SARDRICS

Accelerating ADCP screening using the iQue[®] Advanced Flow Cytometry Platform

log [Truxima] g/mL

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Introduction

- Antibody-dependent cellular phagocytosis
 The iQue[®] Human Antibody Dependent (ADCP) is monoclonal antibody (mAb)stimulated engulfment and clearance of specific cellular targets such as tumor cells.
- Evaluation of mAbs in drug development requires broad MoA characterization, including effects on ADCP.
- Here we present an *in vitro* assay for guantification of ADCP in 96- or 384-well plates using the iQue[®] advanced flow cytometry platform.
- Assay Concept



- Cellular Phagocytosis Kit measures colocalization between live target cells and CD14+ effector cells to provide a readout for ADCP response.
- These data exemplify the power of iQue[®] ADCP assay to generate pharmacological outputs for mAb ADCP activity with the potential to enhance drug discovery or biological research applications.
 - Encoder dye labeled target cells are incubated with test antibody prior to the addition of unlabeled effector cells.
 - Live and dead cells are separated using the iQue[®] Cell Membrane Integrity (R/Red) Dye.
 - ADCP is quantified as the % live, CD14+ effector cells that are positive for the target cell encoder.



Experimental Approach



Donor 2 ADCP

0 to 1

E:T

Control

• Pre-set template provided with the iQue[®] Human Antibody Dependent Cellular Phagocytosis Kit. • The singlet gate is omitted to count co-localized encoded target cells and CD14+ monocytes.

 $10^3 10^4 10^5 10^6 10^7$

Pre-set gating from All Events through to the CD14+ Encoder+ Live cell population

Viability dye (RL1-H)

10² 10³ 10⁴ 10⁵ 10⁶ B/Green Encoder (BL1-H)

10⁴ 10⁵ 10⁶

CD14 (BL3-H)

and Raji cells, respectively.

Results- Response differs between effector cell donors



 Ramos cells were incubated with PBMCs from 2 donors at a range of E:Ts and treated with Truxima. Wells with no antibody were included as a negative control.

At 20:1 E:T, maximal ADCP (%) was similar between donors 1 and 2, at $41 \pm 5\%$ and $43 \pm 2\%$. As the E:T was reduced, the difference in response between donors became more pronounced, with maximal ADCP at 5:1 E:T of 28 ± 8% for donor 1 and 19 ±6 % for donor 2.





Results – mAb profiling with adherent and non-adherent targets



- anti-HER2 mAb isotypes, respectively.
- induce ADCP.
- IgG1 and IgA2 isotypes of Trastuzumab induced an increase in ADCP. The potency of response to the IgG1 was five times greater than the IgA2 isotype.



Results – M2 but not M1 macrophages elicit ADCP response



- Trastuzumab.
- compared to M1s.

Simplifying Progress

Variable region	lsotype	ADCP ¹	EC ₅₀ (ng/mL)
Rituximab (anti-CD20)	lgG1	+++	27.4
	lgG1NQ	-	EC ₅₀ > 1000
	lgG2	+/-	EC ₅₀ > 1000
Trastuzumab (anti-HER2)	lgG1	+++	6.6
	lgA2	+	35.4

• Non-adherent Ramos cells or adherent AU565 cells were incubated with PBMCs (20:1 E:T) and a range of anti-CD20 and ADCP of Ramos cells induced by Rituximab IgG1 was lost with the IgG1NQ (non-glycosylated) mutant. Native IgG2 did not

Results – Target antigen expression correlates with ADCP response ADCP response to HER2 expression 120 35-<u>Trastuzumab</u> 100-30-80 25-(%) 20-d) 15-(x10 NCF-1 BTATA KBR'3

• HER2 expression on 5 adherent breast cancer cell lines using a fluorescently conjugated HER2 antibody and the iQue 3. • Each cell type was then used as a target for an ADCP assay stimulated with Trastuzumab (17 ng/mL). • HER2 expression on the target cells correlated with increased ADCP response. SK-BR-3s and BT474s had 15-20-fold higher HER2 expression and 3-5-fold higher ADCP than the lower expressing cell types (MDA-MB-231, HCC38 and MCF-7).

• After 7 days, macrophages were lifted and used in an ADCP assay containing AU565 target cells and stimulated with

• An antibody concentration dependent increase in ADCP was observed with M2 but not M1 macrophages. • Basal levels of cellular phagocytosis (at the lowest Trastuzumab concentrations) were also much higher with M2s

1. Invivogen: Reagents And Tools For Cell Biology Research. [online] Available at: https://www.invivogen.com