ApoE mouse model

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Disclosures

Presenter: Alex Yi is an employee of Bitterroot Bio.

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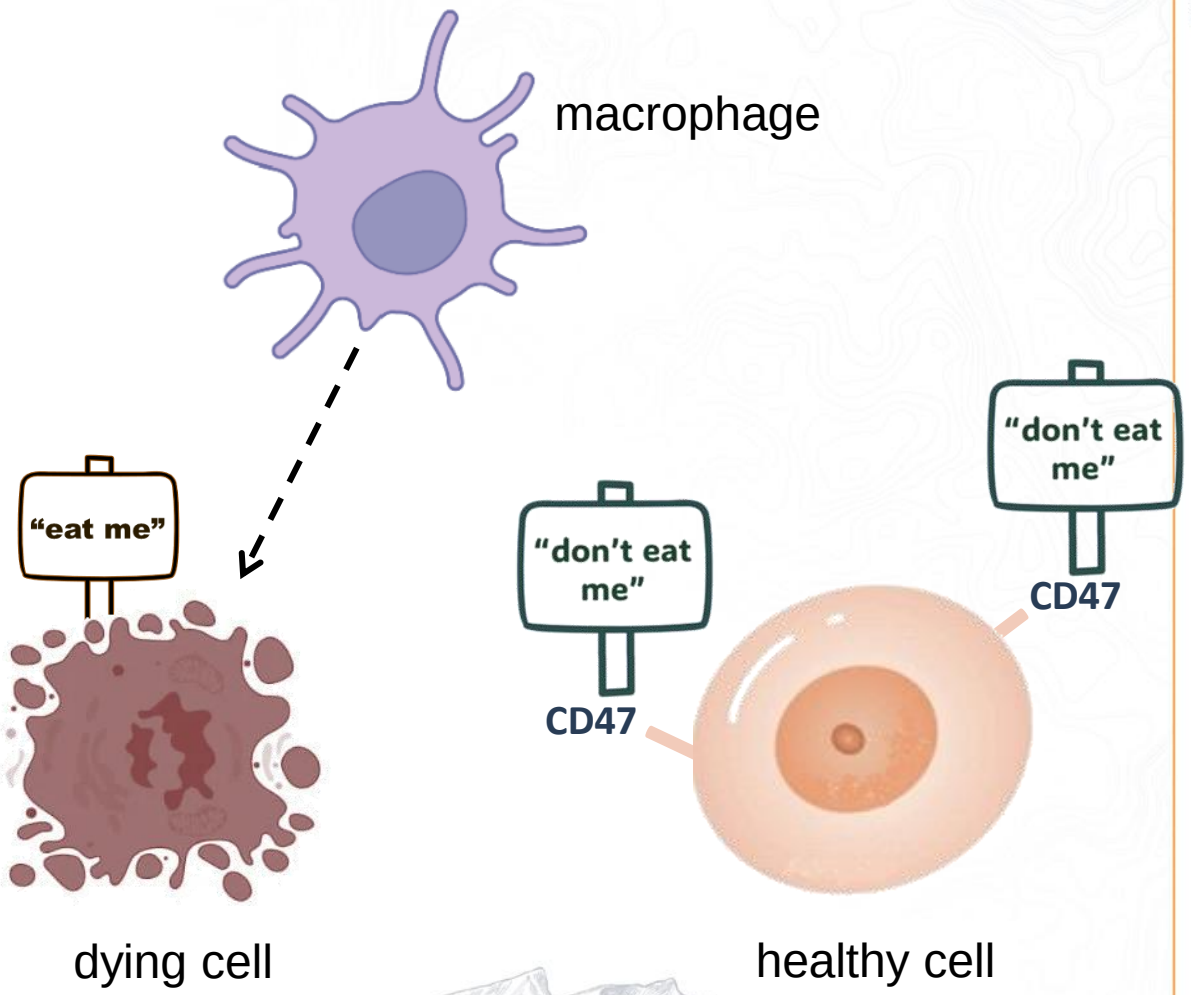
CD47 acts as a “don’t eat me” signal to prevent programmed cell removal by phagocytes

Background

- ~200 billion cells die and turnover every day in the human body as part of normal tissue homeostasis
  - Yet few apoptotic cells are found in healthy individuals suggesting that this debris is rapidly and efficiently cleared
- Programmed cell removal, or ***efferocytosis***, is often carried out by macrophages in a highly regulated fashion
- For instance, the predominant “don’t eat me” signal, **CD47**, is expressed by healthy cells

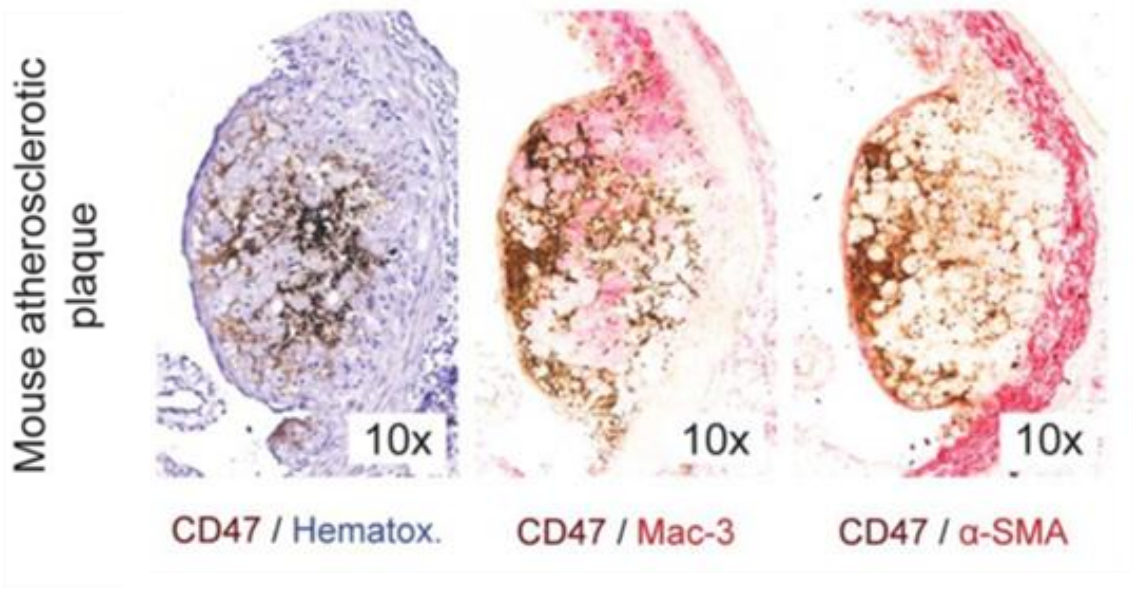


Morioka S et al. (2019) “Living on the Edge: Efferocytosis at the Interface of Homeostasis and Pathology” *Immunity*. 50:1149
Cabrera JTO and Makino A (2022) “Efferocytosis of vascular cells in cardiovascular disease” *Pharmacol Ther*. 229:07919.

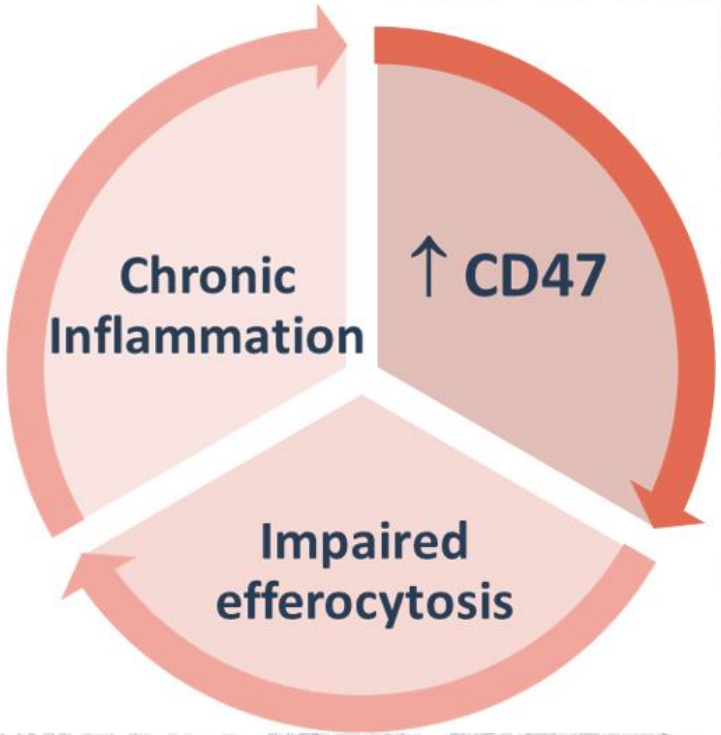
In atherosclerotic plaques, CD47 overexpression may impair efferocytosis and perpetuate inflammation

Hypothesis

CD47 is highly expressed in advanced atherosclerotic plaque



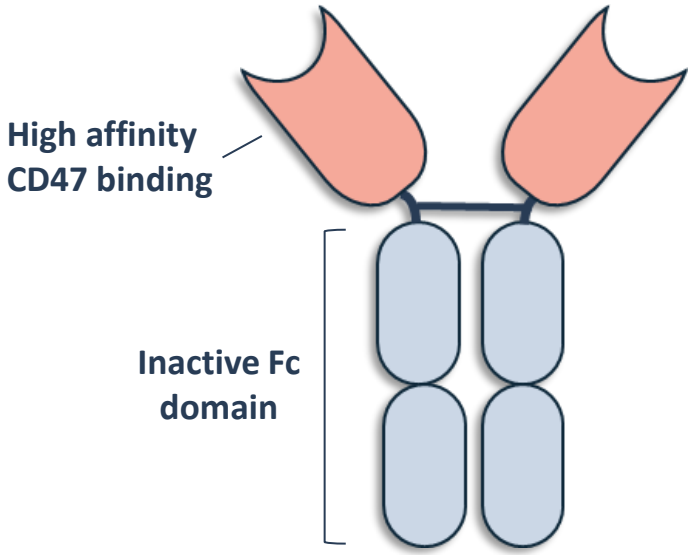
Hypothesis: Stimulate efferocytosis by antagonizing CD47 can demonstrate efficacy in a mouse model of atherosclerosis



Tabas I (2011) "Pulling down the plug on atherosclerosis" *Nat Med.* 17:791
Kojima Y et al (2016) "CD47-blocking antibodies restore phagocytosis and prevent atherosclerosis" *Nature*. 536:86.

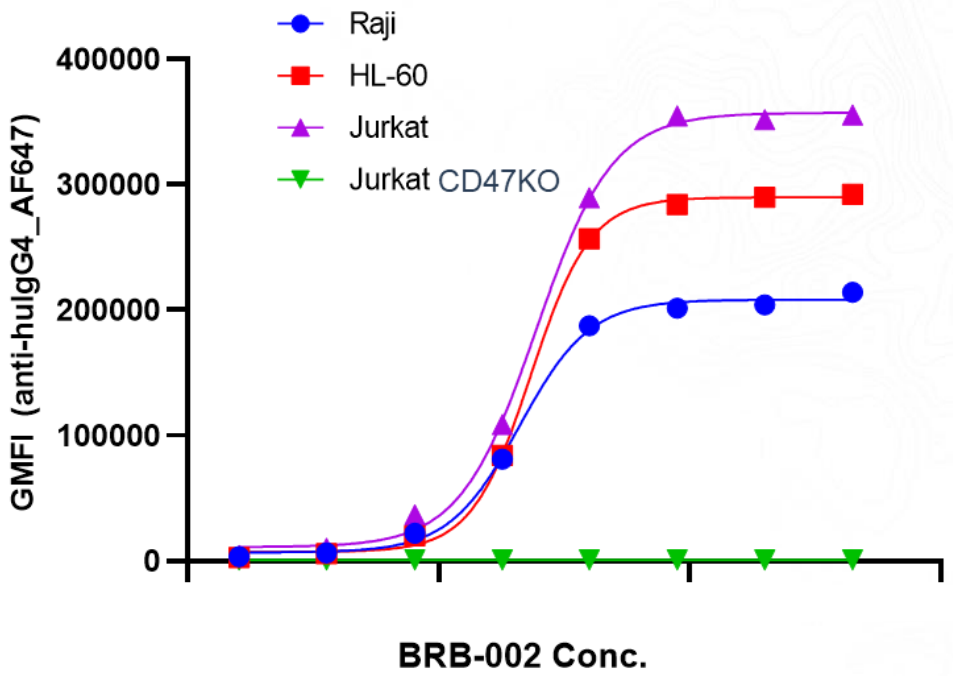
BRB-002 is an engineered recombinant protein designed to bind CD47 with high affinity

BRB-002



- Fc region engineered with mutations for enhanced neonatal receptor (FcRn) binding for improved molecule half-life
- Fc region designed to eliminate Fc-dependent effector functions

BRB-002 binds CD47 with high affinity



Can BRB-002 reduce plaque burden in a mouse model of established atherosclerosis?

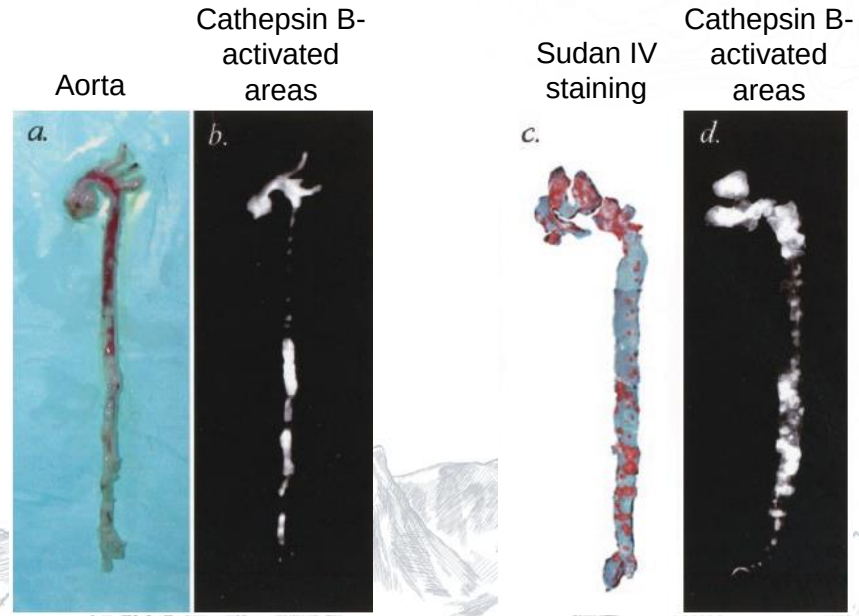
Methods and Study Design

1) Iso-type control
2) BRB-002 2.5 mg/kg i.p. 3x a week
3) BRB-002 10 mg/kg i.p. 3x a week

Readout: Assessment of plaque burden

- aortic roots (histomorphometry)
- descending aorta (cathepsin B activity based probe[1])

Fig 2. from Chen et al. (2002)



Atherosclerotic plaque burden co-localizes with cathepsin B activity in ApoE-/- mice fed a high fat Western diet [2]

i.p. intraperitoneal

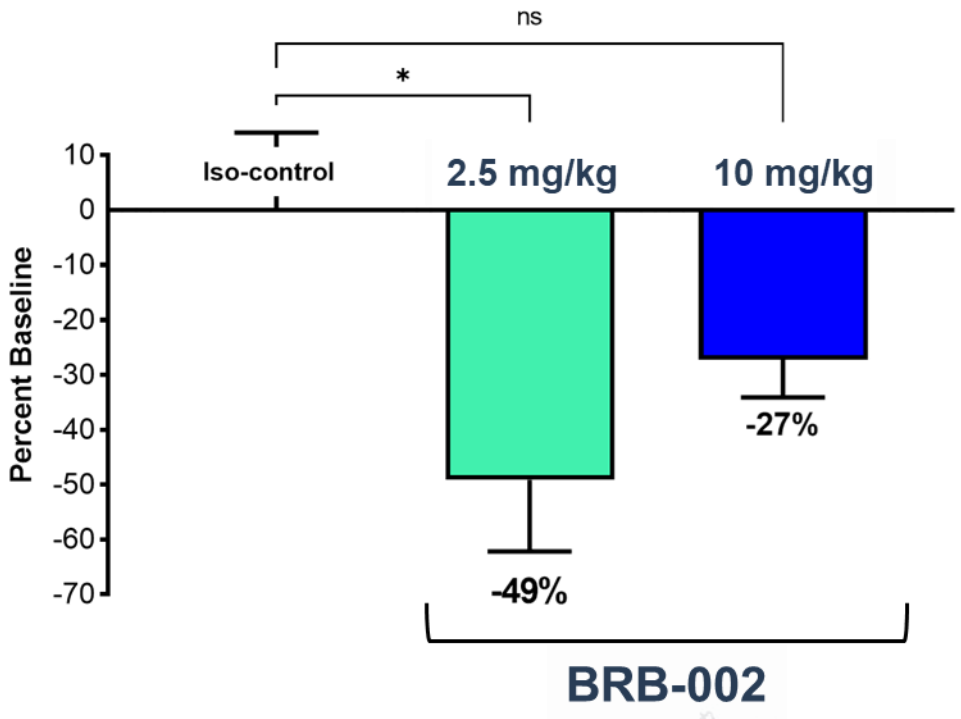
[1] IVISense Cat B Fast;

[2] Chen J et al (2002) "In vivo imaging of proteolytic activity in atherosclerosis" *Circulation* 105:2766

BRB-002 significantly reduced plaque burden in mouse model of established atherosclerosis

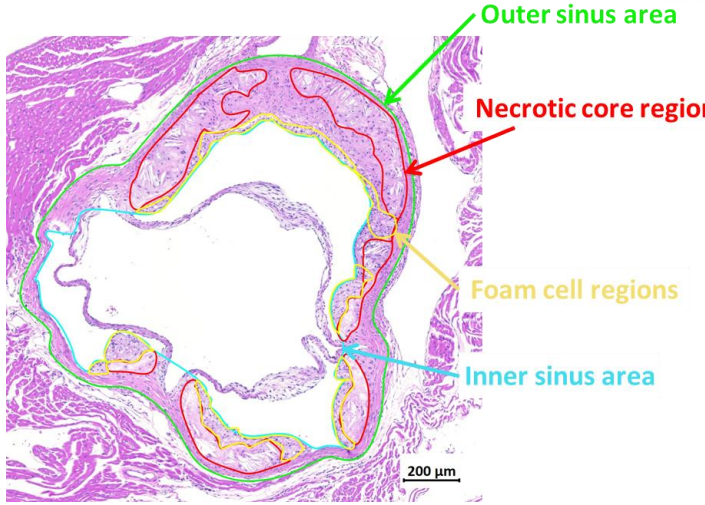
Results

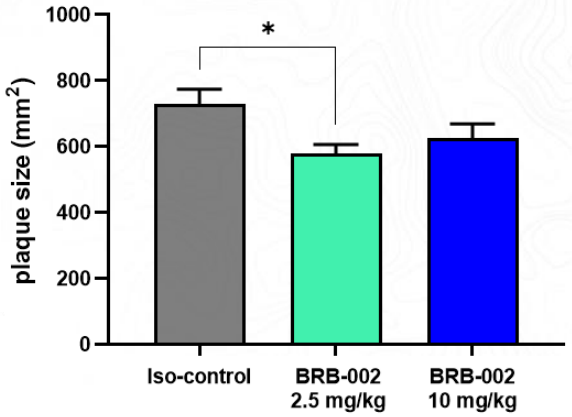
Significant reduction in plaque burden in the descending aorta

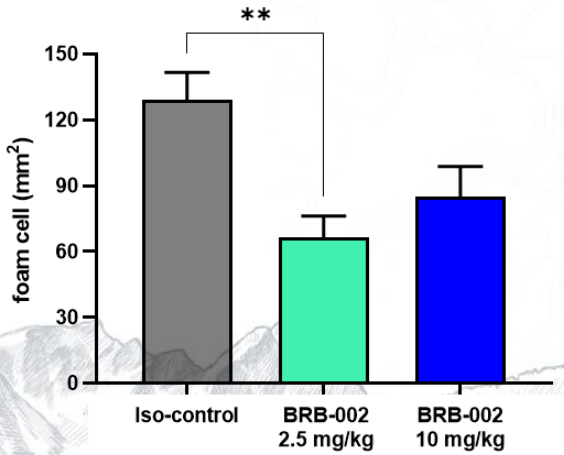


*, p=0.01
ns, not significant

Significant reduction in plaque size and regions that contain foam cells at the aortic root







*, p=0.02; **, p=0.003

BRB-002 demonstrated efficacy in a mouse model of atherosclerosis prevention

Experiment with subcutaneous administration

ApoE-/-/C57B6, male
4 weeks old
N=45

12 weeks

High fat diet

Treatment

1) Iso-type control s.c. 3x a week
2) BRB-002 1 mg/kg s.c. 3x a week
3) BRB-002 2.5 mg/kg s.c. 3x a week
4) BRB-002 10 mg/kg s.c. 3x a week
5) BRB-002 30 mg/kg s.c. 3x a week

Readout

– Plaque burden in descending aortas (cathepsin B activity-based probe)

s.c., subcutaneous

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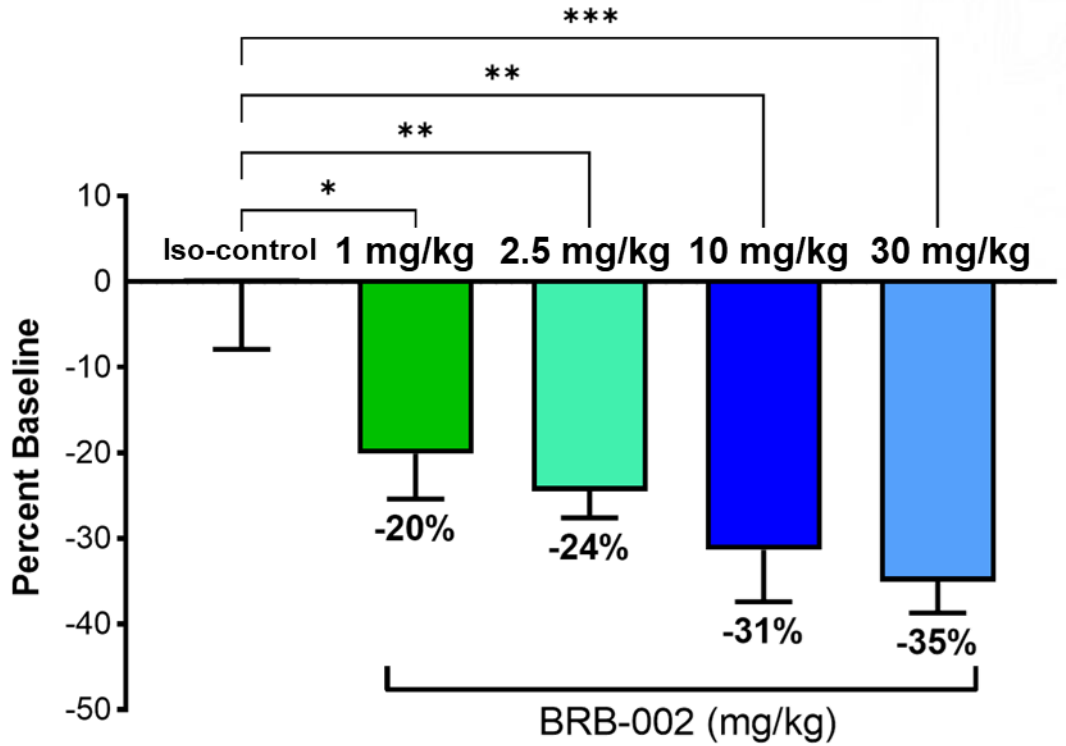
BRB-002 demonstrated efficacy in a mouse model of atherosclerosis prevention

Results



*, p<0.05; **, p<0.01; ***, p<0.001

Results

- Repeated s.c. administration of BRB-002 was well-tolerated in mice
- Significant reductions in plaque burden in the descending aortas at all BRB-002 doses tested

Conclusions

- Repeated administration of BRB-002 in these studies was associated with stable hematologic parameters
- Antagonizing CD47 with BRB-002 demonstrated efficacy in apoE-deficient mouse model of established atherosclerosis and prevented atherogenesis in apoE-deficient mice
- Targeting CD47 has the potential to target the chronic inflammation associated with atherosclerotic lesions

Acknowledgements

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