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Effect of second-generation BTK inhibitors on the functionality of macrophages and neutrophils from CLL patients.

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Disclosures

- Research funding from Microsules, Argentina.



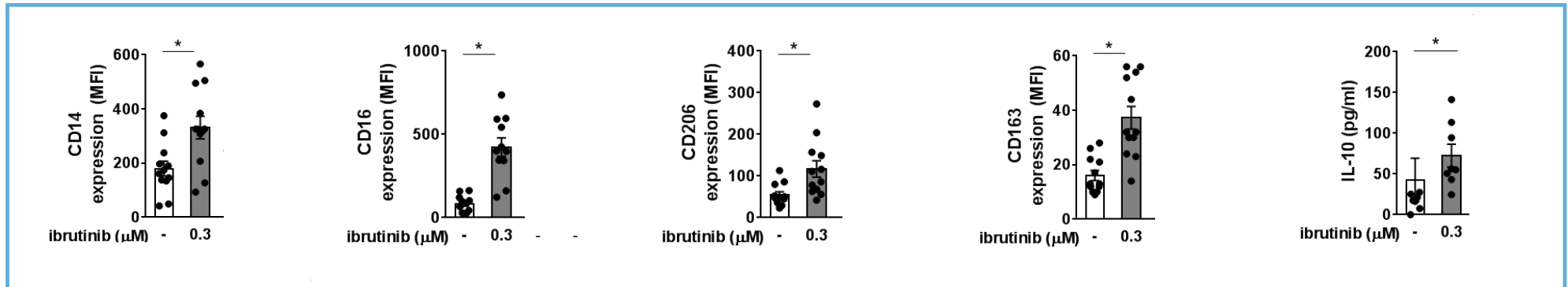
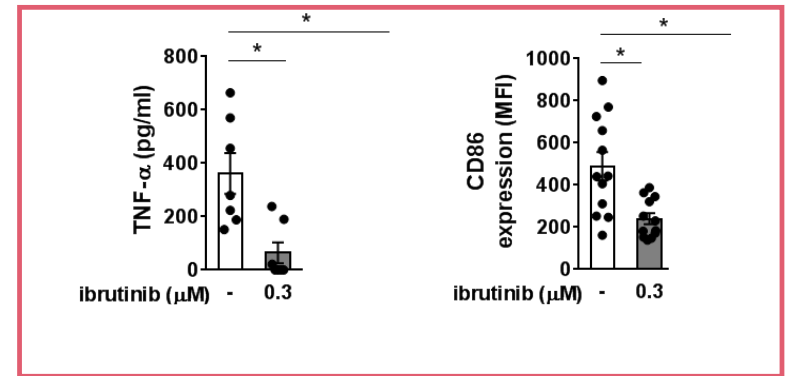
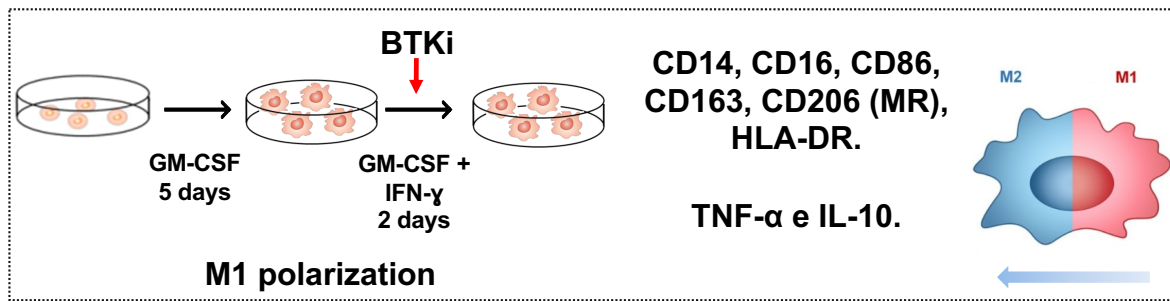
Background

- To date, **ibrutinib**, is the only BTK inhibitor (BTKi) approved for CLL treatment.
- **Second generation BTKi with greater selectivity** for BTK, such as acalabrutinib, approved for MCL, and spebrutinib, are being evaluated in clinical trials.
- Besides its effects on leukemic B-cells, **ibrutinib** also affects key innate immune cells such as **macrophages and neutrophils**.
- **Infection rates** during ibrutinib treatment are lower or similar compared to other treatments (*Ball S, et al. European journal of haematology. 2018; O'Brien S, Clinical Lymphoma, Myeloma & Leukemia. 2018; Mato et al., Haematologica. 2018*).
- They are still a **frequent grade ≥ 3 adverse events** (*O'Brien S, et al. Blood. 2018*), and they are among the most common toxicities leading to drug discontinuation in **relapsed/refractory patients** (*Mato A, et al. Haematologica. 2018*).
- To better understand the impact of new agents on immune cells may help to improve CLL patients' care.

Our aim was to investigate how second generation BTKi , acalabrutinib and spebrutinib, affect macrophage and neutrophil functions.

Effect of second generation BTKi on macrophage polarization


- Previously reported that ibrutinib impairs macrophage-M1 polarization (Colado A, et al. *Blood Cancer Journal*. 2018, Fiorcari, S et al. *Oncotarget*. 2016).



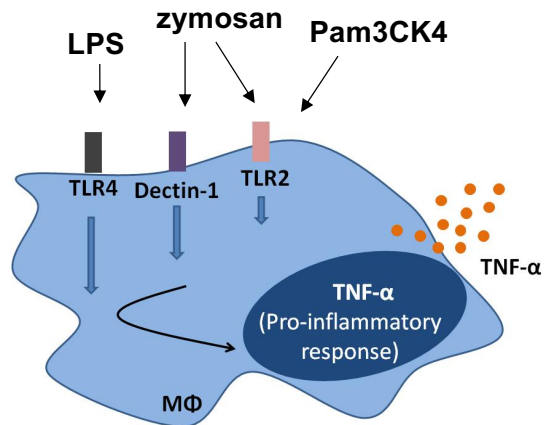
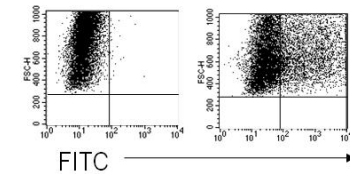
* $p < 0.05$, Friedman test, Dunn's post-test.

Effect of second generation BTKi on macrophage activation and phagocytosis

- *C. albicans* yeast \emptyset
- *A. fumigatus* conidia \emptyset
- *M. tuberculosis*

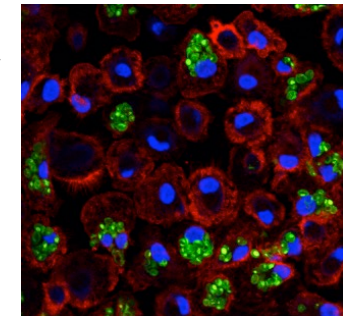
second-generation
BTKi
 *phagocytosis*

- Flow Cytometry



 *TNF-α secretion* • ELISA

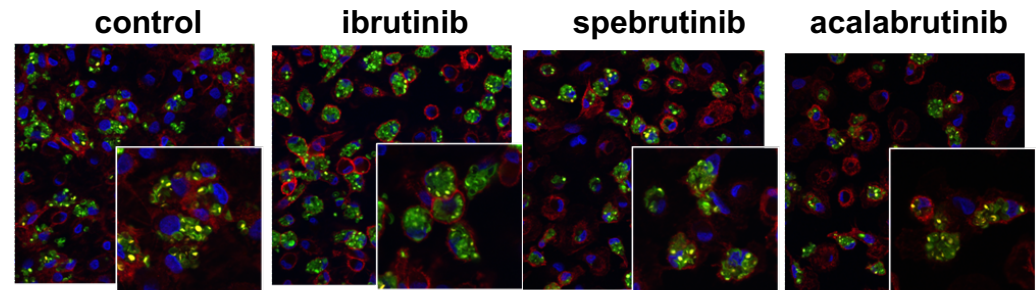
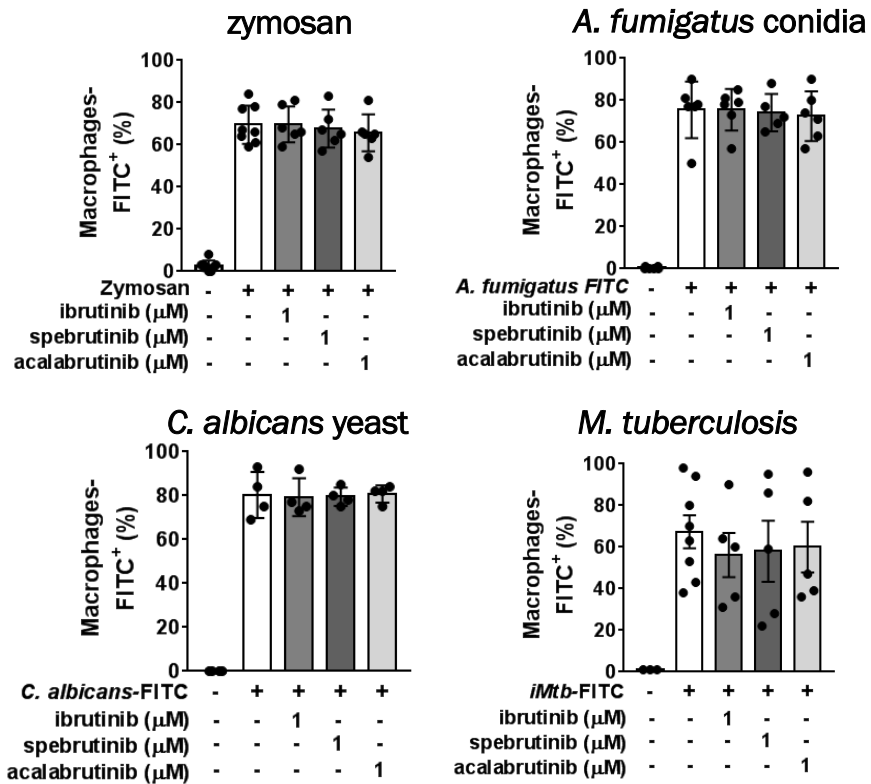
- Confocal microscopy



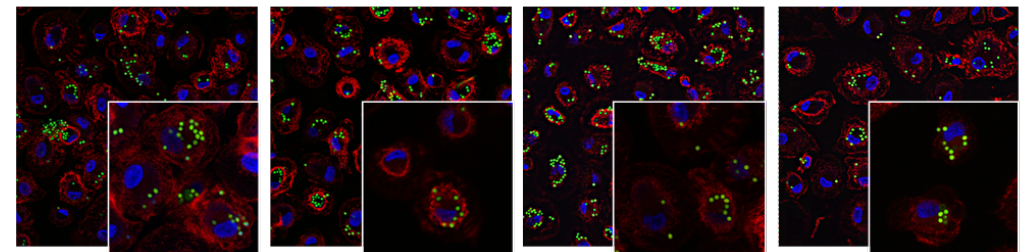
- ELISA

Bercusson, et al. *Blood*. 2018 Nov 1;132(18):1985-1988.
 Colado, et al. *Blood Cancer J*. 2018 Nov 5;8(11):100.
 Lionakis et al. *Cancer Cell*. 31, 833-843.e5 (2017).
 Fiorcari, et al. *Oncotarget*. 2016 Oct 4;7(40):65968-65981

Effect of second generation BTKi on macrophage phagocytosis



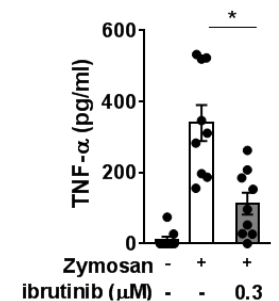
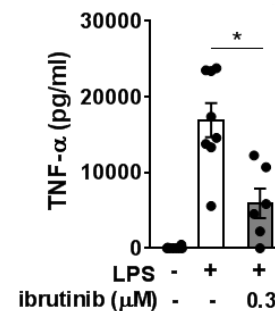
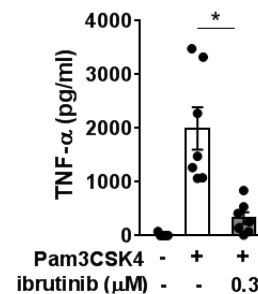
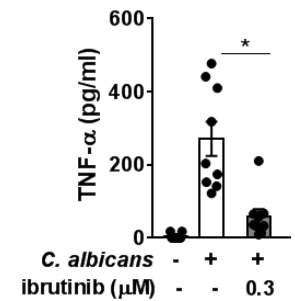
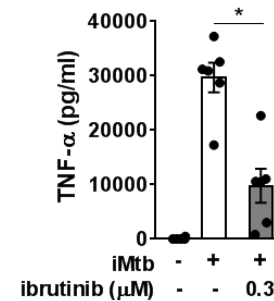
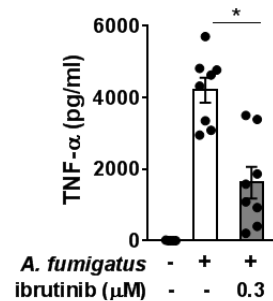
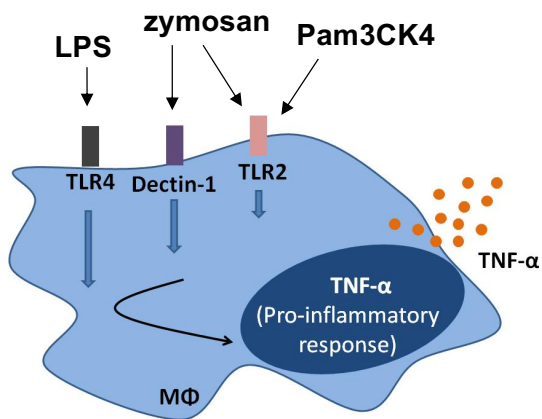
Zymosan-FITC; CD11b PE; DNA (TOPRO-3)



Conidias *A. fumigatus*-FITC; CD11b PE; DNA (TOPRO-3)

Effect of second generation BTKi on cytokine secretion by stimulated-macrophages

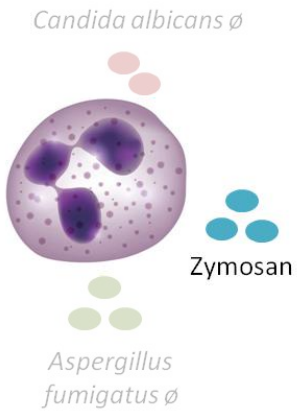
- *C. albicans* yeast Ø
- *A. fumigatus* conidia Ø
- *iM. tuberculosis*



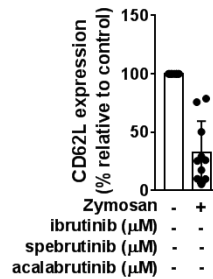
*p<0.05, Friedman test, Dunn's post-test.

Ibrutinib and acalabrutinib impaired TNF-α secretion in response to *A. fumigatus*, *C. albicans* and *Mtb*, while spebrutinib only affected TNF-α secretion in response to *C. albicans*.

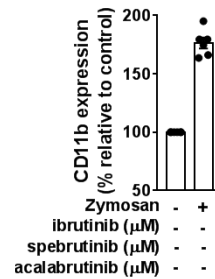
Effect of second generation BTKi on neutrophil activation



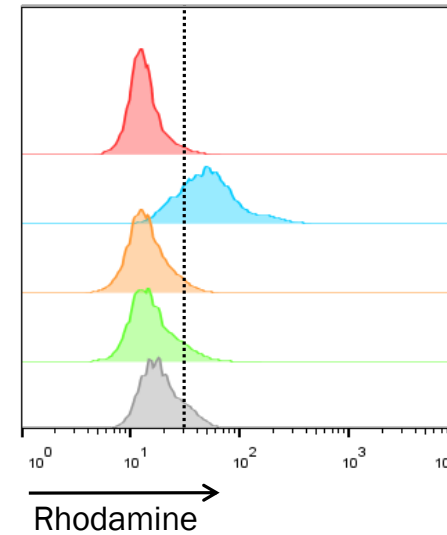
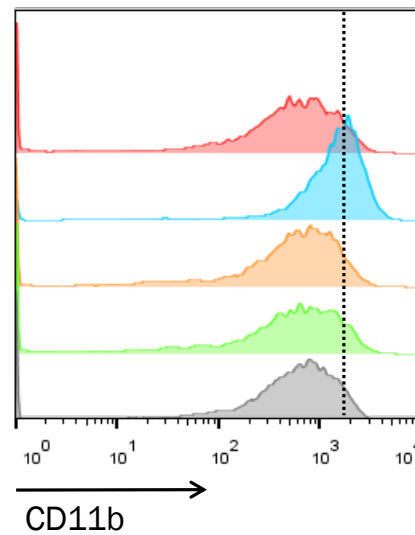
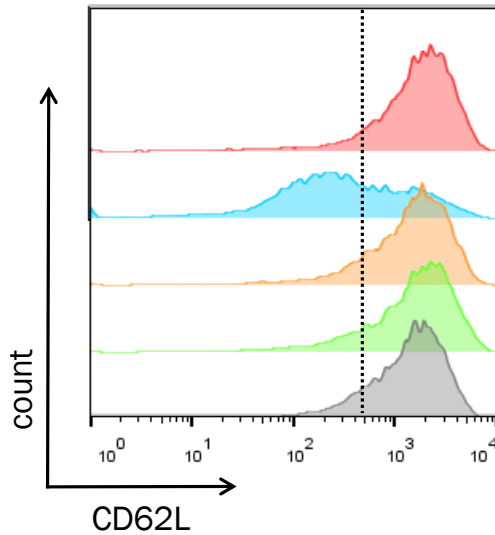
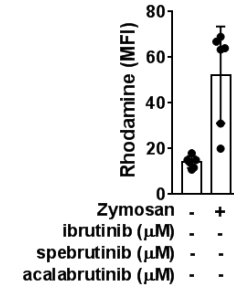
CD62L down-regulation



CD11b up-regulation

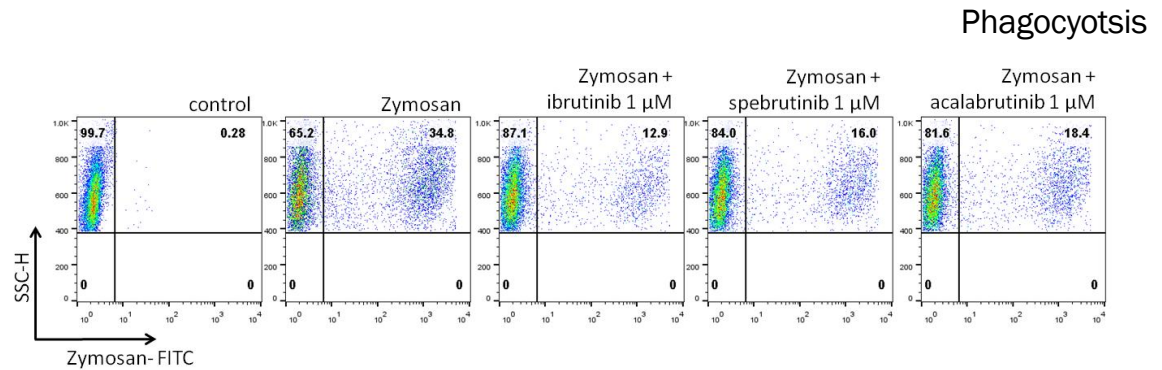
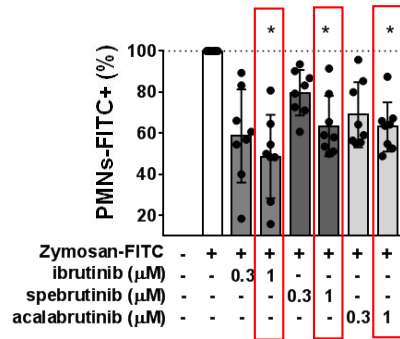
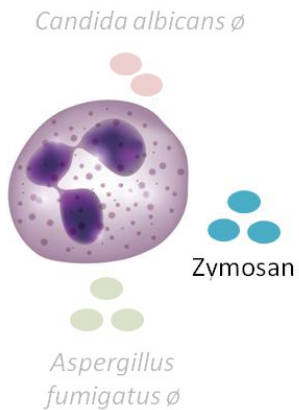


ROS (reactive oxygen species) production



basal
zyzo
zyzo + ibru
zyzo + spebru
zyzo + acala

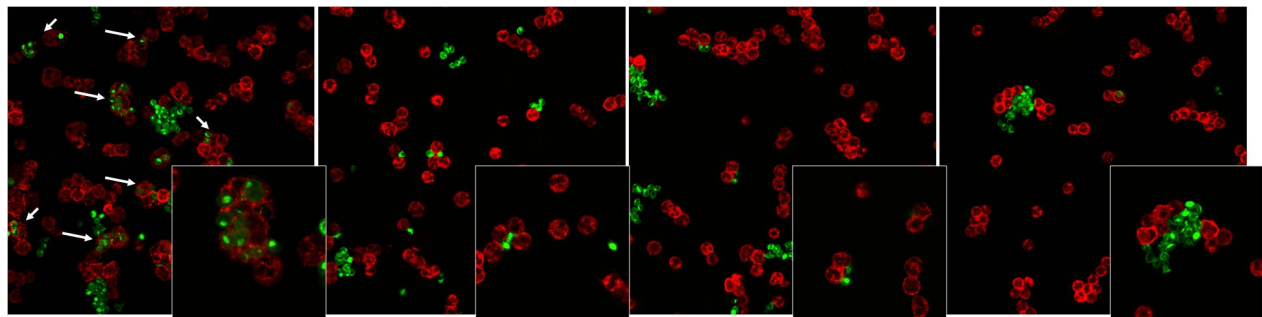
Effect of second generation BTKi on neutrophil activation



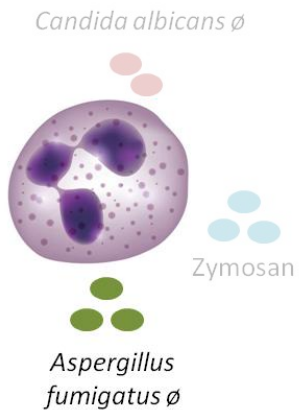
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Zymosan-FITC

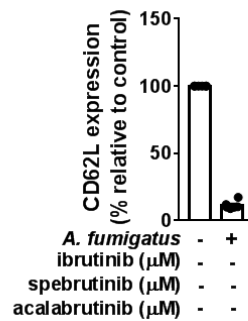
CD11b-PE



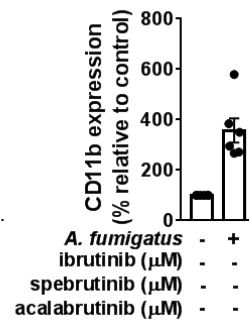
Effect of second generation BTKi on neutrophil activation



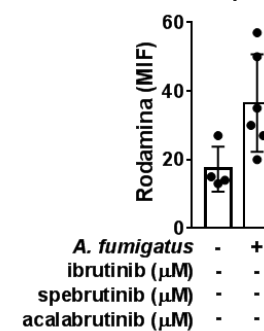
CD62L downregulation



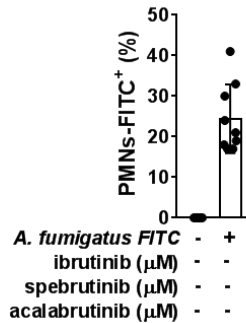
CD11b up-regulation



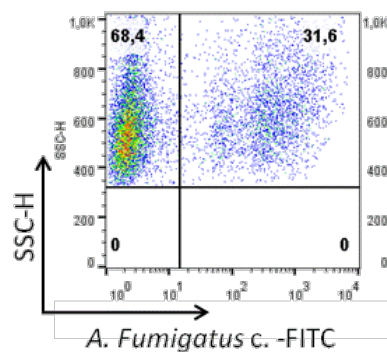
ROS production



Phagocytosis



control



• Similar results were recently reported with ibrutinib (D. Blez et al., *Haematologica*, 2019).

*p<0.05, Friedman test, Dunn's post-test.

Ibrutinib, acalabrutinib and spebrutinib impaired ROS production and phagocytosis in response to *A. fumigatus* conidia in neutrophils.

Summary

In conclusion our results show that BTKi have inhibitory effects on key innate immune cells.

In vitro macrophage polarization and activation in response to microbial stimulation were mostly affected by acalabrutinib and ibrutinib, and not by spebrutinib. While neutrophil activation was similarly affected by the three BTKi.

We speculate that this inhibition may impact on the anti-microbial immune response *in vivo and should be taken into account especially in the presence of other risk factors or in heavily pre-treated patients.*

¡Gracias!

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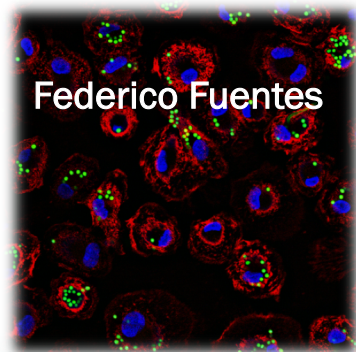
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