Clinical and Laboratory Characterization of Platelet Dysfunction Caused by Ibrutinib Treatment in Patients with Chronic Lymphocytic Leukemia

Maria Adele Alberelli*, Idanna Innocenti°, Francesco Autore°, Luca Laurenti°, Erica De Candia*

*Unità Operativa Malattie Emorragiche e Trombotiche
°Istituto di Ematologia
Fondazione Policlinico Universitario Agostino gemelli
Università Cattolica del Sacro Cuore

Gemelli
Fondazione Policlinico Universitario A. Gemelli
Università Cattolica del Sacro Cuore

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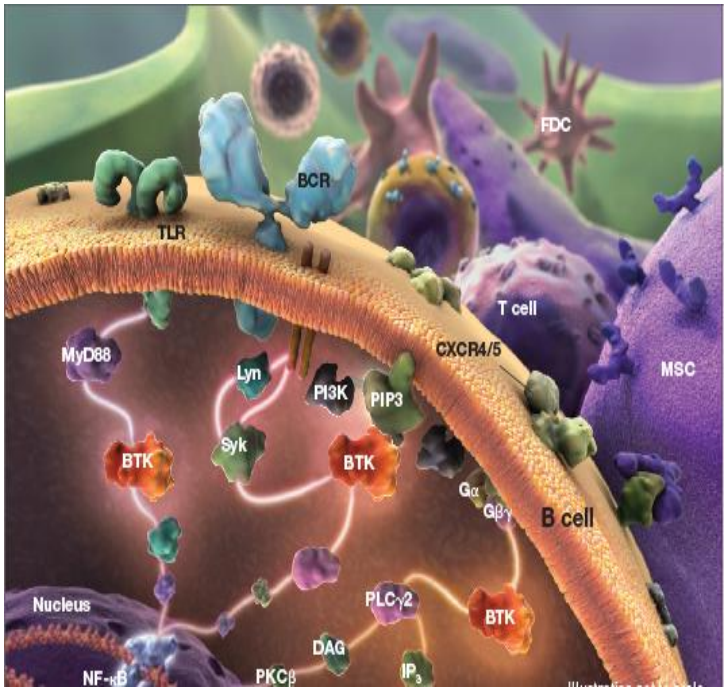
Background.I

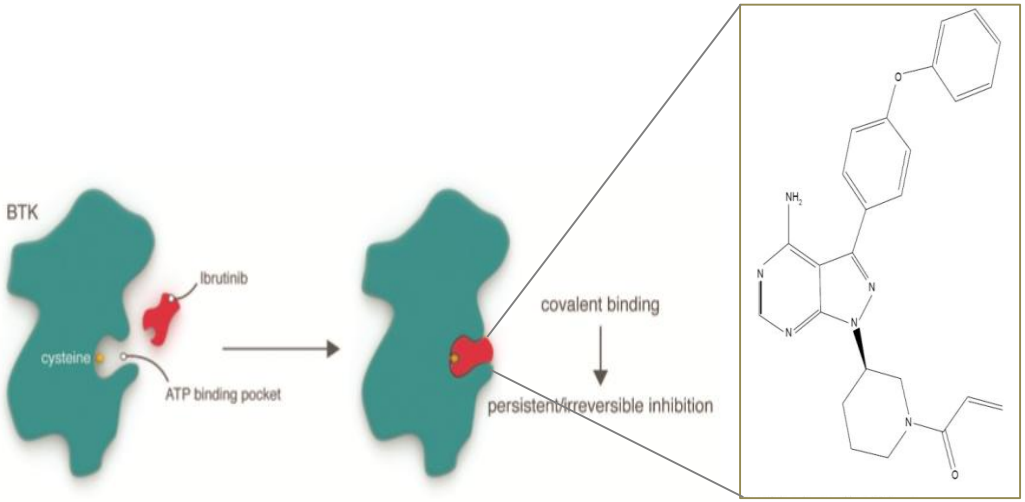
- Chronic lymphocytic leukemia (CLL) is the most common leukemia occurring among aged adults, who have often several comorbidities

- Bruton tyrosine kinase (BTK) inhibitors are effective drugs for the treatment of CLL and other lymphoproliferative diseases, showing high response rates and prolonged disease-free survival

- Ibrutinib, the first BTK inhibitor used in clinical trials for CLL therapy, is now approved as first line therapy in CLL with deletion 17p or TP53 gene mutation and in patients with refractory recurrent CLL

Mechanism of BTK inhibition by ibrutinib





Ibrutinib binds covalently and irreversibly to a cysteine residue (Cys 481) in the active site of BTK, an enzyme downstream the signaling of B-cell-receptors (BCR).

(Weistner, Haematologica 2015)

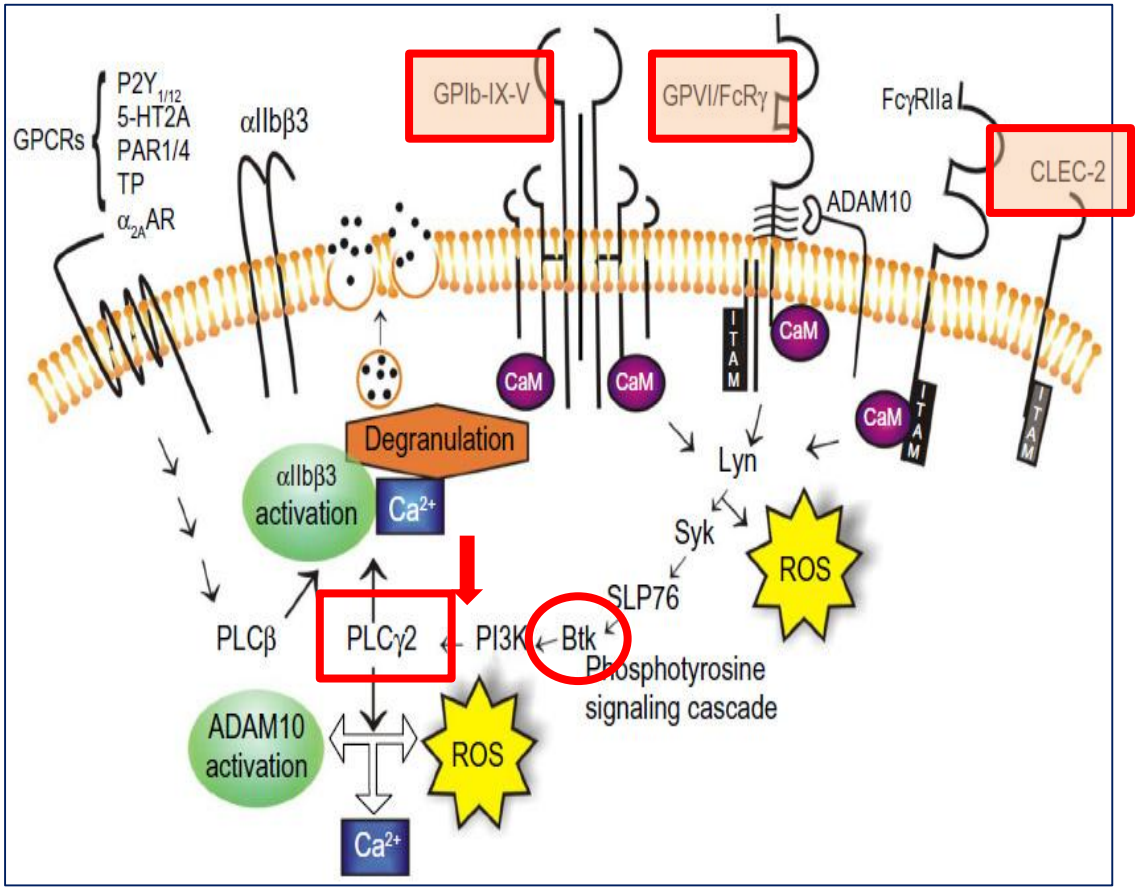
Background.II

- Treatment of CLL patients with ibrutinib is associated with bleeding-related adverse events (61% minor bleedings, 5% major bleedings)

- Bleedings have been attributed to target inhibition of BTK and off-target inhibition of Tec in platelets. Both kinases mediate signalling of GpVI, GpIb and CLEC2 receptors

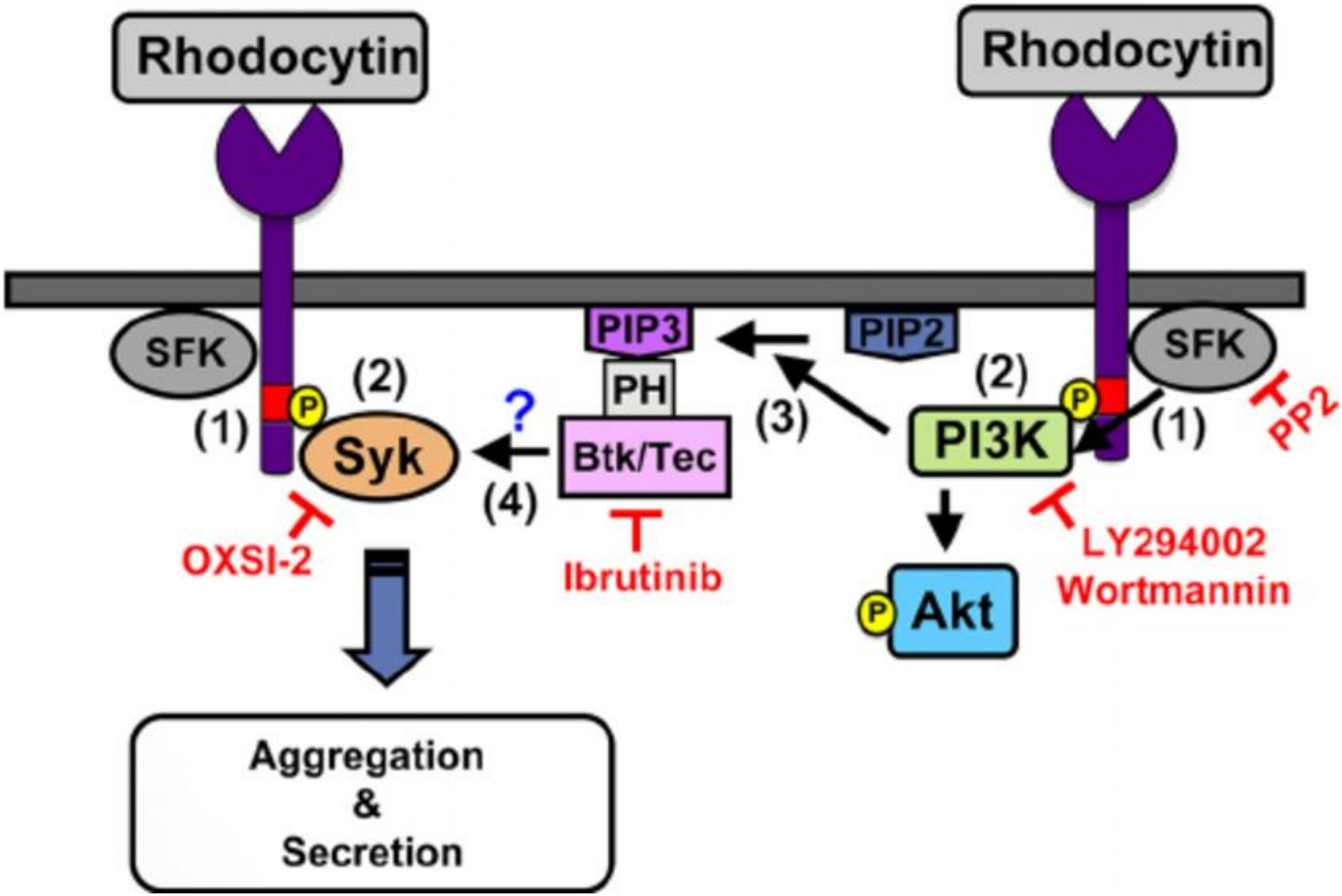
BTK/TEC in Platelets

Platelets



- **BTK has been described in platelets** (Quek et al. 1998; Laffargue et al. 1999).
- **It is activated downstream the GPVI, GPIb-IX-V complex and CLEC-2**
- **The signalling of these receptors is mediated by phosphorylation of phospholipase PLCγ2 by two kinases belonging to the family of Tec *protein tyrosine kinases* , BTK and TEC** (Atkinson BT et al. 2003).

Ibrutinib inhibits both Btk and Tec



Kunapuli JBC, 2015

Ibrutinib and bleedings

Blood. 2014 Dec 18;124(26):3991-5. doi: 10.1182/blood-2014-06-583294. Epub 2014 Oct 10.

Ibrutinib treatment affects collagen and von Willebrand factor-dependent platelet functions.

Levade M[1], David E[2], Garcia C[2], Laurent PA[3], Cadot S[2], Michallet AS[4], Bordet JC[5], Tam C[6], Sié P[1], Ysebaert L[7], Payrastre B[1].

Leukemia. 2015 Apr;29(4):783-7. doi: 10.1038/leu.2014.247. Epub 2014 Aug 20.

Ibrutinib inhibits collagen-mediated but not ADP-mediated platelet aggregation.

Kamel S[1], Horton L[1], Ysebaert L[2], Levade M[3], Burbury K[4], Tan S[1], Cole-Sinclair M[1], Reynolds J[5], Filshie R[1], Schischka S[1], Khot A[4], Sandhu S[4], Keating MJ[6], Nandurkar H[7], Tam CS[8].

Haematologica. 2015 Dec;100(12):1571-8. doi: 10.3324/haematol.2015.126672. Epub 2015 Oct 1.

Incidence and risk factors of bleeding-related adverse events in patients with chronic lymphocytic leukemia treated with ibrutinib.

Lipsky AH[1], Farooqui MZ[2], Tian X[3], Martyr S[2], Cullinane AM[4], Nghiem K[4], Sun C[2], Valdez J[2], Niemann CU[2], Herman SE[2], Saba N[2], Soto S[2], Marti G[2], Uzel G[5], Holland SM[5], Lozier JN[6], Wiestner A[7].

Aims of the study

- **To investigate whether CLL *per se* causes a platelet dysfunction and to characterize ibrutinib-induced platelet dysfunction in lymphoproliferative diseases**

- **To characterize biomarkers for identification of patients at greater risk of bleeding events**

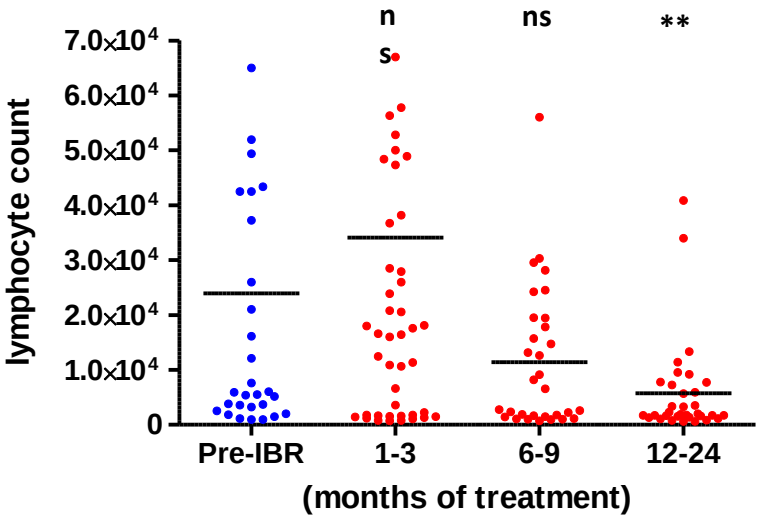
Study Design

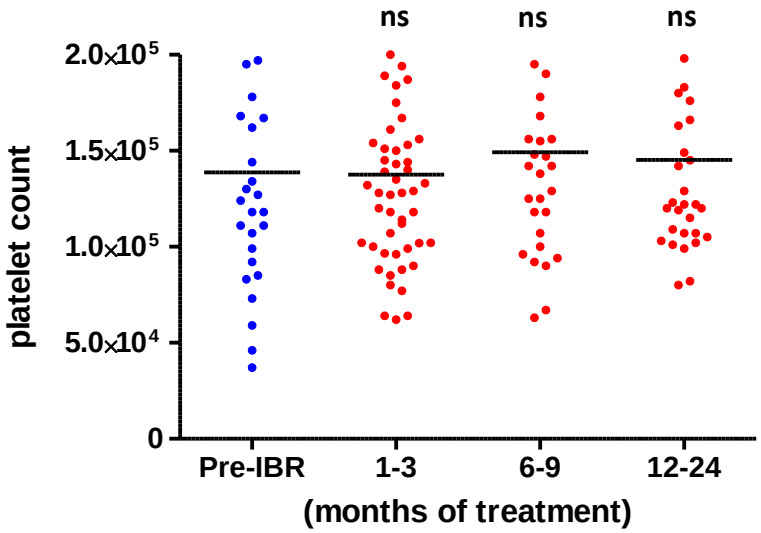
- 23 patients with CLL and 4 patients with Waldenstrom macroglobulinemia treated with 420mg/day oral ibrutinib were enrolled.
- 3 patients with CLL treated with acalabrutinib (ACP-196) 200 mg/die were studied
- 5 patients treated with different chemio-immunotherapy protocols were studied as control group
- Patients under antithrombotic treatment not included in the study
- Clinical and laboratory assessment was performed at baseline and after 1,3,6,9,12,24 months of treatment
- The following tests were performed:
  - platelet aggregation by 2-3.3-10 ug/mL collagen , 2-4 uM ADP, 25 uM PAR1-AP, arachidonic acid 1 mM, ristocetin 0.6, 1.2 mg/mL
  - Whole blood filtroaggregometry (PFA collagen/epinefrine and collagen/ADP)
  - vonWillebrand factor antigen and ristocetin cofactor measurements

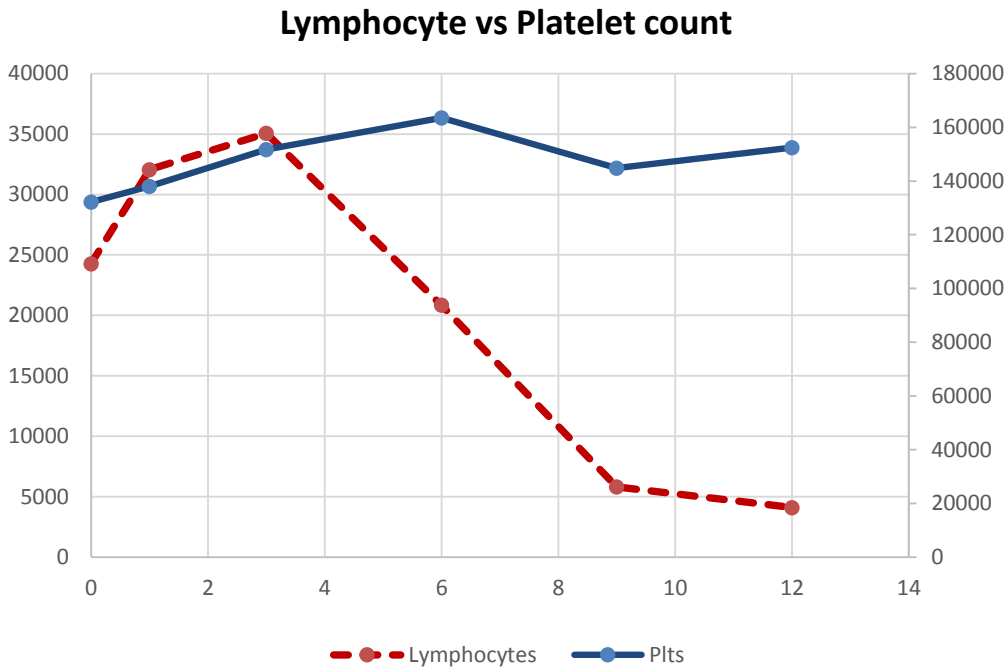
Bleedings in BTK inhibitors treated patients

ID PATIENT	SEX	AGE	DISEASE	TREATMENT	NATURE OF BLEEDING
# 1	M	65	CLL	Ibrutinib	Bruising
# 2	M	62	CLL	Ibrutinib	–
# 3	M	68	CLL	Ibrutinib	Petechiae
# 4	F	71	CLL	Ibrutinib	Bruising
# 5	M	57	CLL	Ibrutinib	Bruising
# 6	M	75	CLL	Ibrutinib	Bruising
# 7	M	68	CLL	Ibrutinib	–
# 8	M	68	CLL	Ibrutinib	Gastrointestinal Bleeding
# 9	M	69	CLL	Ibrutinib	Petechiae
# 10	M	82	CLL	Ibrutinib	Bruising
# 11	M	80	CLL	Ibrutinib	Bruising
# 12	M	61	CLL	Ibrutinib	Petechiae
# 13	M	68	CLL	Ibrutinib	–
# 14	F	63	CLL	Ibrutinib	Bruising
# 15	M	75	CLL	Ibrutinib	Bruising
# 16	M	36	CLL	Ibrutinib	Bruising
# 17	M	69	CLL	Ibrutinib	–
# 18	F	71	CLL	Ibrutinib	Bruising
# 19	M	61	CLL	Ibrutinib	–
# 20	M	63	CLL	Ibrutinib	–
# 21	M	79	CLL	Ibrutinib	–
# 22	M	70	CLL	Ibrutinib	Bruising
# 23	M	57	CLL	Ibrutinib	Petechiae
# 24	M	65	CLL	Acalabrutinib (ACP-196)	–
# 25	M	55	CLL	Acalabrutinib (ACP-196)	–
# 26	M	75	CLL	Acalabrutinib (ACP-196)	–
# 27	F	50	Waldenstrom	Ibrutinb	–
# 28	M	84	Waldenstrom	Ibrutinib	–
# 29	M	64	Waldenstrom	Ibrutinib	–
# 30	M	78	Waldenstrom	Ibrutinib	–

Effect of ibrutinib treatment on lymphocyte and platelet count





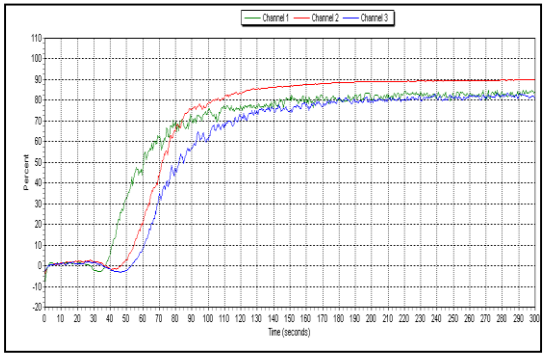


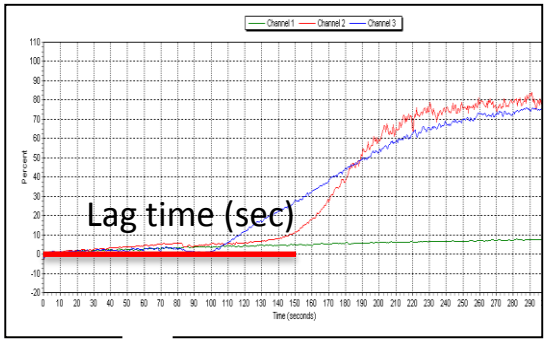
Effect of ibrutinib on collagen induced aggregation and bleedings

Basal

On treatment

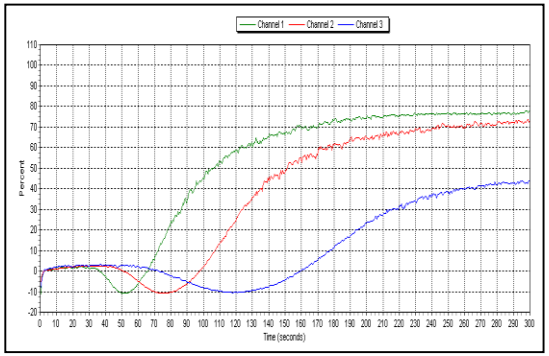
Pt #3

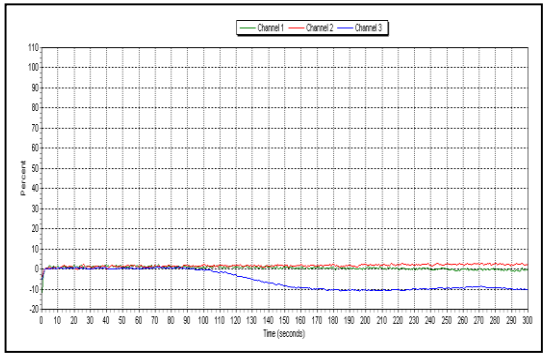




NO BLEEDING

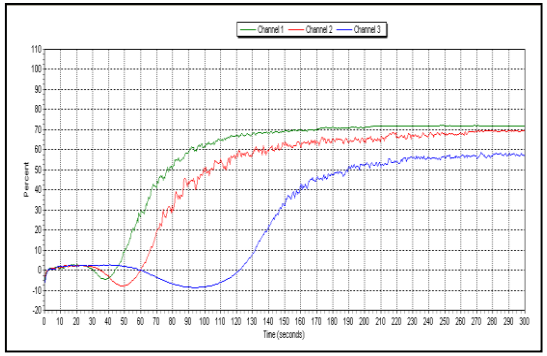
Pt #14

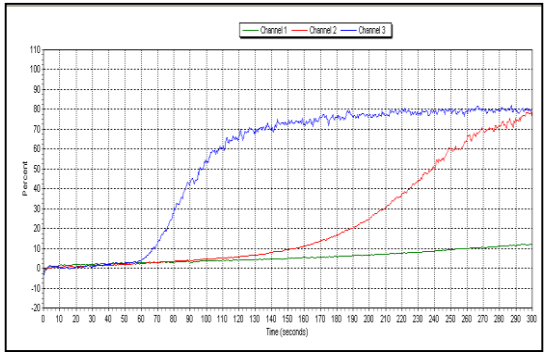




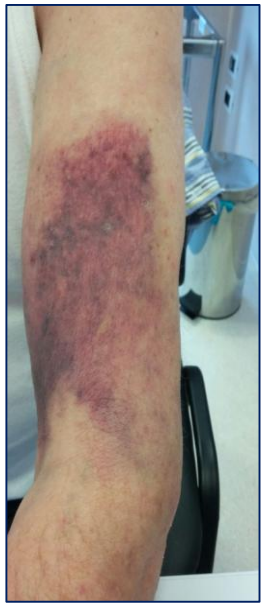
NO BLEEDING

Pt #10



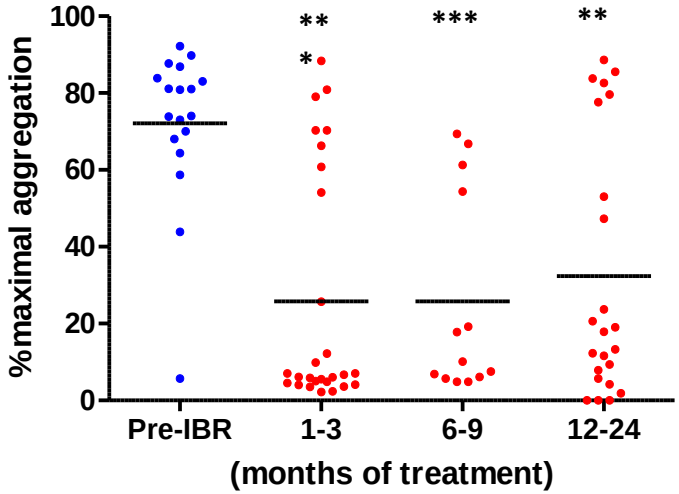


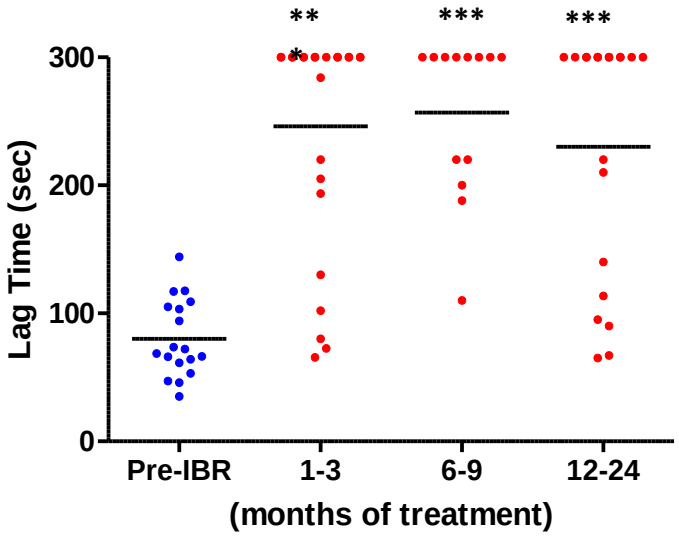
BLEEDING



Effect of ibrutinib on collagen induced aggregation

Collagen 2 µg/mL



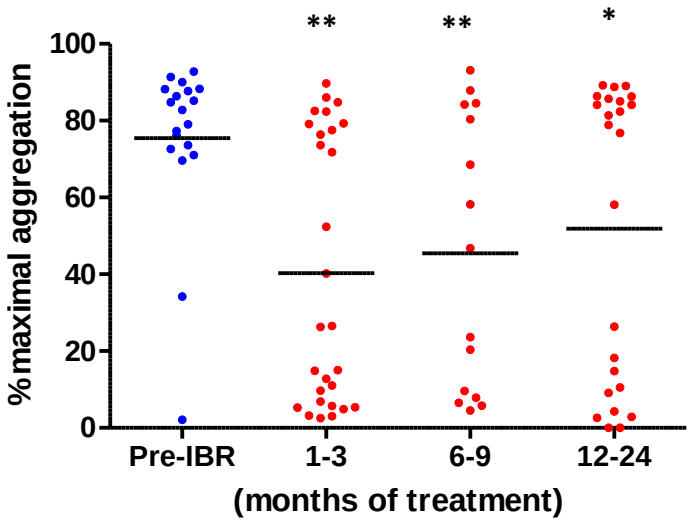


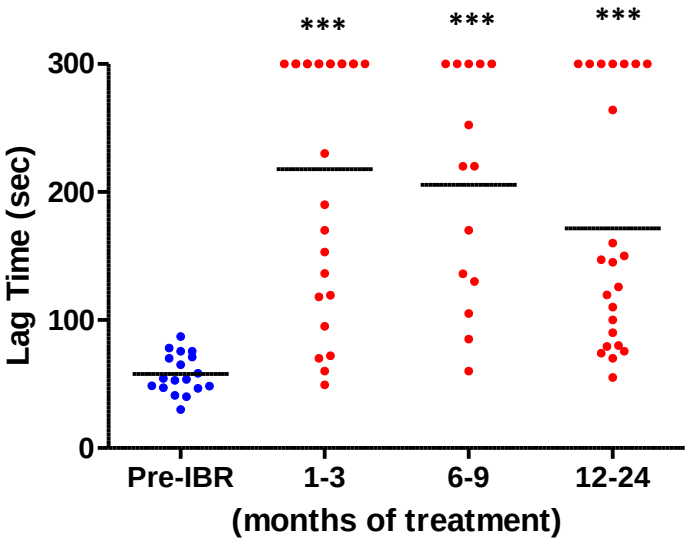
* p< 0.01

** p< 0.005

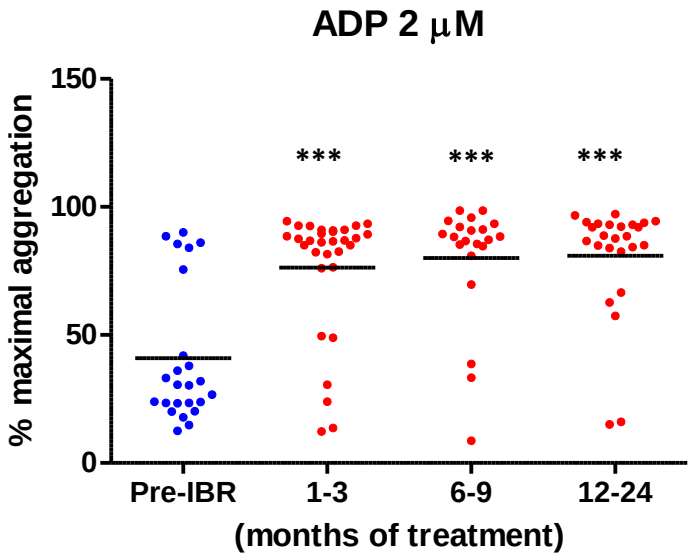
*** p< 0.0001

Collagen 3.3 µg/mL

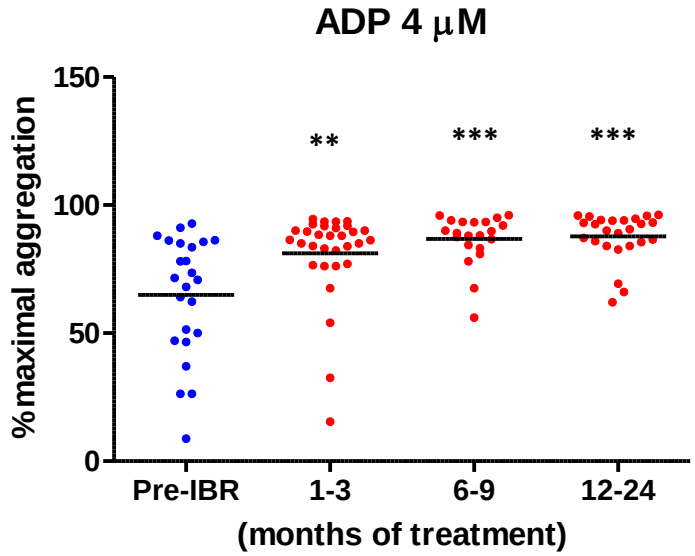




Effect of ibrutinib on ADP induced aggregation



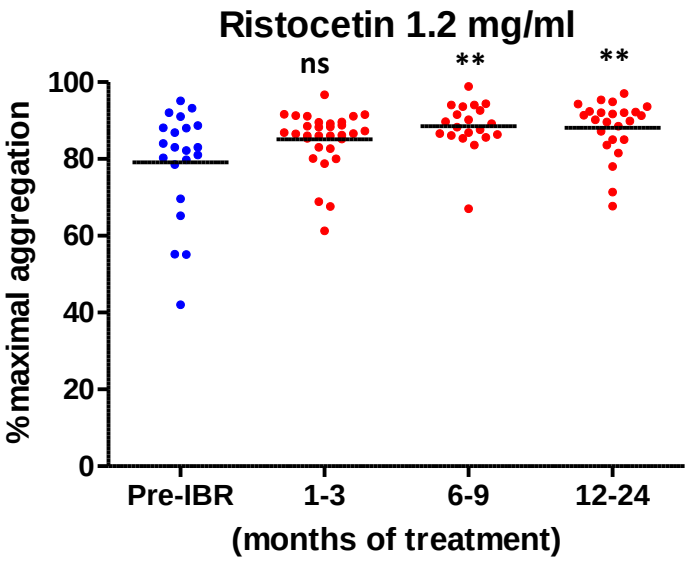
p <0.0001

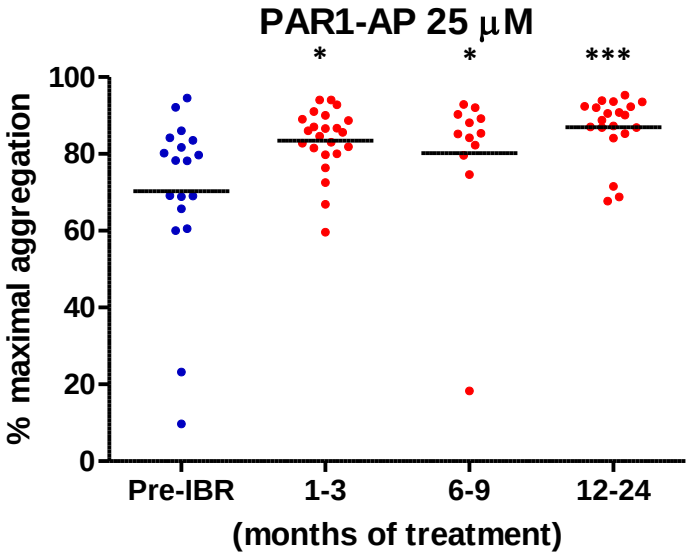


** p< 0.005

*** p< 0.0001

Effect of ibrutinib on aggregation by other agonists





* p< 0.01

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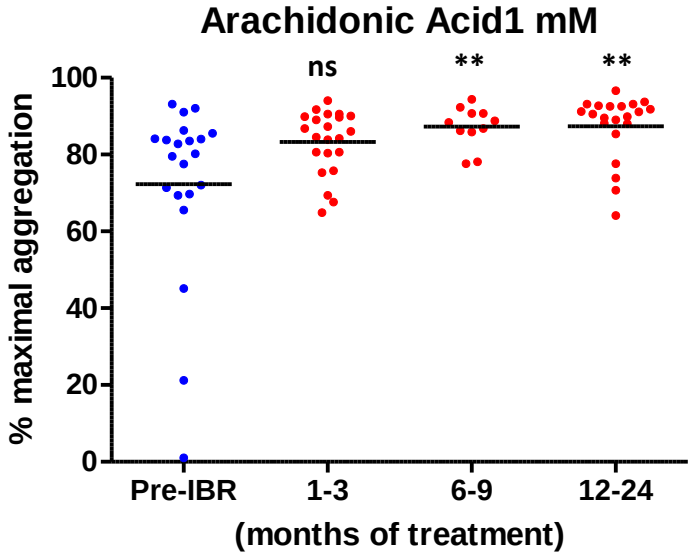
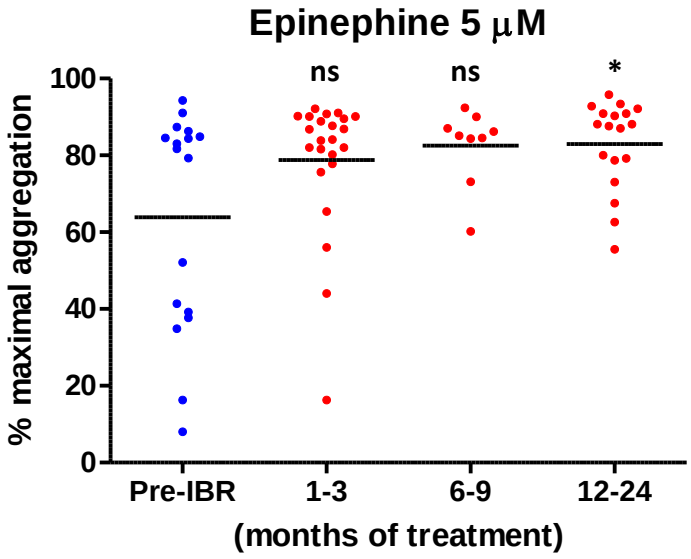
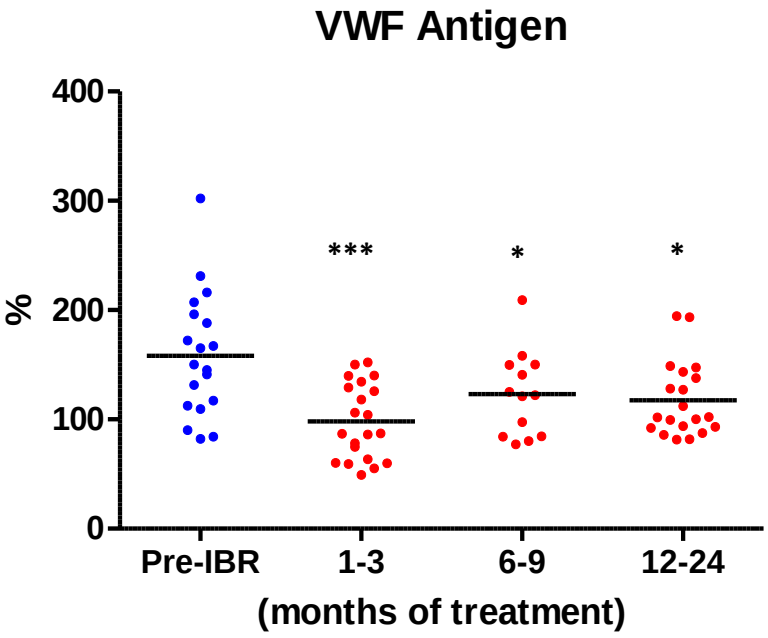
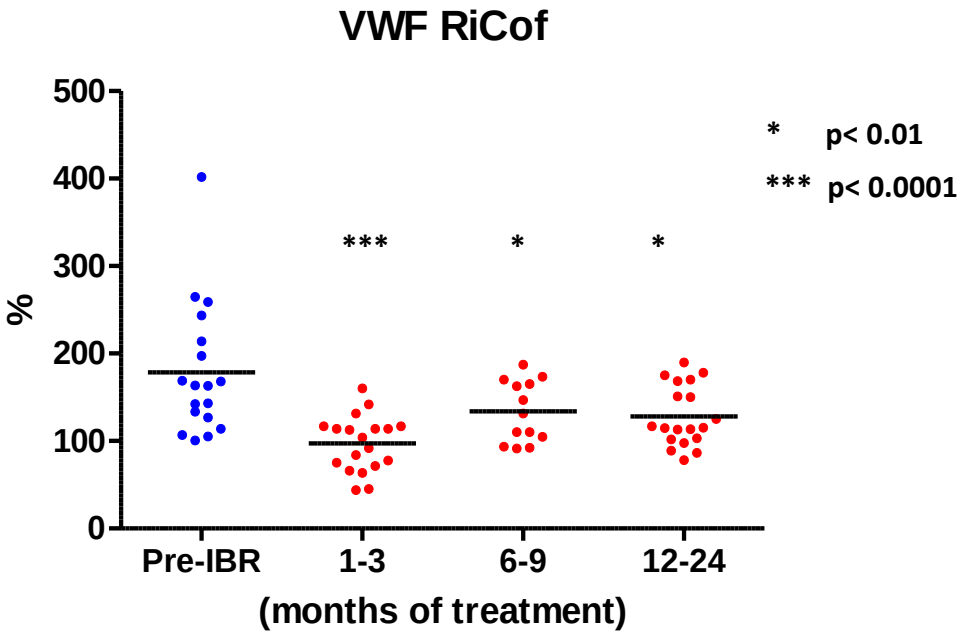


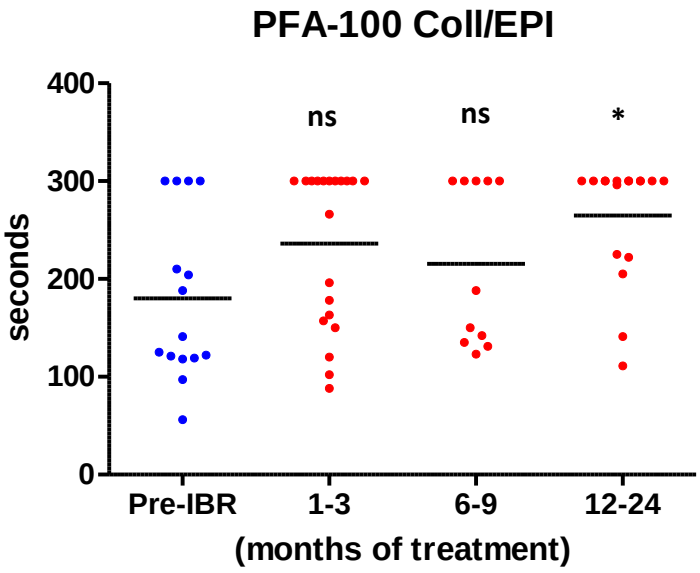


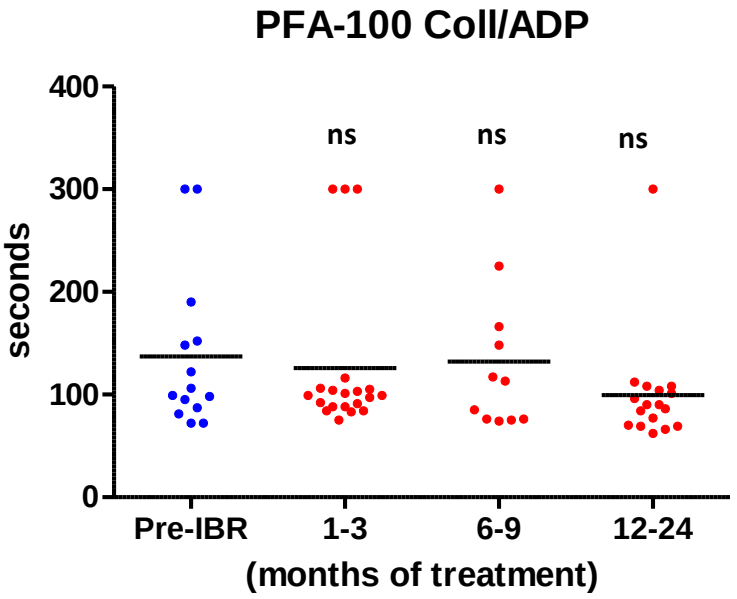
Plasma VonWillebrand factor in CLL





PFA-100 coll/epi and coll/ADP





- CLL patients have mild platelet dysfunction characterized by reduced platelet aggregation by ADP

- Under ibrutinib, the majority of CLL patients display specific abnormality of collagen-induced platelet aggregation

Is platelet dysfunction only a drug effect or there is a disease-related effect ?

- CLL vs Waldenstrom macroglobulinemia
- Stable CLL vs progressive CLL

Bleedings in BTK inhibitors treated patients

ID PATIENT	SEX	AGE	DISEASE	TREATMENT	NATURE OF BLEEDING
# 1	M	65	CLL	Ibrutinib	Bruising
# 2	M	62	CLL	Ibrutinib	–
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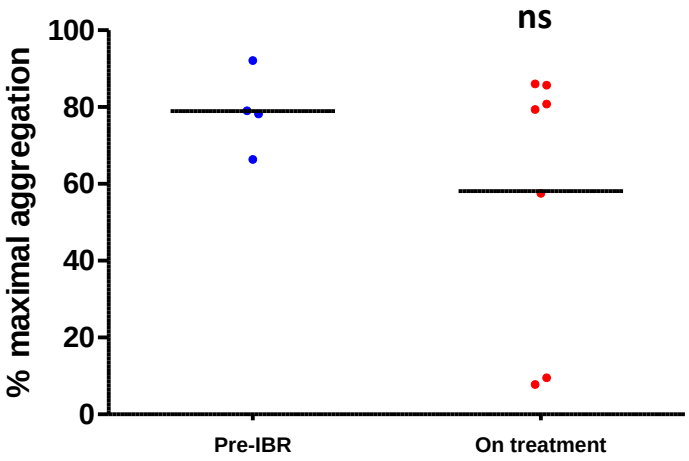
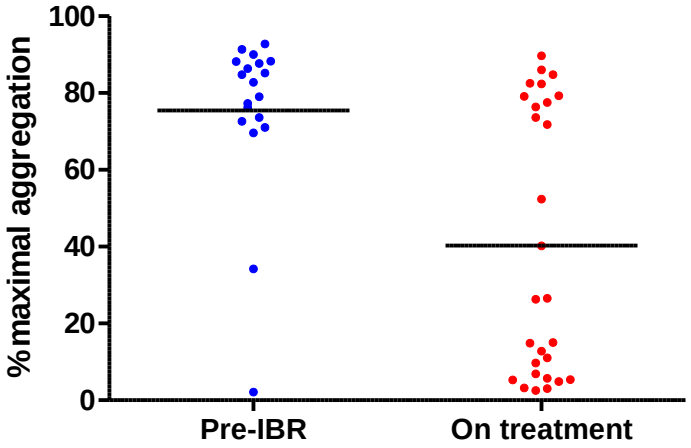
# 15	M	75	CLL	Ibrutinib	Bruising
# 16	M	36	CLL	Ibrutinib	Bruising
# 17	M	69	CLL	Ibrutinib	–
# 18	F	71	CLL	Ibrutinib	Bruising
# 19	M	61	CLL	Ibrutinib	–
# 20	M	63	CLL	Ibrutinib	–
# 21	M	79	CLL	Ibrutinib	–
# 22	M	70	CLL	Ibrutinib	Bruising
# 23	M	57	CLL	Ibrutinib	Petechiae
# 24	M	65	CLL	Acalabrutinib (ACP-196)	–
# 25	M	55	CLL	Acalabrutinib (ACP-196)	–
# 26	M	75	CLL	Acalabrutinib (ACP-196)	–
# 27	**F**	**50**	**Waldenstrom**	**Ibrutinb**	**–**
# 28	**M**	**84**	**Waldenstrom**	**Ibrutinib**	**–**
# 29	**M**	**64**	**Waldenstrom**	**Ibrutinib**	**–**
# 30	**M**	**78**	**Waldenstrom**	**Ibrutinib**	**–**

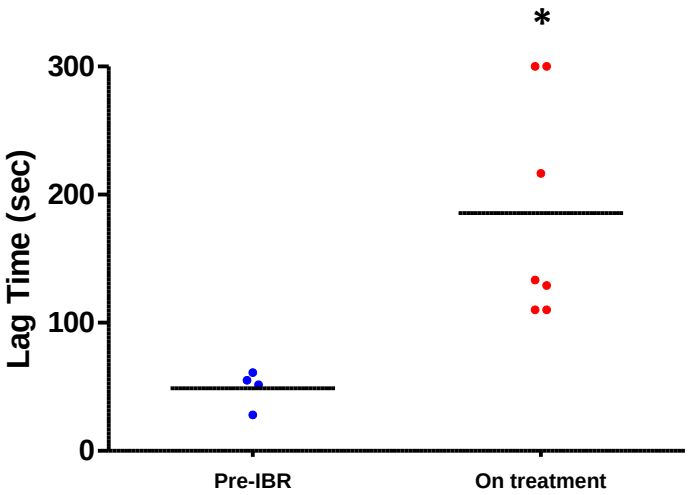
Waldenstrom Macroglobulinemia

Chronic Lymphocytic Leukemia

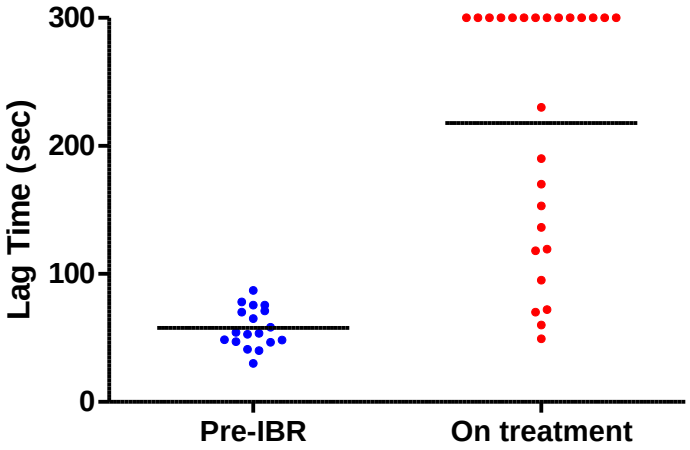
Collagen 3.3 ug/mL



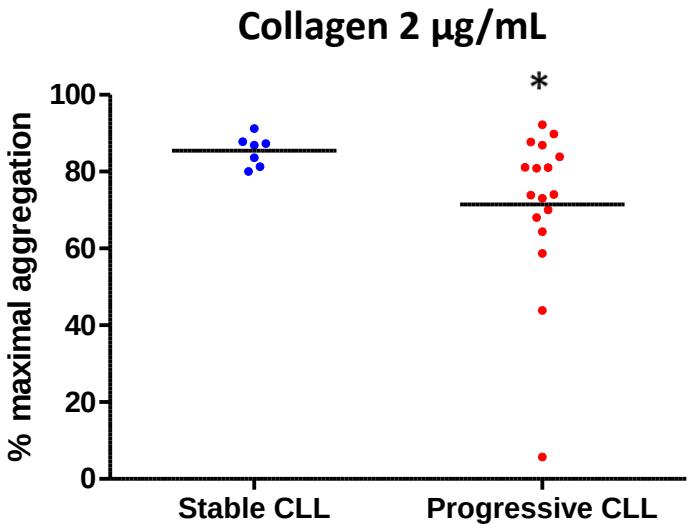


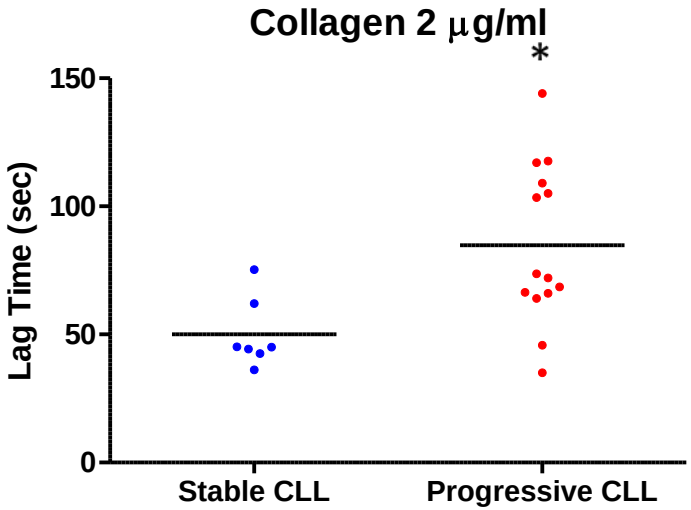


* P<0.05

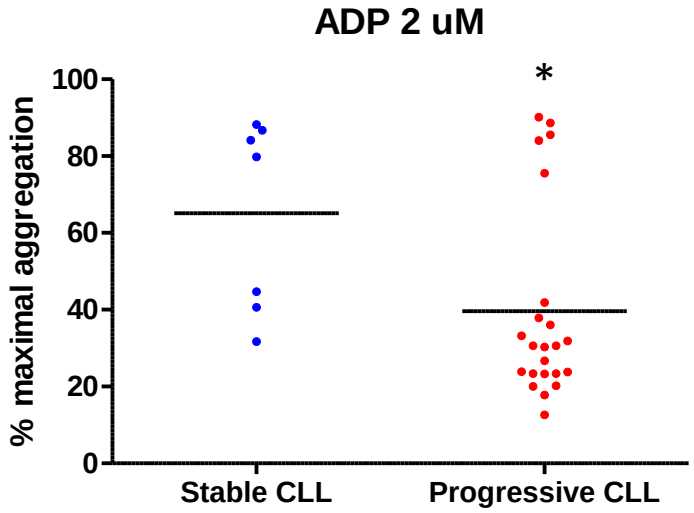


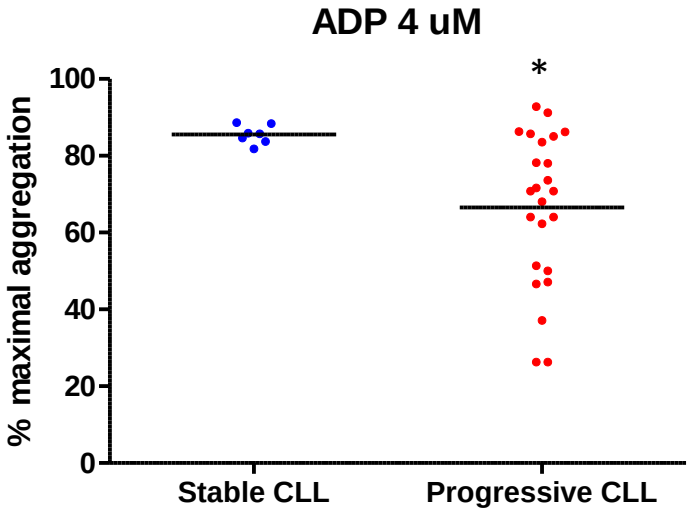
Stable vs progressive CLL (pre –therapy)



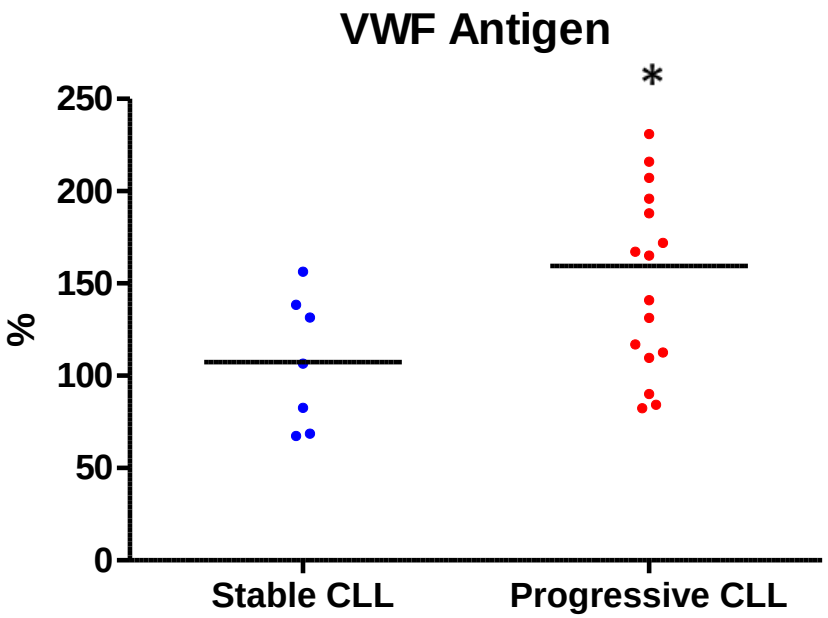


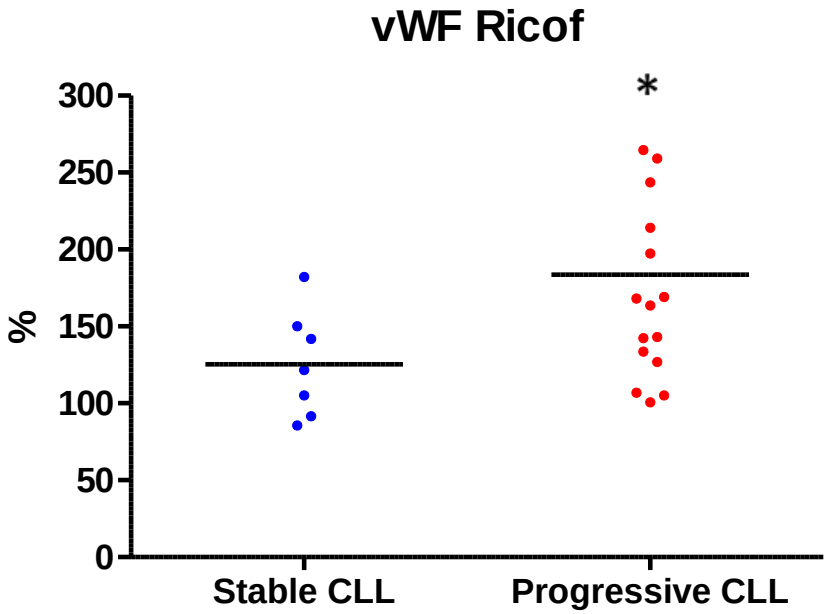
* P<0.05





Plasma vonWillebrand levels
Stable vs progressive CLL





Acalabrutinib

Acalabrutinib, which specifically inhibits Btk without affecting Tec and other SFKs, causes about 12% of mild-moderate bleedings

Is the collagen related platelet function less affected by acalabrutinib?

REGULAR ARTICLE

blood advances

Severe platelet dysfunction in NHL patients receiving ibrutinib is absent in patients receiving acalabrutinib

Alexander P. Bye,[1] Amanda J. Unsworth,[1] Michael J. Desborough,[2,3] Catherine A. T. Hildyard,[4] Niamh Appleby,[4,5] David Bruce,[4,5] Neline Kriek,[1] Sophie H. Nock,[1] Tanya Sage,[1] Craig E. Hughes,[1,*] and Jonathan M. Gibbins[1,*]

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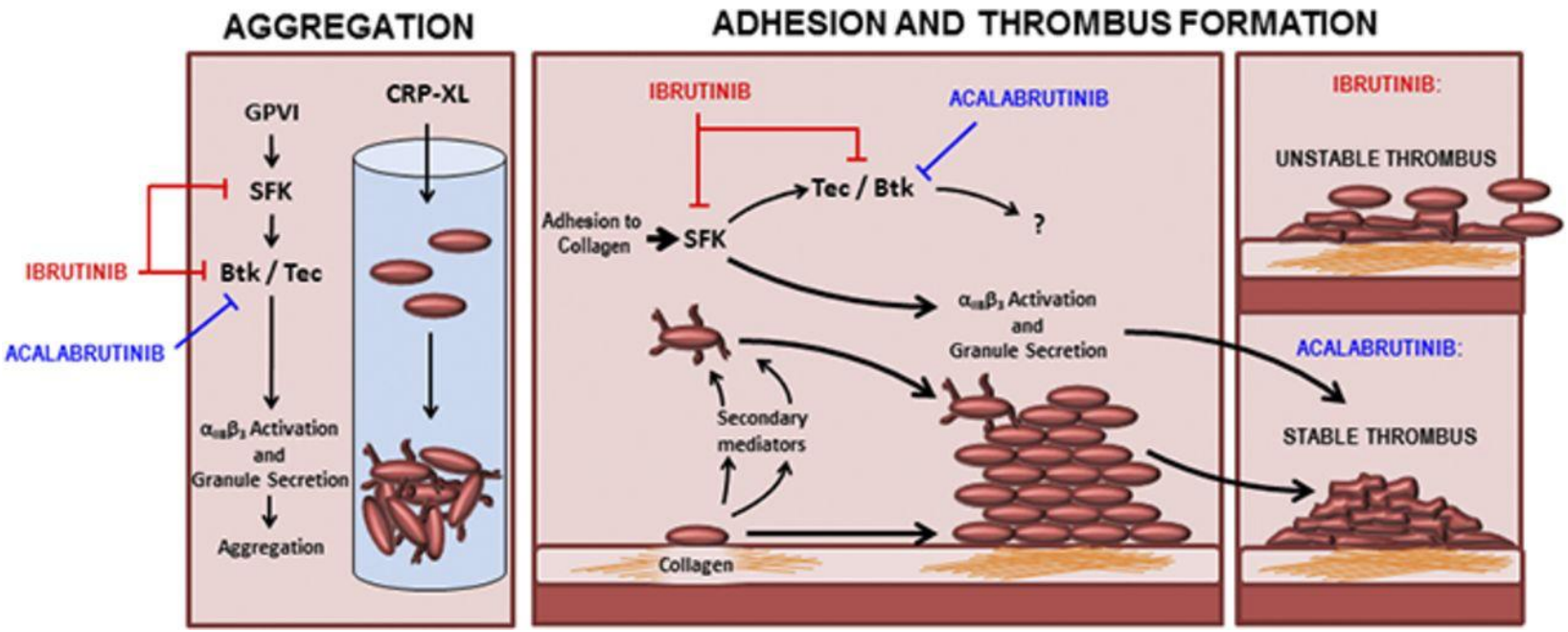
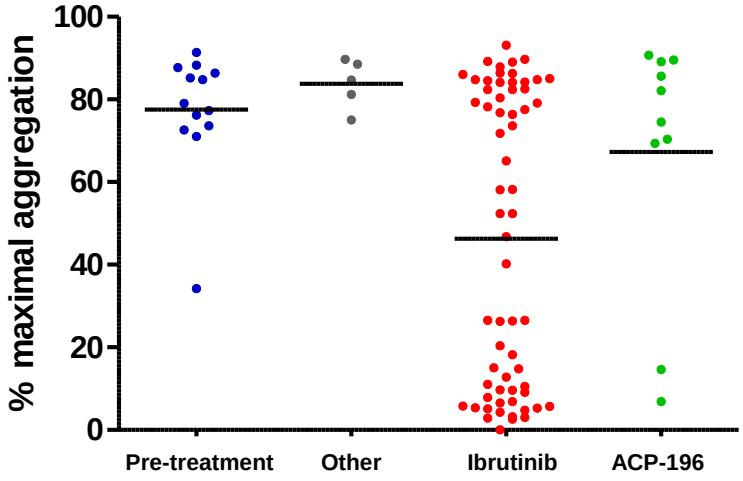


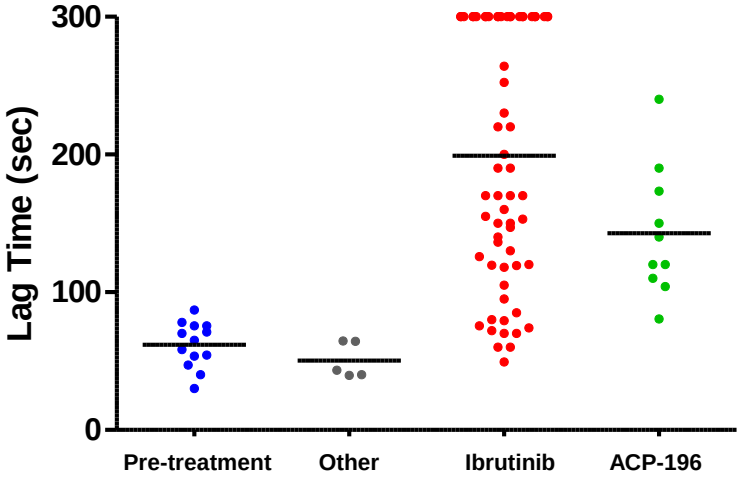
Bleedings in BTK inhibitors treated patients

ID PATIENT	SEX	AGE	DISEASE	TREATMENT	NATURE OF BLEEDING
# 1	M	65	CLL	Ibrutinib	Bruising
# 2	M	62	CLL	Ibrutinib	–
# 3	M	68	CLL	Ibrutinib	Petechiae
# 4	F	71	CLL	Ibrutinib	Bruising
# 5	M	57	CLL	Ibrutinib	Bruising
# 6	M	75	CLL	Ibrutinib	Bruising
# 7	M	68	CLL	Ibrutinib	–
# 8	M	68	CLL	Ibrutinib	Gastrointestinal Bleeding
# 9	M	69	CLL	Ibrutinib	Petechiae
# 10	M	82	CLL	Ibrutinib	Bruising
# 11	M	80	CLL	Ibrutinib	Bruising
# 12	M	61	CLL	Ibrutinib	Petechiae
# 13	M	68	CLL	Ibrutinib	–
# 14	F	63	CLL	Ibrutinib	Bruising
# 15	M	75	CLL	Ibrutinib	Bruising
# 16	M	36	CLL	Ibrutinib	Bruising
# 17	M	69	CLL	Ibrutinib	–
# 18	F	71	CLL	Ibrutinib	Bruising
# 19	M	61	CLL	Ibrutinib	–
# 20	M	63	CLL	Ibrutinib	–
# 21	M	79	CLL	Ibrutinib	–
# 22	M	70	CLL	Ibrutinib	Bruising
# 23	M	57	CLL	Ibrutinib	Petechiae
# 24	**M**	**65**	**CLL**	**Acalabrutinib (ACP-196)**	**–**
# 25	**M**	**55**	**CLL**	**Acalabrutinib (ACP-196)**	**–**
# 26	**M**	**75**	**CLL**	**Acalabrutinib (ACP-196)**	**–**
# 27	F	50	Waldenstrom	Ibrutinb	–
# 28	M	84	Waldenstrom	Ibrutinib	–
# 29	M	64	Waldenstrom	Ibrutinib	–
# 30	M	78	Waldenstrom	Ibrutinib	–

Effect of Ibrutinib vs Acalabrutinib (ACP-196) vs Immuno/Chemotherapy

Collagen 3.3 ug/mL

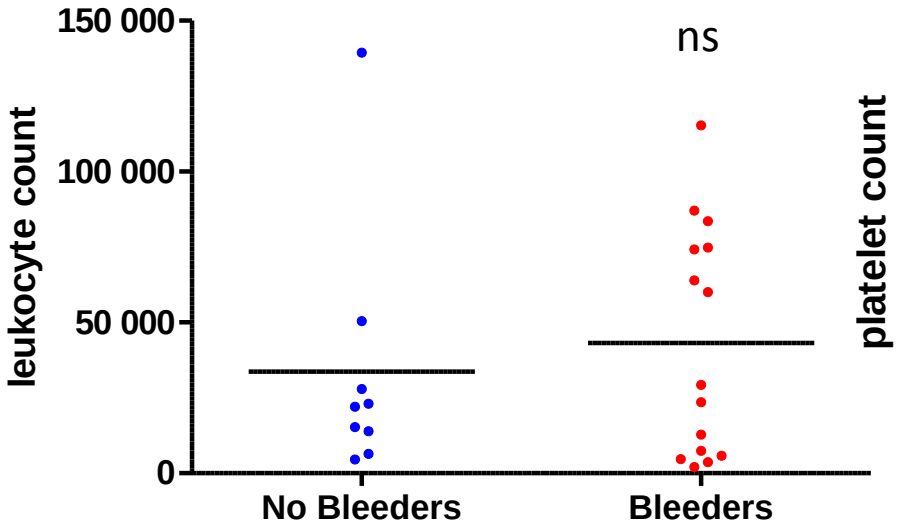


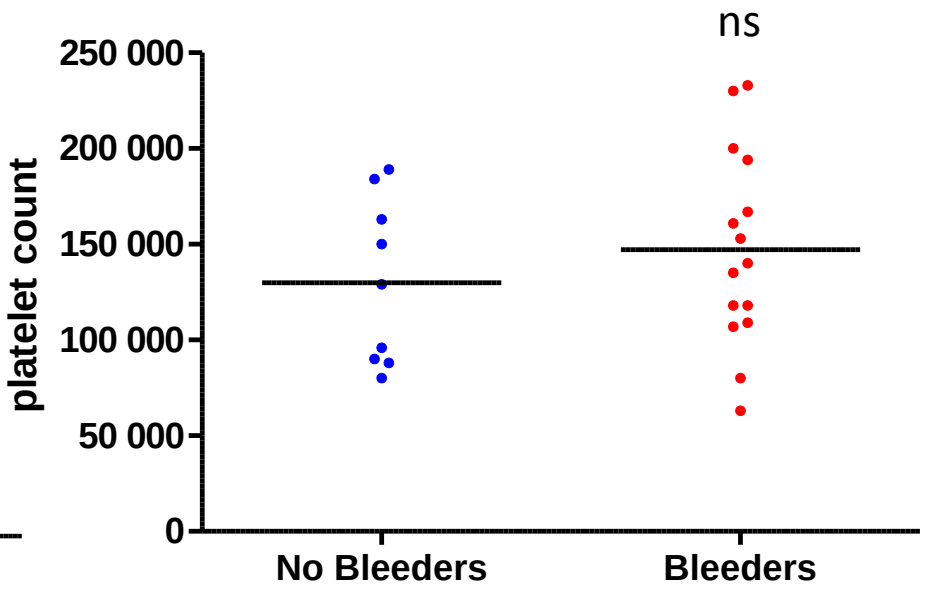


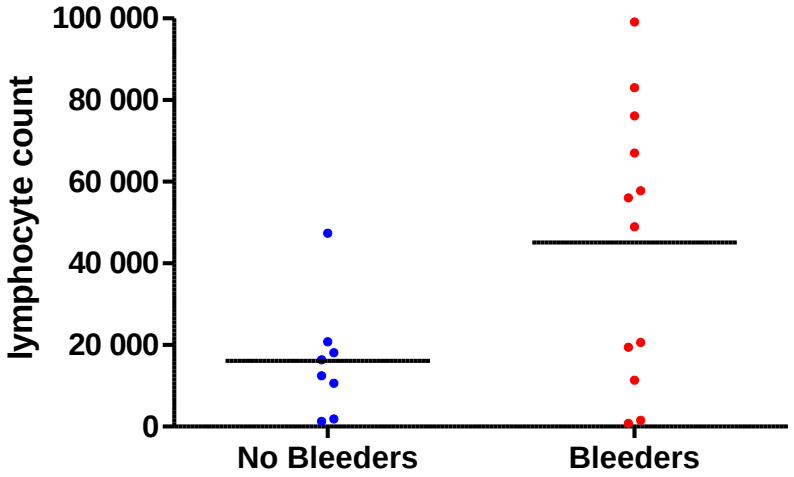
Other:

- Fludarabin +endoxan+rituximab
- Bendamustin+rituximab
- clorambucil-obinutuzamb

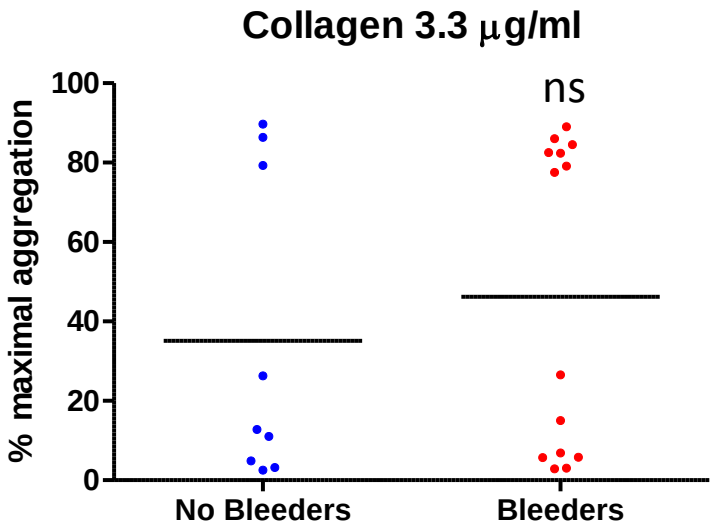
Are there biomarkers correlated to the bleedings?

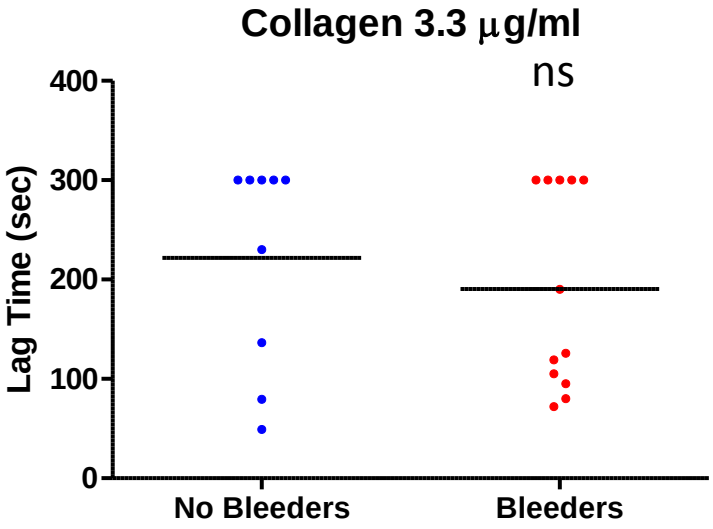

150 000
100 000
50 000
0
leukocyte count
ns
No Bleeders
Bleeders

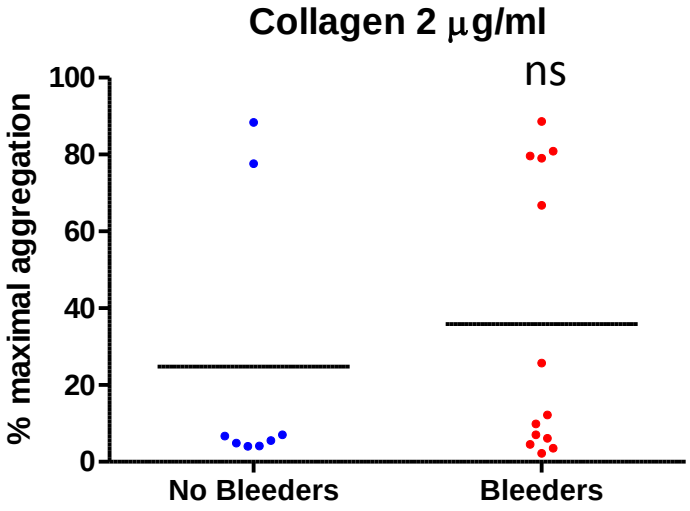

250 000
200 000
150 000
100 000
50 000
0
platelet count
ns
No Bleeders
Bleeders

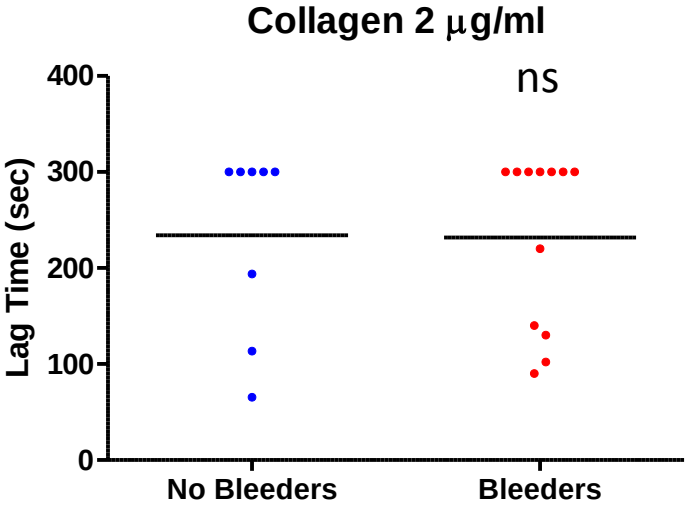

100 000
80 000
60 000
40 000
20 000
0
lymphocyte count
No Bleeders
Bleeders

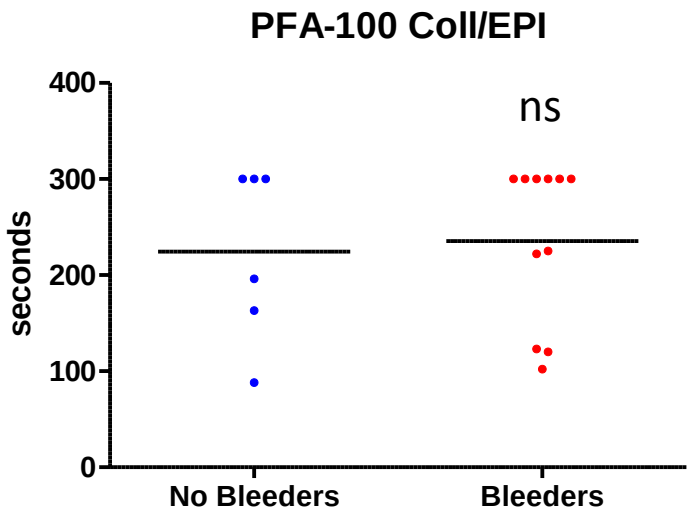
P= 0.069

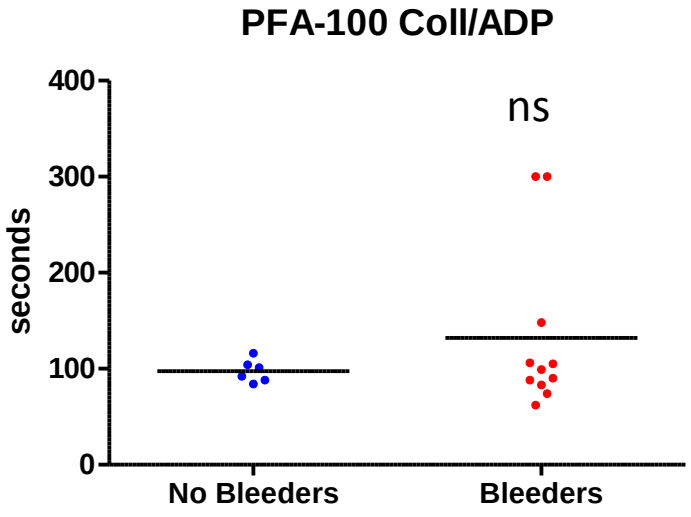

Collagen 3.3 μg/ml
ns
% maximal aggregation
100
80
60
40
20
0
No Bleeders
Bleeders

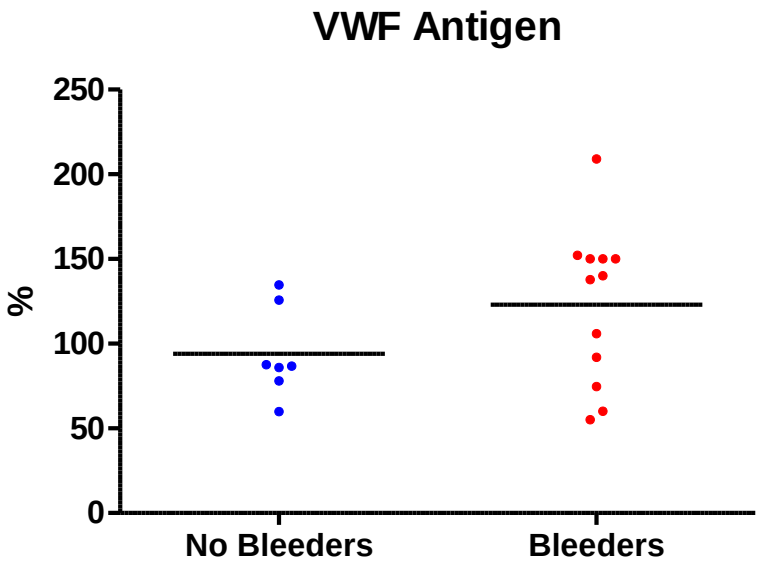

Collagen 3.3 μg/ml
ns
Lag Time (sec)
400
300
200
100
0
No Bleeders
Bleeders

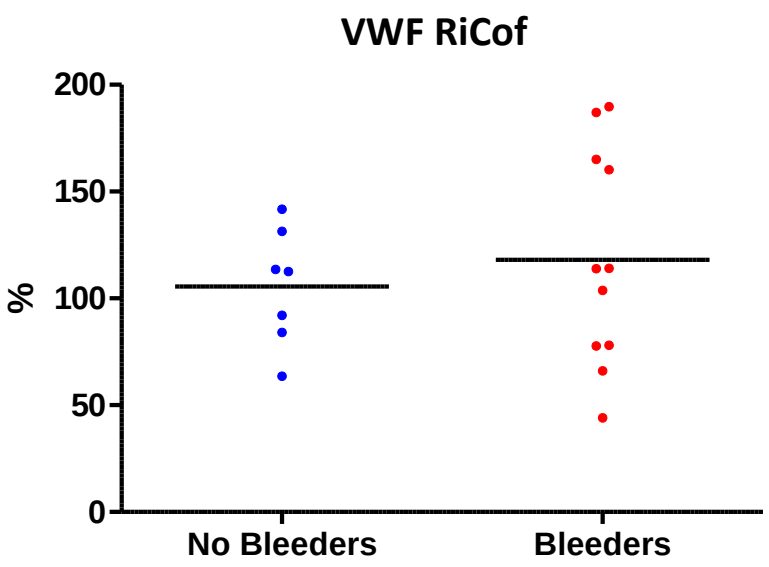

Collagen 2 μg/ml
ns
% maximal aggregation
100
80
60
40
20
0
No Bleeders
Bleeders


Collagen 2 μg/ml
ns
Lag Time (sec)
400
300
200
100
0
No Bleeders
Bleeders


PFA-100 Coll/EPI
ns
seconds
400
300
200
100
0
No Bleeders
Bleeders


PFA-100 Coll/ADP
ns
seconds
400
300
200
100
0
No Bleeders
Bleeders


VWF Antigen
%
250
200
150
100
50
0
No Bleeders
Bleeders


VWF RiCof
%
200
150
100
50
0
No Bleeders
Bleeders

Conclusions.I

- Large majority of ibrutinib treated CLL patients display severe inhibition of collagen induced platelet aggregation
- About 60-65 % of IBR treated CLL patients have mild-moderate bleedings
- The defect of platelet function does not correlate with the bleedings

Conclusions.II

- CLL patients display also disease-related platelet dysfunction, which seems correlated to the severity of the disease

- CLL patients display increased levels of plasma VWF (inflammation/endothelial dysfunction?) which are rapidly reversed by ibrutinib treatment

- A combination of CLL/lymphocytosis related factors, reduction of plasma VWF, ibrutinib-related platelet dysfunction might contribute to the occurrence of bleedings in CLL patients

Conclusion.III

- Acalabrutinib is associated with less bleedings and with reduced platelet dysfunction

- In the future, acalabrutinib might become the drug of choice in patients with comorbidities requiring antithrombotic treatments