BALANCING IMMUNE DYSFUNCTION IN ACLF: ASK1 INHIBITOR SRT-015 MITIGATES INFLAMMATORY RESPONSES WHILE ENHANCING IMMUNE ANTIBACTERIAL DEFENSES

GENFIT
TOWARDS BETTER MEDICINE

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FRI-412

BACKGROUND & AIM

- Patients with cirrhosis develop significant immune dysfunction, characterized by the simultaneous presence of systemic inflammation and immune paralysis. Systemic inflammation correlates with disease progression, while immune paralysis increases susceptibility to bacterial infections, leading to severe complications such as acute-on-chronic liver failure (ACLF)[1]
- Currently, there are limited therapeutic options addressing the underlying pathogenic mechanisms of ACLF. While systemic inflammation is a key driver of tissue damage, broad anti-inflammatory strategies (corticosteroids, anti-TNF or NSAIDs) have failed or resulted in adverse outcomes in advanced liver disease, notably by increasing infection risk through further aggravation of immune dysfunction[2,3]. These limitations underscore the need for targeted anti-inflammatory therapies that control inflammation while preserving host immune defenses[4]
- Apoptosis-signal-regulating kinase 1 (ASK1) is a key mediator of the inflammatory response, activated by reactive oxygen species (ROS) produced after recognition of pathogen associated molecular patterns (PAMPs). Its phosphorylation results in activation of JNK and p38 that regulate cell death and immune response[5]
- SRT-015, an investigational ASK1 inhibitor, has demonstrated efficacy in reducing systemic inflammation and liver injury in preclinical models of liver failure and ACLF[6,7,8]

The aim of this study was to further characterize the effects of SRT-015 on immune cells, not only to confirm its anti-inflammatory activity but also to evaluate its effects on immune cell functions important for host defense. The studies were performed in blood cells isolated from healthy donors as a proof-of-concept

1) **SRT-015 effects on degranulation, migration and bacterial phagocytosis were evaluated in polymorphonuclear leukocytes (PMNs)**
2) **To evaluate the effects of SRT-015 on inflammation and immune reprogramming, cytokine production and immunophenotyping of B cells and monocytes were assessed in peripheral blood mononuclear cells (PBMCs)**
3) **SRT-015 effects on cytokine secretion and polarization were tested in monocyte-derived macrophages (MDM)**

METHODS

Evaluation of SRT-015 effect on neutrophils' functions

Polymorphonuclear leukocytes (PMNs) were isolated from blood of 5-7 healthy donors by Ficoll-Hypaque density centrifugation

- Degranulation was assessed by quantification of the myeloperoxidase (MPO) release with Human ELISA kit (Abcam) and activity by using the Neutrophil MPO Activity Assay kit (Cayman Chemical, ann Arbor, MI) after 2-hour incubation with phorbol 12-myristate 13-acetate (PMA) in presence of SRT-015 (2 and 10 µM) or vehicle
- Chemotaxis was assessed in a Transwell assay: neutrophils were seeded at 1.5 x 10^6 cells/mL into the upper chamber and incubated with SRT-015 (2 µM or 10 µM) or vehicle, while the lower chamber was filled with medium supplemented with leukotriene B4 (LTB4). After 90 minutes of incubation, neutrophils that migrated into the lower chamber were measured using Trypan blue and Countess Automated Cell Counter 3 (Invitrogen, Waltham, MA)
- Phagocytic activity was quantified by measuring the fluorescence intensity of internalized opsonized fluorescent-labeled *Escherichia coli* (Thermo Fisher Scientific) using the FLUOstar Optima plate reader (Ortenberg) after 2-hour incubation in presence of SRT-015 (2 and 10 µM) or vehicle

Evaluation of SRT-015 effect on cytokine production and B cell and monocyte immunophenotype in peripheral blood mononuclear cells (PBMCs)

Human PBMCs were isolated from whole blood of six healthy donors by Ficoll-Hypaque density centrifugation

- LPS stimulation: PBMCs were stimulated with lipopolysaccharide (LPS *Escherichia coli* O111:B4, 10 ng/mL, Sigma-Aldrich) in presence of SRT-015 (2 and 10 µM) or vehicle as indicated on the figure. Cells and supernatants were collected to measure cytokine expression and release
- Immunophenotyping: After incubation in presence of SRT-015 (2 and 10 µM) or vehicle, cells were incubated with antibodies targeting B-cells (CD19, CD38, CD24, CD27, CD21, IgD, IgM) or monocytes (CD14, CD16, HLA-DR). Samples were analyzed using a Cytek Aurora spectral cytometer (Cytek Biosciences, Fremont, CA)

Evaluation of SRT-015 effect on peripheral blood monocyte-derived macrophages (MDM)

Human Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood of 5 healthy donors by Ficoll Hypaque density centrifugation. PBMCs were seeded to allow attachment of monocytes and treated with macrophage colony stimulating factor (M-CSF, 20ng/mL) for 6 days to obtain MDM

- LPS stimulation: MDM were stimulated with LPS (10 ng/mL; *Escherichia coli* O111:B4, 10 ng/mL, Sigma-Aldrich) in presence of SRT 015 (2 µM or 10 µM) or vehicle. Cytokine and chemokine were quantified in the supernatant
- M2 polarization: MDM were treated with IL-4 in presence of SRT 015 (2 µM or 10 µM) or vehicle. At day 8, cells were collected and expression of M2 marker CD206 was analyzed

Gene expression analyses

- Total RNA were isolated using TRIzol reagent and RT-qPCR was performed (Applied Biosystems). RNA and RT-qPCR were performed to measure gene expression by QuantStudio 7 Pro Real-Time PCR System (Applied Biosystems). Gene expression levels were normalized to β-actin as an endogenous control and expressed relative to a calibrator sample (vehicle-treated cells). Raw data were standardized to allow comparisons across different ranges, and results were expressed as the percentage of mRNA expression

Cytokine and chemokine measurement in supernatant

- Cytokines/chemokines were quantified using bead–based immunoassay Luminex® technology (MilliplexMAP Human Cytokine/Chemokine Magnetic Bead Plex Kit, Merck Millipore). Data analysis was conducted with Belysa™ immunoassay curve-fitting software

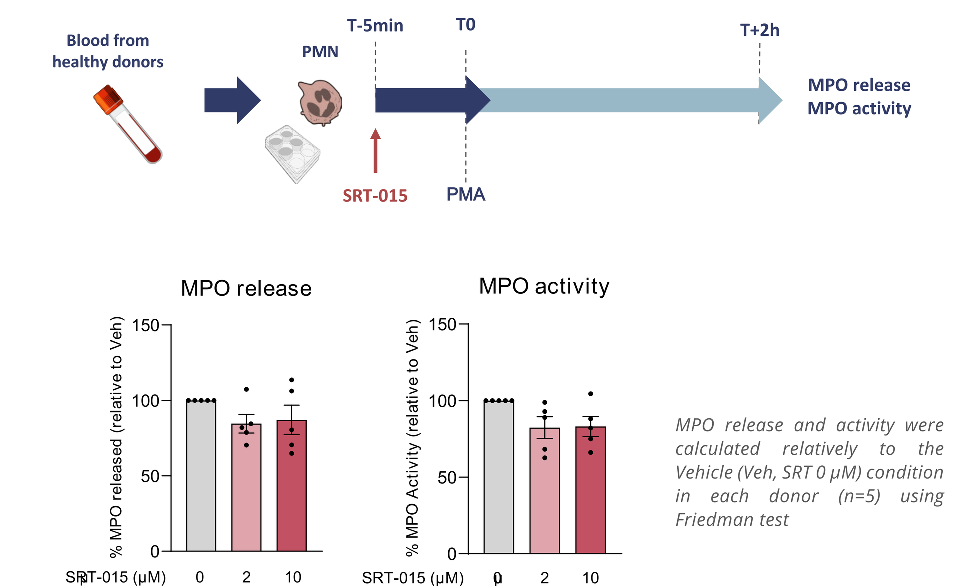
Statistical analyses

- Normality of the data was evaluated, and parametric or non-parametric test was applied accordingly (GraphPad Prism). Statistical tests are indicated in each figure

RESULTS

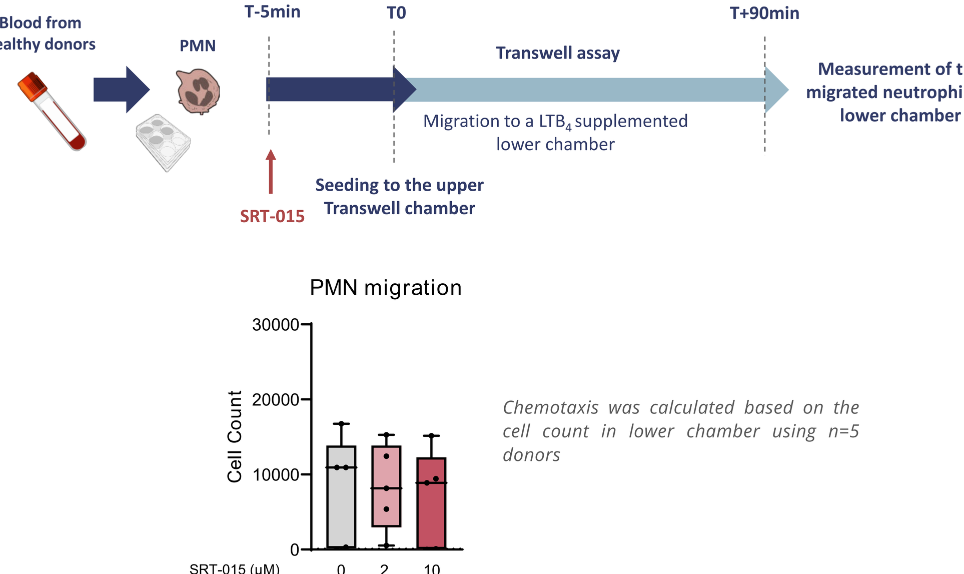
IN PMNs, SRT-015 PRESERVES DEGRANULATION AND CELL MIGRATION WHILE INCREASING PHAGOCYTIC CAPACITY

EVALUATION OF SRT-015 EFFECT ON NEUTROPHIL DEGRANULATION



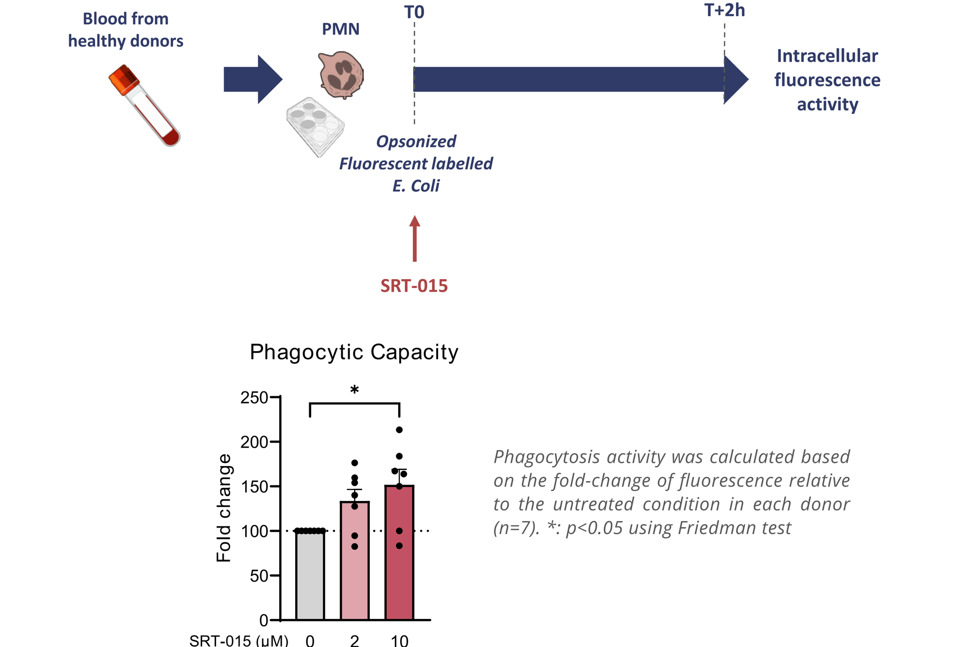
SRT-015 does not impair degranulation of neutrophils

EVALUATION OF SRT-015 EFFECT ON NEUTROPHIL MIGRATION



SRT-015 does not impair migration of neutrophils

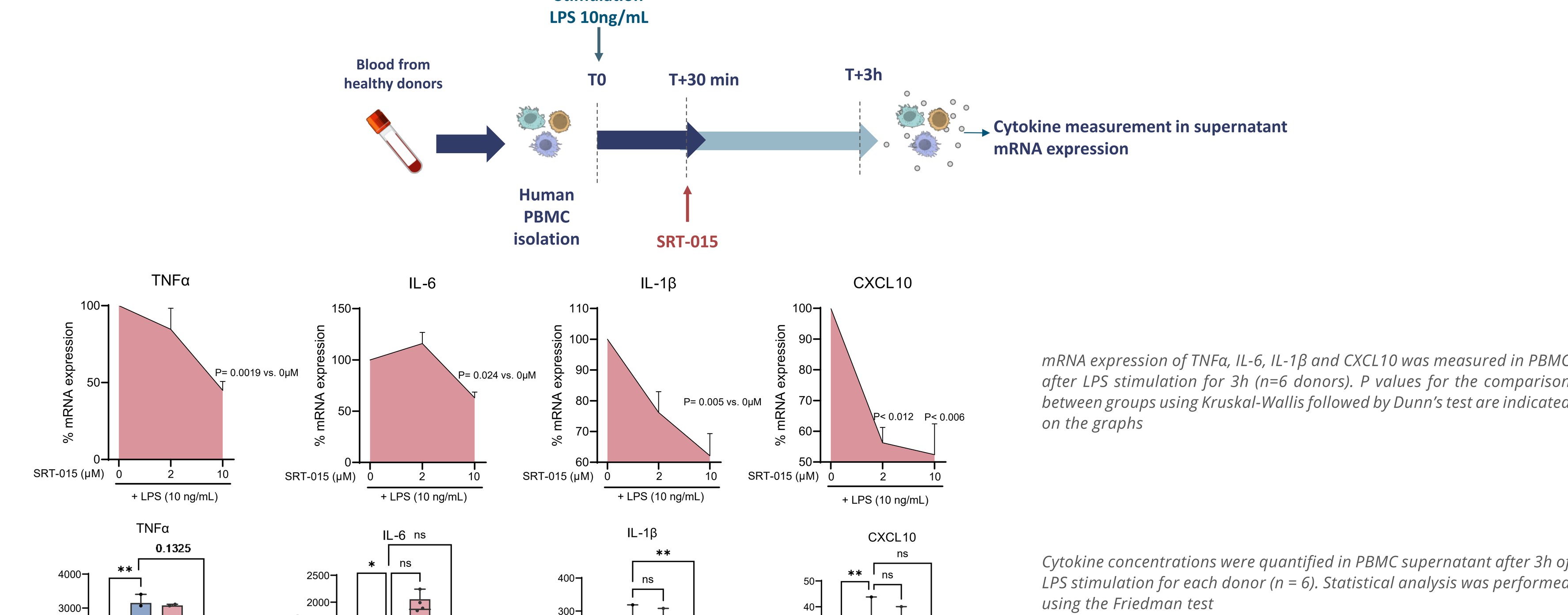
EVALUATION OF SRT-015 EFFECT ON NEUTROPHIL PHAGOCYTOSIS



SRT-015 significantly increased neutrophil bacterial phagocytosis in a concentration-dependent manner

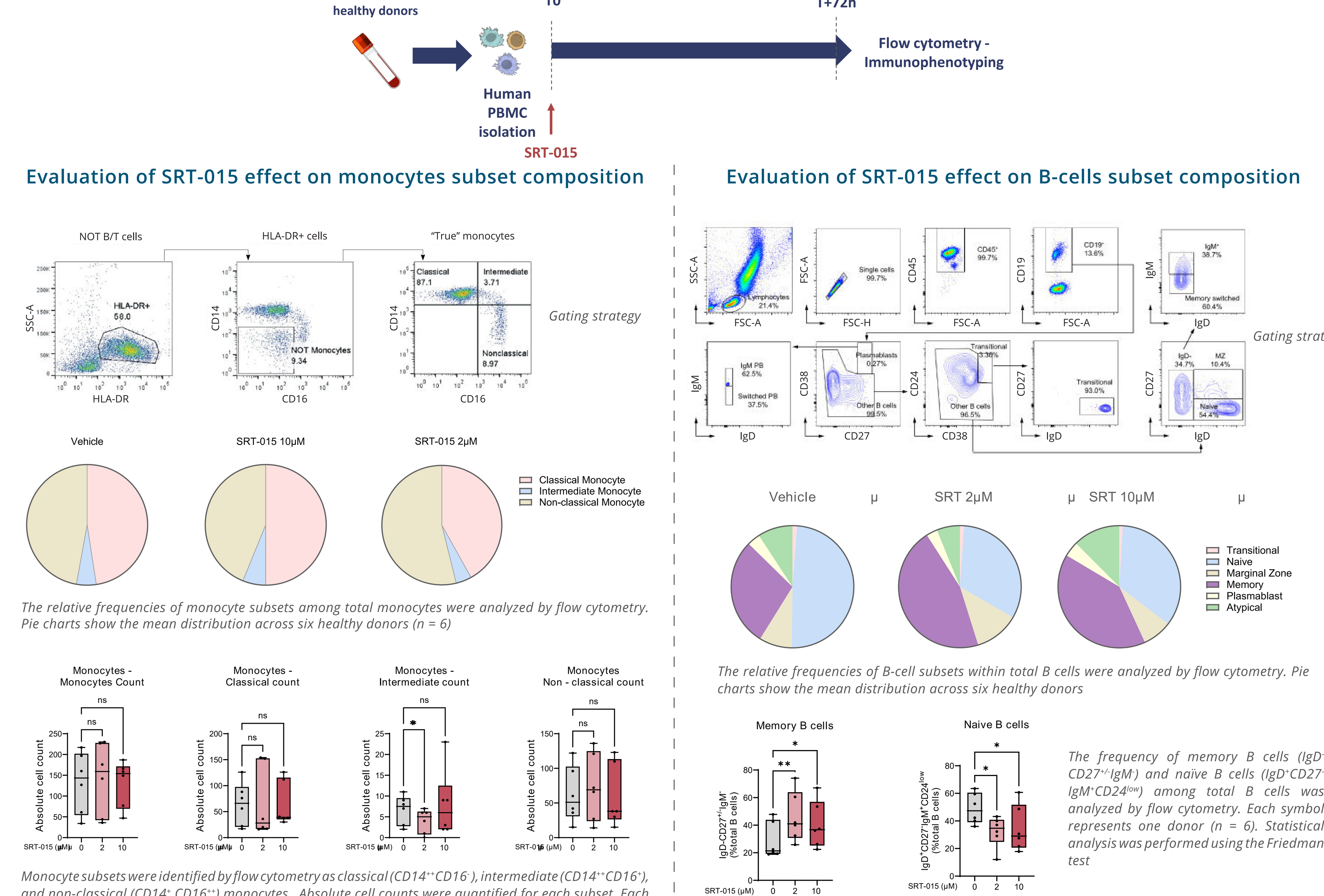
IN PBMCs, SRT-015 ATTENUATES LPS-INDUCED PRO-INFLAMMATORY CYTOKINE PRODUCTION, WITH NO IMPACT ON MONOCYTE POPULATIONS, WHILE FAVORING A MEMORY B-CELL PHENOTYPE

EVALUATION OF SRT-015 EFFECT ON LPS-INDUCED CYTOKINE PRODUCTION IN PBMCs



SRT-015 decreases LPS-induced production of pro-inflammatory cytokines and chemokine in a concentration-dependent manner

EVALUATION OF SRT-015 EFFECT ON MONOCYTES AND B-CELLS SUBSET COMPOSITION

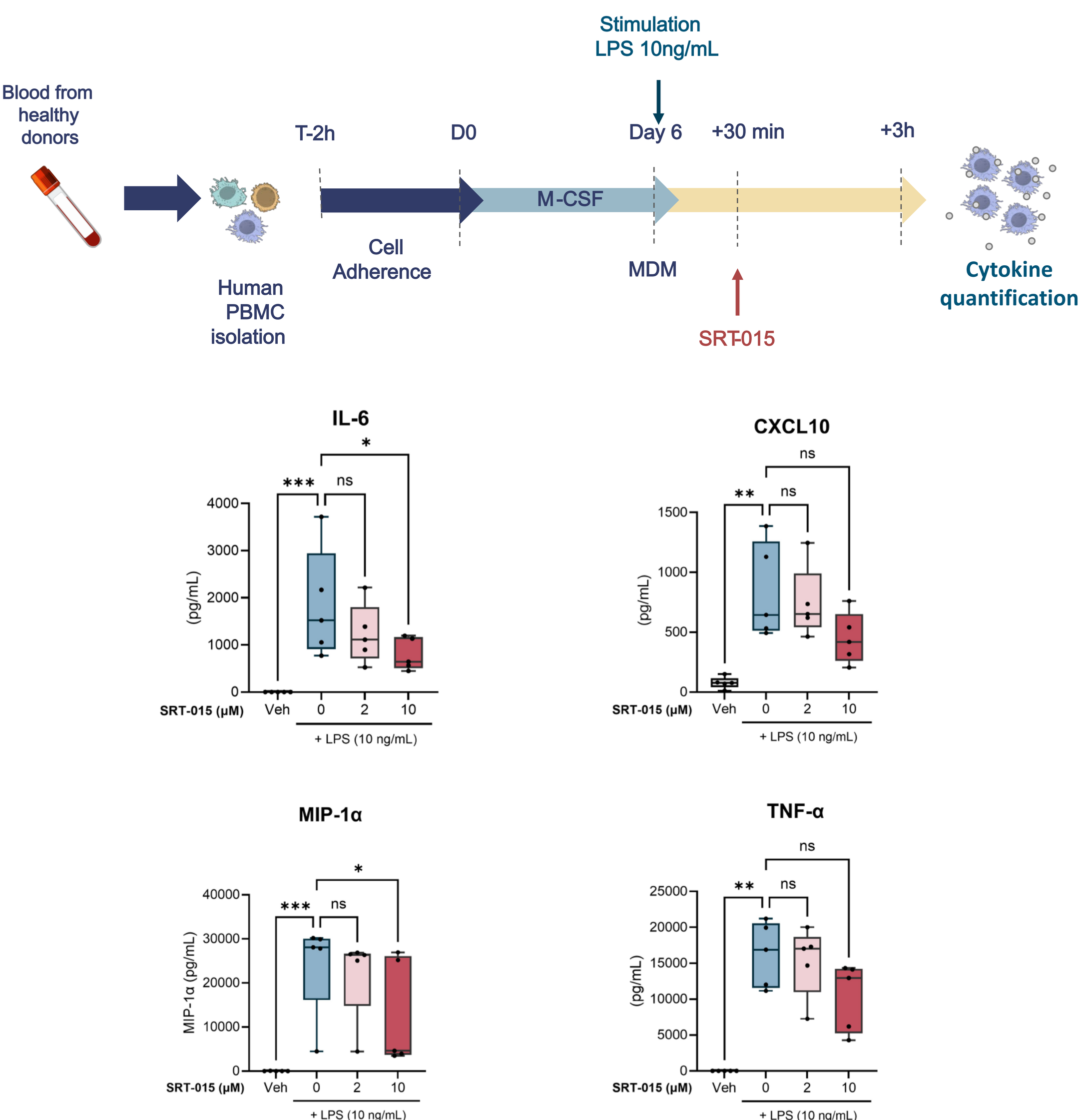


SRT-015 had no effect on the composition of classical, intermediate or non-classical monocytes

SRT-015 shifts the B-cell composition toward a memory phenotype, increasing memory B cells while decreasing naive B cells

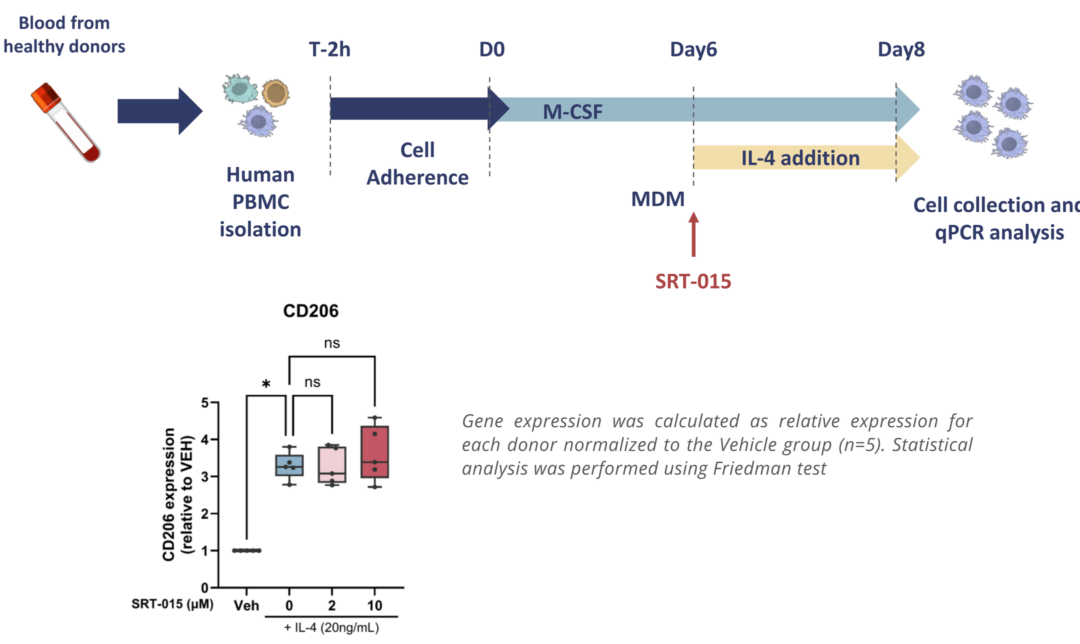
IN MDM, SRT-015 ATTENUATES LPS-INDUCED PRODUCTION OF CYTOKINES WHILE PRESERVING M2 POLARIZATION

EVALUATION OF SRT-015 EFFECT ON LPS-INDUCED CYTOKINE AND CHEMOKINE PRODUCTION IN MDM



SRT-015 decreases LPS-induced production of pro-inflammatory cytokines and chemokine in a concentration-dependent manner

EVALUATION OF SRT-015 EFFECT ON MDM POLARIZATION



Induction of CD206 expression was not altered by SRT-015, suggesting that SRT-015 preserves IL-4–driven M2 polarization

CONCLUSION

This proof-of-concept study demonstrates the potential of the investigational drug SRT-015 to reduce systemic and tissular inflammation while enhancing antibacterial activity and preserving immune cell function, supporting its further development for advanced liver disease and ACLF

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