Effects of Pyruvate Kinase Activators on Red Blood Cell Rheology, Sickling and Senescence in Sickle Cell Disease

Philippe Joly[1,2,3], Elie Nader[2,3], Flora Ketels[2,3], Camille Boisson[1,2,3], Romain Carin[2,3], Céline Renoux[1,2,3], Alexandra Gauthier[2,3,4], Solene Poutrel[2,3,5], Yves Bertrand[4] and Philippe Connes[2,3]

[1] *Laboratoire de Biochimie et de Biologie Moléculaire, UF de Biochimie des Pathologies Erythrocytaires, Centre de Biologie et de Pathologie Est, Hospices Civils de Lyon, Bron, France*

[2] *Laboratoire interuniversitaire de Biologie de la Motricité (LIBM) EA7424, Team « Vascular Biology and Red Blood Cell » Universié Claude Bernard Lyon 1, Université de Lyon, Lyon, France.*

[3] *Laboratoire d'Excellence du Globule Rouge (Labex GR-Ex), PRES Sorbonne, 79015 Paris, France.*

[4] *Institut d'Hématologique et d'Oncologique Pédiatrique, Hospices Civils de Lyon, Lyon, France.*

[5] *Service de Médecine Interne, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, France.*

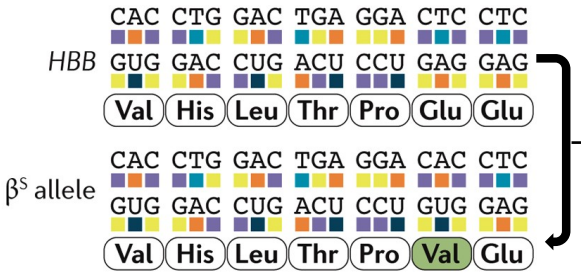
Email: pconnes@yahoo.fr ; philippe.connes@univ-lyon1.fr

UNIVERSITÉ DE LYON | Université Claude Bernard Lyon 1 | LIBM Laboratoire Interuniversitaire Biologie de la Motricité | VBRBC Vascular Biology & Red Blood Cell | HCL Hospices Civils de Lyon

Sickle cell disease

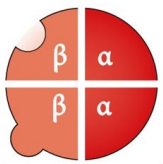
VBRBC
Vascular Biology & Red Blood Cell

β-globine gene mutation



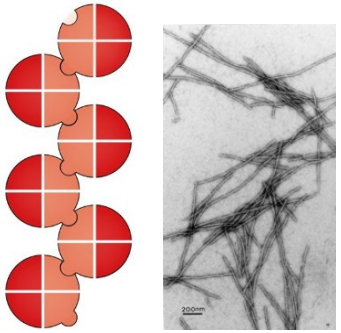
HbA

HbS



O_2

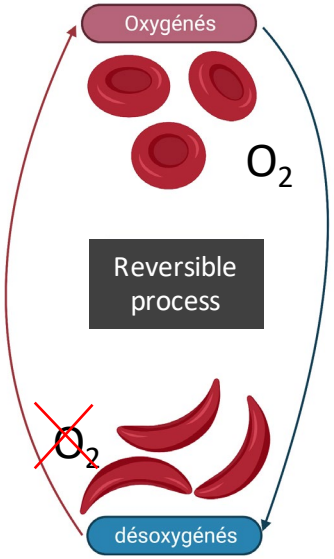
HbS polymerization



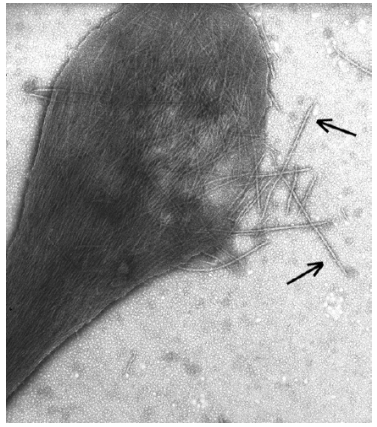
RBC sickling

Fragility

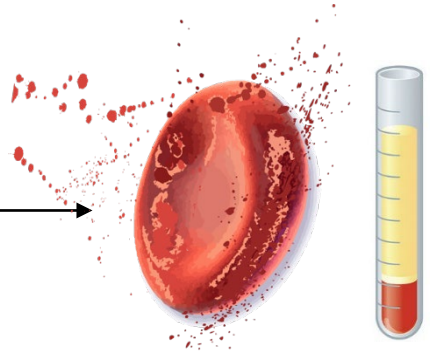
Deformability



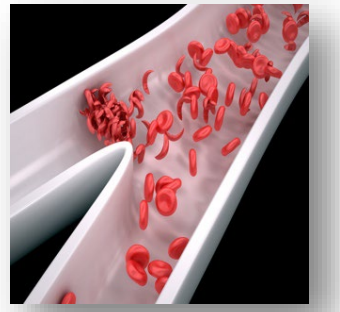
Damaged RBCs



Intravascular hemolysis



Chronic hemolytic anemia

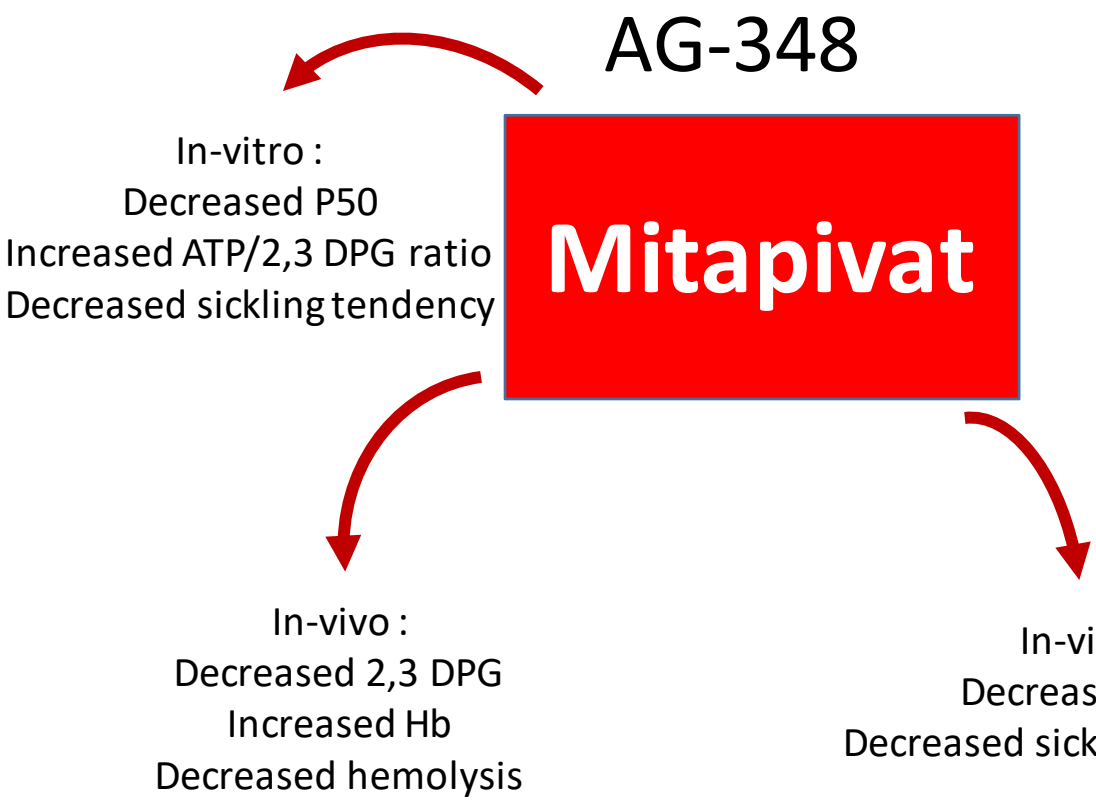


Vaso-occlusive crises

Sickle cell disease and pyruvate kinase activators

VBRBC
Vascular Biology & Red Blood Cell

Increased 2,3 DPG – increased sickling tendency
Decreased ATP – impaired membrane integrity



AG-946 ?

Novel PK activator with a different chemotype

2021 Rab et al. – Decreased activity and stability of pyruvate kinase in sickle cell disease: a novel target for mitapivat therapy

2022 Xu et al. - A Phase 1 Dose Escalation Study of the Pyruvate Kinase Activator Mitapivat (AG-348) in Sickle Cell Disease

2022 Van Dijk et al. - Safety and efficacy of mitapivat, an oral pyruvate kinase activator, in sickle cell disease: A phase 2, open-label study

Aims

VBRBC
Vascular Biology & Red Blood Cell

1) To evaluate the effects of two PK activators (AG-348 and AG-946) on:

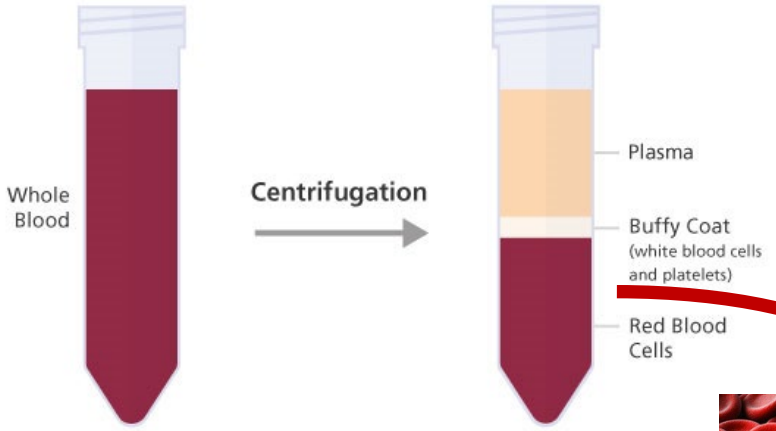
- RBC deformability and sickling

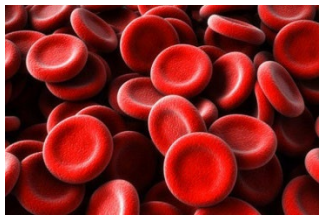
- RBC senescence markers

2) To test the effects of two PK activators (AG-348 and AG-946) on RBC deformability, sickling and senescence under conditions where oxidative stress is elevated.

Protocol

VBRBC
Vascular Biology & Red Blood Cell





+ **PBS**

Washing

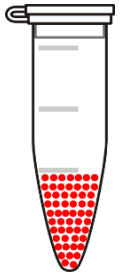
CTL AG-348 AG-946

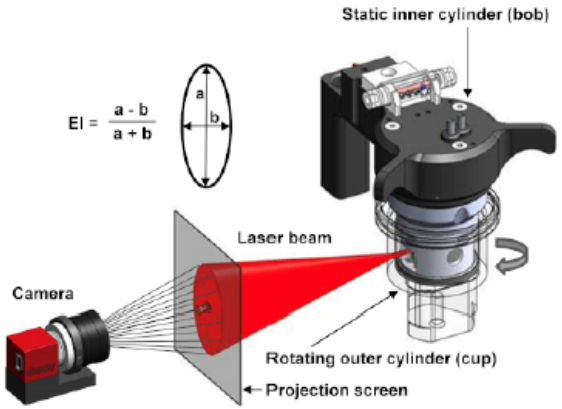
CTL AG-348 AG-946

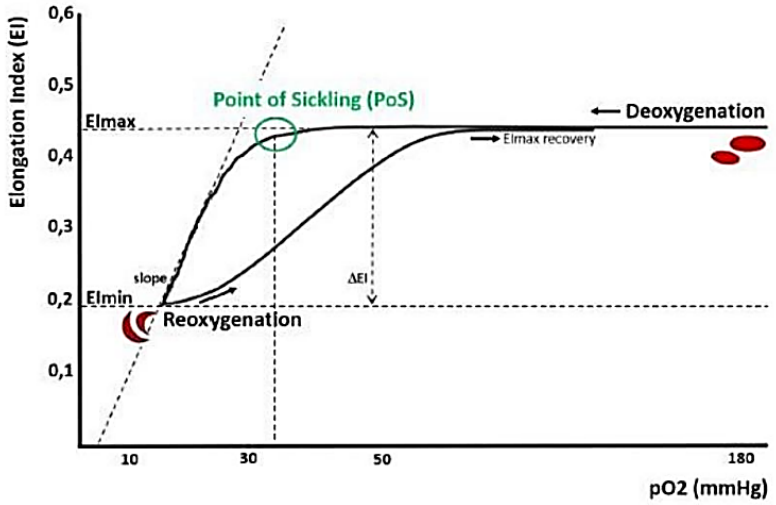
+ Cumene Hydroperoxyde (0.4 mM)

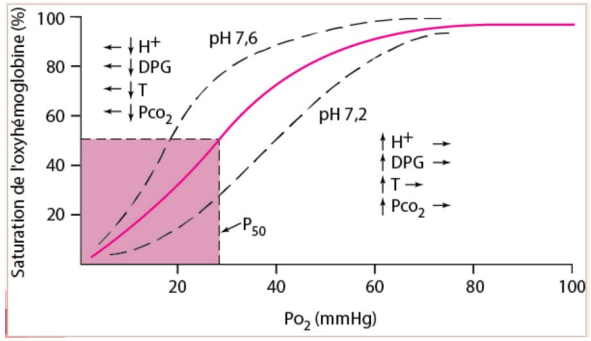
Incubation: 4 hrs at 37°C

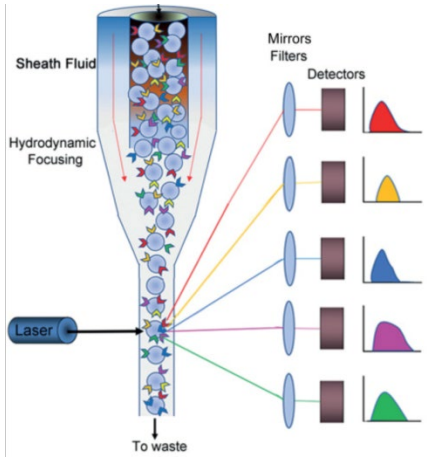
Protocol

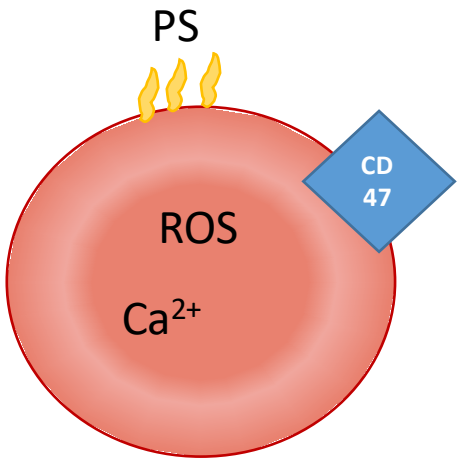










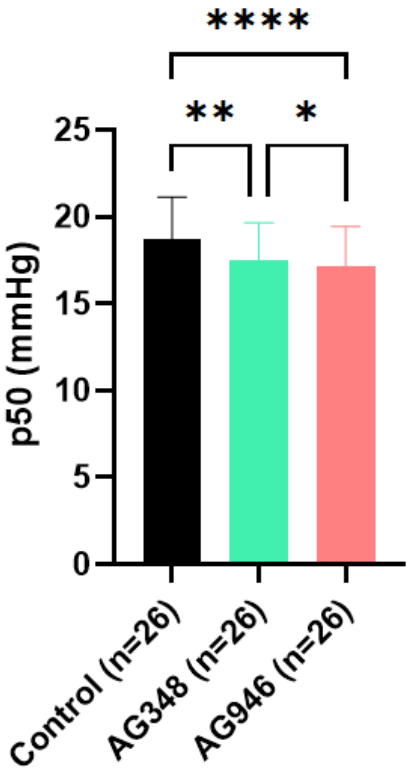


+/- Oxydative Stress

Results without oxidative stress

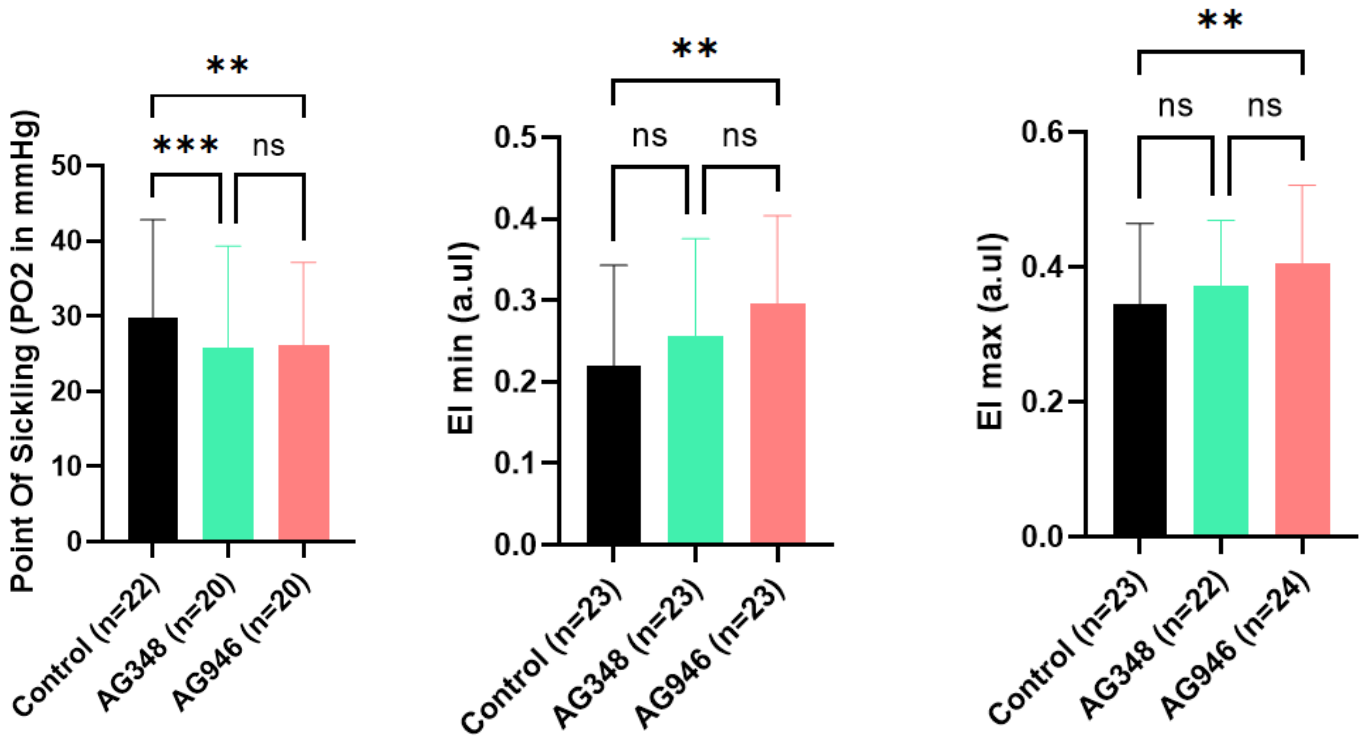
VBRBC
Vascular Biology & Red Blood Cell

Oxygen affinity



PK activators increase HbS affinity for O_2

RBC deformability and sickling

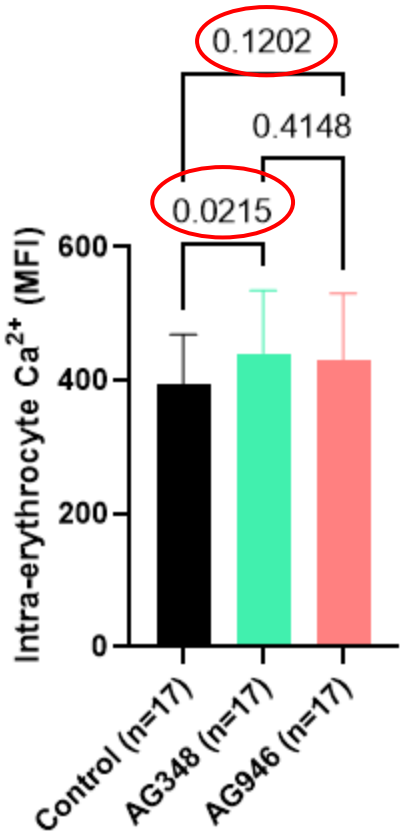


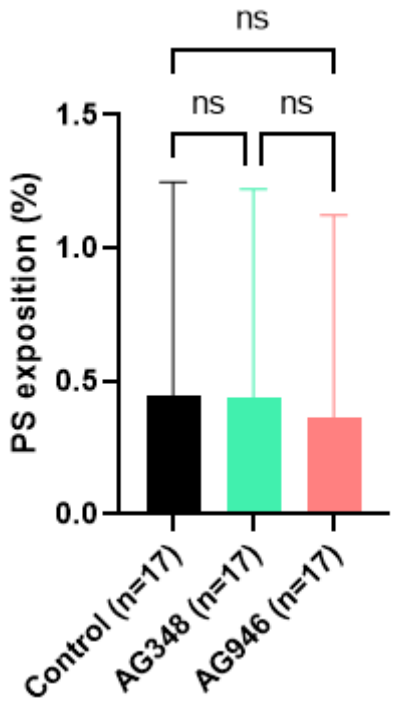
Both PK activators decrease PoS.
AG-946 increases Eimin and Eimax.

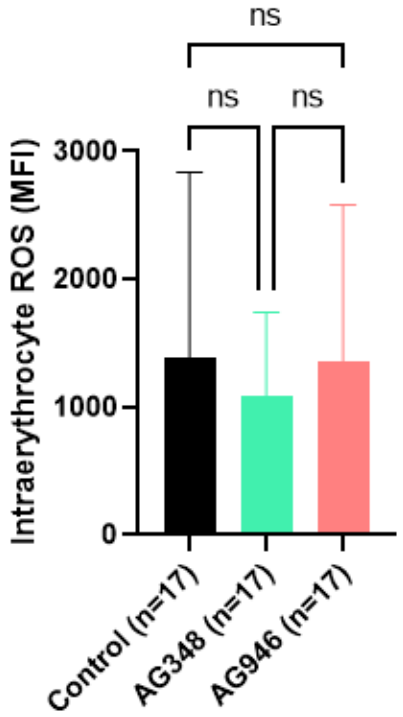
Results without oxidative stress

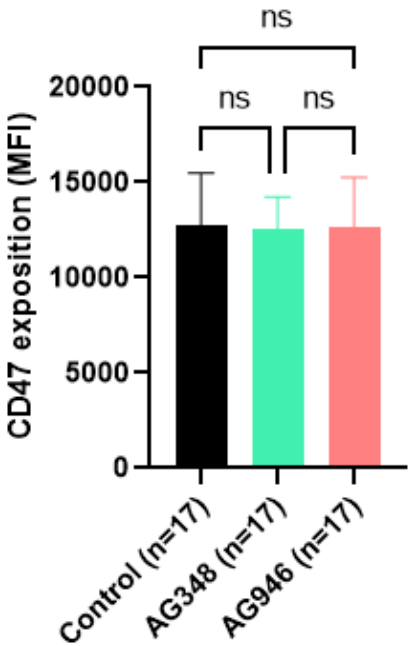
VBRBC
Vascular Biology & Red Blood Cell

RBC senescence markers





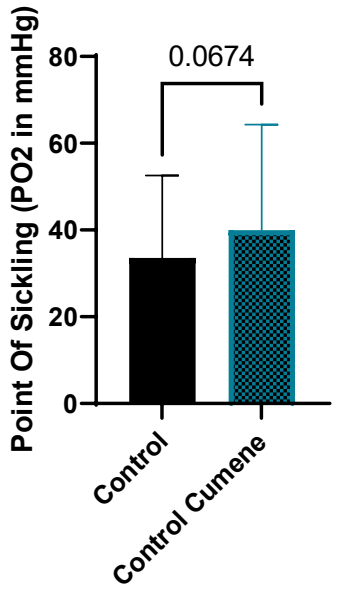


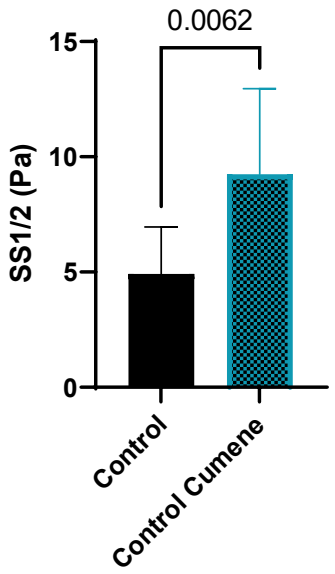


Slight increase of RBC Ca^{2+} (related with increase of PK activity ??)

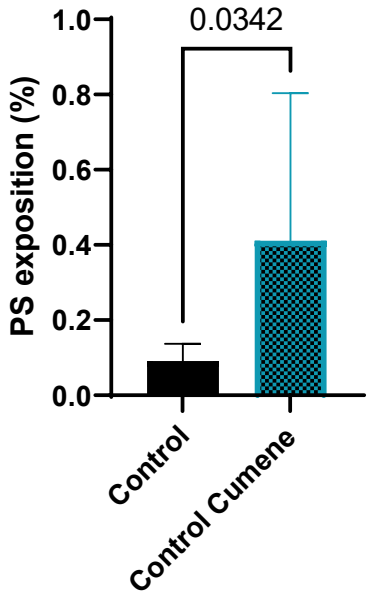
….. but without any consequences on the other senescence markers

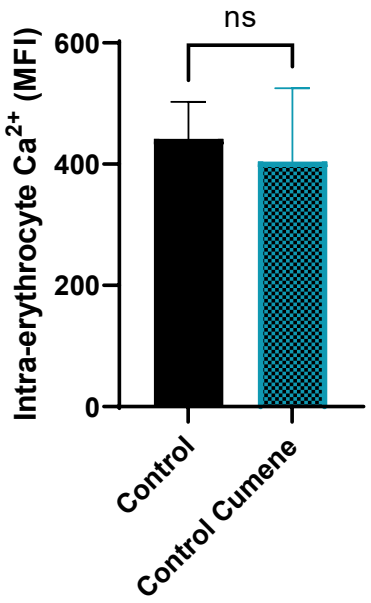
Results with oxidative stress: effects of cumene hydroperoxyde alone

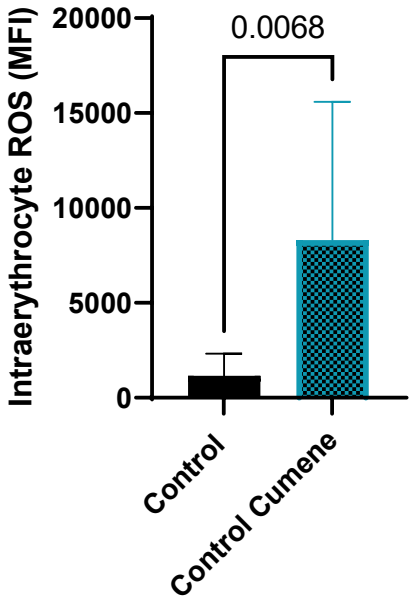


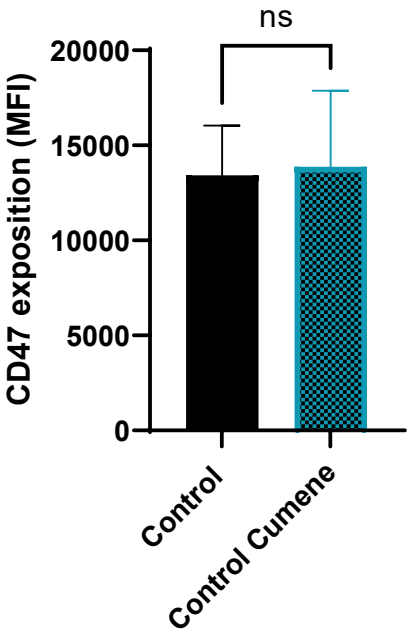


Increased sickling and decreased RBC deformability







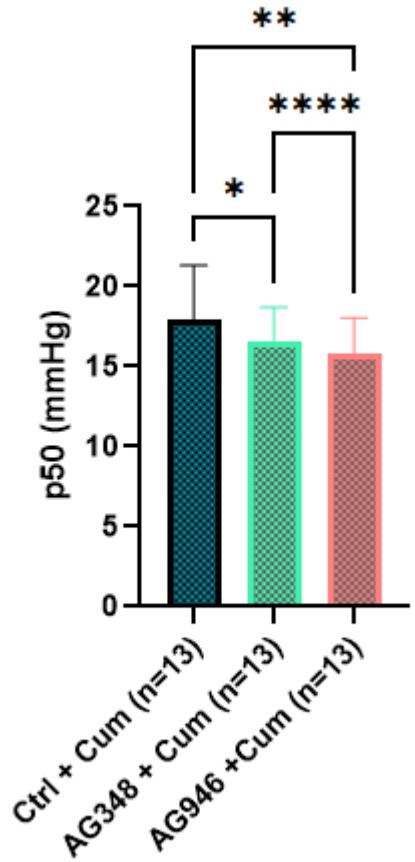


Increased PS externalization and ROS

Results with oxidative stress

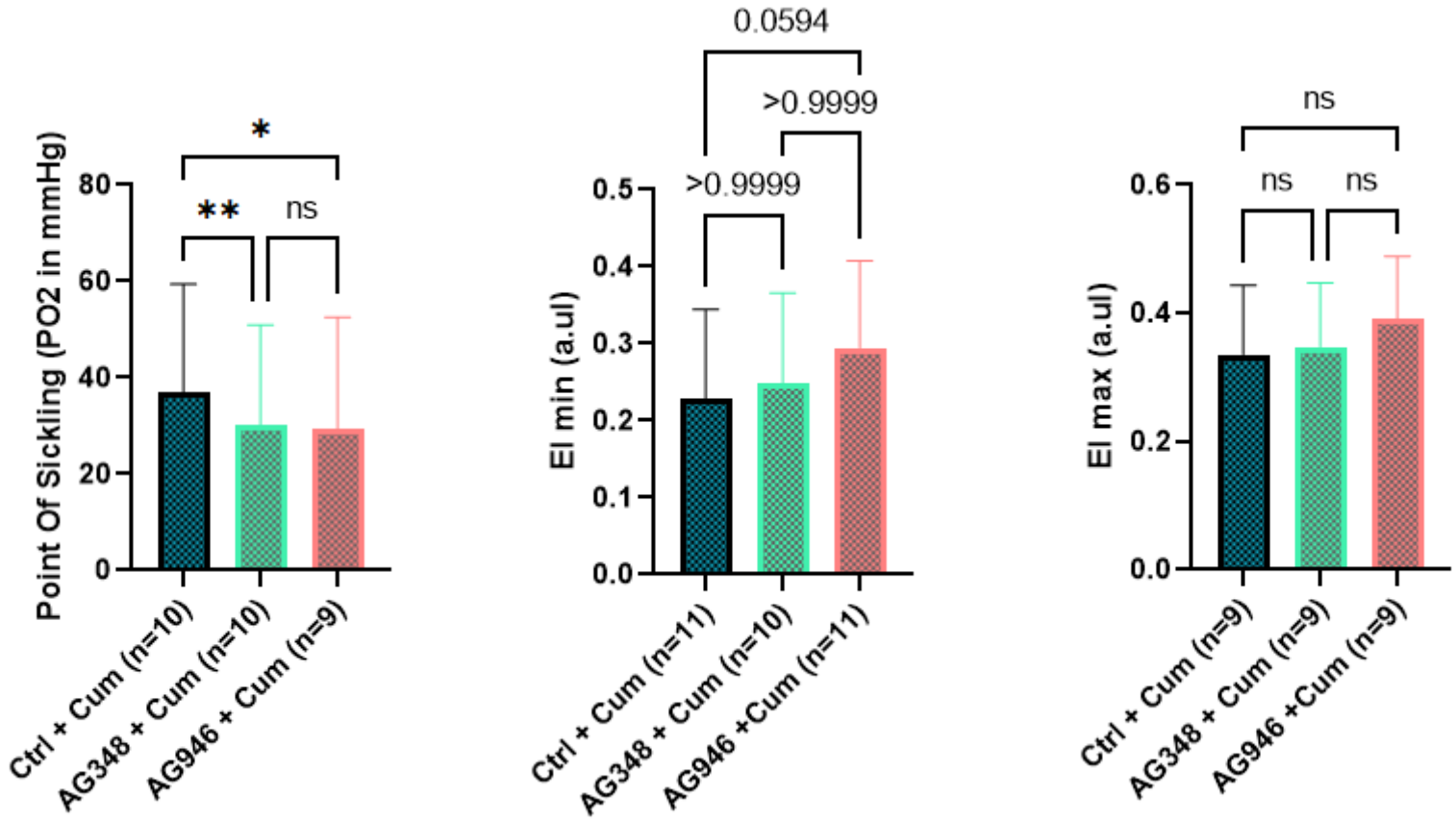
VBRBC
Vascular Biology & Red Blood Cell

Oxygen affinity



The increase of HbS oxygen affinity by PK activators is preserved in case of elevated oxidative stress.

RBC deformability and sickling

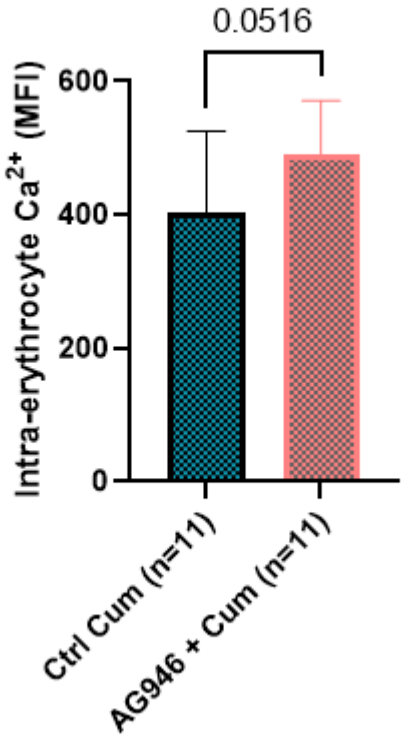


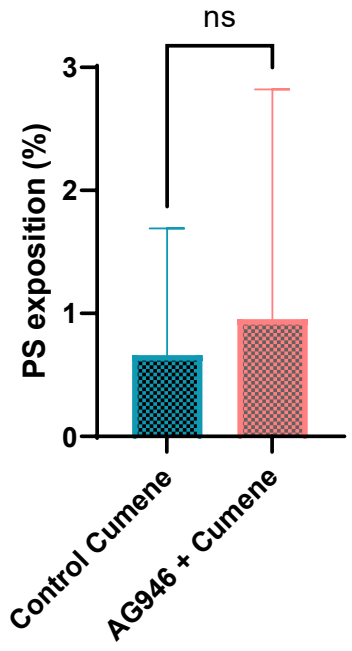
PK activators retain the ability to reduce RBC sickling after exogenous oxidative stress.

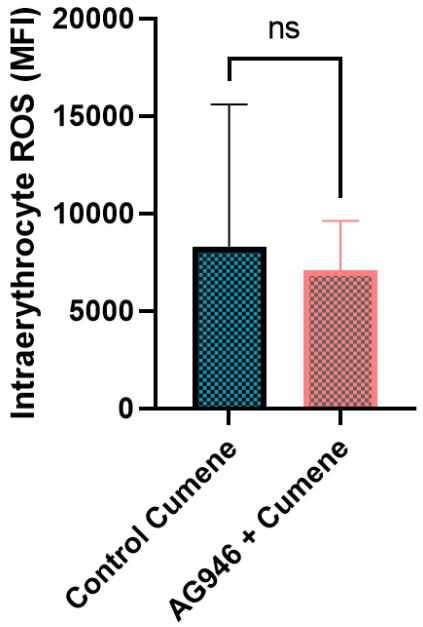
Results with oxidative stress

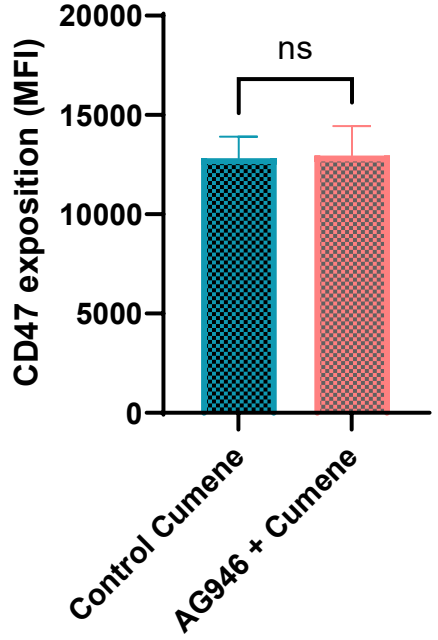
VBRBC
Vascular Biology & Red Blood Cell

RBC senescence markers for AG-946









Still a tendency for more RBC Ca^{2+} with AG-946

…. but no further increase of ROS and PS externalization compared to cumene alone

Discussion-conclusion

VBRBC
Vascular Biology & Red Blood Cell

Both PK activators increase the oxygen affinity and reduce the sickling propensity of RBCs from patients with SCD in vitro in the presence or absence of increased oxidative stress.

The observed small increase of intracellular Ca^{2+} upon drug treatment may be a result of increased overall cellular metabolic activity, as a consequence of PK activation.

Interestingly, the increased intracellular Ca^{2+} is not accompanied by increased RBC senescence.

These data support to continue testing PKA in prospective clinical trials (RISE UP) for the reduction of vaso-occlusive crisis and/or decrease the severity of anemia in patients with SCD.

UNIVERSITÉ DE LYON

Université Claude Bernard Lyon 1

LIBM Laboratoire Interuniversitaire Biologie de la Motricité

VBRBC Vascular Biology & Red Blood Cell

HCL HOSPICES CIVILS DE LYON