CD47 antibody-induced engulfment of human leukaemic T-cells by bone-marrow derived macrophages

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Overview
- CD47 is a cell-surface marker of self, or “don’t-eat-me” signal. Expression of CD47 enables tumour cells to evade clearance by host macrophages.
- Blocking CD47 holds great promise as a therapeutic strategy for both solid and haematologic cancers.
- Here, we have developed and validated an automated image-based 96-well assay for anti-CD47 antibody-mediated cellular phagocytosis.

**Anti-CD47 Ab induces phagocytosis of CCRF cells**

Labelling CCRF-CEM cells were treated with increasing concentrations of anti-CD47 antibody (86H12.2, Abcam; ab3283) and added to human bone marrow-derived macrophages (BMDM), values shown are mean ± SEM, n=4.

**Anti-CD47 engulfment does not involve target cell apoptosis**

- Anti-CD47 antibody induces phagocytosis of CCRF-CEM cells by J774A.1 mouse macrophages without inducing apoptosis. Data shown are for 5 ng/mL anti-CD47- and camptothecin-treated (apoptotic) CCRF-CEM cells.

**Anti-CD47 directly inhibits proliferation at later time points**

- Anti-CD47 antibody exerts a direct anti-proliferative effect on CCRF-CEM cells in mono-culture; no effect observed after 3 h, whereas a marked reduction seen at 24 h. Data shown are for 5 μg/mL anti-CD47- and camptothecin-treated (apoptotic) CCRF-CEM cells.

Phagocytosis time-course is effector-cell dependent

Differential time-courses observed for anti-CD47-induced phagocytosis in different effector cells.

**Continuous Live-Cell Analysis: Methodology**

IncuCyte® S3 Live-Cell Analysis System
A flexible assay platform that enables high content and high throughput screening with a multi-parametric readout of cell shape, movement, migration, or secreted protein production. Used in microplates, dishes, or flasks.

IncuCyte® Software
Fast, flexible, and powerful control hub for continuous live-cell analysis comprising image acquisition, processing, and data visualization.

IncuCyte® Reagents & Consumables
A suite of non-perturbing cell labelling and reporter reagents. Includes nuclear-targeted GFP and RFP for cell counting; co-wash reporters with apoptosis, cell cycle, and cell kits for engagement.

Cellular phagocytosis assay principle

- Target cells are labelled using the IncuCyte pHrodo Red Cell Labelling Kit which covalently attaches a pH-sensitive dye to proteins on the exterior of the cell.
- Labelled target cells are added to a standard 96-well microtitre plate containing effector phagocytes (e.g. macrophages, dendritic cells), then placed in an IncuCyte live-cell analysis system within an incubator.
- Phase contrast and fluorescence images are taken at regular intervals.
- Fluorescence (area or intensity) increases as the target cells are engulfed into the effector cell lysosome (low pH).
- A kinetic profile showing real-time cell engulfment is generated using the IncuCyte software.

Validation of labelling step:
- pHrodo-labelled Jurkat cells display red fluorescence in low pH buffer (pH5).
- Fluorescence intensity and the number of cells (objects) exceeding threshold brightness were determined using an IncuCyte live-cell analysis system.
- Intensity increase is proportional to the label concentration.
- The number of labelled cells exceeding the intensity analysis threshold plateaus.

Imaging and quantification

- Engulfed cells are observed inside the macrophages and have high fluorescence intensity.
- The fluorescence (total fluorescent area or intensity) is proportional to the amount of cell phagocytosis.
- Rapid imaging (2 minute intervals) can be performed to create movies showing individual engulfment events.

Automated 96-well assay and analysis

- Inhibitors with varying mechanisms of action (actin polymerisation inhibitor, microtubule disruptor, PKC inhibitor) were applied to the effector cells (J774A.1 mouse macrophages).
- Four concentration-response curves generated (triplicate data, 7 point curves) from a single 96-well plate.