Asundexian in atrial fibrillation

Vassart, Julie; Didembourg, Marie; Morimont, Laure; Brisbois, Clotilde; Jamart, Laurent; Lebreton, Aurélien; Mullier, Francois; Donis, Nathalie; Favresse, Julien; Dogne, Jean-Michel; Douxfils, Jonathan

Published in:
Thrombosis Research

DOI:
10.1016/j.thromres.2024.03.001

Publication date:
2024

Document Version
Publisher's PDF, also known as Version of record

Thrombosis Research 236 (2024) 236–239

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Letter to the Editors-in-Chief

Asundexian in atrial fibrillation: Can pharmacodynamic data explain the failure?

To the Editors,

The phase 3 OCEANIC-AF trial (NCT05643573) of the investigational oral activated factor XI (FXIa) inhibitor asundexian (Bayer, Leverkusen, Germany) tested at the dose of 50 mg has been stopped early due to an inferior efficacy of asundexian versus the control arm (apixaban) for the prevention of strokes and systemic embolisms in patients with atrial fibrillation (AF). This decision is based on the recommendation of the study's independent data monitoring committee (IDMC) as part of ongoing surveillance [1]. No additional details were available when this manuscript was written. The Phase 3 trial LIBREXIA-AF (NCT05757869), a randomized, double-blind study that evaluates the efficacy and safety of milvexian versus apixaban in AF is ongoing. Results are expected in 2027.

In the PACIFIC-AF trial (NCT04218266), asundexian 50 mg resulted in a 92 % reduction in FXIa activity at trough concentrations and a 94 % reduction at peak concentrations. The investigators used this surrogate endpoint to support dose selection [2]. The comparison of the pharmacodynamic profile of asundexian and apixaban has not yet been directly assessed. Several coagulation tests could be used to demonstrate the inhibitory activity of a drug on hemostasis coagulation factors such as FXIa in a well-targeted system. However, a global coagulation test like thrombin generation could better reflect the interaction between the different coagulation proteins and be used as a more adapted pharmacological tool to assess and compare anticoagulant therapies inhibiting distinctive targets [3]. In this study, we aim to compare the inhibitory profile of asundexian, milvexian and apixaban on thrombin generation to highlight potential differences in their anticoagulant activity.

Asundexian and milvexian were purchased at Bio-Connect (Huissen, The Netherlands) and apixaban at AlsaChim (Strasbourg, France). As the three studied inhibitors exhibit molecular weights in the same range (i.e., asundexian 592.1 g/mol; milvexian 625.1 g/mol; apixaban 459.5 g/mol), concentrations are reported in ng/mL to facilitate comparison with the literature. Each molecule was diluted in dimethyl sulfoxide to obtain 1 mg/mL stock solutions. Stock solutions underwent serial dilution in phosphate buffer saline (PBS) without calcium or magnesium to prepare the intermediate solutions. Normal pooled plasma (NPP) from the Namur Biobank Exchange (NAB-X, Namur, Belgium) was spiked with these intermediate solutions to obtain plasmatic solutions (dilution factor = 20). For the baseline condition, the NPP was spiked with PBS without calcium or magnesium at the same dilution factor to ensure identical plasma dilution.

Corn Trypsin Inhibitor (CTI) was purchased at Enzyme Research Laboratories, Ltd. (Swansea, United Kingdom) and diluted in hydroxyethylpiperazine ethane sulfonic acid (Hepes) buffer to obtain 1.4 mg/mL stock solution. This solution of CTI was spiked into a second set of intermediate solutions of asundexian, milvexian and apixaban to obtain a final CTI concentration of 40 μg/mL (2.9 μM) in plasma [4].

Thrombin generation was assessed using a Calibrated Automated Thrombogram (CAT) (Stago Diagnostica, Asnières-sur-Seine, France). Investigations were carried out at QUALIblood s.a. (Namur, Belgium). In brief, 80 μL of each plasmatic solution was added to 20 μL of platelet-poor plasma (PPP) reagent in a 96-well plate. Three different reagents were used: PPP Reagent Low (1 pM tissue factor (TF) [0.16 pM in final concentration]; 4 μM phospholipids (PL)), PPP Reagent (5 pM TF [0.80 pM in final concentration]; 4 μM phospholipids) and PPP Reagent High (20 pM TF [3.2 pM in final concentration]; 4 μM phospholipids). A fourth reagent was prepared by diluting the PPP Reagent Low 10-fold (0.1 pM TF [0.016 pM in final concentration]; 0.4 μM phospholipids). A set of wells was dedicated to the calibration (80 μL of 0 ng/mL plasmatic solution and 20 μL of Thrombin Calibrator reagent). Thrombin generation was triggered by adding 20 μL of FluCa Reagent (fluorogenic substrate, buffer, calcium chloride). All conditions have been tested in triplicate within the same experiment. The relations between the anticoagulants concentration and the parameter have been modeled by one-phase decay equations ($Y = (Y0 - Plateau) * \exp(-K * X) + Plateau$). The concentrations of inhibitors needed to double the lag time and the time to peak (2× parameters), and the concentration needed to halve (IC_{50}) the peak, the endogenous thrombin potential (ETP) and the mean velocity rate index (mVRI) have been calculated. Statistical analyses were performed using GraphPad Prism software version 10.1.1 (GraphPad Prism 10 for macOS®, version 10.1.1, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com).

According to their mechanism of action, none of the activated FXIa inhibitors were able to significantly delay the initiation of the coagulation whatever the triggering reagent used (Fig. 1). However, surprisingly, different profiles were observed between asundexian and milvexian for the ETP, the peak and the mVRI. Globally, no significant difference was observed in the presence or absence of CTI 40 μg/mL (Table 1). The thrombin generation curves in presence versus in absence of CTI with 0.1 and 1 pM of TF are provided as Supplementary material. Milvexian can halve the peak and the ETP at a concentration of ≅ 400 to 650 ng/mL with PPP Reagent Low and at ≅ 200 to 500 ng/mL with PPP Reagent "very Low" (0.1 pM TF). As per comparison, 400 ng/mL corresponds to the maximal concentration observed after a dose of 60 mg fasted [5]. Importantly, milvexian is currently tested at a dose of 100 mg twice daily in atrial fibrillation, leading to peak plasma concentrations comprised between 350 ng/mL and 1000 ng/mL. With a half-life of approximately 10 h, the drug should remain sufficiently active between two intakes, similarly to apixaban 5 mg twice daily. On the other hand, asundexian is only able to halve the peak and the mVRI in PPP Reagent Low at concentrations around 1500 ng/mL (and above 2000 ng/mL with 0.1 pM TF). According to available pharmacokinetic data, the Cmax of asundexian after oral doses ranging from 25 mg to 100 mg once daily was 358 ng/mL to 1230 ng/mL. Subsequent studies have confirmed

https://doi.org/10.1016/j.thromres.2024.03.001
Received 8 January 2024; Received in revised form 18 February 2024; Accepted 1 March 2024
Available online 7 March 2024

these pharmacokinetic parameters [6]. Unfortunately, these concentrations were lower than the concentration needed to halve the peak or the mVRI. Even if it exhibits a longer half-life than milvexian (i.e., ≅ 20 h for asundexian versus ≅ 10 h for milvexian), its capacity to inhibit thrombin generation sufficiently during the entire dose interval is lower and probably insufficient for AF patients. Also, the Ki of asundexian (Ki = 1.0 nM for FXIa) [7] is higher than the one of milvexian (Ki = 0.11 nM for FXIa) [8], explaining, at least in part, its lower capacity to inhibit thrombin generation triggered at low TF concentration.

Previous investigations also showed a low capacity of asundexian to inhibit TF-induced thrombin generation [7]. Heitmeier et al. had to reduce TF concentration to 0.1 pM in presence of 4 μM of PL to show an acceptable dose-dependent decrease of the peak with an IC_{50} of 0.3 μM (178 ng/mL). In presence of lower PL concentrations (i.e. 0.4 μM), we were not able to replicate this observation. Nevertheless, at such low TF concentrations, there is still a difference between asundexian and milvexian (Table 1). Nevertheless, the physiopathology of AF includes abnormal changes in the atrial tissue, inflammation and endothelial damage/dysfunction [9] leading to an overexpression of tissue factor [9]. Although no consensus exists on the TF concentration that has to be used in such a pharmacological model, previous investigations have demonstrated that 0.16 pM in final TF concentration permits the appreciation of the activation of FXI by thrombin [10]. Therefore, the involvement of TF to trigger thrombogenesis in AF seems more reliable than the contact system that activates FXI via FXII.

Consequently, to support their use in AF on a pharmacological basis, FXIa inhibitors like asundexian and milvexian need to be able to inhibit the amplification process triggered by the activation of FXI by thrombin, which itself has been initially activated via the extrinsic pathway.

When developing a new anticoagulant agent, we are of the opinion that the assessment of its impact on thrombin or fibrin generation, using appropriate triggering reagents mimicking the pathophysiology of the thrombotic disease, brings more value than the evaluation of FXIa activity which is less relevant in the setting of AF.

In conclusion, the weak capacity to inhibit thrombin generation amplification at the dose tested in the OCEANIC-AF study may at least partially explain why asundexian failed to demonstrate non-inferiority compared to apixaban. Milvexian, either by its higher potency on FXIa compared to asundexian and/or by off-target mechanisms at the doses currently used in the LIBREXIA-AF may be more successful in demonstrating a non-inferior antithrombotic effect compared to apixaban 5 mg twice daily.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2024.03.001.

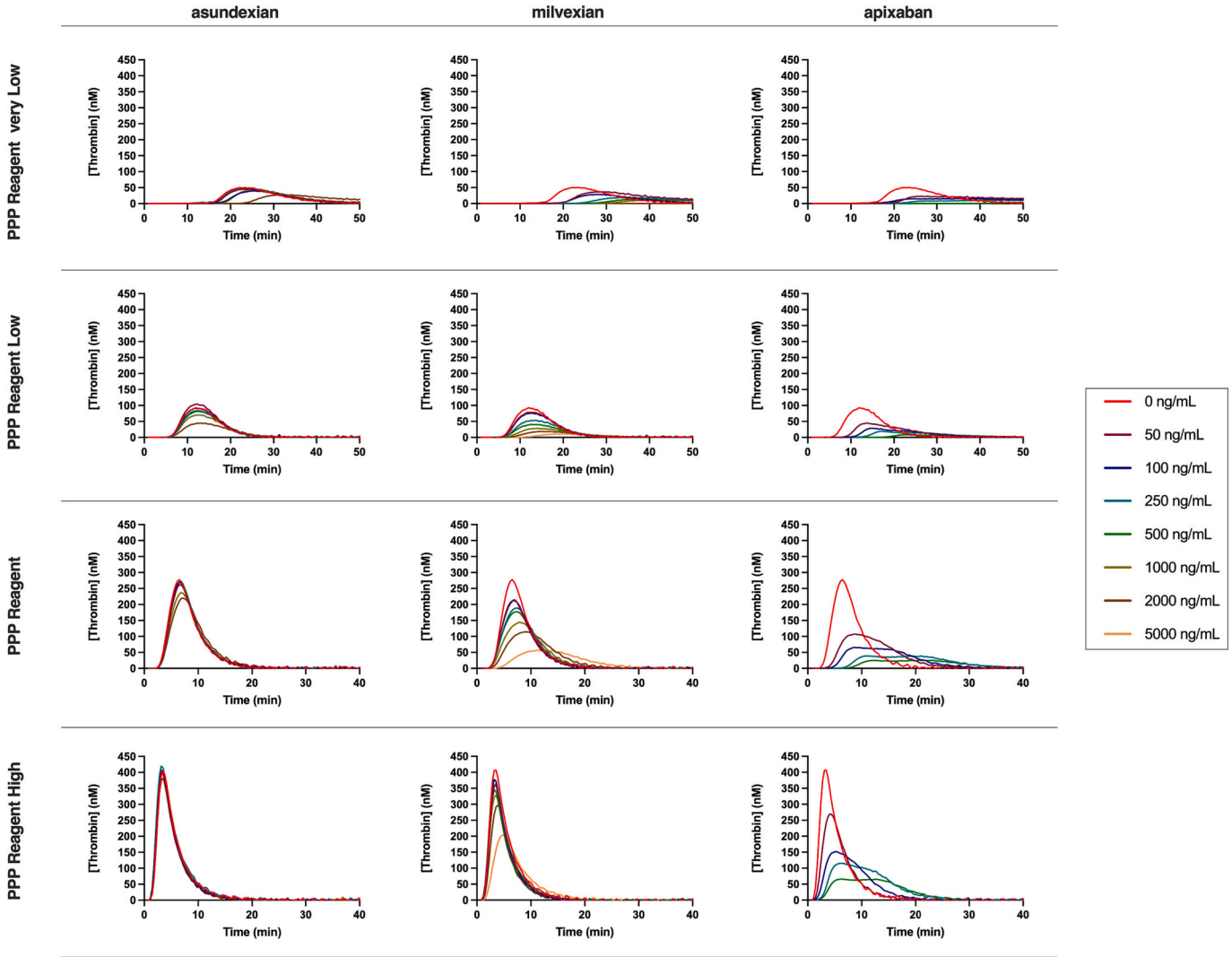

Fig. 1. Thrombin generation curves for normal pooled plasma spiked with several concentrations of asundexian, milvexian or apixaban. The selected concentrations were 50; 100; 250; 500; 1000; 2000 ng/mL for asundexian, 50; 100; 250; 500; 1000; 2000; 5000 ng/mL for milvexian and 50; 100; 250; 500 ng/mL for apixaban, based on the available pharmacokinetics data for the molecules mentioned above.

Table 1
Two-fold times and inhibitory concentrations 50 (IC_{50}) of the thrombin generation assay parameters.

	Reagent	Asundexian		Milvexian		Apixaban	
		Without CTI	CTI 40 μg/mL	Without CTI	CTI 40 μg/mL	Without CTI	CTI 40 μg/mL
2× Lag time (ng/mL) [95 % CI]	PPP very Low	n.c. (>2000)	n.c. (>2000)	n.c. (>5000)	1109.3 [n.c.]	289.9 [182.7–398.9]	n.c. (>500)
	PPP Low	n.c. (>2000)	n.c. (>2000)	n.c. (>5000)	n.c. (>5000)	304.2 [250.2–358.5]	193.4 [180.4–207.1]
	PPP	n.c. (>2000)	/	n.c. (>5000)	/	209.4 [194.4–225.1]	/
	PPP High	n.c. (>2000)	/	n.c. (>5000)	/	279.6 [228.9–372.3]	/
IC_{50} ETP (ng/mL) [95 % CI]	PPP very Low	n.c. (>2000)	n.c. (>2000)	484.0 [255.8–1001.3]	300.3 [209.1–448.4]	279.9 [239.5–320.2]	91.9 [84.8–99.7]
	PPP Low	n.c. (>2000)	n.c. (>2000)	643.8 [558.2–742.4]	839.6 [656.8–1073.5]	100.3 [80.6–124.4]	85.4 [68.0–107.9]
	PPP	n.c. (>2000)	/	n.c. (>5000)	/	151.1 [130.7–174.0]	/
	PPP High	n.c. (>2000)	/	n.c. (>5000)	/	n.c. (>500)	/
IC_{50} Peak (ng/mL) [95 % CI]	PPP very Low	n.c. (>2000)	n.c. (>2000)	200.1 [149.2–274.8]	293.0 [234.6–372.5]	47.1 [37.4–58.9]	71.9 [51.4–100.4]
	PPP Low	1876.0 [1598.1–>2000]	n.c. (>2000)	419.4 [360.4–489.9]	580.9 [422.7–809.9]	51.5 [42.7–61.9]	58.2 [53.6–63.1]
	PPP	n.c. (>2000)	/	1893.8 [1633.4–2188.7]	/	40.5 [37.5–43.7]	/
	PPP High	n.c. (>2000)	/	n.c. (>5000)	/	80.5 [69.3–93.5]	/
2× Time to peak (ng/mL) [95 % CI]	PPP very Low	n.c. (>2000)	n.c. (>2000)	n.c. (>5000)	962.6 [867.8 – n.c.]	210.6 [183.6–240.2]	192.1 [89.9–n.c.]
	PPP Low	n.c. (>2000)	n.c. (>2000)	n.c. (>5000)	n.c. (>5000)	n.c. (>500)	226.1 [171.9–273.8]
	PPP	n.c. (>2000)	/	n.c. (>5000)	/	164.4 [121.8–222.1]	/
	PPP High	n.c. (>2000)	/	n.c. (>5000)	/	408.2 [262.7–>500.0]	/
IC_{50} mVRI (ng/mL) [95 % CI]	PPP very Low	n.c. (>2000)	1798.2 [1643.3–>2000]	92.9 [76.9–112.5]	232.4 [145.2–378.9]	39.0 [31.0–48.8]	57.2 [31.9–97.0]
	PPP Low	1428.4 [1053.6–1855.3]	n.c. (>2000)	342.0 [287.3–409.8]	492.5 [346.1–718.8]	88.1 [70.3–110.7]	90.6 [82.4–99.8]
	PPP	n.c. (>2000)	/	1008.6 [833.6–1225]	/	34.6 [29.4–40.6]	/
	PPP High	n.c. (>2000)	/	3791.9 [3558.8–4227.5]	/	58.8 [50.9–67.9]	/

Abbreviations: n.c.: not calculable as the value of the parameter corresponding to 50 % of inhibition or the two-fold time does not appear on the graph (no possibility of interpolation); 2×: two-fold; 95 % CI: 95 % confidence interval; IC_{50}: inhibitory concentration 50; ETP: endogenous thrombin potential; mVRI: mean velocity rate index; PPP: platelet-poor plasma; [n.c.] the 95 % confidence interval was (partially) not calculable due to the low number of measurable values for the related parameter (only for the PPP Reagent very Low i.e. 0.1 pM TF, where flat curves were obtained for the highest concentrations in inhibitors); /: the condition was not investigated.

Funding

Published with the support of the Fondation Universitaire de Belgique.

CRediT authorship contribution statement

Marie Didembourg: Methodology, Investigation. **Laure Morimont:** Writing – review & editing, Validation, Data curation. **Clotilde Brisbois:** Methodology, Investigation. **Laurent Jamart:** Writing – review & editing, Validation. **Aurélien Lebreton:** Writing – review & editing, Validation, Conceptualization. **François Mullier:** Investigation, Writing – review & editing. **Nathalie Donis:** Data curation, Formal analysis, Investigation, Project administration, Writing – review & editing. **Julien Favresse:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Jean-Michel Dogné:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition. **Jonathan Douxfils:** Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Bayer, OCEANIC-AF study stopped early due to lack of efficacy. https://www.bayer.com/media/en-us/oceanic-af-study-stopped-early-due-to-lack-of-efficacy/, 2023. (Accessed 19 November 2023).
[2] J.P. Piccini, V. Caso, S.J. Connolly, K.A.A. Fox, J. Oldgren, W.S. Jones, Safety of the oral factor XIa inhibitor asundexian compared with apixaban in patients with atrial fibrillation (PACIFIC-AF): a multicentre, randomised, double-blind, double-dummy, dose-finding phase 2 study, Lancet 399 (10333) (2022) 1383–1390.
[3] S. Robert, J. Ghiotto, B. Pirotte, J.L. David, B. Masereel, L. Pochet, J.M. Dogne, Is thrombin generation the new rapid, reliable and relevant pharmacological tool for the development of anticoagulant drugs? Pharmacol. Res. 59 (3) (2009) 160–166.
[4] H.M. Spronk, A.W. Dielis, M. Panova-Noeva, R. van Oerle, J.W. Govers-Riemslag, K. Hamulyák, A. Falanga, H.T. Cate, Monitoring thrombin generation: is addition of corn trypsin inhibitor needed? Thromb. Haemost. 101 (6) (2009) 1156–1162.
[5] V. Perera, Z. Wang, J. Luettgen, D. Li, M. Desouza, M. Cerra, D. Seiffert, First-in-human study of milvexian, an oral, direct, small molecule factor XIa inhibitor, Clin. Transl. Sci. 15 (2) (2022) 330–342.
[6] F. Kanefendt, C. Brase, N. Jungmann, R. Fricke, A. Engelen, S. Schmitz, Pharmacokinetics of asundexian with combined CYP3A and P-gp inhibitors and an inducer: target in vitro and in vivo studies, Br. J. Clin. Pharmacol. (2023), https://doi.org/10.1111/bcp.15981.
[7] S. Heitmeier, M. Visser, A. Tersteegen, J. Dietze-Torres, J. Glunz, C. Gerdes, V. Laux, J. Stampfuss, S. Roehrig, Pharmacological profile of asundexian, a novel, orally bioavailable inhibitor of factor XIa, J. Thromb. Haemost. 20 (6) (2022) 1400–1411.
[8] A.K. Dilger, K.B. Pabbisetty, J.R. Corte, I. De Lucca, T. Fang, W. Yang, D.J.P. Pinto, Y. Wang, Y. Zhu, A. Mathur, J. Li, X. Hou, D. Smith, D. Sun, H. Zhang, S. Krishnananthan, D.-R. Wu, J.E. Myers, S. Sheriff, K.A. Rossi, S. Chacko, J. J. Zheng, M.A. Galella, T. Ziemba, E.A. Dierks, J.M. Bozarth, Y. Wu, E. Crain, P. C. Wong, J.M. Luettgen, R.R. Wexler, W.R. Ewing, Discovery of milvexian, a high-affinity, orally bioavailable inhibitor of factor XIa in clinical studies for antithrombotic therapy, J. Med. Chem. 65 (3) (2022) 1770–1785.
[9] Y. Nakamura, K. Nakamura, K. Fukushima-Kusano, K. Ohta, H. Matsubara, T. Hamuro, C. Yutani, T. Ohe, Tissue factor expression in atrial endothelia associated with nonvalvular atrial fibrillation: possible involvement in intracardiac thrombogenesis, Thromb. Res. 111 (3) (2003) 137–142.
[10] D.V. Kravtsov, A. Matafonov, E.I. Tucker, M.F. Sun, P.N. Walsh, A. Gruber, D. Gailani, Factor XI contributes to thrombin generation in the absence of factor XII, Blood 114 (2) (2009) 452–458.

Julie Vassart[a], Marie Didembourg[a], Laure Morimont[a,b], Clotilde Brisbois[b], Laurent Jamart[c], Aurélien Lebreton[d], François Mullier[e,f], Nathalie Donis[b], Julien Favresse[a,g], Jean-Michel Dogné[a], Jonathan Douxfils[a,b,d,*,**,***]

[a] *Clinical Pharmacology and Toxicology Research Unit (URPC), Faculty of Medicine, Namur Research Institute for Life Sciences (NARILIS), University of Namur, Namur, Belgium*
[b] *QUALIresearch, QUALIblood s.a., Namur, Belgium*
[c] *Service de cardiologie interventionnelle, Centre Hospitalier Régional Sambre et Meuse (CHRSM), Namur, Belgium*
[d] *Department of Biological Hematology, Centre Hospitalier Universitaire Clermont-Ferrand, Hôpital Estaing, Clermont-Ferrand, France*
[e] *Namur Thrombosis and Hemostasis Center (NTHC), CHU UCL NAMUR, Université catholique de Louvain*
[f] *Institut de Recherche Expérimentale et Clinique (IREC), Pôle Mont, Université catholique de Louvain, Yvoir, Belgium*
[g] *Department of Clinical Biology, Clinique Saint-Luc Bouge (SLBO), Bouge, Belgium*

[*] Corresponding author at: Clinical Pharmacology and Toxicology Research Unit, Faculty of Medicine, NARILIS, Université de Namur, Namur, Belgium.

[**] Corresponding author at: QUALIresearch, QUALIblood s.a., Namur, Belgium.

[***] Corresponding author at: Department of Biological Hematology, Centre Hospitalier Universitaire Clermont-Ferrand, Hôpital Estaing, Clermont-Ferrand, France.
E-mail address: jonathan.douxfils@unamur.be (J. Douxfils).