SARTORIUS

Simplifying Progress

High-Throughput Quantification of Antibody-Dependent Phagocytosis using Live-Cell Analysis

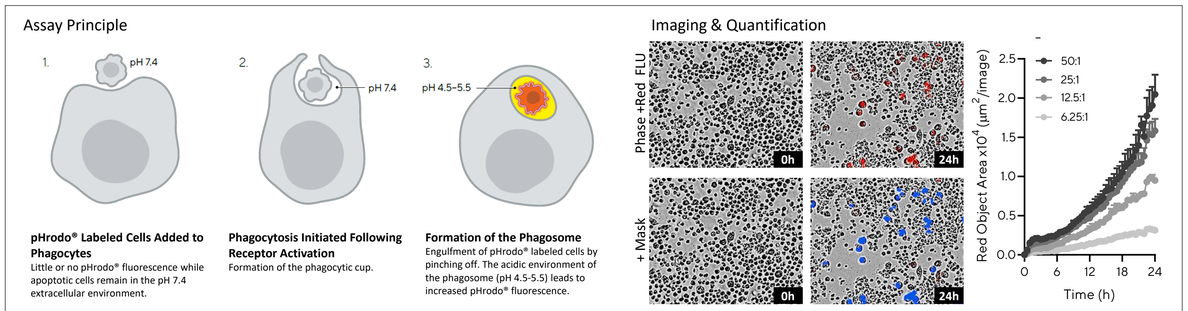
J. Trigg*, G. Lovell, N. Bevan, and T. Dale

Sartorius Corporation, Royston, Hertfordshire, UK
*Corresponding author: Jasmine.Trigg@sartorius.com

Introduction

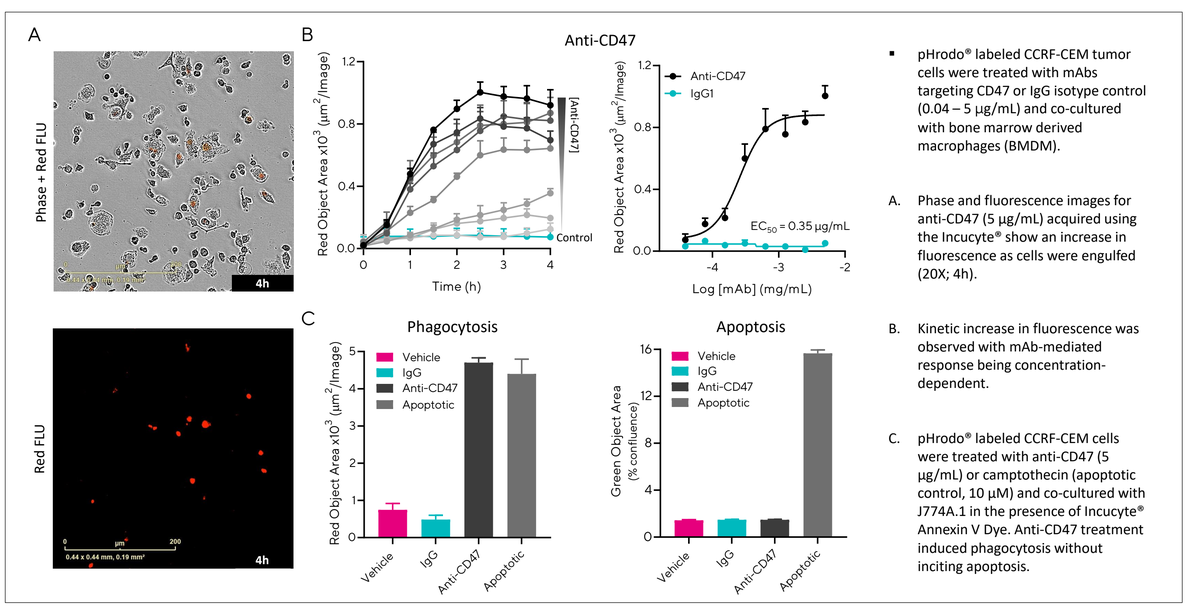
- Phagocytosis, a specific form of endocytosis, is a critical component of innate and adaptive immune responses.
- The uptake and clearance of viable tumor cells can be promoted with monoclonal antibodies (mAbs) via antibody-dependent phagocytosis (ADCP) or through the blockade of "don't-eat-me" signals, such as CD47. These mechanisms hold immunotherapeutic promise and are studied extensively in drug development.
- Here, we have developed and validated an *in vitro* assay for the high-throughput quantification of phagocytosis using the Incucyte® Live-Cell Analysis System.
- The Incucyte® Phagocytosis Assay combines pHrodo® for Incucyte® reagents and integrated image-based fluorescent measurements in a simple mix-and-read protocol.
- These data exemplify that live-cell analysis is a powerful tool for quantitative morphological and functional assessment of ADCP, which is amenable to screening for therapeutic candidates.

Visualize and quantify phagocytosis in real-time



- pHrodo® for Incucyte® is a pH sensitive fluorophore that increases in fluorescence when target cells are engulfed and enables high-throughput visualization and quantification of phagocytosis in real-time.
- pHrodo® labeled apoptotic Jurkat cells were co-cultured with J774A.1 mouse macrophages at a range of target to effector ratios. Phase and fluorescent images (20X) were acquired using the Incucyte® Live-Cell Analysis System and phagocytosis quantified using a fluorescent segmentation mask (blue). Timecourse shows a density-dependent increase in red fluorescence following engulfment over 24h.

Anti-CD47 mAb stimulates macrophage-mediated phagocytosis



- pHrodo® labeled CCRF-CEM tumor cells were treated with mAbs targeting CD47 or IgG isotype control (0.04 – 5 µg/mL) and co-cultured with bone marrow derived macrophages (BMDM).

A. Phase and fluorescence images for anti-CD47 (5 µg/mL) acquired using the Incucyte® show an increase in fluorescence as cells were engulfed (20X; 4h).

B. Kinetic increase in fluorescence was observed with mAb-mediated response being concentration-dependent.

C. pHrodo® labeled CCRF-CEM cells were treated with anti-CD47 (5 µg/mL) or camptothecin (apoptotic control, 10 µM) and co-cultured with J774A.1 in the presence of Incucyte® Annexin V Dye. Anti-CD47 treatment induced phagocytosis without inciting apoptosis.

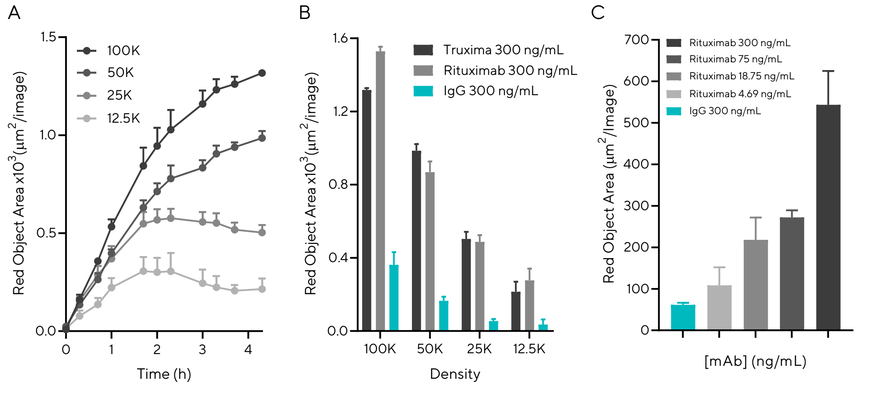
Clinical anti-CD20 mAbs Truxima® and Rituximab promote ADCP

- pHrodo® labeled Ramos target cells (100 – 12.5K/well) were treated with anti-CD20 mAbs, Rituximab or biosimilar Truxima®, or IgG1 isotype control (300 ng/mL), co-cultured with BMDM.

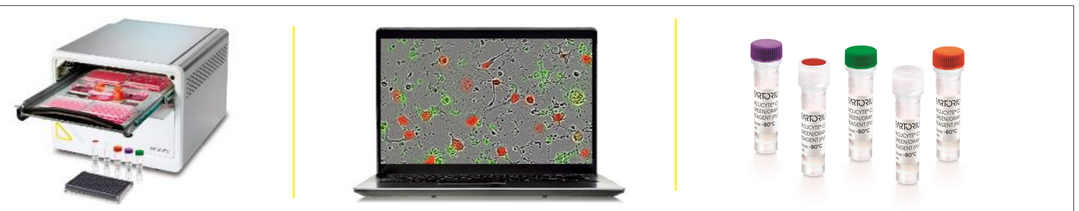
A. Timecourse shows Truxima® (300 ng/mL) induced ADCP response is time- and target cell density-dependent.

B. Results show increase in fluorescence for Truxima® and Rituximab compared to IgG control at all target cell densities (4h).

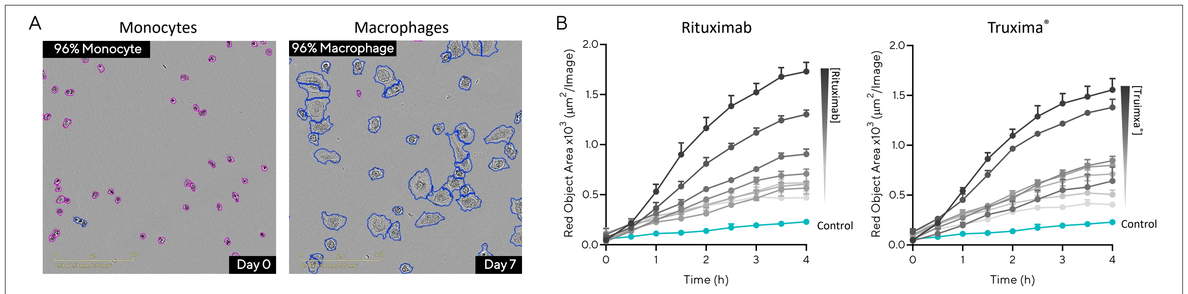
C. Rituximab treatment showed a concentration-dependent increase in ADCP (50K cells/well)(2h).



Incucyte® Live-Cell Imaging and Analysis Solutions



ADCP increases with anti-CD20 concentration in primary macrophages

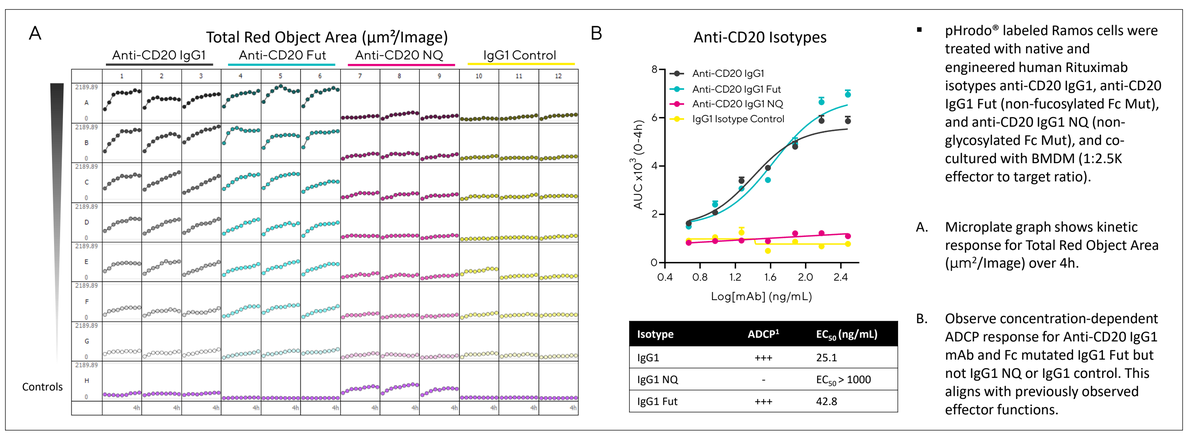


- Monocytes isolated from whole blood were differentiated into macrophages using cytokines. pHrodo® labeled Ramos cells were treated with mAbs targeting anti-CD20, Rituximab or Truxima, or IgG isotype control (300 ng/mL) and co-cultured with macrophages.

A. Macrophage differentiation was monitored and quantified using Incucyte® Advanced Label-Free Classification Analysis, images show classification masks for monocytes (pink outline) and macrophages (blue outline) on day 0 and day 7.

B. Timecourses show Rituximab and Truxima® induced ADCP in a concentration-dependent manner, with similar kinetic profiles being observed over 4h.

High-throughput assessment of native and engineered Rituximab Fc Mutants



Isotype	ADCP[1]	EC_{50} (ng/mL)
IgG1	+++	25.1
IgG1 NQ	-	EC_{50} > 1000
IgG1 Fut	+++	42.8

- pHrodo® labeled Ramos cells were treated with native and engineered human Rituximab isotypes anti-CD20 IgG1, anti-CD20 IgG1 Fut (non-fucosylated Fc Mut), and anti-CD20 IgG1 NQ (non-glycosylated Fc Mut), and co-cultured with BMDM (1:2.5K effector to target ratio).

A. Microplate graph shows kinetic response for Total Red Object Area (µm²/Image) over 4h.

B. Observe concentration-dependent ADCP response for Anti-CD20 IgG1 mAb and Fc mutated IgG1 Fut but not IgG1 NQ or IgG1 control. This aligns with previously observed effector functions.

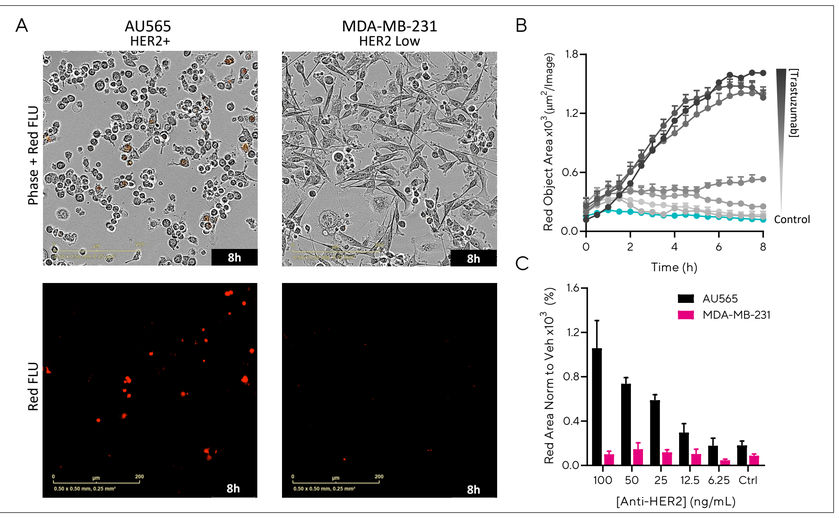
Target HER2 antigen expression correlates with ADCP response

- ADCP was examined using HER2-positive AU565 or HER2-low MDA-MB-231 adherent target cells. Cells were labeled with pHrodo®, treated with anti-HER2 mAb (Trastuzumab) and co-cultured with BMDM.

A. Anti-HER2 induced an increase in fluorescence for AU565 but little-to-no fluorescence was observed for MDA-MB-231 cells (100 ng/mL; 8h).

B. Timecourse shows ADCP response for AU565 cells occurs in a time- and concentration-dependent manner.

C. Anti-HER2 induced ADCP for AU565 cells but not MDA-MB-231 cells, consistent with established correlations between HER2 expression and ADCP response. Red area was normalized to vehicle to account for differences in background intensity of target cells.



1. Invivogen: Reagents And Tools For Cell Biology Research. [online] Available at: https://www.invivogen.com; Data shown as Mean ± SEM, 3 replicates.