Novel Rapid Excreting Immunothrombosis Inhibitors

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Introduction

Immunothrombosis

- A process that describes the prevalence of excess blood clots due to the intricate network between haemostasis and immune system,[1]
- Venous thromboembolism represents the prototypical response, affecting 100, 000 Canadians each year with a mortality rate of 10 %.[2]
- Current antithrombotic therapeutics target both haemostasis and coagulation, resulting in a high haemorrhagic risk.[3]

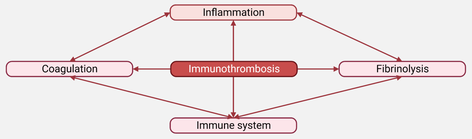


Figure 1. Schematic representation of immunothrombosis as a result of complex dysregulation between haemostasis and immune system.

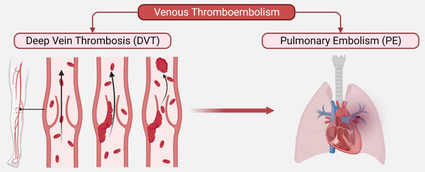


Figure 2. Venous thromboembolism manifests in two forms: DVT where clots formed in lower extremities; PE where blood supply to lungs are cut off by blood clots.

Procoagulants

- Natural biopolymers present in blood circulation capable of augmenting coagulation.[4]
- Elevated levels of cell-free DNAs linked to numerous diseases, e.g., cancer, VTE, sepsis, autoimmune diseases, etc.[5]

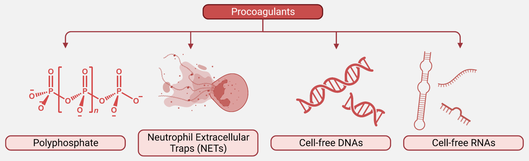


Figure 3. Examples of pathophysiologic activators of coagulation.

Nanotoxicity

- Toxicity results from prolonged exposure of nanoparticles due to accumulation in organs.[6]
- Major obstacle for clinical translations.

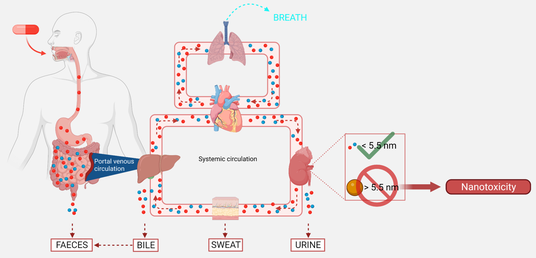


Figure 4. Nanotoxicity due to accumulation of drugs or their metabolites having sizes above renal clearance limit.

Objectives

i. Design and development of selective nucleic acid inhibitors (PNBI) which inhibit cell-free nucleic acids (cf-NAs) to protect against disseminated intravascular coagulation in immunothrombosis.

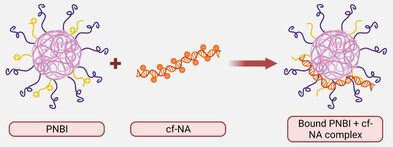


Figure 5. Schematic representation of cf-NA inhibition by PNBI.

ii. Design, development and evaluation of PNBIs with molecular architecture supporting the active and rapid hepatobiliary excretion of PNBI-cf-NAs complexes (R-PNBIs).

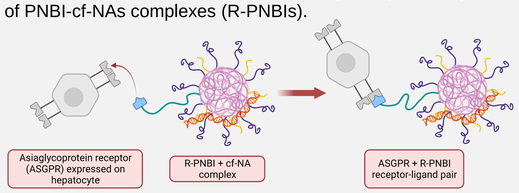


Figure 6. Proposed ASGPR-mediated targeting for active hepatobiliary clearance.

Synthetic Strategies

- HPG-mPEG as polymer scaffold will be functionalised with:
  - i. cation binding groups, R showing excellent cf-NA neutralisation activity identified from high-throughput screening, and
  - ii. ASGPR-targeting ligand via a poly(ethylene glycol) (PEG) linker.

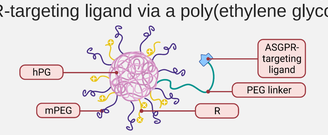
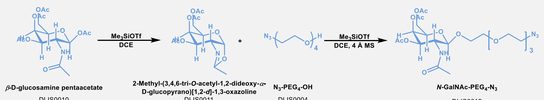
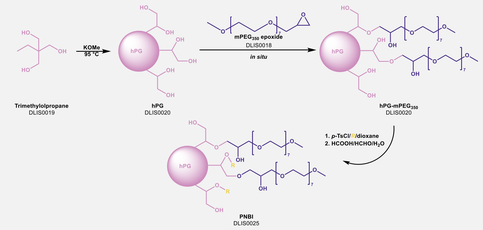


Figure 7. Illustration of proposed structure of R-PNBI.

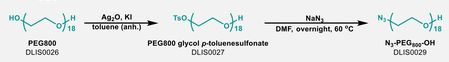
- The synthesis is divided into three parts:
  - i. ASGPR-targeting ligand – N-GalNAc-PEG$_4$-N$_3$.



  - ii. PNBI core – hPG-mPEG$_{350}$ (M_r 23 kDa) functionalised with R.



  - iii. PEG$_{800}$ linker to conjugate PNBI to ASGPR-targeting ligand.



Results

ASGPR-targeting ligand

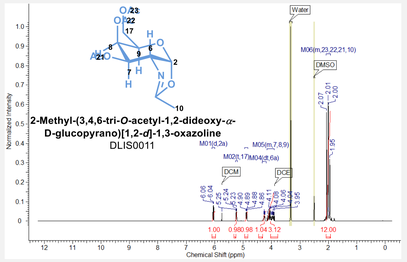


Figure 8. ^{1}H NMR data of DLIS0011 in DMSO.

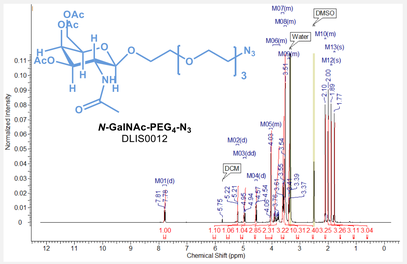


Figure 9. ^{1}H NMR data of DLIS0012 in DMSO.

PNBI

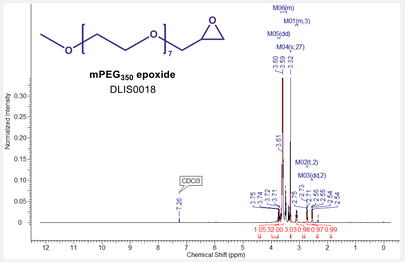


Figure 10. ^{1}H NMR data of DLIS0018 in $CDCl_3$.

PEG$_{800}$ linker

Exp. No.	Condition	Et_3N, DCM	Catalysis (eq.) Ag_2O	Catalysis (eq.) KI	Temperature (°C)	Duration	Precipitation[a]	1st LLE[b]	2nd LLE[c]	Column	Yield (%)
S0029 A1-1		✓	×	×	rt	2 d	×	×	×	✓	< 6
S0029 A1-2		×	1.50	0.20	rt	overnight	✓	✓	×	×	< 15
S0029 A1-3		×	1.50	0.20	40	overnight	✓	✓	×	×	< 11
S0029 A1-4		×	1.50 × 3[d]	0.20 × 3[d]	0 – rt	5 d	✓	✓	✓	×	< 38
S0029 A1-5		×	7.50	1.00	rt	2 d	×	✓	×	×	< 58

Table 1. Optimisation attempts at more selective mono-tosylation of PEG$_{800}$.

a. To remove unreacted *p*-TsCl.
b. LLE = Liquid-liquid extraction. To remove unreacted PEG$_{800}$.
c. To remove bi-tosylated PEG$_{800}$.
d. Two more equivalents of catalysts were added, each equivalent on a separate day.

Conclusions and Future Directions

- A panel of the R-PNBIs will be synthesised from the structural optimisation on the type and density of R to optimise the Cf-NAs inhibition activity.
- Isothermal titration calorimetry (ITC) based screening will be employed to identify the lead R-PNBI candidates with high binding affinity to cf-NAs.
- *In vitro* studies, e.g., thrombin generation (TG) assay and thromboelastography will be performed to evaluate their broad-spectrum inhibition activity. Intracellular distribution of R-PNBIs and their cf-NAs complexes will be studied to investigate their uptake and effects on cellular responses.
- *In vivo* studies, e.g., polymicrobial sepsis and cecal ligation and puncture (CLP) models will be performed to study their effects at different doses. Their biodistribution and excretion profiles will be studied to investigate their vascular residency, organ accumulation, and clearance specificity.
- This project presents a novel strategy that targets and neutralises cf-NAs, which could be the key in developing antithrombotic therapeutics for the treatment and/ or prevention of immunothrombosis, while maintaining haemostasis, resulting in safer and more effective therapies.

References

[1] Palankar, R., et al., *Blood* (2019).
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[4] Gould, T. J., et al., *Journal of Thrombosis and Haemostasis* (2015).
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Acknowledgements

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