Hepatic plasminogen lowering with RNA interference for the treatment of bleeding disorders is unlikely to pose thrombotic risk based on UK Biobank analyses and mouse models of provoked thrombosis

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Disclosures

Presenter: Rodney Camire, PhD

Conflict	Disclosure
Alnylam Pharmaceuticals	Research Funding

ALN-6400:

ALN-6400 is an investigational drug being studied for the treatment of bleeding disorders. ALN-6400 is not approved by any health authority, and the safety and efficacy of ALN-6400 have not been established.

Funding:

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Large Unmet Need in the Treatment of Bleeding Disorders

A substantial number of patients are affected by bleeding disorders, with global recognition and prevalence on the rise[1]

Bleeding disorders have a major impact on quality of life and lead to life-threatening complications for patients[2-5]

Current treatment options are inadequate[6]

- Antifibrinolytics (e.g. TXA) are well established as reasonably effective for bleeding disorders but are burdensome
- Other therapies have various challenges, e.g. some are administered intravenously and some are associated with an increased risk of thrombosis

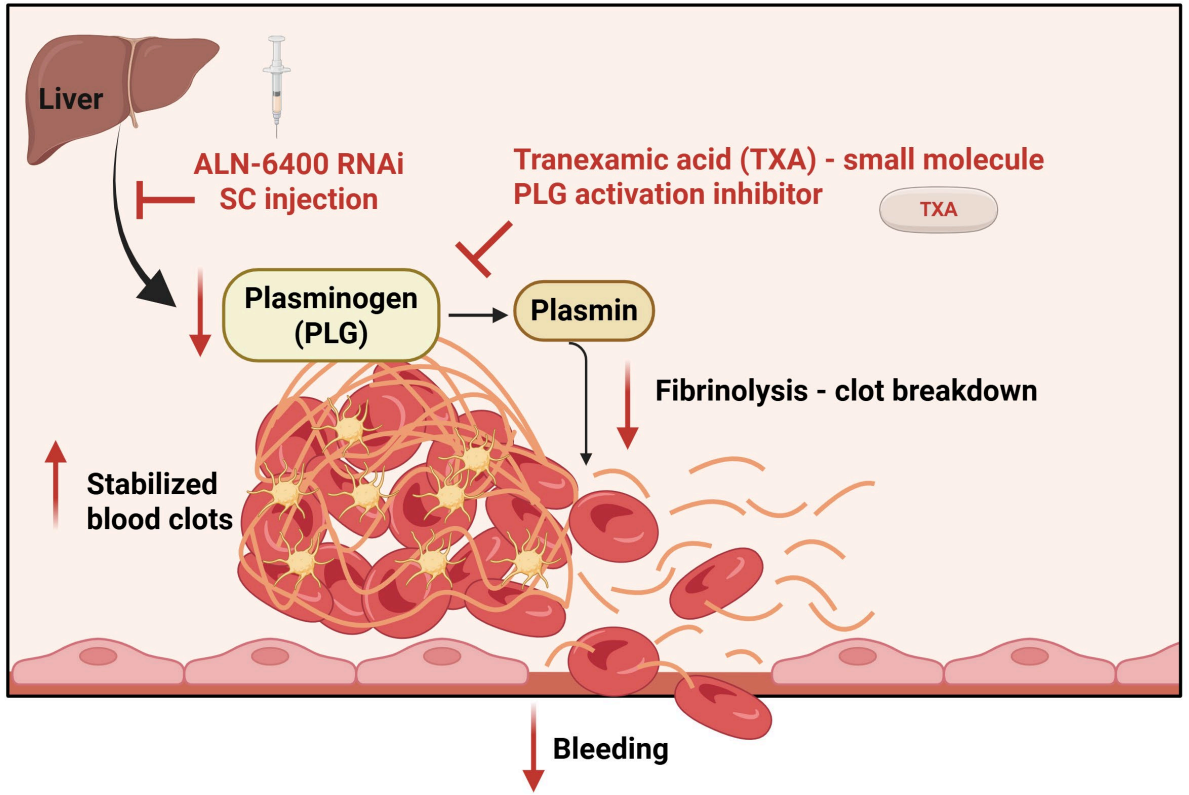
TXA, tranexamic acid.

1. World Federation of Hemophilia (WFH), *Annual Global Survey* 2023. Available at: https://www1.wfh.org/publications/files/pdf-2525.pdf. 2. Gong AJ, et al. *Orphanet J Rare Dis*. 2025;20(1):109. 3. Castaman G, et al. *Haemophilia*. 2023;29(2):411-422. 4. van Hoorn ES, et al. *Haemophilia*. 2022;28(2):197-214. 5. Holm E, et al. *Haemophilia*. 2018;24(4):628-633. 6. Kim DJ, Cho SY, Jung KT. *Korean J. Anesthesiol*. 2024;77(4):411-422.

Plasminogen is a Key Player in Fibrinolysis and Hemostasis

- Plasminogen (PLG) is produced primarily in the liver[1]
- Lowering hepatic PLG with RNA interference has potential to be a longer lasting, more effective, and safer approach than current antifibrinolytics, and may act as a universal hemostatic agent for bleeding disorders
- The balance between clot formation and degradation, however, is essential to maintain hemostasis

Therapeutic Hypothesis



Aim

To assess whether thrombotic risk, a potential safety concern with antifibrinolytics, is associated with lower PLG levels, either in humans (via genetics and proteomics) or in mouse injury models

PLG, plasminogen; RNAi, RNA interference; SC, subcutaneous; TXA, tranexamic acid.

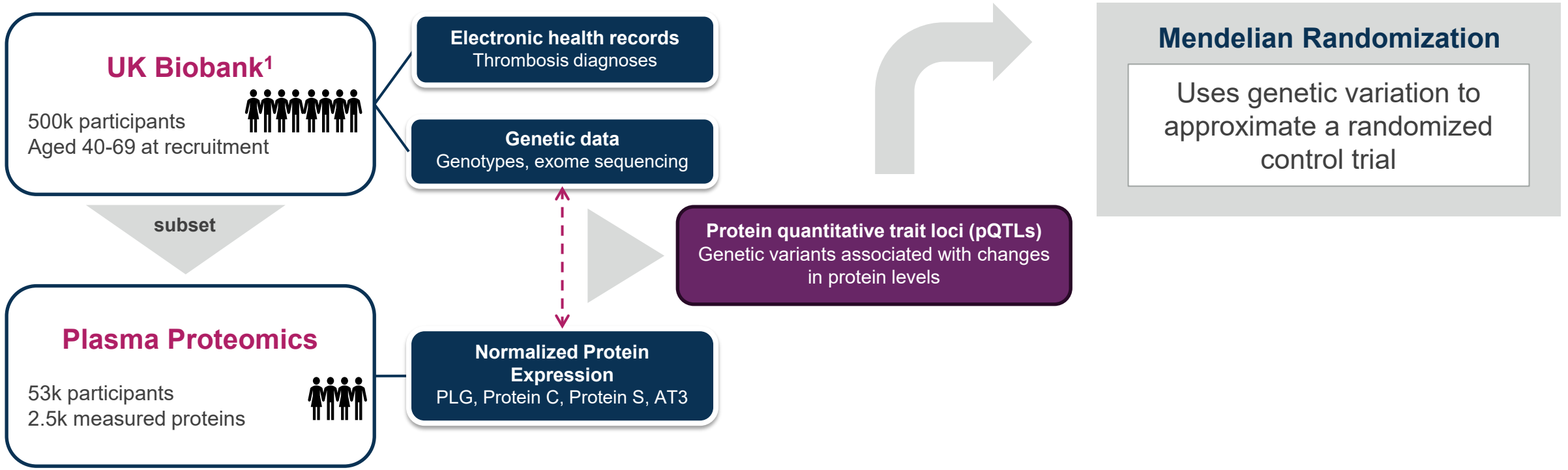
1. Cesarman-Maus G, Hajjar KA. *Br J Haematol*. 2005;129(3):307-321.

UK Biobank Analyses

Methodology for Computational Analyses in the UK Biobank

Using human genetics and proteomics data via the UK Biobank, we assessed whether low plasma PLG protein levels were associated with increased risk for thrombosis

- Linear regression used to test whether lower PLG levels were **correlated** with thrombosis diagnoses
- Mendelian Randomization (MR) applied to assess whether lower PLG **causally** increases thrombotic risk
- Analyses repeated with known thrombotic factors Protein C, Protein S, and Antithrombin (AT3) as comparators

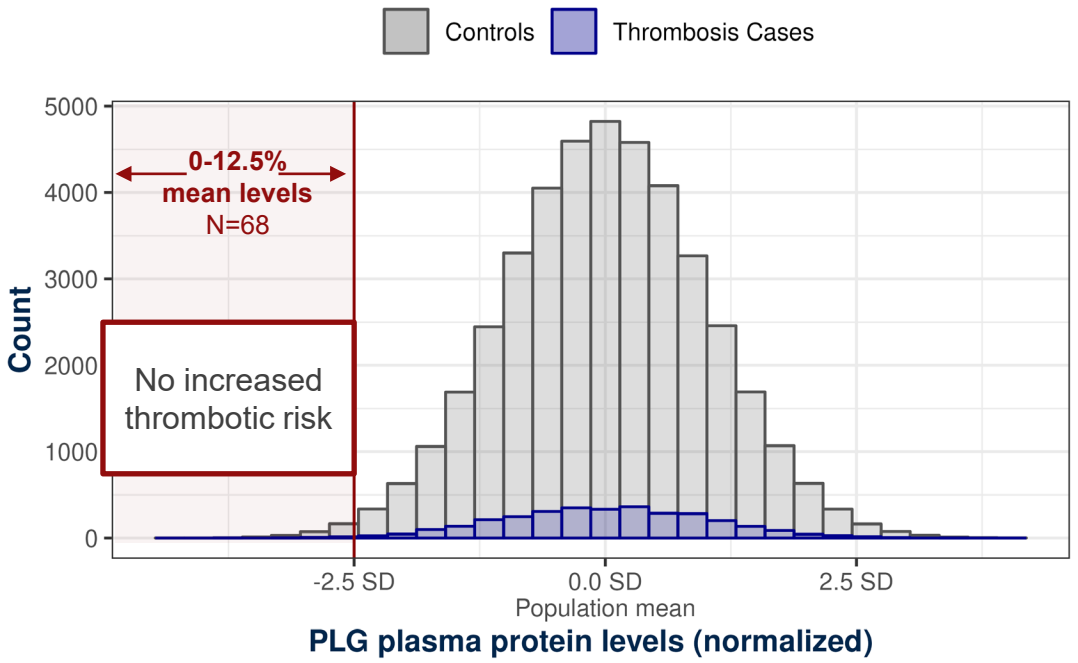


PLG, plasminogen.

1. Allen, N. *et al.* UK Biobank: Current status and what it means for epidemiology. *Health Policy and Technology* 2012;**1**:123-126.

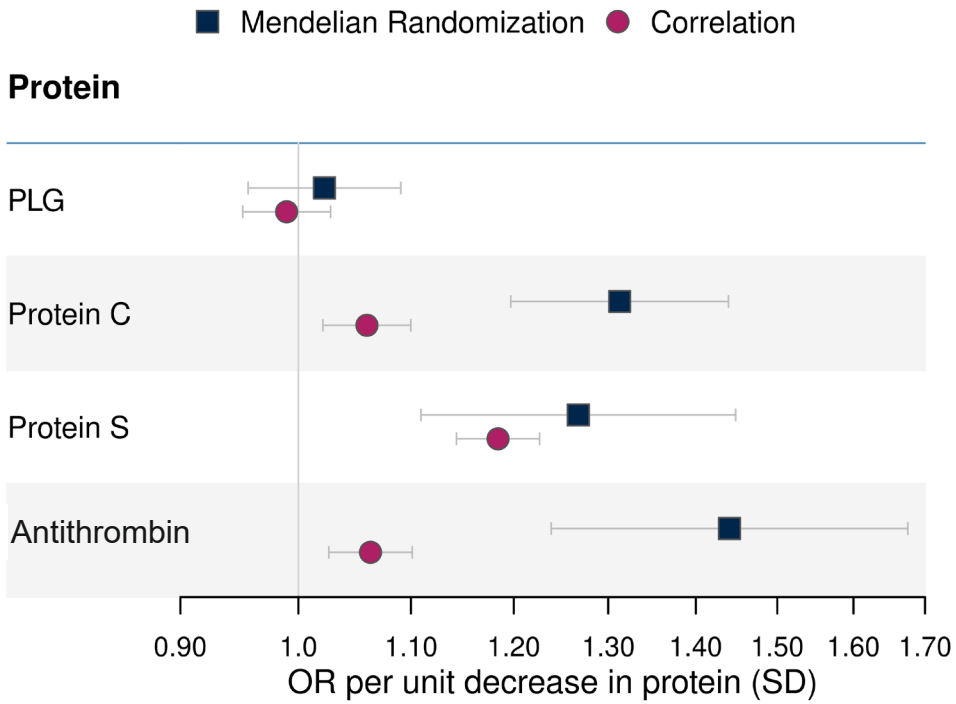
Low Levels of PLG are Not Associated with Thrombosis

PLG plasma protein levels are not correlated with thrombosis



- Thrombosis incidence is not higher among subset of participants with very low plasma PLG levels (PLG reduced at least 87.5% from the mean, p>0.05)

MR results support no causal effect of lower PLG on thrombotic risk (p>0.05)



- Lower levels of known thrombotic factors are associated with thrombosis in both correlative and causal estimates (p<0.006)[a]

MR, Mendelian Randomization; PLG, plasminogen.

[a]Bonferroni corrected p-value threshold accounting for 4 proteins, 2 tests each (0.05/8)

RNAi Harnesses an Endogenous Process to Lower Target Protein Production

Synthetic double-stranded small interfering RNA (siRNA) is designed to target specific mRNA[1–3]

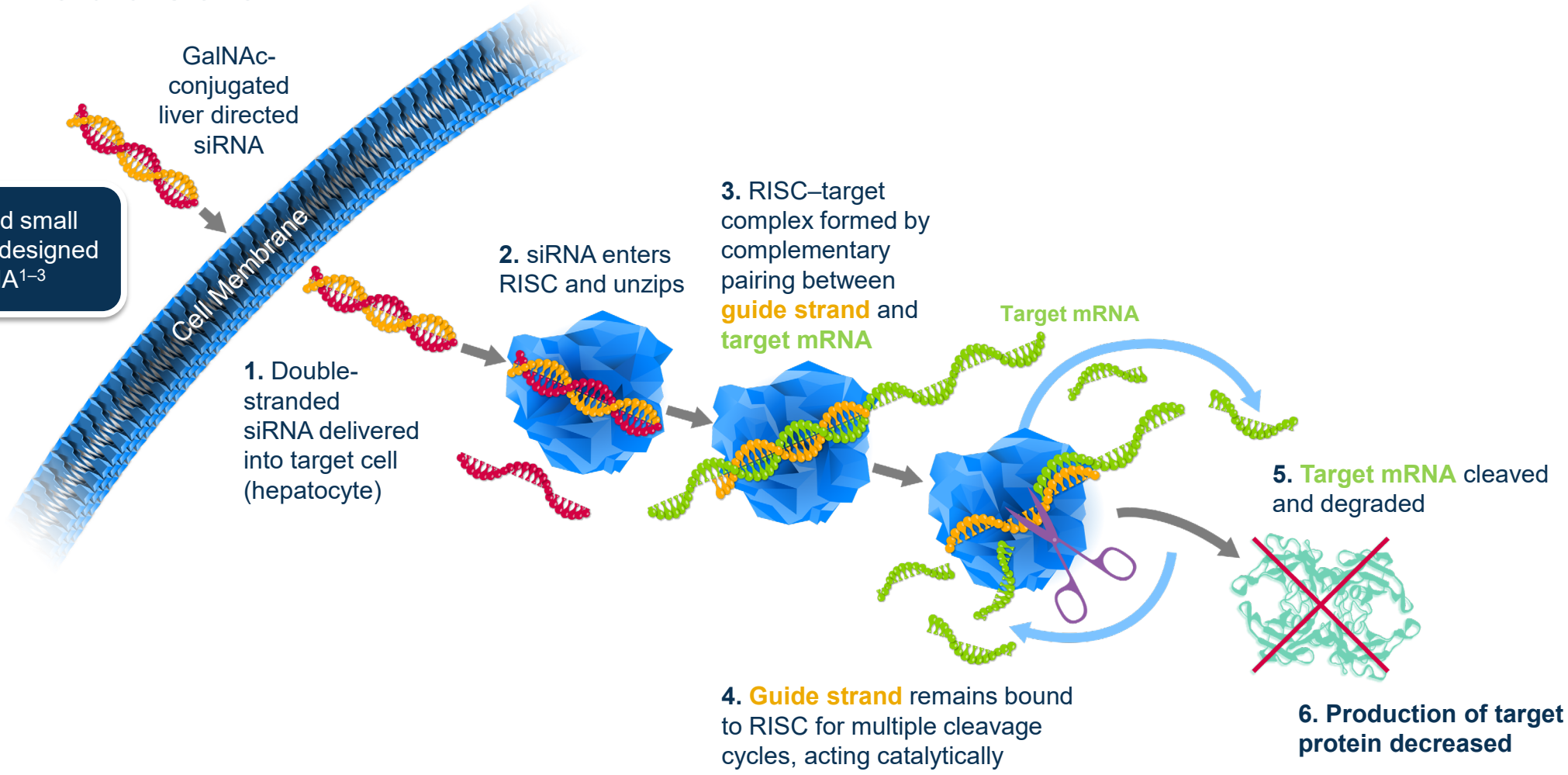


Image credit: Alnylam Pharmaceuticals. Figure adapted from information in Friedrich & Aigner, and Niemietz et al., plus data published in Coelho et al.[1,5,6]

GalNAc, N-acetylgalactosamine; mRNA, messenger RNA; RISC, RNA-induced silencing complex; RNAi, RNA interference; siRNA, small interfering RNA.

1. Niemietz C et al. Molecules 2015;20(10):17944–75. 2. An G. J Clin Pharmacol 2024;64(1):45–57. 3. Aagaard L, Rossi JJ. Adv Drug Deliv Rev 2007;59(2-3):75–86. 4. Hutvágner G, Zamore PD. Science 2002;297:205–60. 5. Friedrich M, Aigner A. BioDrugs 2022;36:549–71. 6. Coelho et al. N Engl J Med 2013;369:819–29.

Mouse Injury Model Analyses

Evaluation of Thrombus Formation with Hepatic PLG Lowering

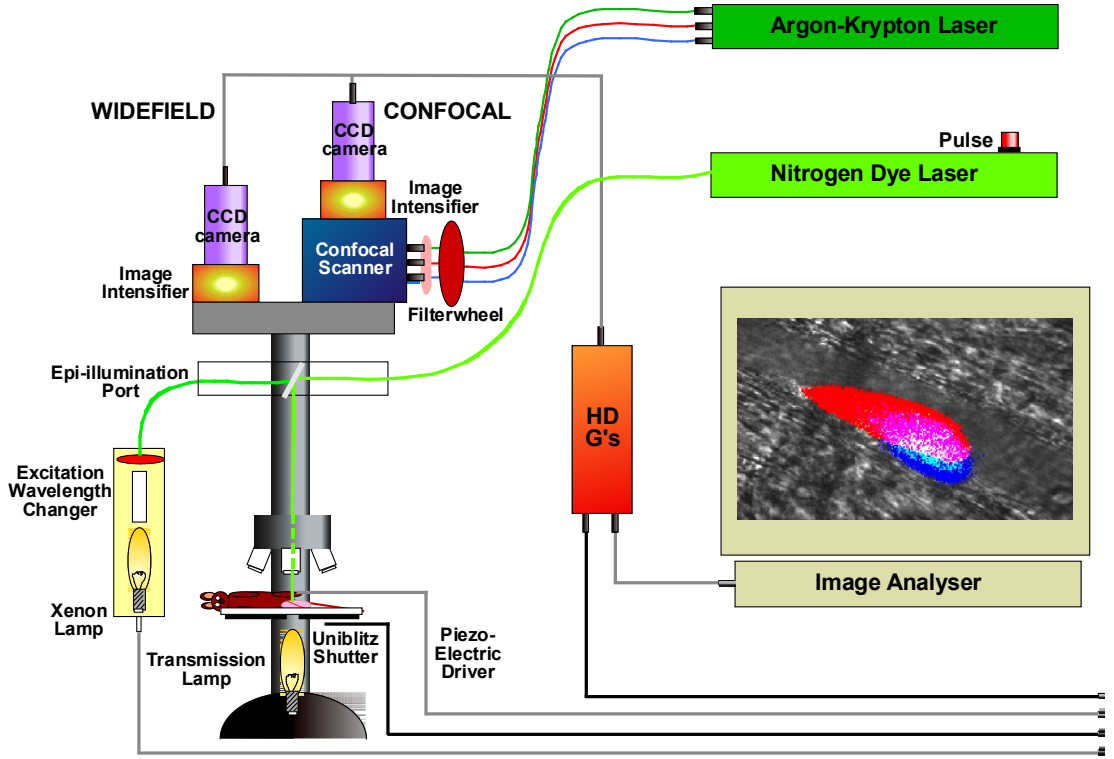
Methodology Using the Cremaster Arteriole Laser-Induced Injury Mouse Model

1. Wild-type mice received a single subcutaneous injection of one of:
    - PLG siRNA
    - Antithrombin (AT3) siRNA (positive control)
    - Vehicle (PBS)

2. 14 days post-dose:
    a) Antibodies injected to quantify thrombus formation kinetics: $Alexa_{555}$-labeled platelet marker and $Alexa_{647}$-labeled fibrin marker
    b) Laser injury applied to cremaster artery (heat injury to vessel wall)

3. Accumulation of labeled platelets and fibrin (thrombus formation) monitored over time

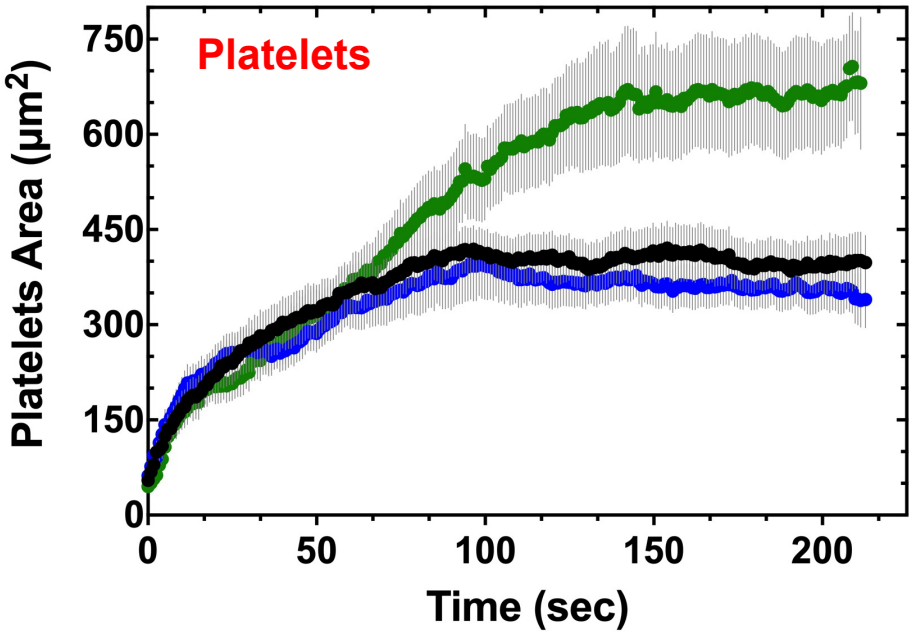
4. Liver knockdown (of AT3 or PLG) mRNA confirmed by RT-qPCR



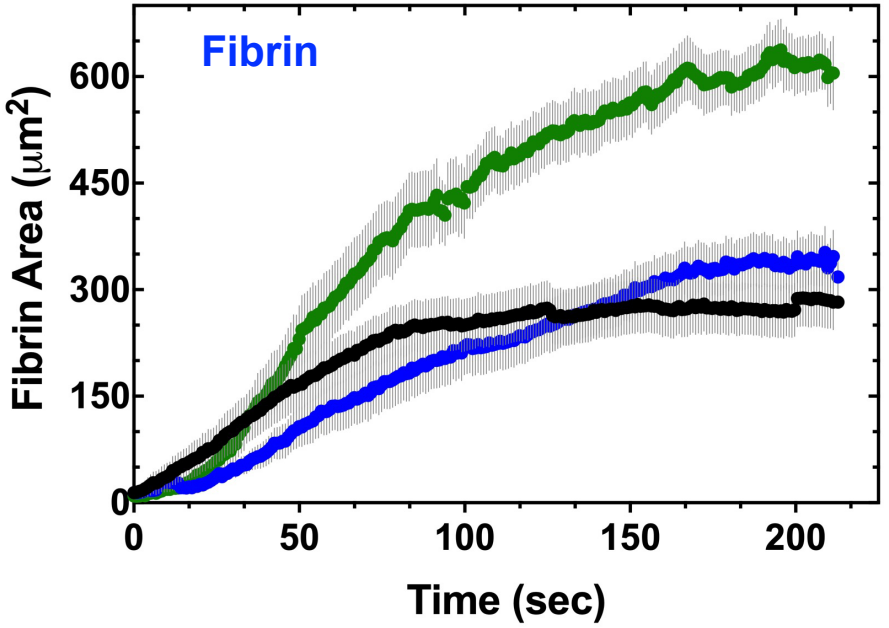
PBS, phosphate buffered saline; PLG, plasminogen. AT3, antithrombin; KD, knockdown; mRNA, messenger RNA; RT qPCR, quantitative reverse transcription PCR; siRNA, small interfering RNA.

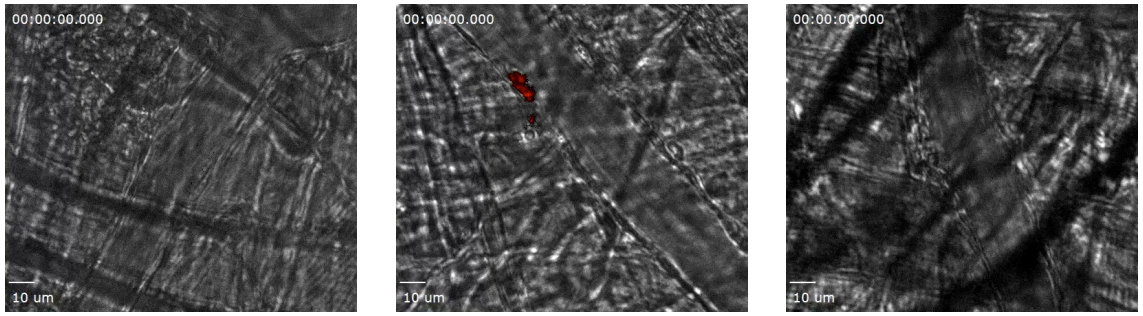
No Increase in Thrombus Formation with ~99% PLG Lowering

Comparable Platelet/Fibrin Accumulation between PLG siRNA and Vehicle Treated Mice



AT3 siRNA (~96% KD of AT3)
PLG siRNA (~99% KD of PLG)
Vehicle (PBS)





AT3 siRNA | PLG siRNA | PBS treated

Platelets/Fibrin

AT3, antithrombin; KD, knockdown; mRNA, messenger RNA; PBS, phosphate buffered saline; PLG, plasminogen; RT qPCR, quantitative reverse transcription PCR; siRNA, small interfering RNA.
Liver mRNA KD was confirmed by RT qPCR. Data are presented as mean ± SEM from n = 4-5 wild-type mice; 20-25 injuries per group.

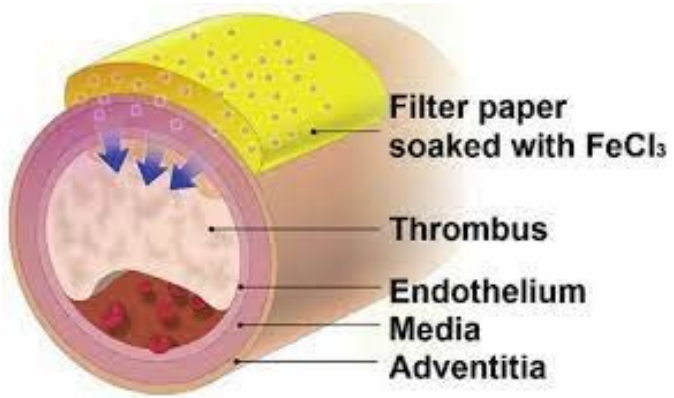
Evaluation of Vessel Occlusion with Hepatic PLG Lowering

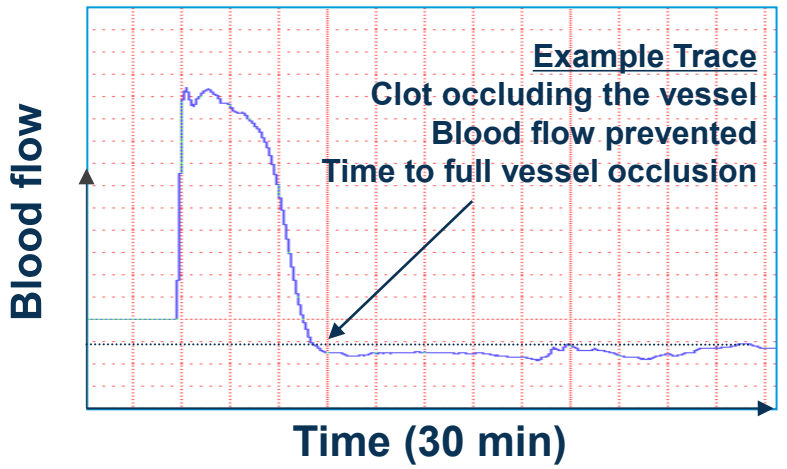
Methodology Using the $FeCl_3$-Induced Carotid Arterial Thrombosis Mouse Model

1. Wild-type mice received a single subcutaneous injection of one of:
    - PLG siRNA
    - Negative control (non-targeting) siRNA

2. 14 days post-dose:
    a) Carotid artery in anesthetized mice exposed
    b) Doppler probe placed
    c) Baseline blood flow recorded for 3 min
    d) Filter paper soaked with 5% $FeCl_3$ placed on top of carotid artery for 2 min, then washed away
    e) Blood flow monitored for 30 min and vessel occlusion time recorded

3. Liver knockdown of PLG mRNA confirmed by RT-qPCR

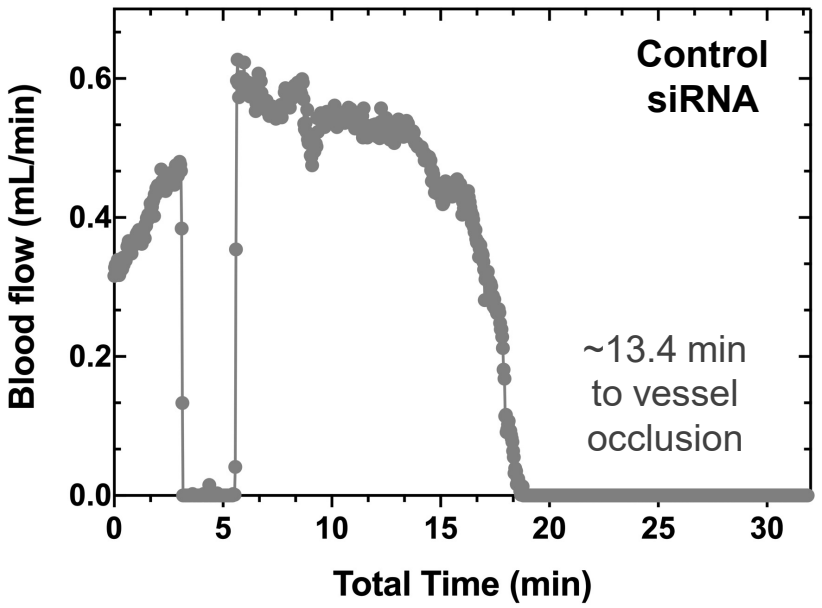


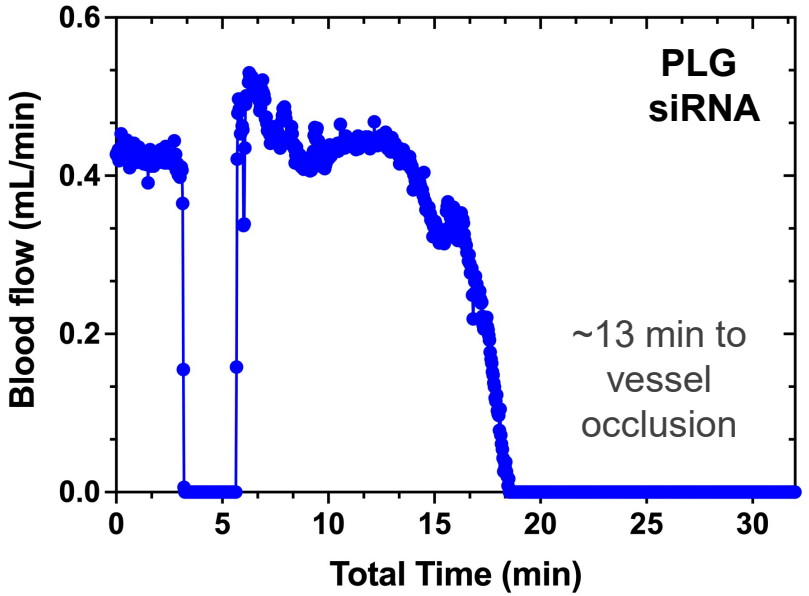


$FeCl_3$, ferric chloride; PLG, plasminogen; siRNA, small interfering RNA. KD, knockdown; mRNA, messenger RNA; RT qPCR, quantitative reverse transcription PCR

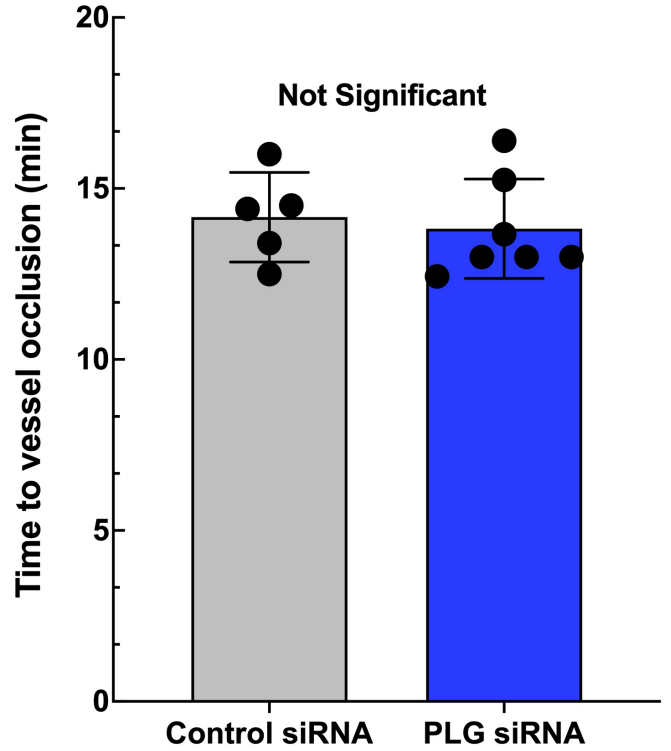
No Difference in Speed of Thrombus Formation with ~99% PLG Lowering

Comparable Time to Vessel Occlusion between PLG siRNA and Control siRNA Treated Mice





Non-Significant Difference in Time to Occlusion Between Groups

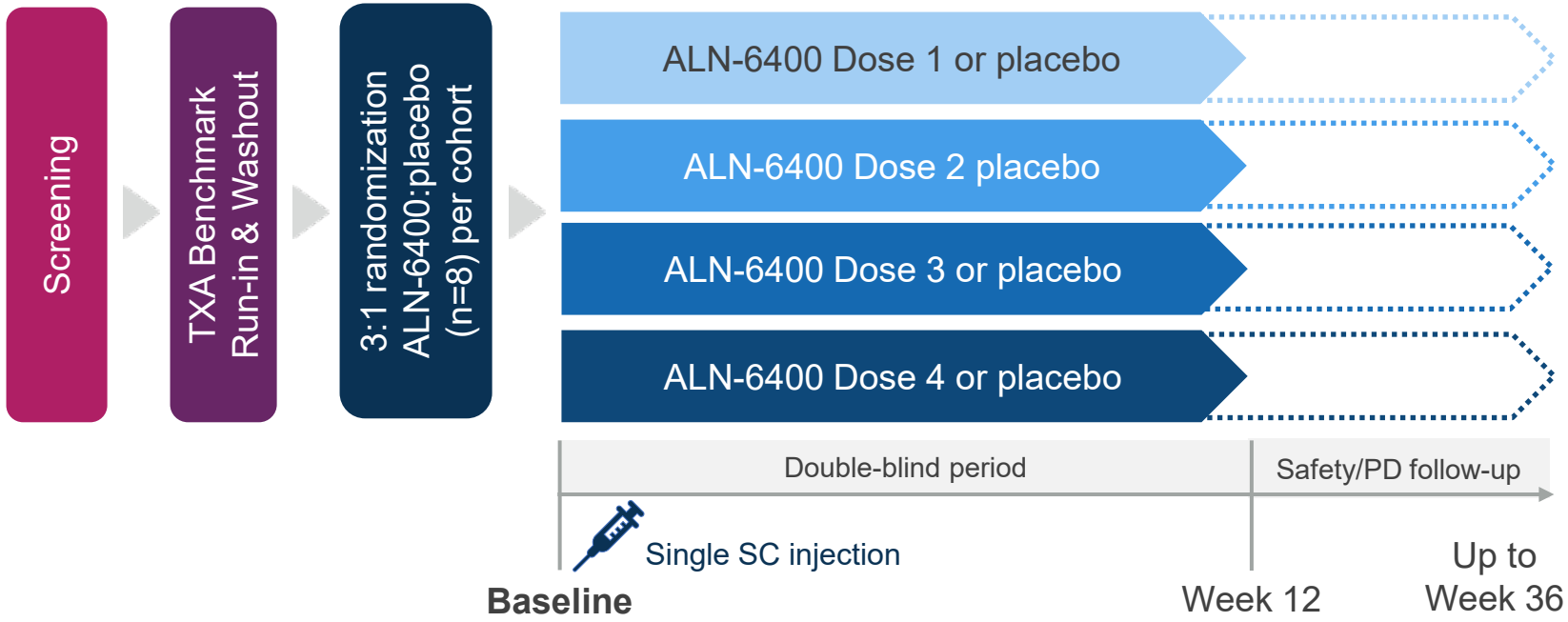


Representative traces.

KD, knockdown; mRNA, messenger RNA; PLG, plasminogen; siRNA, small interfering RNA.

Results Support Clinical Development of ALN-6400, an Investigational RNAi Therapeutic for Bleeding Disorders

A Phase 1, Double-Blind, Placebo-Controlled, Single Ascending Dose Study of ALN-6400 is Ongoing in Healthy Adult Volunteers



Primary Objective
- Safety and tolerability

Secondary Objectives
- Characterize PK and PD

Includes standard-of-care oral TXA dosing prior to administration of ALN-6400/placebo to allow comparison of intra-individual antifibrinolytic response with ALN-6400 relative to TXA by tPA-ROTEM

NCT06659640. PD, pharmacodynamics; PK, pharmacokinetics; RNAi, RNA interference; SC, subcutaneous; tPA-ROTEM, tissue plasminogen activator-rotational thromboelastography; TXA, tranexamic acid.

Summary

- In this analysis of UK Biobank data, low levels of plasma PLG protein were not associated with thrombotic risk
- Additionally, in two different mouse injury models, thrombus formation was similar in controls compared to those with ~99% lowering of hepatic PLG using a liver-directed siRNA
- These results suggest that hepatic targeting of PLG with RNAi is unlikely to confer thrombotic risk, which will be further evaluated in clinical trials
- This early evidence supports clinical development of ALN-6400, an investigational RNAi therapeutic designed to target hepatic PLG for the treatment of bleeding disorders (NCT06659640)

PLG, plasminogen; RNAi, RNA interference; siRNA, small interfering RNA

Back Up

No Increase in Thrombus Formation with ~99% PLG Lowering

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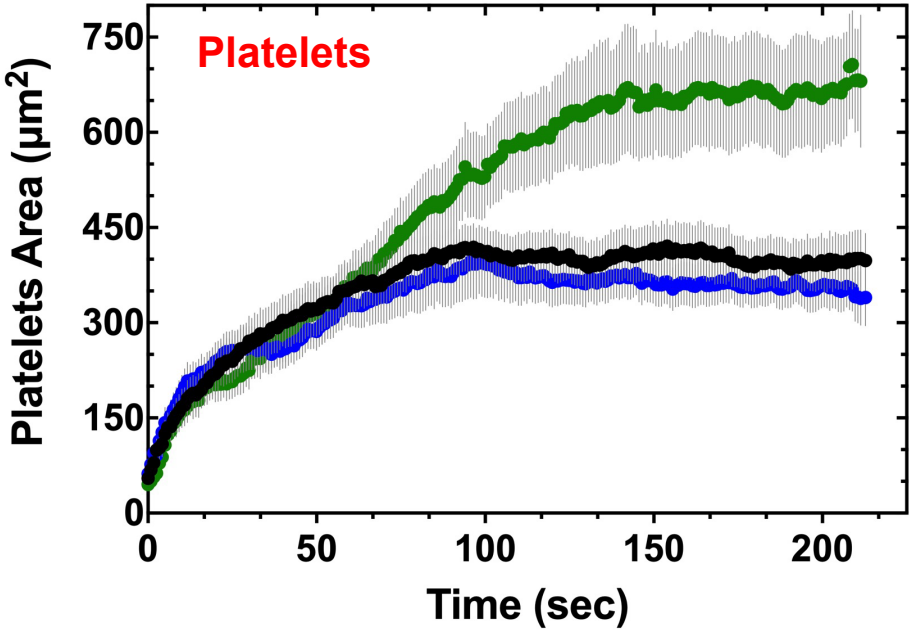

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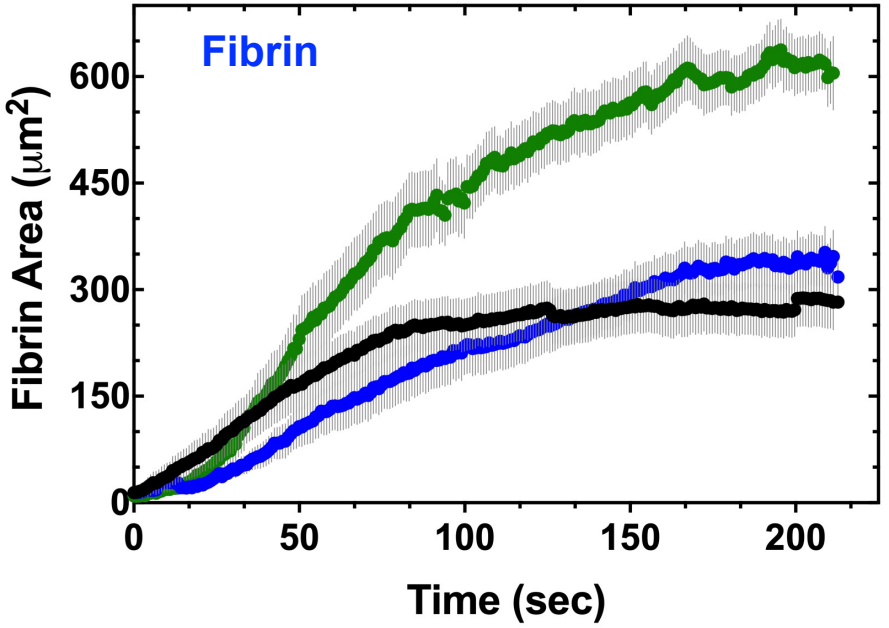

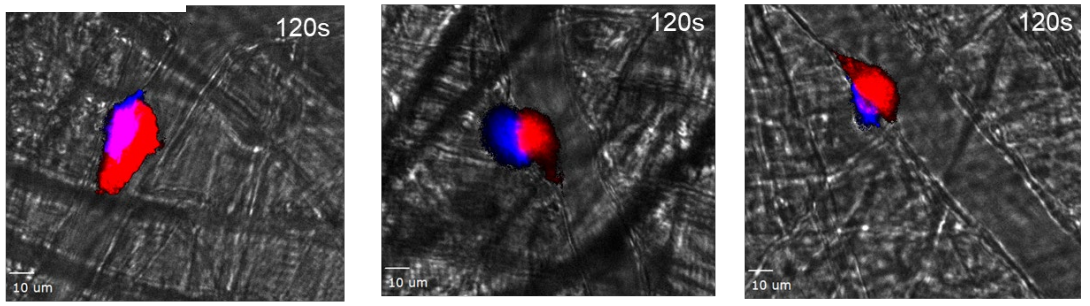

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Platelets/Fibrin

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