SSC Subcommittee Project Template

<u>Subcommittee Names:</u> Models of Thrombosis & Hemostasis and Platelet Physiology

<u>Project Name:</u> Evaluating procedural of mouse models of platelet depletion and transfusion for the *in vivo* assessment of platelet function.

Project Lead/PI: Beatrice HECHLER (Models of Thrombosis and Hemostasis SSC)

Co-investigator - Laura Gutiérrez

Template Form Date: 11/02/2022

Project Description/Abstract:

State the project's broad, long-term objectives and specific aims, making reference to the proposed health/scientific impact as a result of the project. Suggested length is 2-3 paragraphs.

<u>Long-term objectives</u>:

To provide unifying guidelines for the use and interpretation of mouse models of platelet depletion and transfusion (or the combination of both) for the *in vivo* assessment of platelet function.

<u>Specific aims</u>:

- To define the optimal methods for *in vivo* platelet depletion in mice, or alternatively, to highlight the characteristics and suitability of each model for specific biological questions (pros and cons).
- To define the optimal methodology for platelet transfusion into mice, and to consider different scenarios to answer different biological questions, as well as an accurate description of capacities as a model, pros and cons.
- To establish experimental guides when these mouse models should be combined, pros and cons.
- To identify the more appropriate, or currently recommended, readout tests for platelet functionality, or platelet half-life as to assess the normal or abnormal response on the platelet depletion and transfusion models.

Experimentally-induced severe thrombocytopenia in mice has become a very common approach in order to identify whether platelets are critical in a particular pathophysiological process. In addition, to investigate the molecular mechanisms by which platelets contribute to a complex physiological or pathophysiological setting in mice *in vivo*, one possibility is transfusion

of exogenous 'donor' platelets into platelet-depleted mice. The donor platelets can be genetically or pharmacologically modified. This allows rapid and efficient generation of mice with platelet-specific defects in multiple signaling molecules. This is a good alternative to the use of the loxP PF4cre system to induce deletion of genes specifically in the MK-platelet lineage, since crossing of mice is expensive and time-consuming, and loxP-flanked genes of interest, are not available in all cases.

Several approaches are available for *in vivo* depletion of platelets in mice: antiplatelet antibody-mediated clearance of platelets; using transgenic mice expressing the diphtheria toxin (DT) receptor (iDTR) specifically in the megakaryocyte-platelet lineage; using transgenic mice expressing GPIbα/human IL4Receptor α instead of GPIbα on the platelet surface; using mice genetically thrombocytopenic ($mpl^{-/-}$ or $nfe2^{-/-}$ mice); or using chemical compounds.

There are also several approaches available for the adoptive transfer of exogenous platelets into mice, which have been previously depleted of their own platelets. Mouse or human platelets may be transferred into mice. Each of these approaches has strengths and weaknesses and each of these models has characteristics and suitability for specific biological questions.

Project Design and Methodology:

List the data expected to collect, sample size and statistical analysis. Concisely describe the research design and methods for achieving these goals. Suggested length 2-3 paragraphs

Phase 1: Comprehensive review

The objective is to write a comprehensive review of the spectrum of approaches used for platelet removal and platelet transfusion in mice, including how they may be combined for specific studies and understanding the systemic impact of each model. The advantages and limits of each method will be highlighted, along with an analysis of which stages would benefit from improvement. The various readout tests for platelet functionality or platelet half-life will be identified to assess normal or abnormal response on the platelet depletion and transfusion models.

The first step is to engage a team of experts composed of leaders in this field, identified through literature appraisal and expert recommendations. A draft proposal is ready on the key points to consider for the review.

Phase 2: Investigator Questionnaire to identify commonly used approaches via self-reporting of investigators

An investigator questionnaire will be drafted to collect data on procedural practice details on platelet depletion and transfusion models. The Questionnaire will be circulated to corresponding authors of papers identified in Phase 1 (leaders in this field, identified through literature appraisal and expert recommendations) and will also be made more widely available to the field on the SSC Models of Thrombosis & Hemostasis section of the myISTH community website.

Phase 3: To establish experimental guidelines for the use of these models.

The responses to the investigator questionnaire will be analyzed to define the optimal methods and best practice recommendations for current methods of *in vivo* platelet depletion and transfusion models in mice and also identify the more appropriate, or recommended, readout tests for platelet functionality or platelet half-life to assess normal or abnormal response on the platelet depletion and transfusion models. A guidance document will be published. This will include statements of the shortfalls of current methods.

Study Population:

List the inclusion and exclusion criteria, eligibility, patient population; recruitment of participating institutions/physicians and subjects; minimum number needed; expected number:
Suggested length 2-3 paragraphs

Not Applicable

Infrastructure:

Description of project set-up, management, operational requirements and resources.

This project was initiated as joint effort together with the Platelet Physiology SSC (Chairman, Marie Lordkipanidze and Co-Chair Justin Hamilton), and Laura Gutiérrez presented in the ISTH 2020 SSC Session data on the impact of PLT depletion on hematopoiesis. However, Justin Hamilton left the ISTH-SSC and the project has been delayed. Beatrice Hechler took it over as the coordinator, with Laura Gutiérrez participating in the working team, which will be strengthen by a panel of external experts identified by the project leads.

ISTH support may be required for: survey design; facilitation of survey delivery and collection; room bookings for face-to-face (in-person or virtual) meetings at ISTH meetings; Computational resources necessary to integrate responses from the investigator questionnaire.

Timeline and Milestones:

Project stage/set up: July 2022
Launch: January 2023
Duration: 2 years
Finalization/analysis: December 2024
Reporting (annual at minimum): Annually
Publication:

Manuscript 1 - Comprehensive review of the spectrum of approaches used for platelet removal and platelet transfusion in mice, October-December 2023.

Manuscript 2 - Investigator Questionnaire Analysis and Experimental guidelines for the use of these models, July 2024.

Expected Outcomes:

Describe potential for future collaboration, funded research grant, publication (specify type – SSC Communications, Guidance Document, Original Article, etc.):

- This project should lead directly to the submission of 2 manuscripts for publication.

- This project will establish a network of study investigators with expertise in the platelet depletion/platelet transfusion fields that may lead to future collaborations on aspects not only of transfusional medicine, but also on immunothrombosis, etc.

Selected references:

Angenieux C, Maitre B, Eckly A, Lanza F, Gachet C, de la Salle H. Time-dependent decay of mrna and ribosomal rna during platelet aging and its correlation with translation activity. *PLoS One*. 2016;11:e0148064

Boulaftali Y, Hess PR, Getz TM, Cholka A, Stolla M, Mackman N, Owens AP, 3rd, Ware J, Kahn ML, Bergmeier W. Platelet itam signaling is critical for vascular integrity in inflammation. *J Clin Invest*. 2013;123:908-916

Do Sacramento V, Mallo L, Freund M, Eckly A, Hechler B, Mangin P, Lanza F, Gachet C, Strassel C. Functional properties of human platelets derived in vitro from cd34(+) cells. *Sci Rep*. 2020;10:914

Hechler B, Maitre B, Magnenat S, Heim V, El Mdawar MB, Gachet C, de la Salle H. Platelets are dispensable for antibody-mediated transfusion-related acute lung injury in the mouse. *J Thromb Haemost*. 2016;14:1255-1267

Paul DS, Bergmeier W. Novel mouse model for studying hemostatic function of human platelets. *Arterioscler Thromb Vasc Biol*. 2020;40:1891-1904

Salzmann M, Schrottmaier WC, Kral-Pointner JB, Mussbacher M, Volz J, Hoesel B, Moser B, Bleichert S, Morava S, Nieswandt B, Schmid JA, Assinger A. Genetic platelet depletion is superior in platelet transfusion compared to current models. *Haematologica*. 2020;105:2698

Strassel C, Brouard N, Mallo L, Receveur N, Mangin P, Eckly A, Bieche I, Tarte K, Gachet C, Lanza F. Aryl hydrocarbon receptor-dependent enrichment of a megakaryocytic precursor with a high potential to produce proplatelets. *Blood*. 2016;127:2231-2240

Wuescher LM, Nishat S, Worth RG. Characterization of a transgenic mouse model of chronic conditional platelet depletion. *Res Pract Thromb Haemost*. 2019;3:704-712