CONGRÈS FRANÇAIS D'HÉMOSTASE

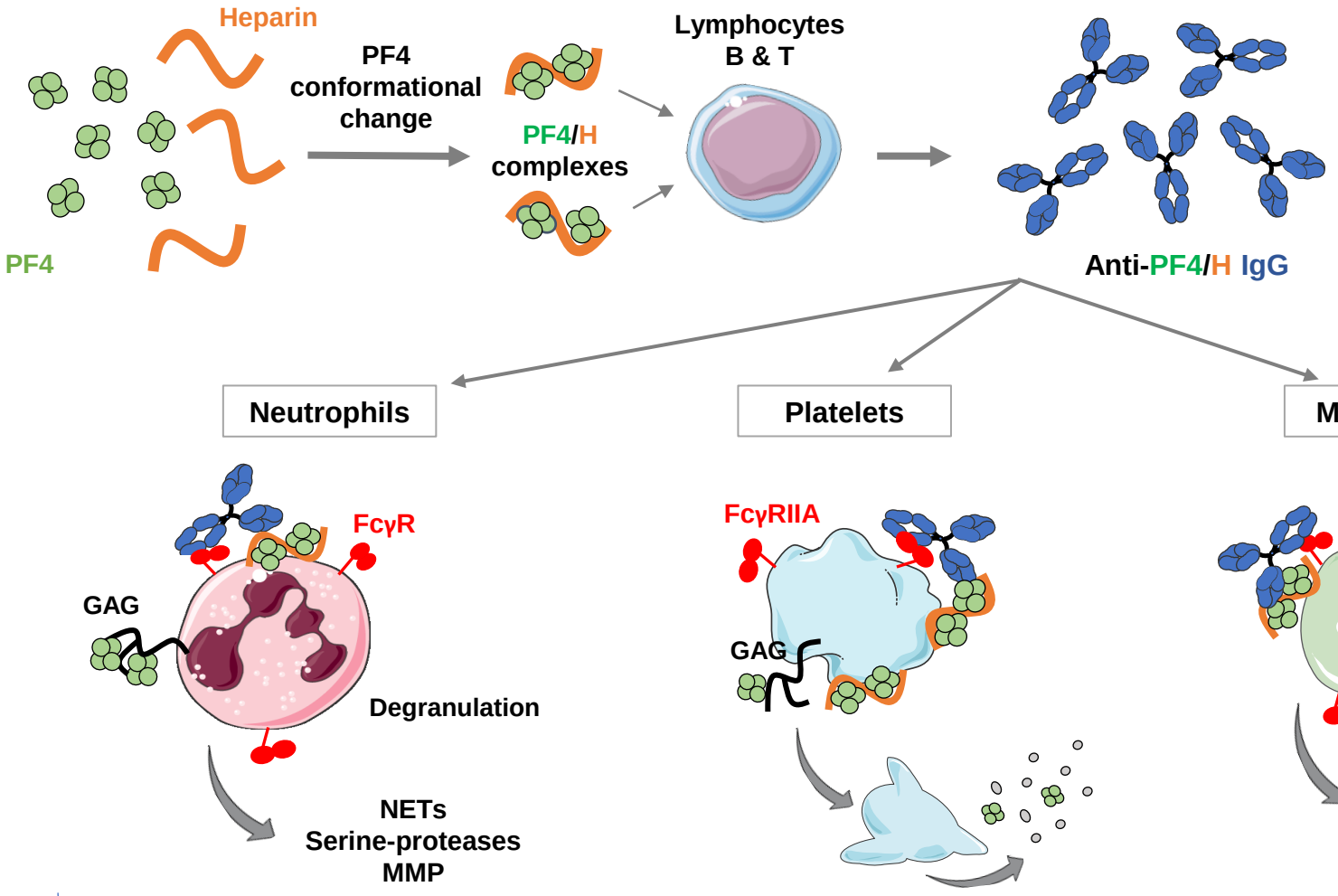
10 -12 MAI 2023

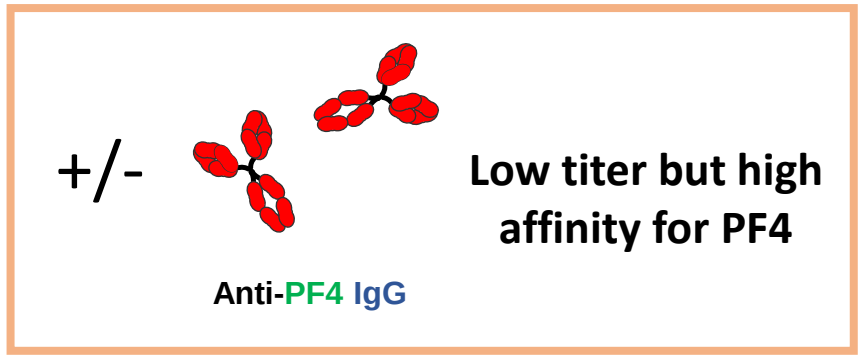
Cooperation between PF4-specific antibodies: a new physiopathological mechanism in Heparin-Induced Thrombocytopenia (HIT) ?

Sandra Billy

May 12th , 2023

Heparin-induced thrombocytopenia (HIT)





Pouplard et al, BJH, 1997
Nguyen et al, Nat Comm, 2017

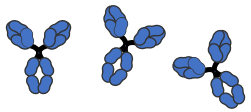
Pouplard et al, Blood, 2001
Rollin et al., Blood, 2015
Rollin *et al*, Thromb Haemost, 2016
Perdomo *et al*, Nat Commun, 2019

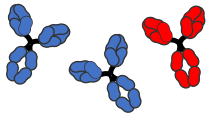
Hypercoagulability → **Thrombosis (30-50%)**

Variable serotonin release assay pattern and specificity of PF4-specific antibodies in HIT, and clinical relevance

Journal of Thrombosis and Haemostasis, 2022

Jérôme Rollin[1,2] | Noémie Charuel[1] | Yves Gruel[1,2] | Sandra Billy[1] | Eve-Anne Guéry[2] | Marc-Antoine May[3,4] | Claire Pouplard[1,2] | Caroline Vayne[1,2]





	Patients with anti-PF4/H only n= 26	**Patients with anti-PF4/H and anti-PF4** n= 21
Thrombopenia median [min-max]	**+** 53 [14-122] G/L	**+++** 35 [6-82] G/L
Platelet count recovery time after heparin withdraw (>150G/L) median [min-max]	**+** 3 [1-21] days	**+++** 6 [2-17] days

Hypothesis = is **anti-PF4 Abs** have a role of in **severity** of HIT ?

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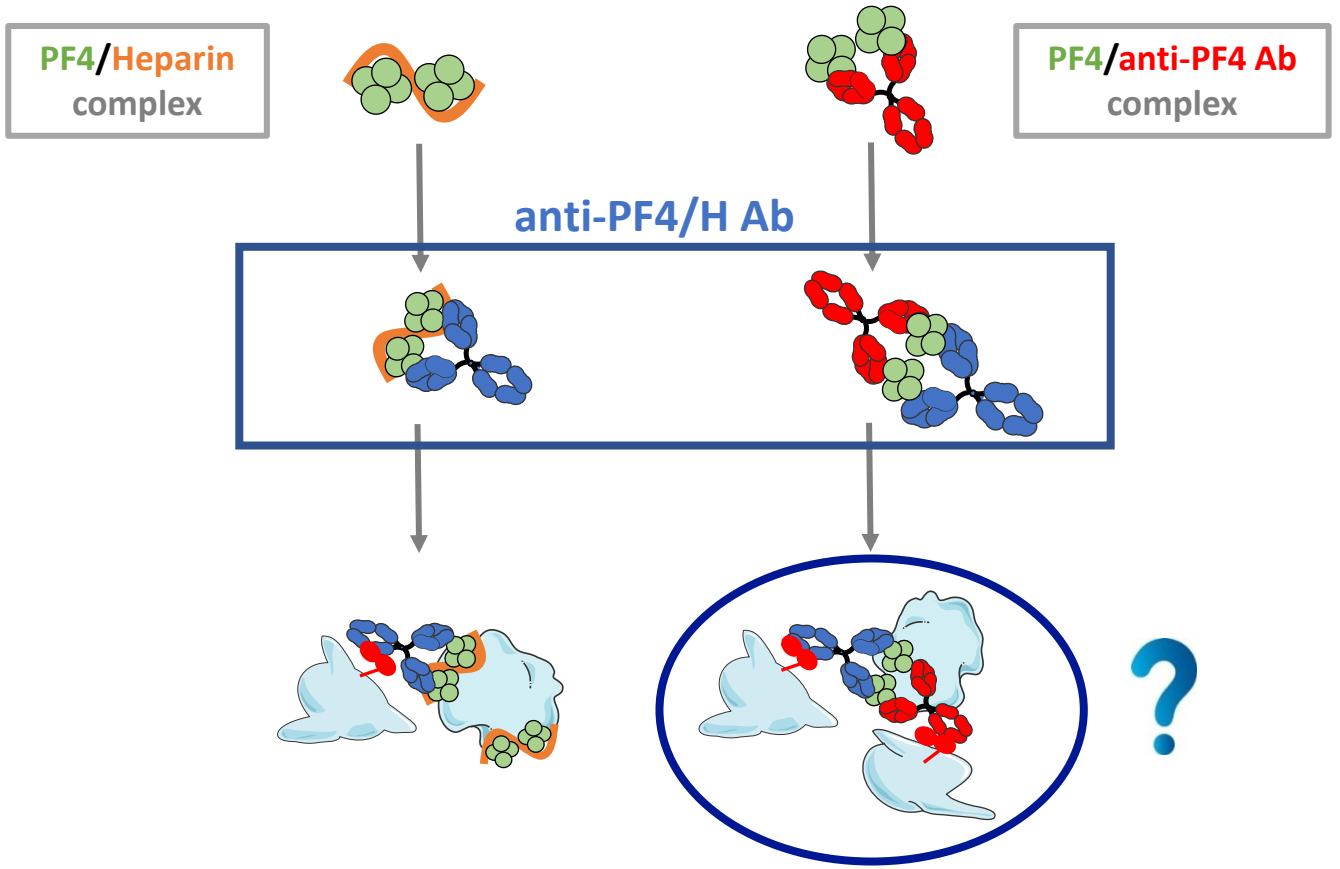
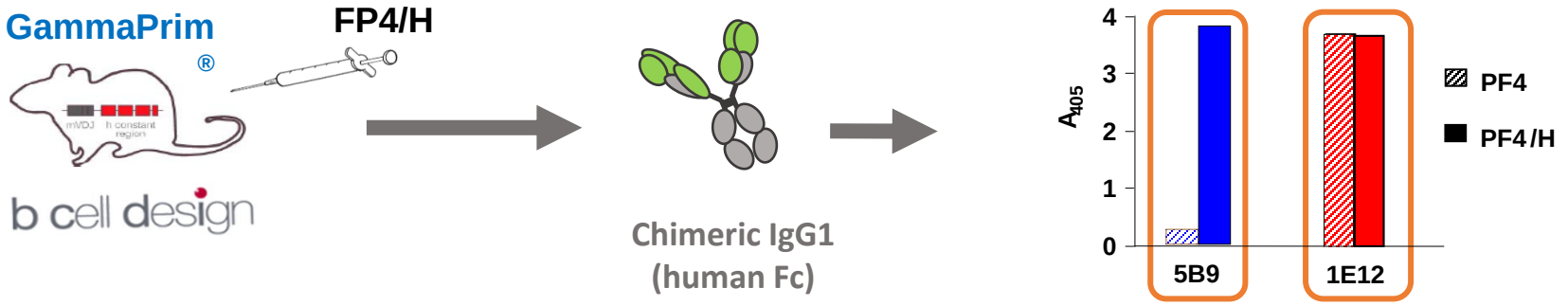
Anti-platelet factor 4/polyanion antibodies mediate a new mechanism of autoimmunity

Thi-Huong Nguyen[1,2], Nikolay Medvedev[2], Mihaela Delcea[1,2] & Andreas Greinacher[1]



Objective:
Evaluate the cooperation between PF4-specific antibodies on platelet activation and thrombus formation

Monoclonal antibodies to study pathophysiology of HIT



5B9, a monoclonal antiplatelet factor 4/heparin IgG with a human Fc fragment that mimics heparin-induced thrombocytopenia antibodies

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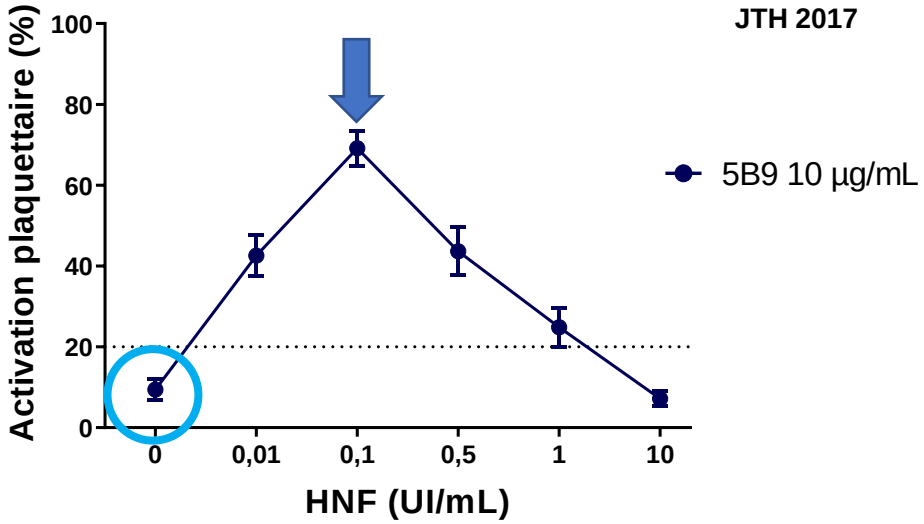
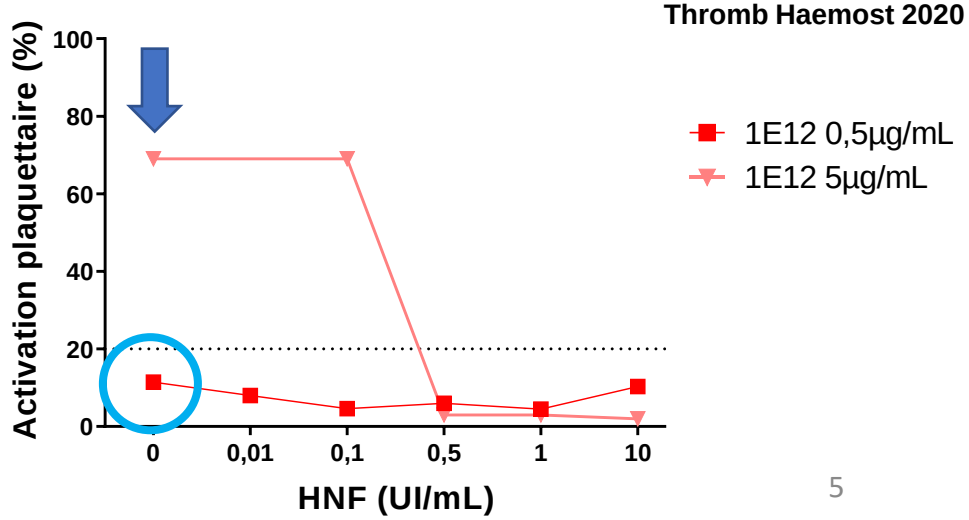
JTH 2017



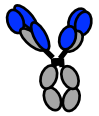
Characterization of New Monoclonal PF4-Specific Antibodies as Useful Tools for Studies on Typical and Autoimmune Heparin-Induced Thrombocytopenia

Caroline Vayne[1,2,*] Thi-Huong Nguyen[3,4,*] Jérôme Rollin[1,2] Noémie Charuel[1] Anne Poupon[5,6] Claire Pouplard[1,2] Nicole Normann[3] Yves Gruel[1,2,**] Andreas Greinacher[3,**]

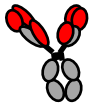
Thromb Haemost 2020



Synergistic effect of 1E12 and 5B9 on platelet activation

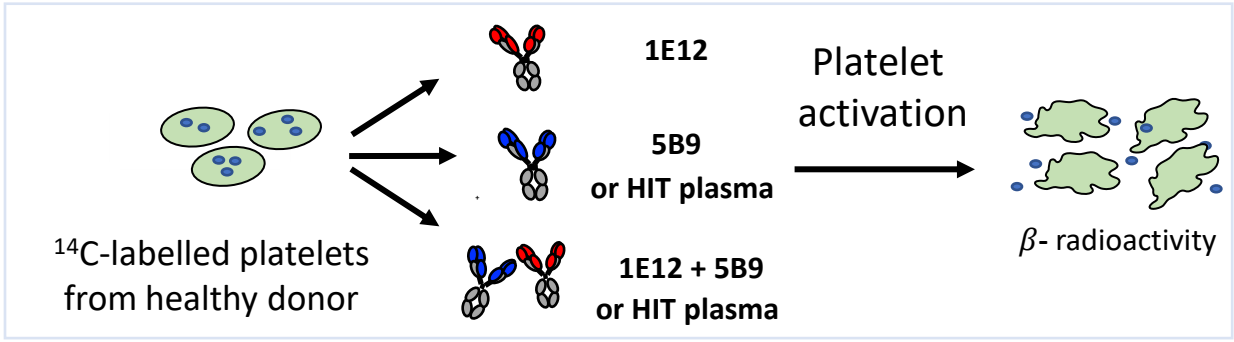


5B9
(Anti-PF4/H Ab)

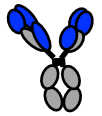


1E12
(Anti-PF4 Ab)

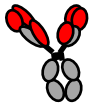
SRA
Serotonin Release Assay



Synergistic effect of 1E12 and 5B9 on platelet activation



5B9
(Anti-PF4/H Ab)



1E12
(Anti-PF4 Ab)

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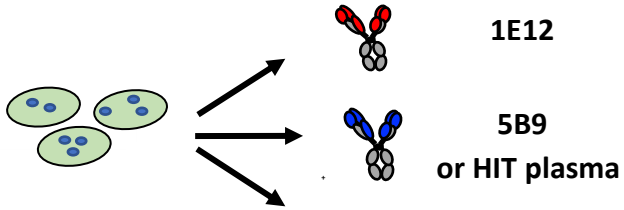
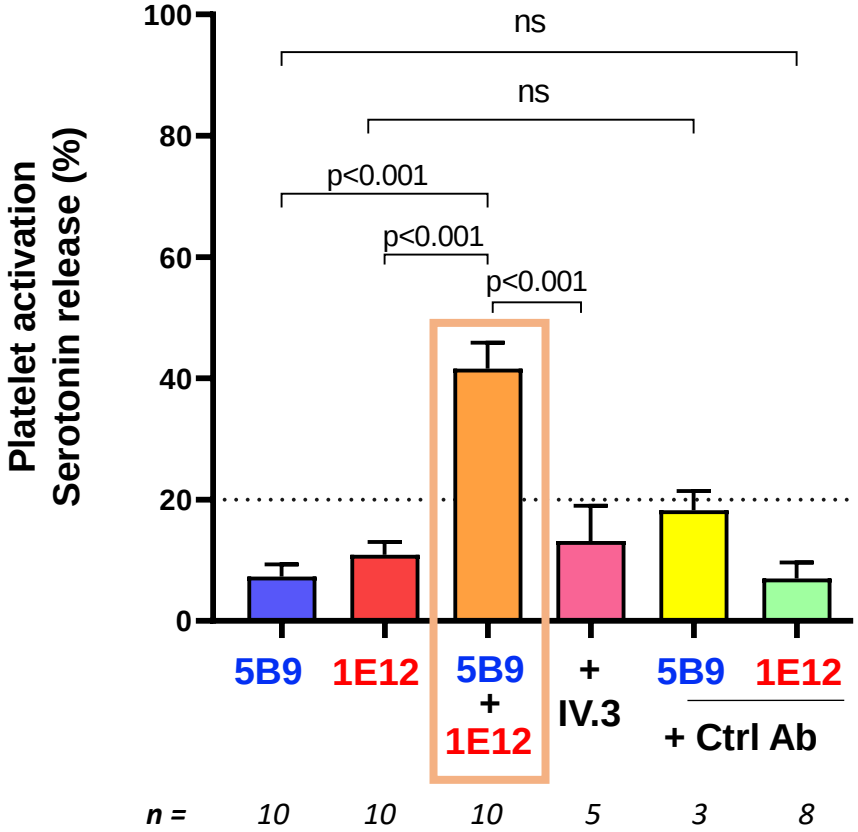
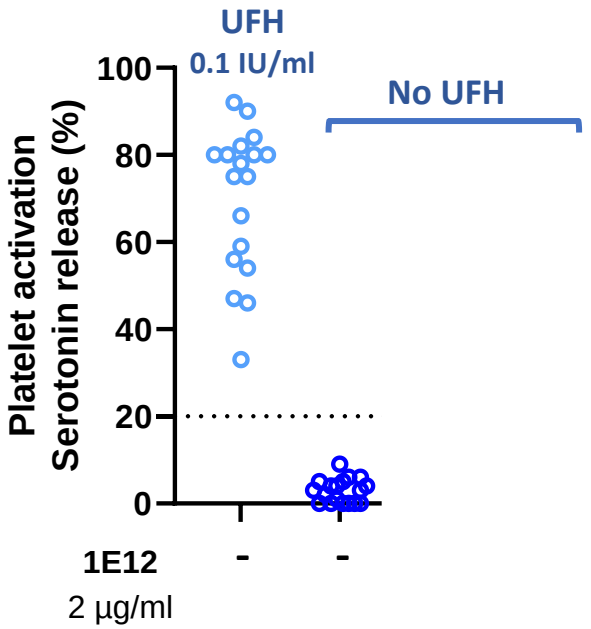
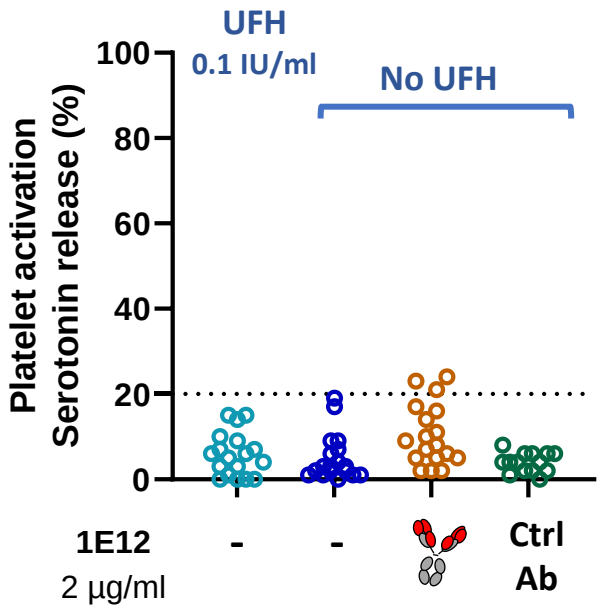




HIT Plasmas
(ELISA + and SRA +) (n=18)

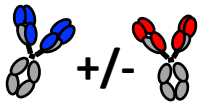
Non-pathogenic plasmas
(ELISA + and SRA -) (n=18)





Synergistic effect of 1E12 and 5B9 on thrombus formation

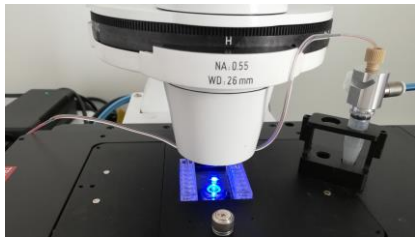
Whole blood from healthy donor



10 min incubation

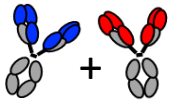
Ca2+

Microfluidic system in flow chamber coated with vWF (Cellix)



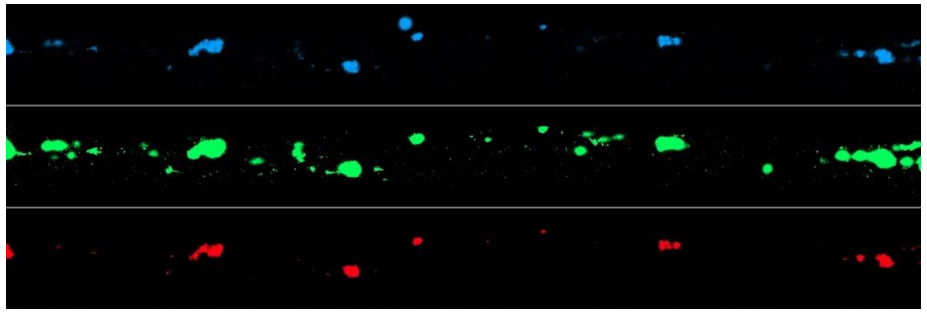
8 min perfusion at 20µl/min (500 s^{-1})

5B9 100µg/ml
+ 1E12 2µg/ml



5B9 (Anti-PF4/H Ab) + **1E12 (Anti-PF4 Ab)**

500 s^{-1}



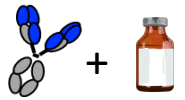
Leucocytes

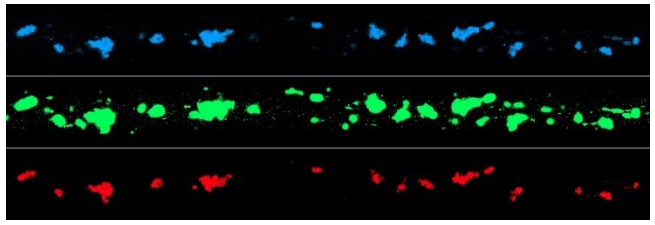
Platelets

Fibrin(ogen)

Fibrin-rich platelet-leukocytes aggregates

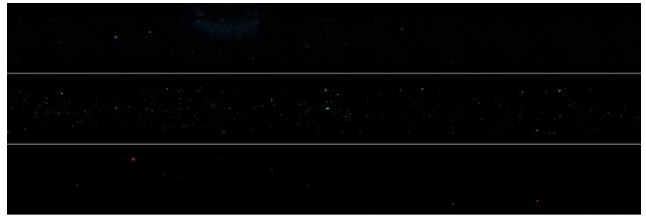
5B9 100µg/ml +
Héparine 1IU/ml



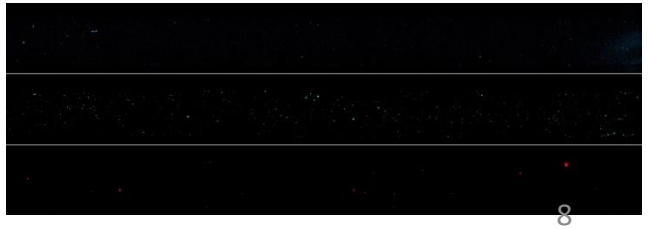


1E12 2µg/ml

5B9 100µg/ml



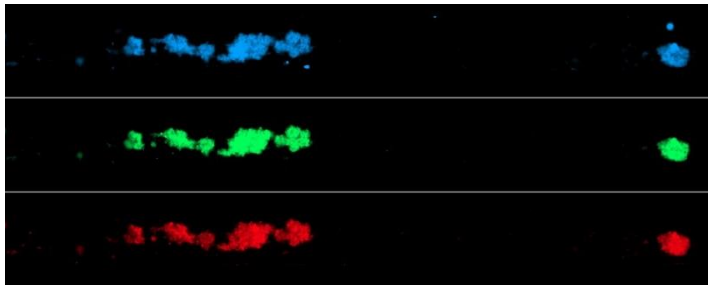
5B9 100µg/ml + Ctrl Ab 2µg/ml



Synergistic effect of 1E12 and 5B9 on thrombus formation prevents by IdeS or IVIg

1E12 2µg/ml
+ 5B9 100µg/ml

500 s^{-1}



+

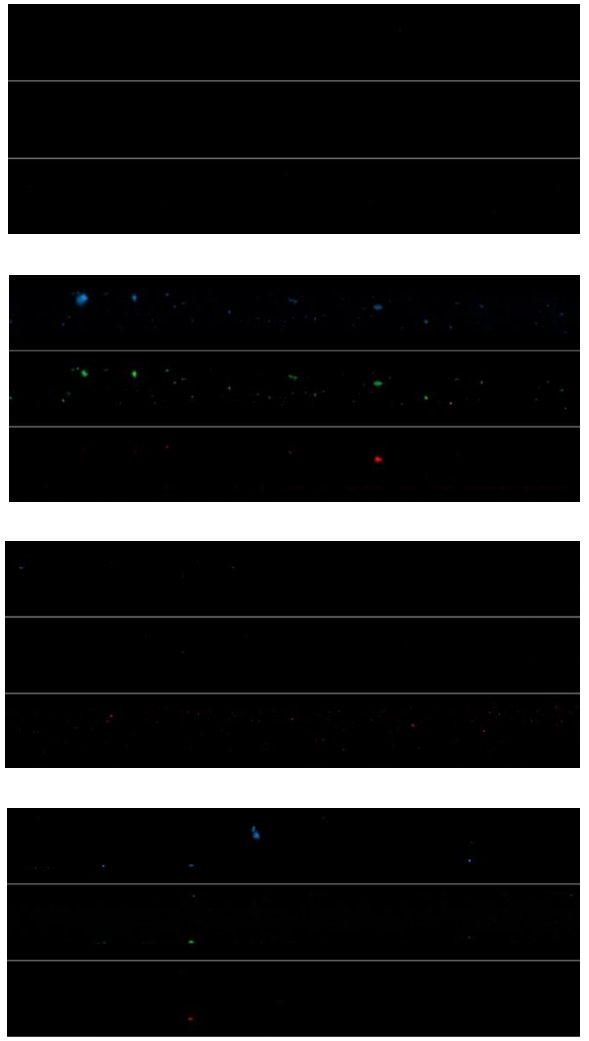
+ UFH
(100 IU/ml)

+ IV.3
(10µg/ml)

+ IVIg
(20mg/ml)

+ IdeS
(0.02 U/mg IgG)

500 s^{-1}

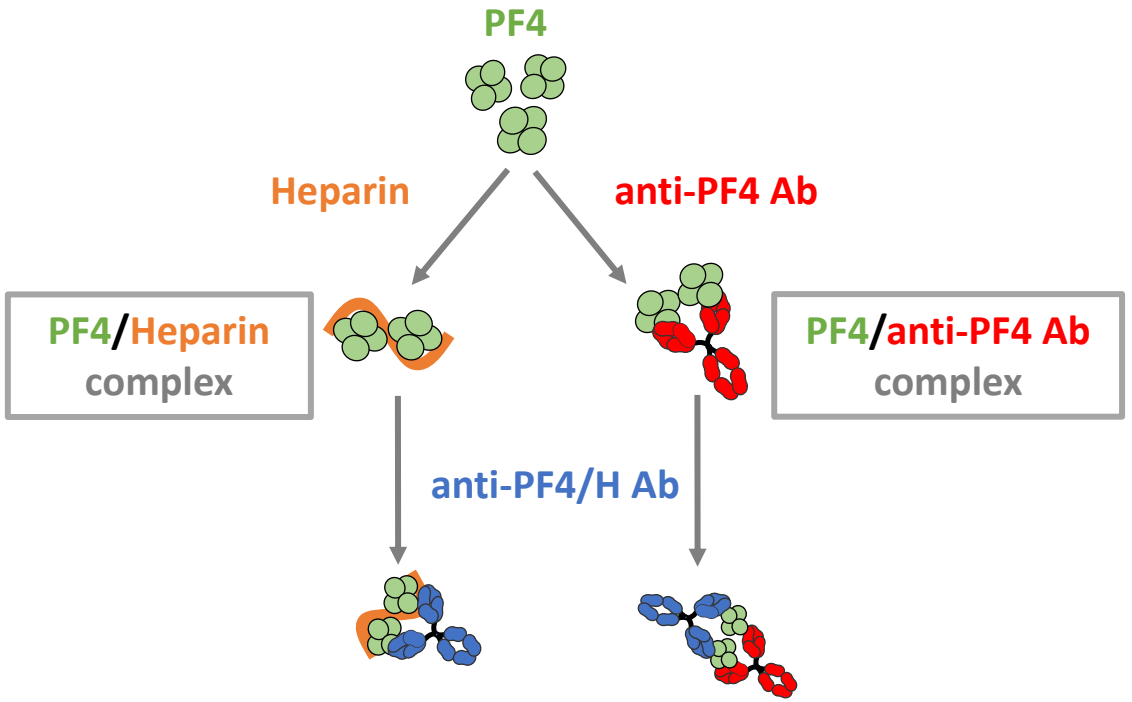


Synergistic effect between 1E12 and 5B9

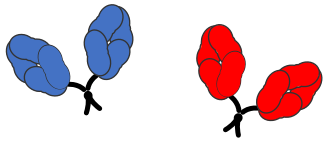
Which mechanism 

Fc fragments of PF4 specific Abs plays an important role in this synergy

1st Hypothesis: **1E12 changes the PF4 conformation** in the same way as heparin



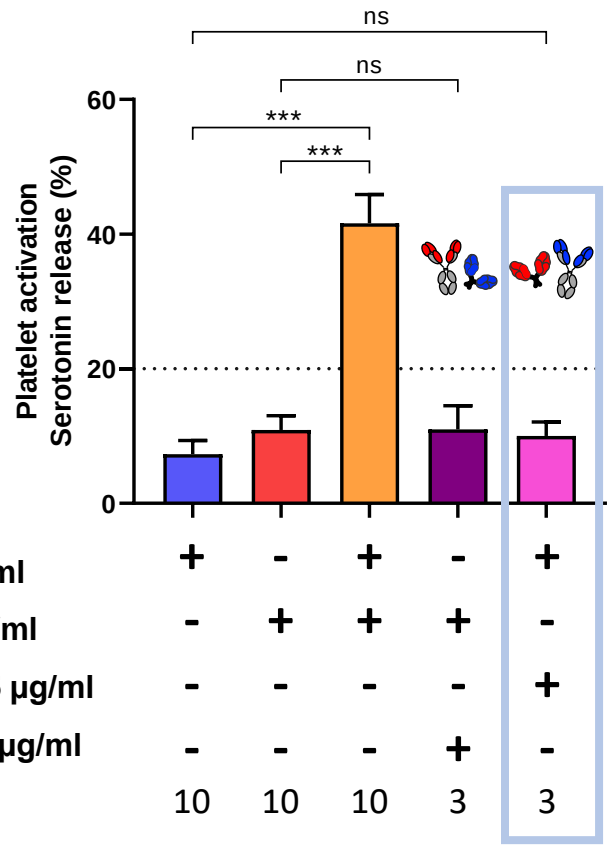
Nguyen, Nat Comm, 2017



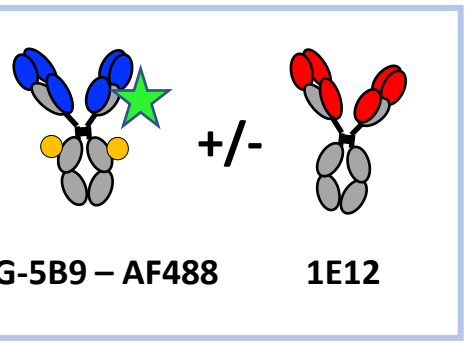
F(ab')2 5B9 and **F(ab')2 1E12**

→ No synergy with F(ab')2 Ab

SRA

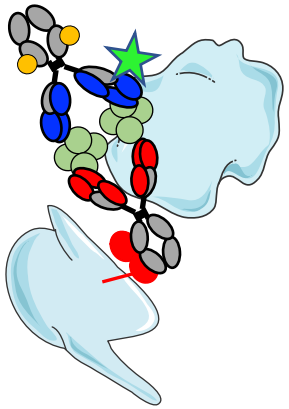


1E12 and 5B9 both increase the other one binding on platelets



Flow cytometry

DG-5B9 fixation on platelets from healthy donor



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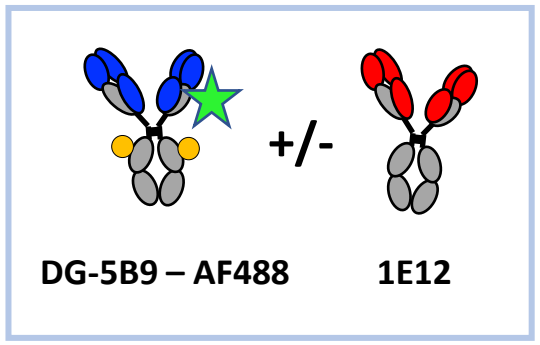
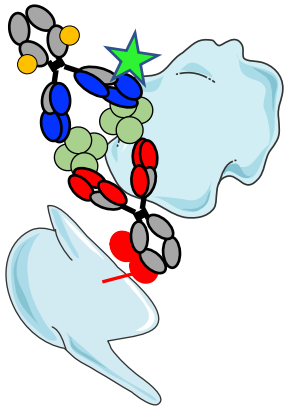
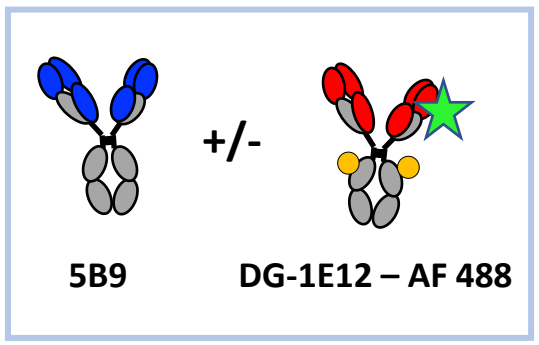


Flow cytometry

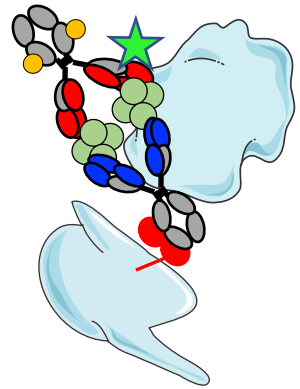
DG-5B9 fixation on platelets from healthy donor

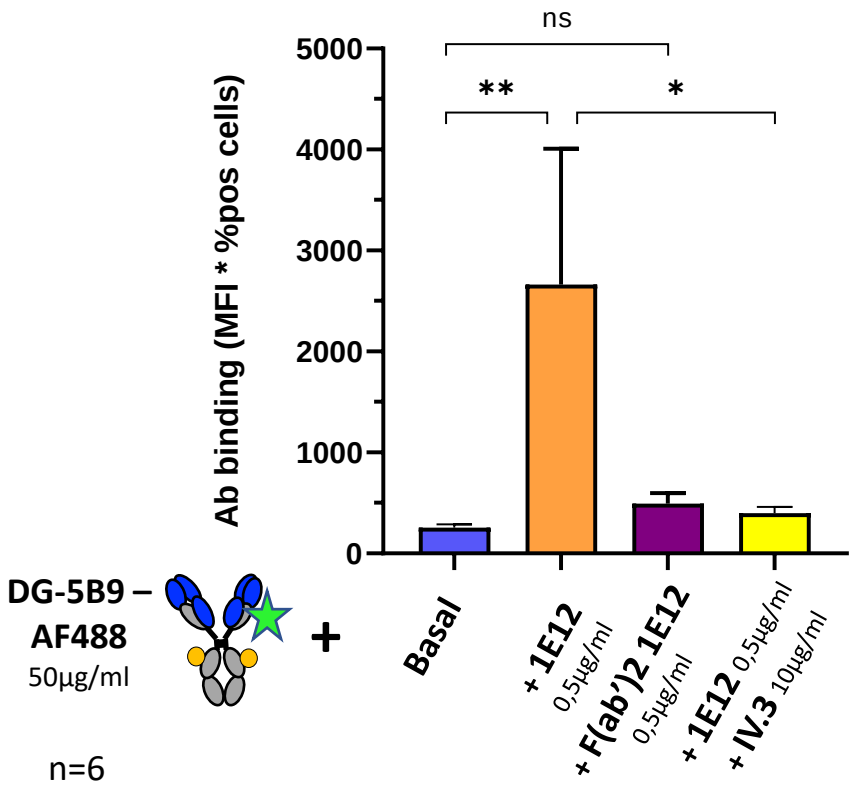




Flow cytometry

DG-1E12 fixation on platelets from healthy donor





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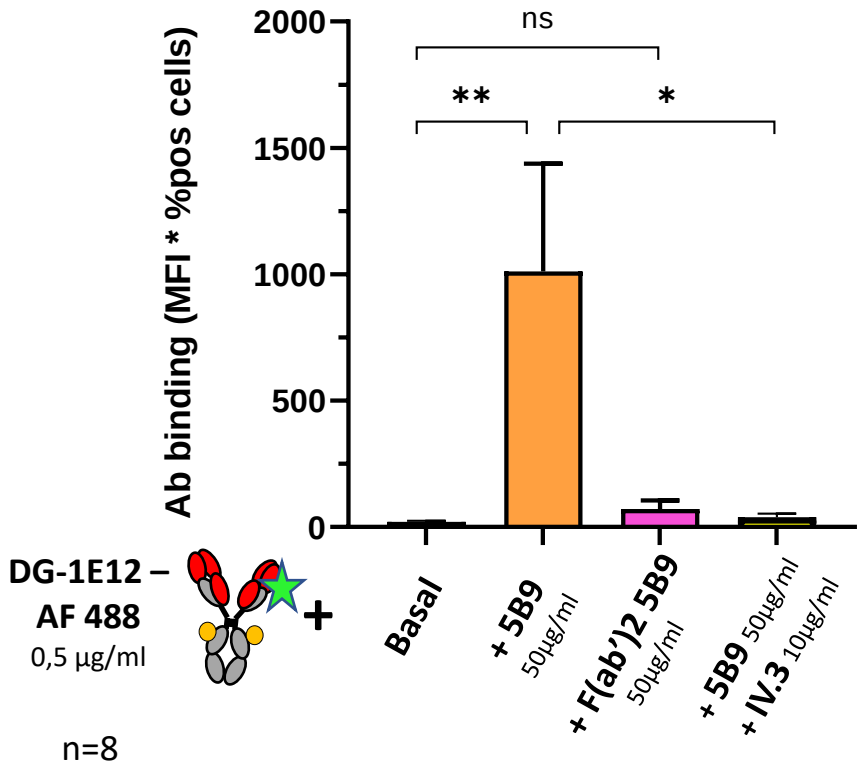
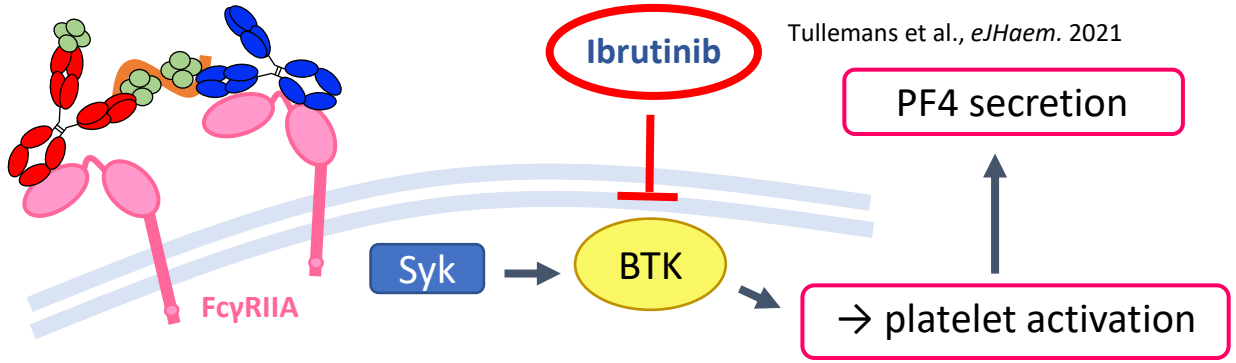
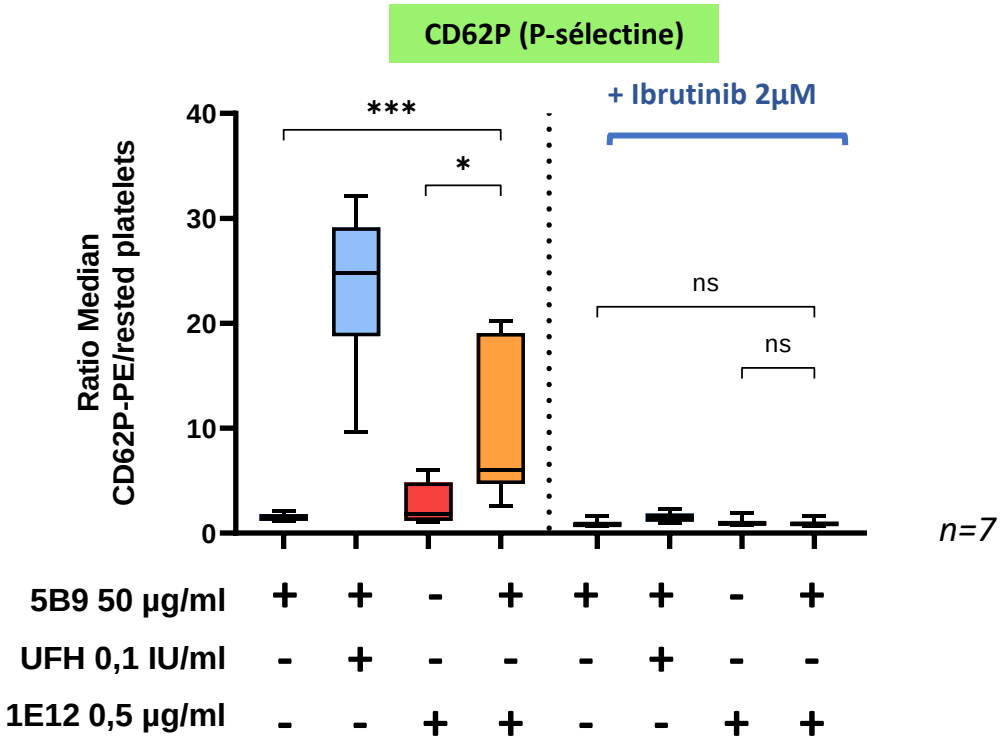
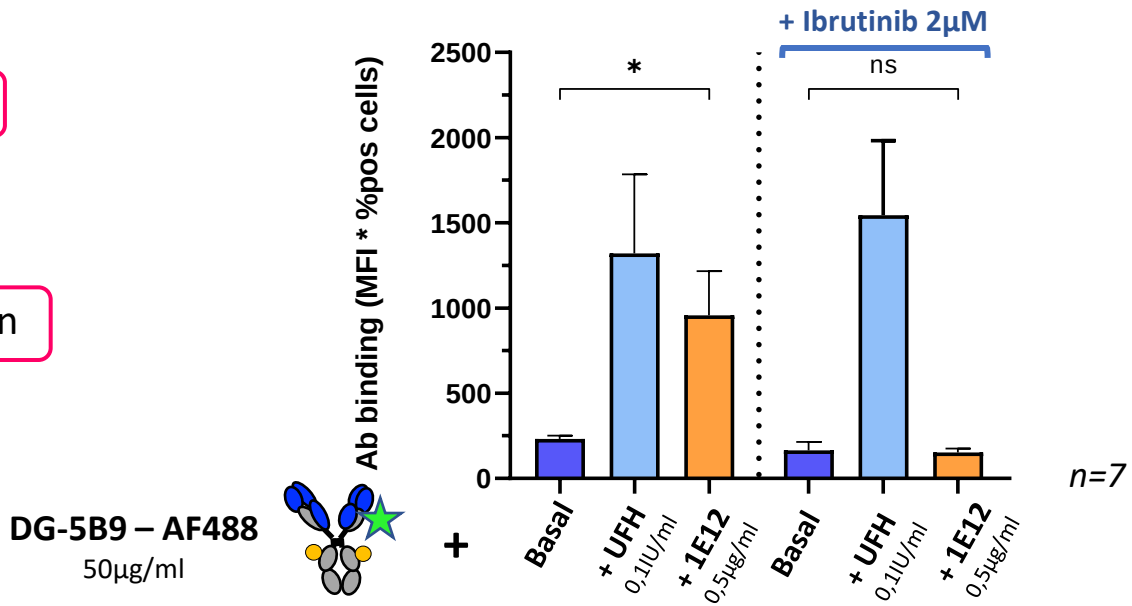


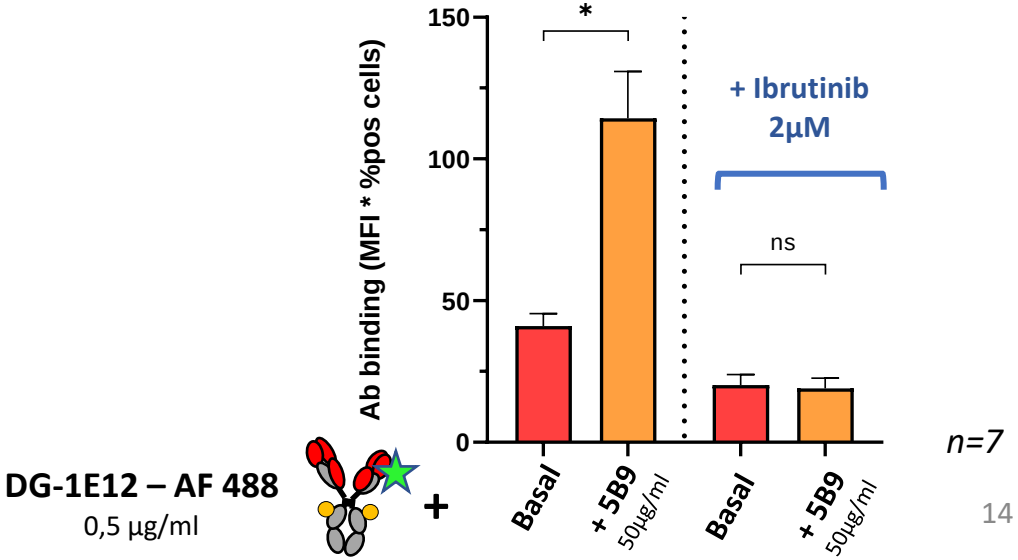
n=8

Mechanism FcgRIIA dependant





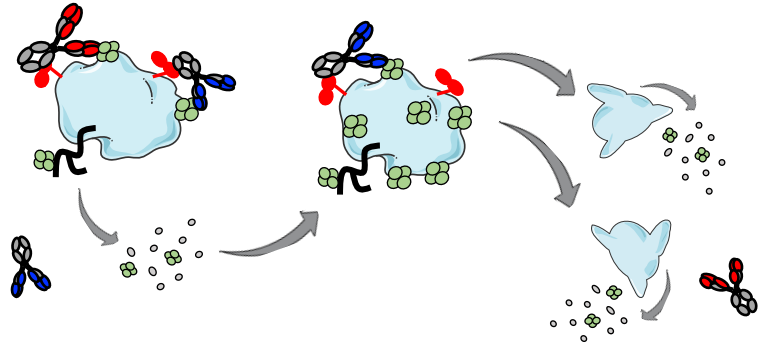




Conclusion

Synergic effect between 2 PF4-specific antibodies without heparin

- Increase platelet activation and promotes thrombosis formation *in vitro*
- Fc fragment of PF4 specific Abs play an important role in this synergy
- PF4 secretion induced by both Abs, even in 'sub-optimal' conditions ?



This effect may promotes platelet activation and increases **thrombotic risk in HIT patients** even after stopping heparin

Thank you

Jérôme Rollin
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Johanna Augros
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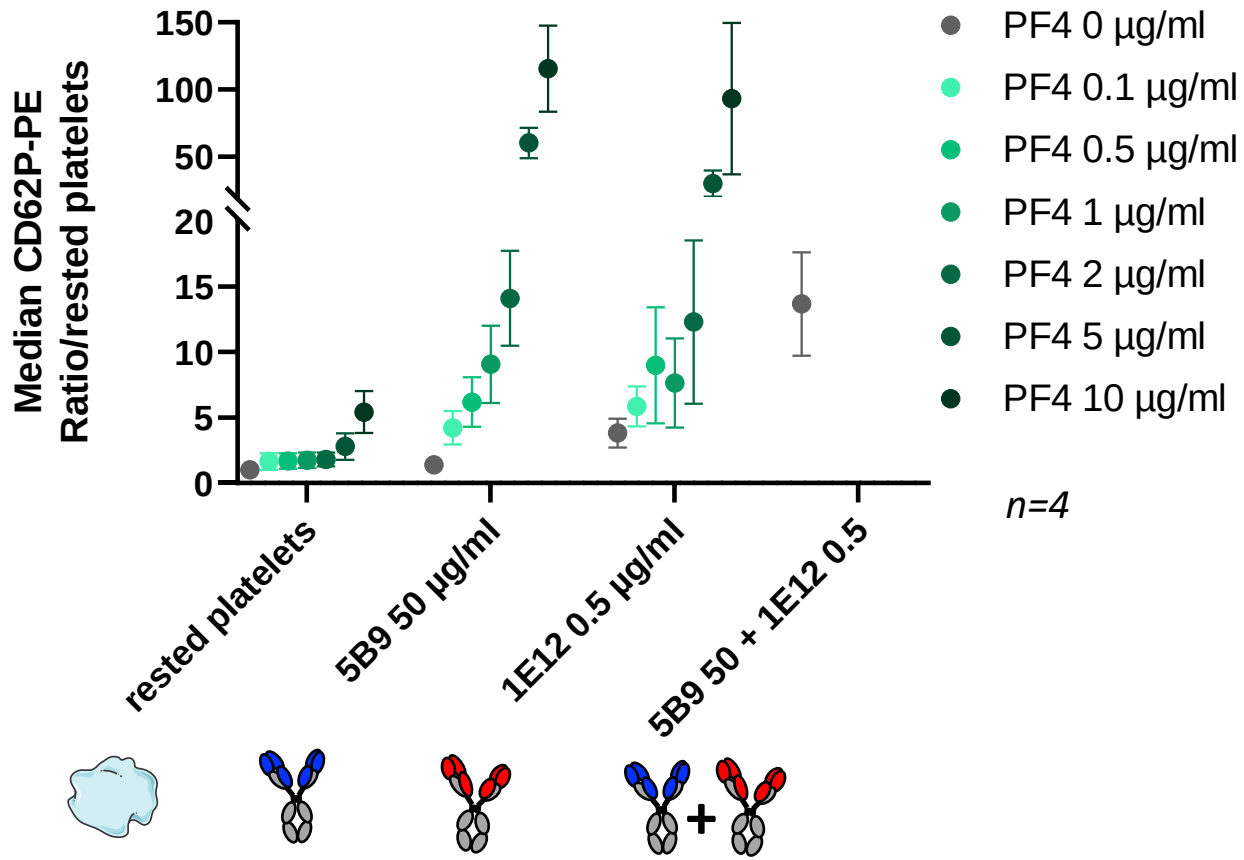
Merveille Atsouawe
Noémie Charuel
Loïs Coënon

Sébastien Roger - T2i team
Valérie Gouilleux - CEPR team

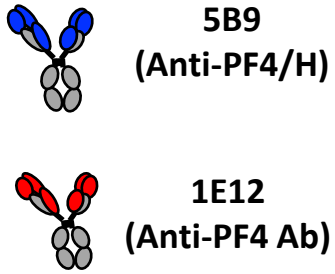
Fundings: & MAbImprove LabEx Tours Montpellier

Small quantities of PF4 released by platelets



Synergic effect between anti-PF4/H 5B9 and anti-PF4 1E12 in 'sub-optimal' conditions induces **PF4 secretion** by platelets, even in the absence of heparin

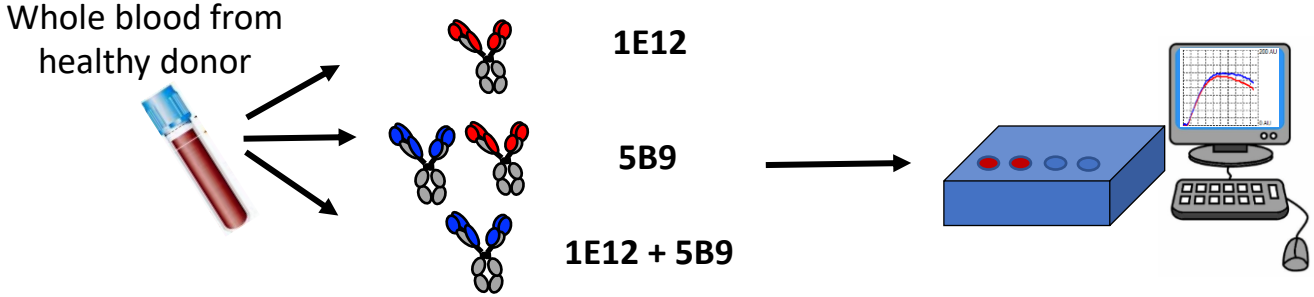
Synergistic effect of 1E12 and 5B9 on platelet aggregation

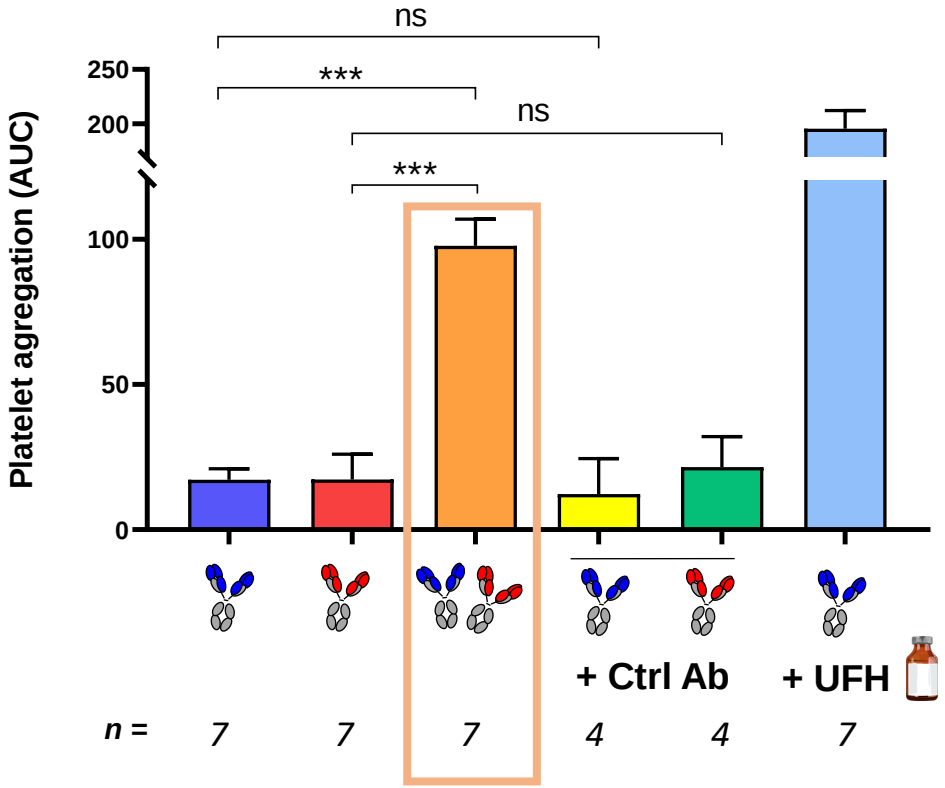


5B9
(Anti-PF4/H)

1E12
(Anti-PF4 Ab)

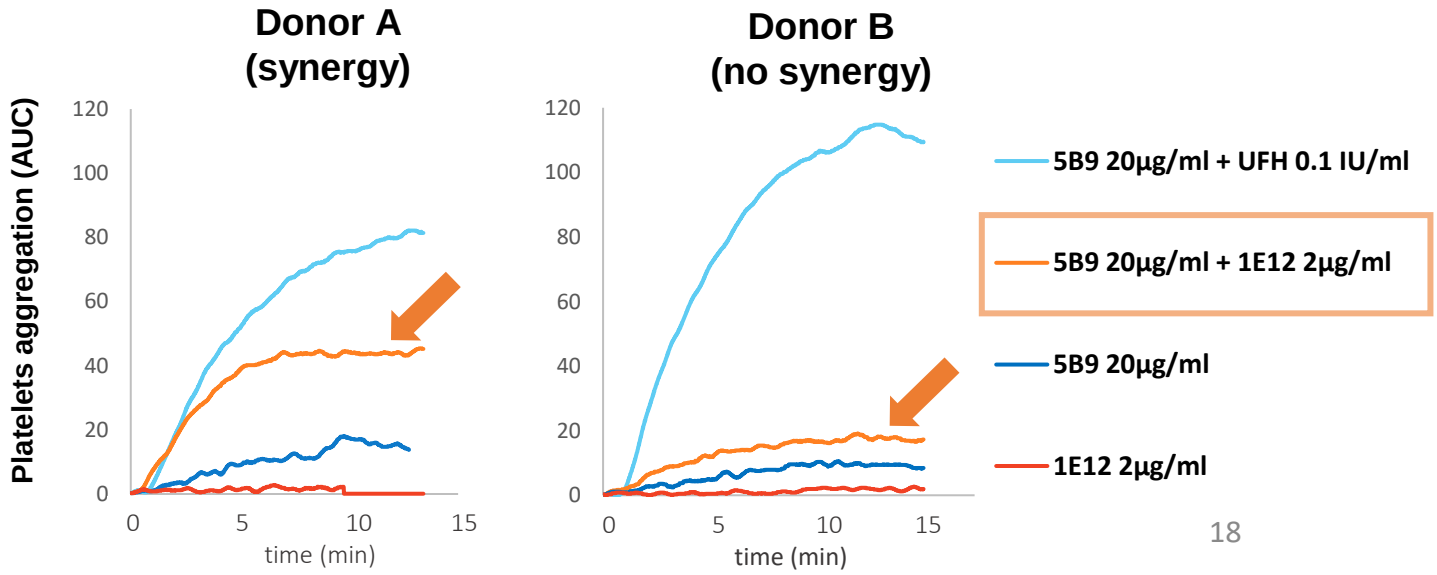
Multiple electrode aggregometry





Synergistic effect observed on 7 over 13 tested donors

→ Variability of response in whole blood



Perspective

Role of this synergy on HIT murin model ?

Human FcγRIIA, hPF4-transgenic, mPF4 knockout

(Collab. Yotis Senis – Inserm U1255, Strasbourg)