# Development of *in vitro* assays for the assessment of potential drugs regulating the migration of blood-derived human monocytes or neutrophils

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## 1 Overview

Monocytes and neutrophils are key 'sentinels' of the innate immune responses and maintain immune surveillance. They are both recruited to inflamed or injured tissues where they eliminate pathogens through phagocytosis or by releasing soluble mediators. Their trafficking across the blood vessel wall to inflamed tissues is mediated and regulated by specific molecules called chemo-attractants or chemokines. *In vivo* and *in vitro* evidences have demonstrated that both monocytes and neutrophils are implicated in different immuno-inflammatory diseases like atherosclerosis, neurodegenerative disorders and also in tumorigenesis. Therefore, understanding the switches that regulate migration of innate cells in pathological settings can be a fundamental approach in the fields of inflammation, auto-immune disease and cancer research. We have developed two optimized, off-the-shelf chemotaxis assays, using human primary blood derived monocytes and neutrophils to evaluate the translational potential of small molecules as novel therapies.

## 2 Chemotaxis assay on Monocytes

Monocytes are innate immune cells involved in the resolution of tissue injuries, inflammation and infections. Monocyte migration is driven by chemokines including the stromal cell-derived factor 1α (SDF-1α), which is released by residential cells upon tissue damage and recognized by cells expressing CXCR4. Several studies have demonstrated that SDF-1α promotes monocytes to migrate through the vascular or lymphatic endothelium to the peripheral injured or inflamed tissue. A validated and robust chemotaxis assay has been developed using monocytes isolated from human blood of healthy donors to evaluate therapeutic candidates. Migration of monocytes is identified by measuring the ATP levels of cells that have migrated in a Boyden Chamber or Transwell® system.

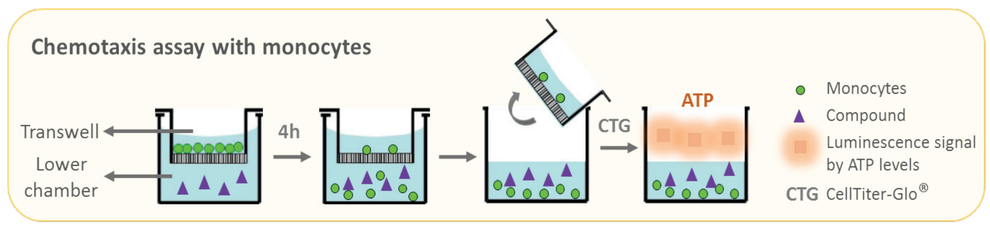


**Figure 1.** Schematic overview of the Chemotaxis assay for human monocytes.

Blood-derived primary human monocytes ($CD14^+$ cells) are isolated from healthy blood donors using Ficoll separation and a bead-based magnetic separation approach (positive selection). Monocytes are then seeded in the upper chamber of a 96-well Boyden chamber that contain 5.0 µm pore polyester membrane in serum-free medium, while chemoattractant and/or compounds are added to the lower chamber. After four (4) hours, monocytes that have migrated through the pores into the lower chamber are detected by measuring their ATP levels through a luminescent-based method (CellTiter-Glo®) and read with a plate reader.

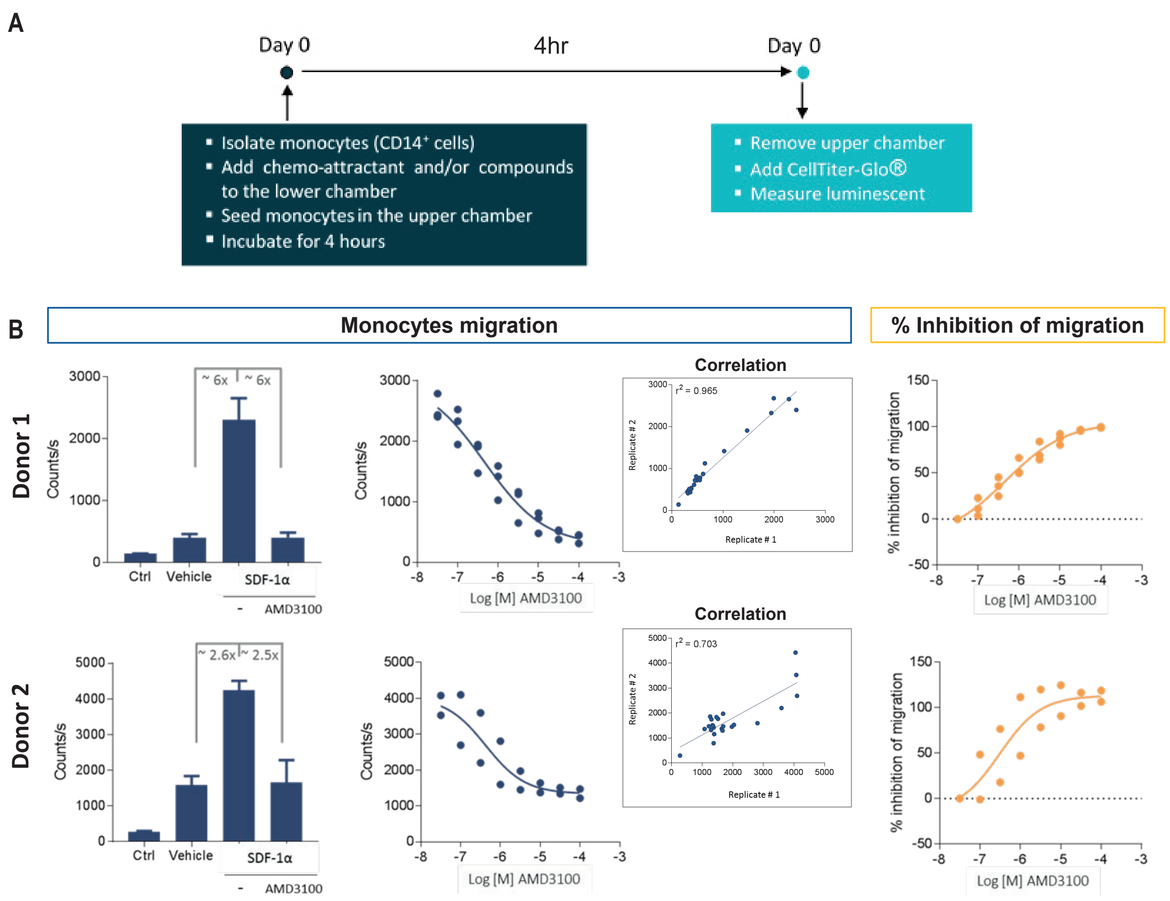


**Figure 2. A.** Outline and timelines of the optimized Chemotaxis Monocytes assay. **B.** SDF-1α-mediated migration of monocytes is strongly inhibited by the treatment with a CXCR4 antagonist (AMD3100). $IC_{50}$ values for AMD3100 were consistent across different donors (not shown) and the Spearman rank correlation denotes high reproducibility between biological replicates.

## 3 Chemotaxis assay on Neutrophils

Neutrophils are one of the first responders during acute inflammation. Neutrophils are recruited to injured in inflamed site to kill extracellular pathogens through phagocytosis or 'degranulation' by releasing anti-microbial mediators such as reactive oxygen species. Numerous studies have demonstrated that IL-8, which is released by macrophages and endothelial/epithelial cells, has chemotactic functions because it triggers neutrophils migration to the inflamed site and promotes their degranulation by binding to CXCR1/CXCR2 molecules. A validated and robust chemotaxis assay has been developed using neutrophils isolated from human blood of healthy donors to evaluate therapeutic candidates. Migration of neutrophils is identified by measuring the ATP levels of cells that have migrated in a Boyden Chamber or Transwell® system.

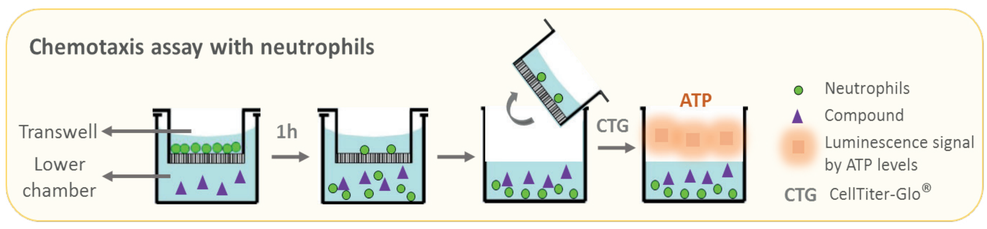


**Figure 3.** Schematic overview of the Chemotaxis assay for human neutrophils.

Blood-derived primary human neutrophils are isolated from healthy blood donors using Ficoll separation and dextran-based sedimentation. Neutrophils are then seeded in the upper chamber of a 96-well Boyden chamber that contain 5.0 µm pore polyester membrane in serum-free medium, while chemoattractant and/or compounds are added to the lower chamber. After one (1) hour, neutrophils that have migrated through the pores into the lower chamber are detected by measuring their ATP levels through a luminescent-based method (CellTiter-Glo®) and read with a plate reader.

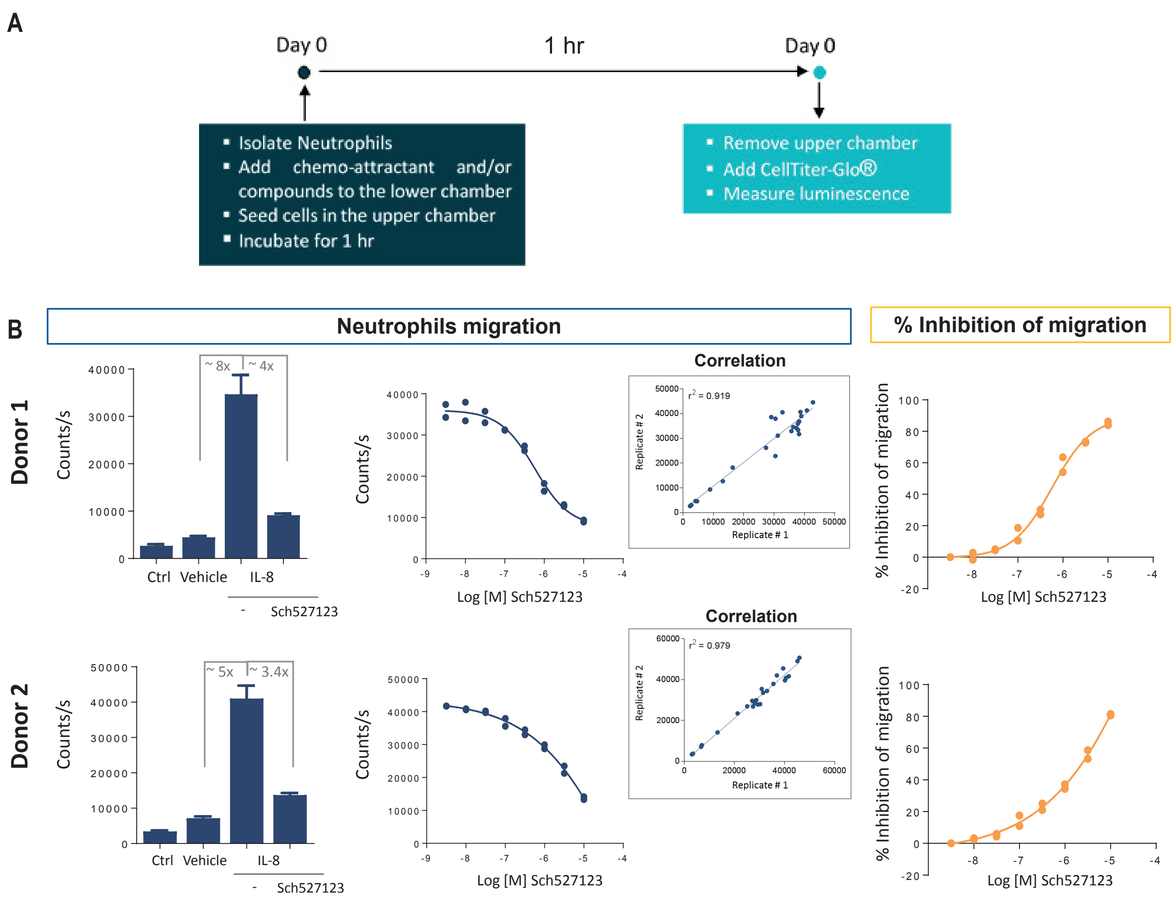


**Figure 4. A.** Outline and timelines of the optimized Chemotaxis Neutrophils assay. **B.** IL-8-mediated migration of neutrophils is strongly inhibited by treatment with a CXCR1/2 antagonist (Sch527123). $IC_{50}$ values for Sch527123 were consistent across different donors (not shown) and the Spearman rank correlation denotes high reproducibility between biological replicates.

## 4 Conclusions

Migration of immune cells is essential for resolving tissue injuries, inflammation and infections, and this process is orchestrated by chemokines that attract immune cells to the injured or inflamed site. Here we showed two optimized in vitro assays for monocytes and neutrophils migration. We demonstrated that SDF-1α, a member of the C-X-C chemokine family, strongly induced (at 10 nM) the migration of human monocytes, and that IL-8, a key chemokine for the recruitment of neutrophils, strongly promoted the migration of human neutrophils at 10nM. Migration of both cell types was inhibited in a dose-dependent fashion by specific chemokine-receptor inhibitors such as AMD3100, a SDF-1α receptor (CXCR4) antagonist used in the monocytes assay, and Sch527123, a IL-8 receptor (CXCR1/2) antagonist used in the neutrophil assay. The $IC_{50}$ values were consistent across different donors (not shown) and strong assay reproducibility was observed. In both assays, therapeutic candidates can be evaluated in 8-point concentration response curves, as is shown for the reference compounds.
These results suggest that both in vitro Chemotaxis Monocytes and Chemotaxis Neutrophils assays can be a useful translatable tool for the evaluation of the potency and efficacy of prospective drugs for the prevention and/or treatment of diseases involving monocytes and neutrophils such as acute and chronic inflammatory conditions, auto-immune and neurodegenerative diseases and cancer.

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