Flow Cytometry for Determination of Efficacy in Phase I

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Drug Development:

- Time Consuming (years)
- Costly
- Termination of a compound in case of
  - Adverse effects
  - Inactivity
- Biomarkers for proper decision making

Ultimate Goal:

- Demonstrate Drug Efficacy as early as possible
- Phase I
  - Safety Volunteer
  - Adverse Effects
  - Pharmacokinetics
  - Efficacy?!

Flow Cytometry

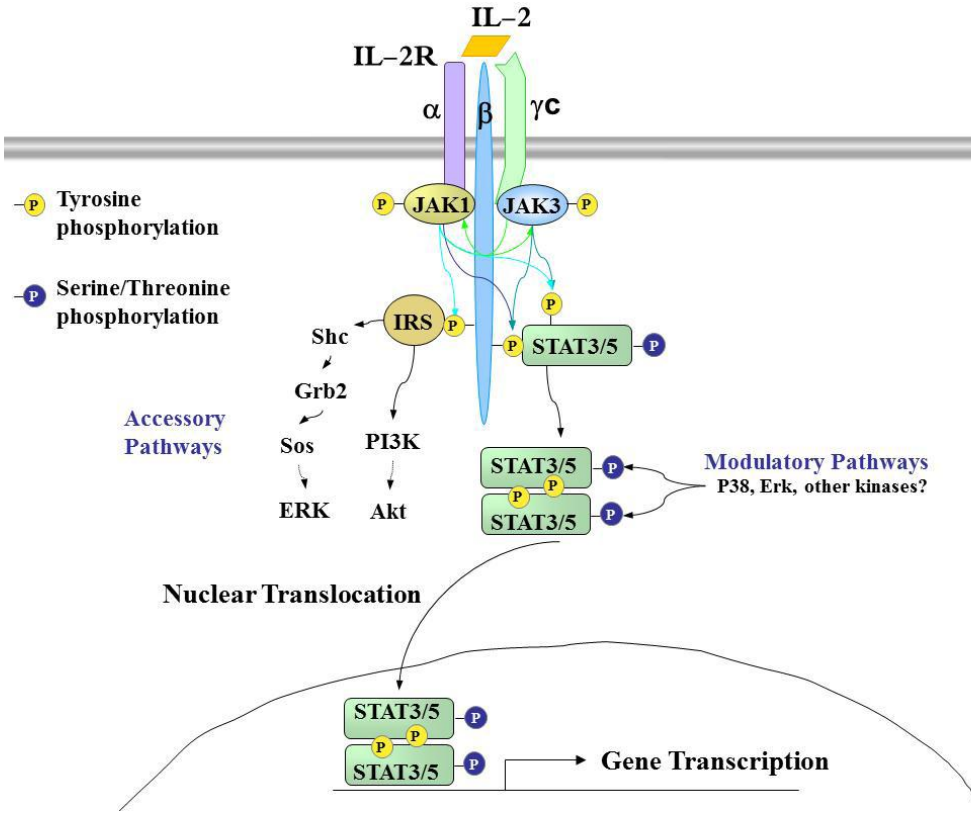
- Flow Cytometry can be useful in early Drug Development:
  - Safety (leukocyte subsets)
  - PK (receptor occupancy)
  - Drug Efficacy (sufficient Drug Levels)

Flow Cytometry for Drug Efficacy

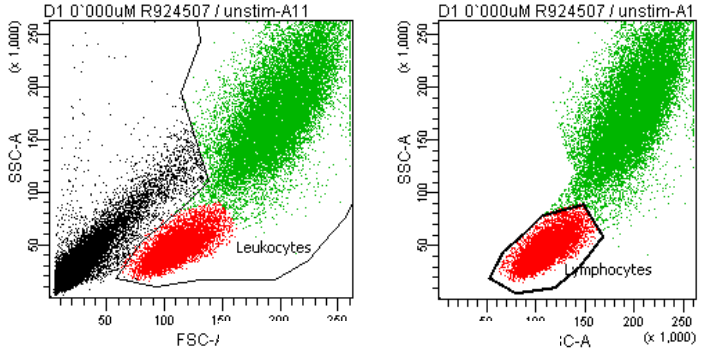
- Case Studies:
  - Method Development/Method Transfer
    - Compound 1: pSTAT5
    - Compound 2: polymerized actin
    - Compound 3: CD63
  - Drug Efficacy *IN VITRO*
    - *Dose-Response Testing*
  - Drug Efficacy EX VIVO during clinical trial
  - Integration with PK results

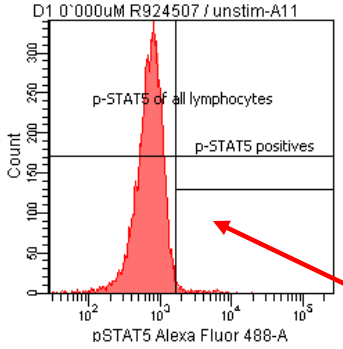
pSTAT5:

- Transcription Factor
- Jak/Stat pathway
- Gene transcription
  - activation
  - Proliferation
- Target for chronic inflammatory reactions:
  - Auto immunity
  - Allograft rejection



pSTAT-5 expression in WB lymphocytes: PBS

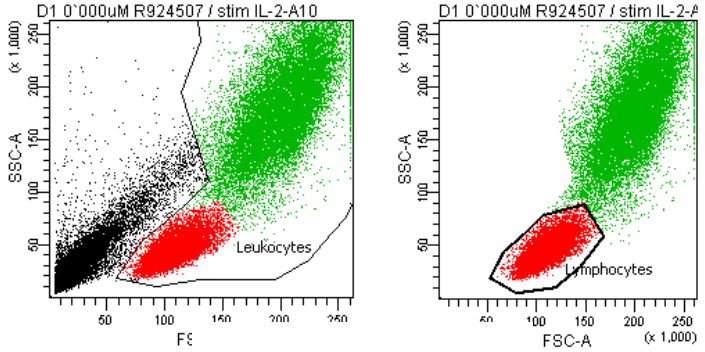




Experiment Name:
Plate Name:
Specimen Name:
Well Name:
Record Date:
$OP: tademahenko

Population	#Events	%Parent	pSTAT5 Al... Geo Mean
Leukocytes	29,776	38.5	####
Lymphocytes	8,385	28.2	####
p-STAT5 positives	124	1.5	2,772
p-STAT5 of all lymphocytes	8,370	99.8	689

pSTAT-5 expression in WB lymphocytes: IL-2



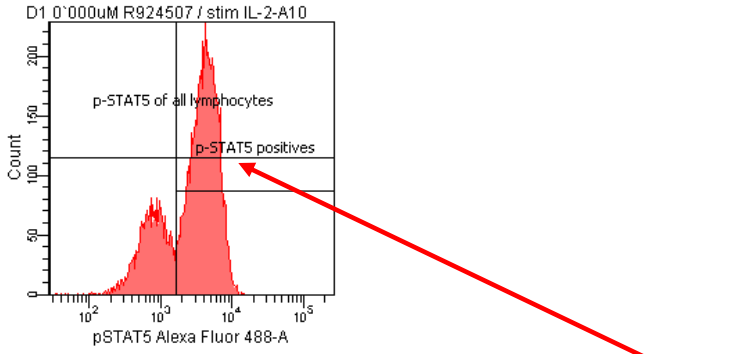


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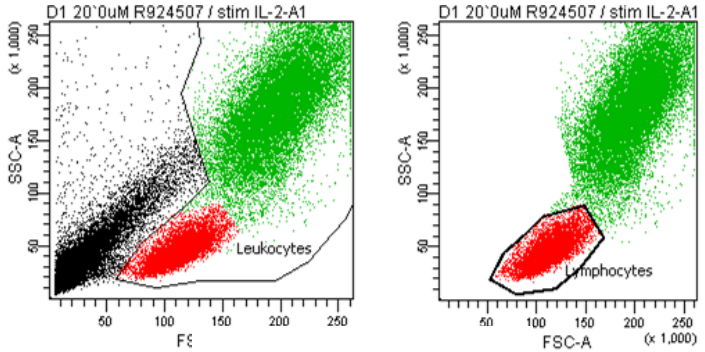
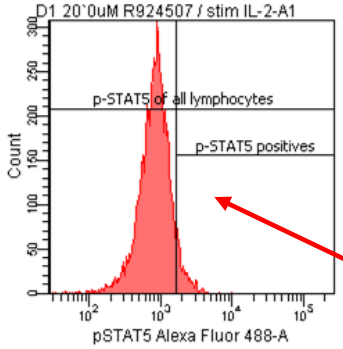
Plate Name:
Specimen Name:
Well Name:
Record Date: 16-Sep-2011 11:36:44
$OP: tademahenko

Population	#Events	%Parent	pSTAT5 Al... Geo Mean
Leukocytes	29,824	40.8	####
Lymphocytes	8,582	28.8	####
p-STAT5 positives	6,229	72.6	4,097
p-STAT5 of all lymphocytes	8,578	100.0	2,534

pSTAT-5 expression in WB lymphocytes: IL-2 + Compound

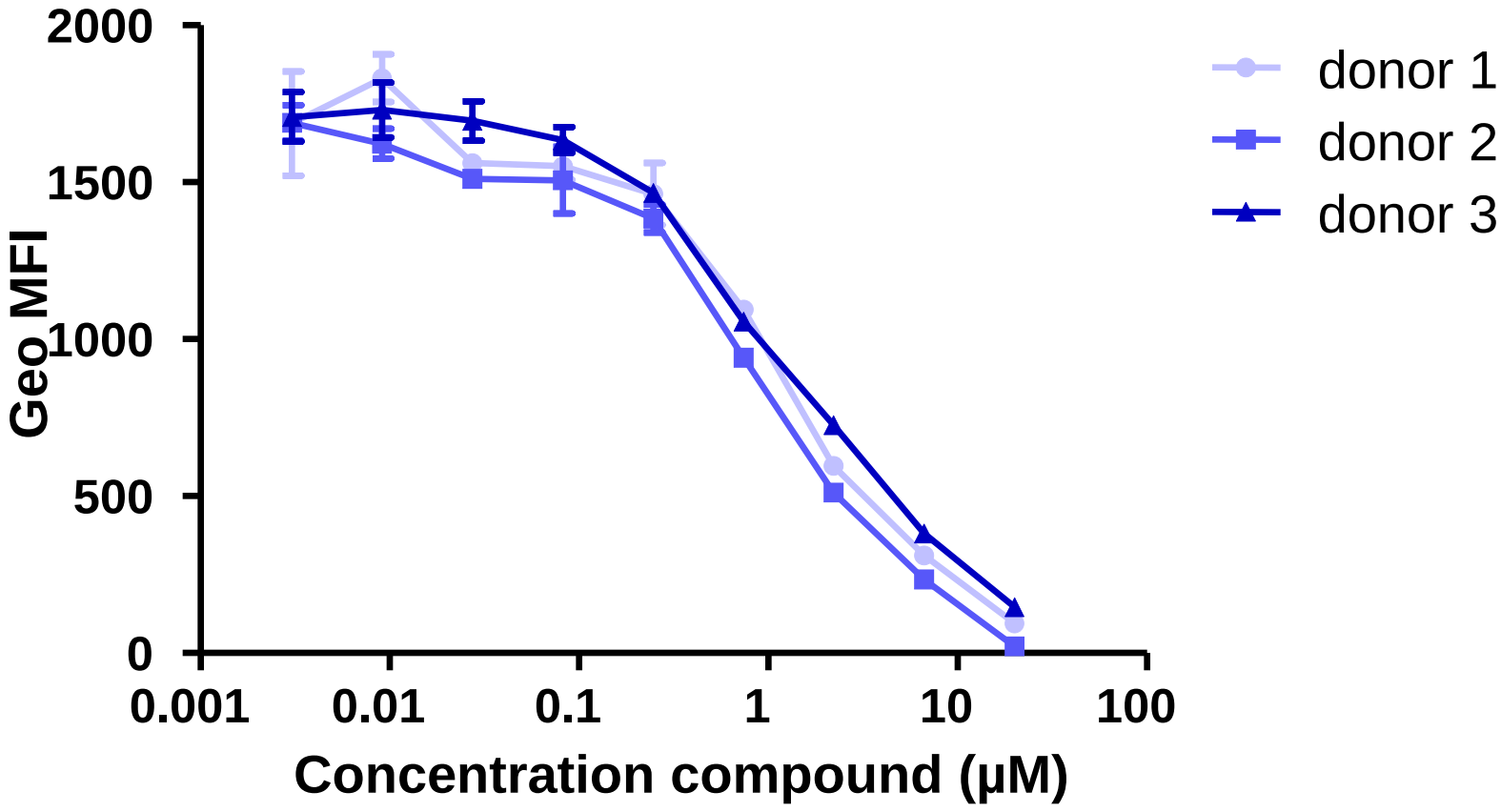




Experiment Name:
Plate Name:
Specimen Name:
Well Name:
Record Date:
$OP: tademahenko

Population	#Events	%Parent	pSTAT5 Al... Geo Mean
Leukocytes	29,805	38.7	####
Lymphocytes	7,761	26.0	####
p-STAT5 positives	556	7.2	2,082
p-STAT5 of all lymphocytes	7,749	99.8	807

Inhibition of pSTAT5 is Dose-Dependent

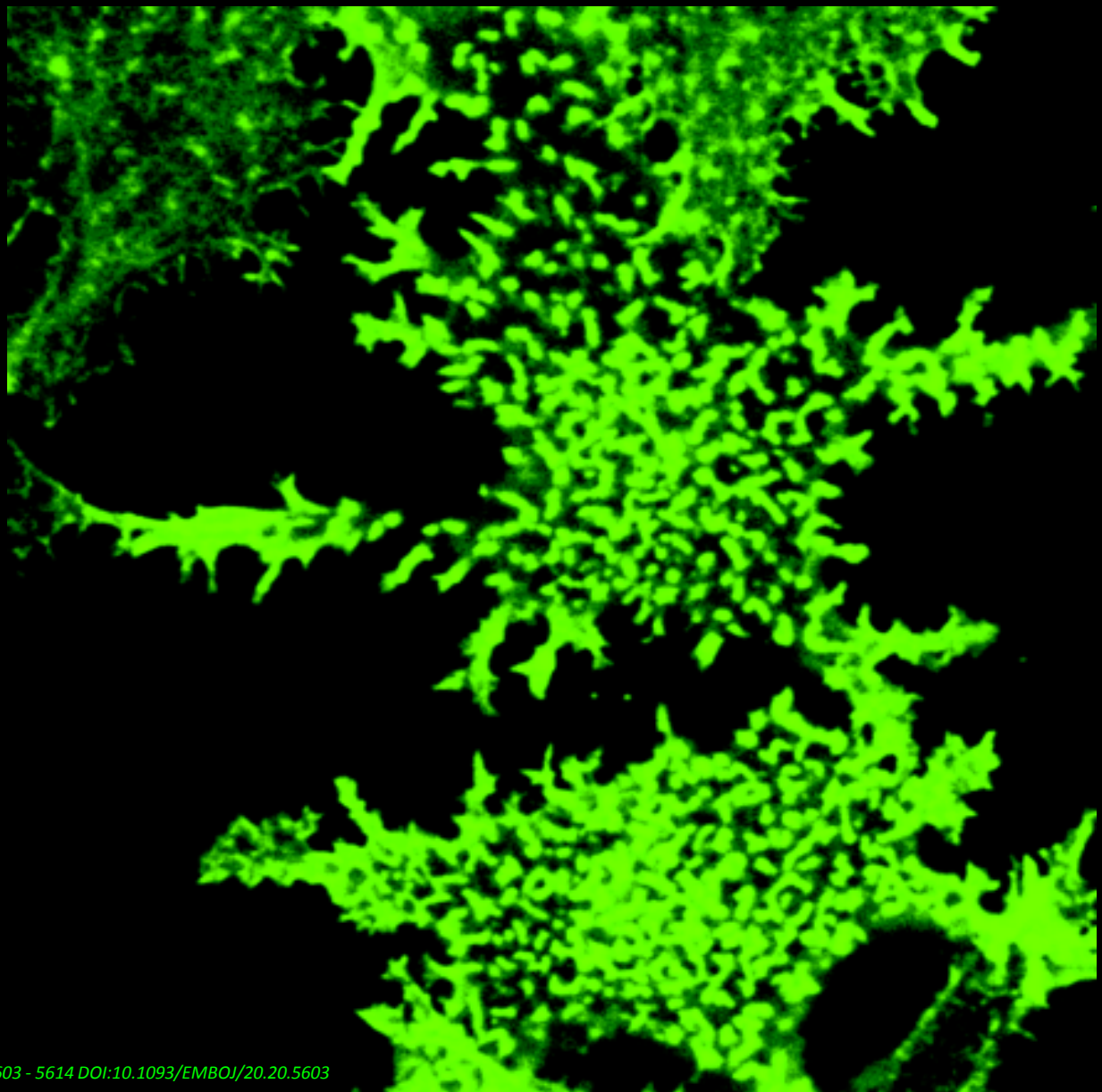


Actin Polymerization: TARC

- CD4+ T cells migrate towards chemokine TARC during inflammatory reactions
- Actin polymerization is involved in migration process
- Interfere in migration of CD4+ T cells in chronic disease

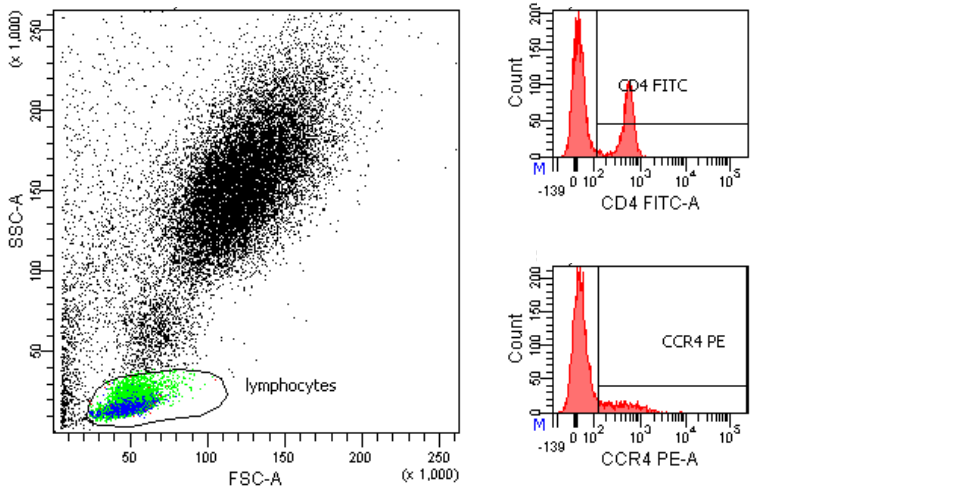
- Phalloidin labels polymerized actin

Phalloidin-FITC labels polymerized actin



Castellano et al THE EMBO JOURNAL (2001) 20, 5603 - 5614 DOI:10.1093/EMBOJ/20.20.5603

Actin polymerisation in CD4+ lymphocytes: PBS

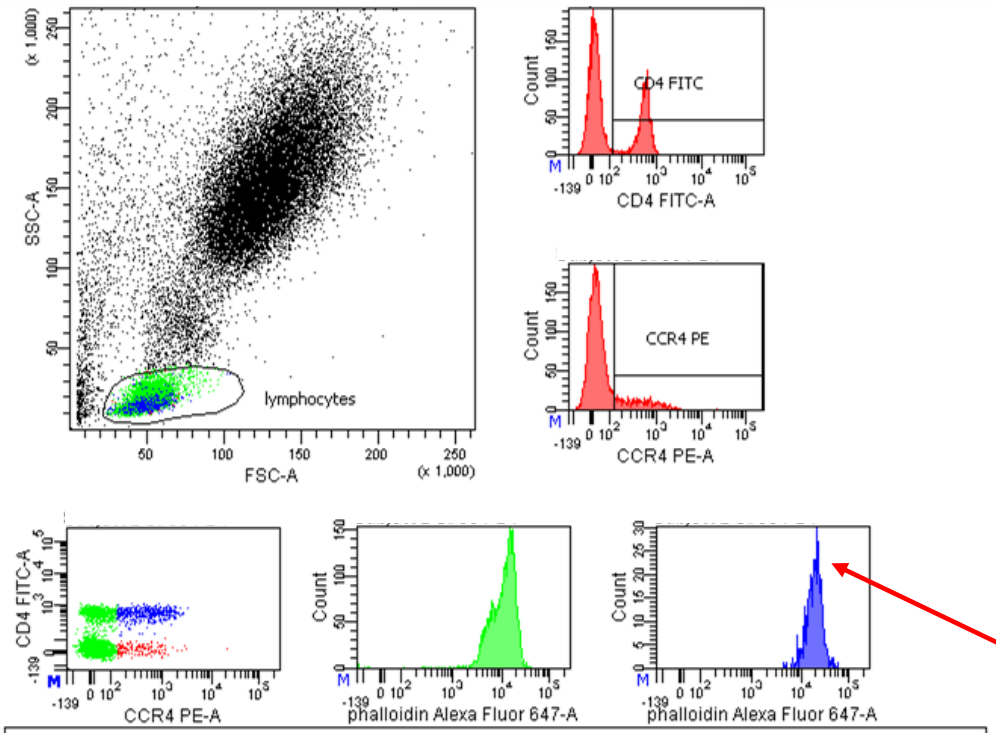


Experiment Name:
Plate Name:
Specimen Name:
Well Name:
Record Date:
$OP:

Population	#Events	%Parent	phalloidin Alexa Fluor 647-A Mean
NOT(CCR4 PE)	####	####	9,198
CD4 FITC AND CCR4 PE	477	9.5	8,430

Ratio = 0.92

Actin polymerisation in CD4+ lymphocytes: TARC

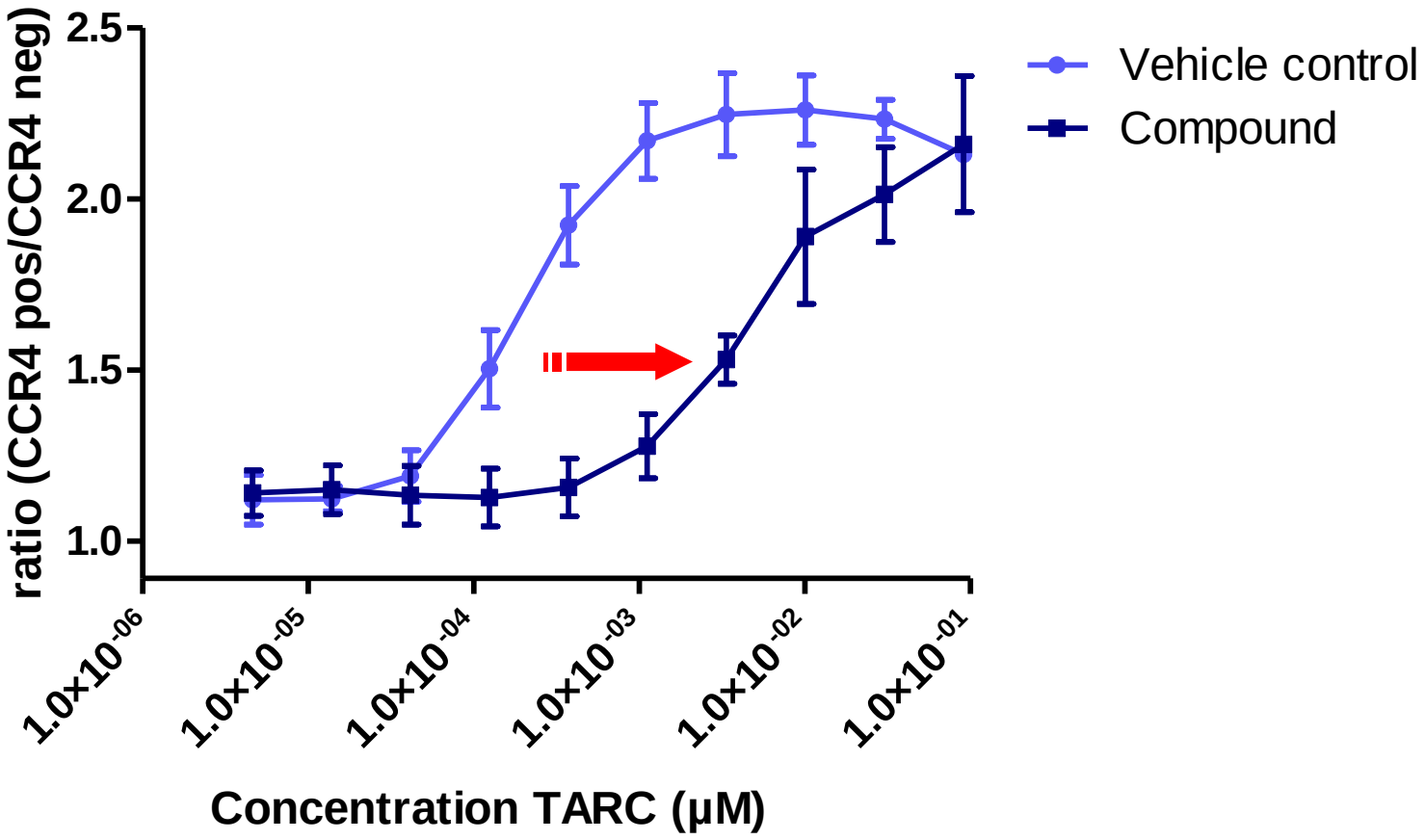


Experiment Name:
Plate Name:
Specimen Name:
Well Name:
Record Date:
$OP:

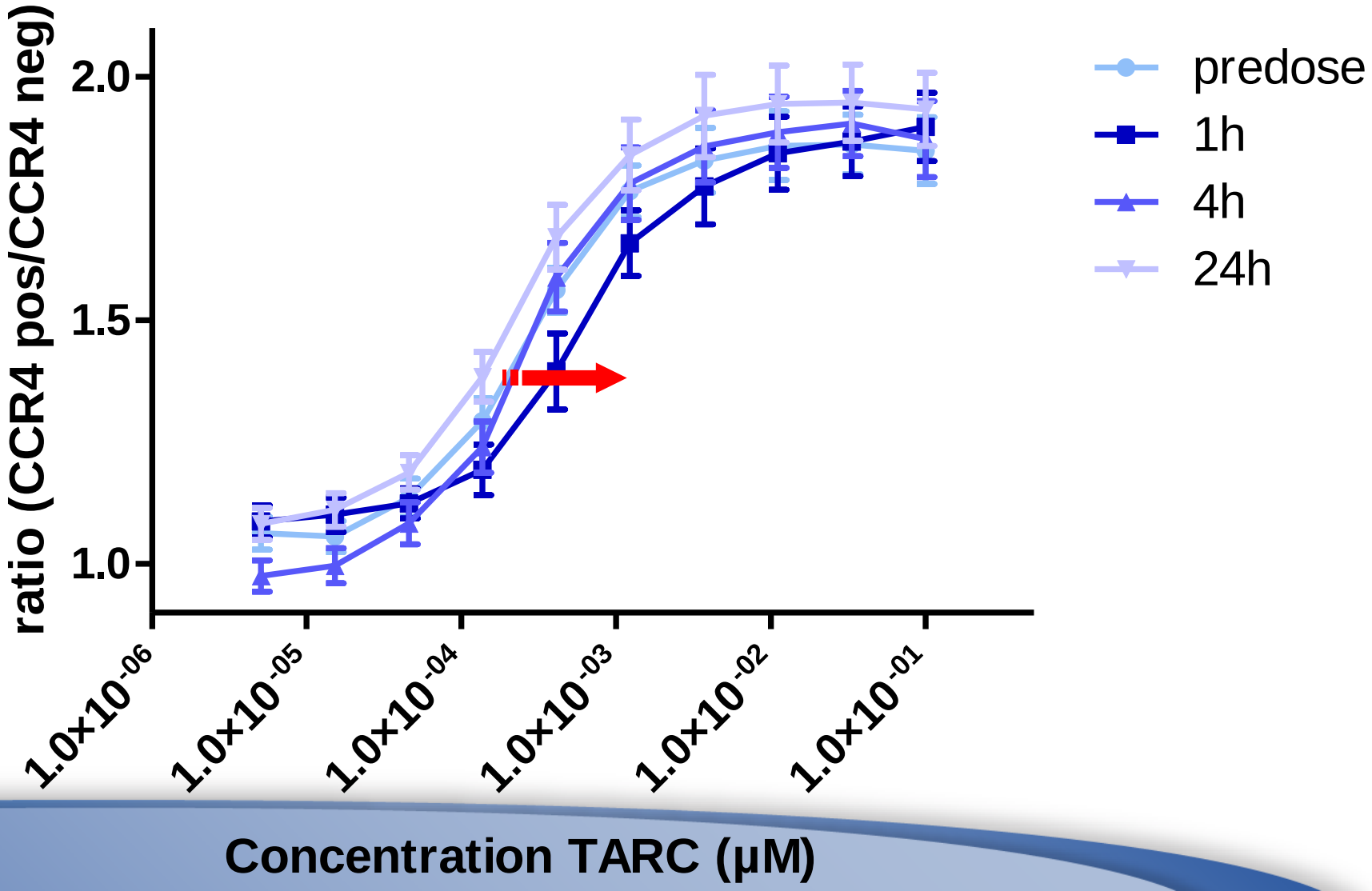
Population	#Events	%Parent	phalloidin Alexa Fluor 647-A Mean
NOT(CCR4 PE)	####	####	11,496
CD4 FITC AND CCR4 PE	460	10.0	19,226

Ratio = 1.67

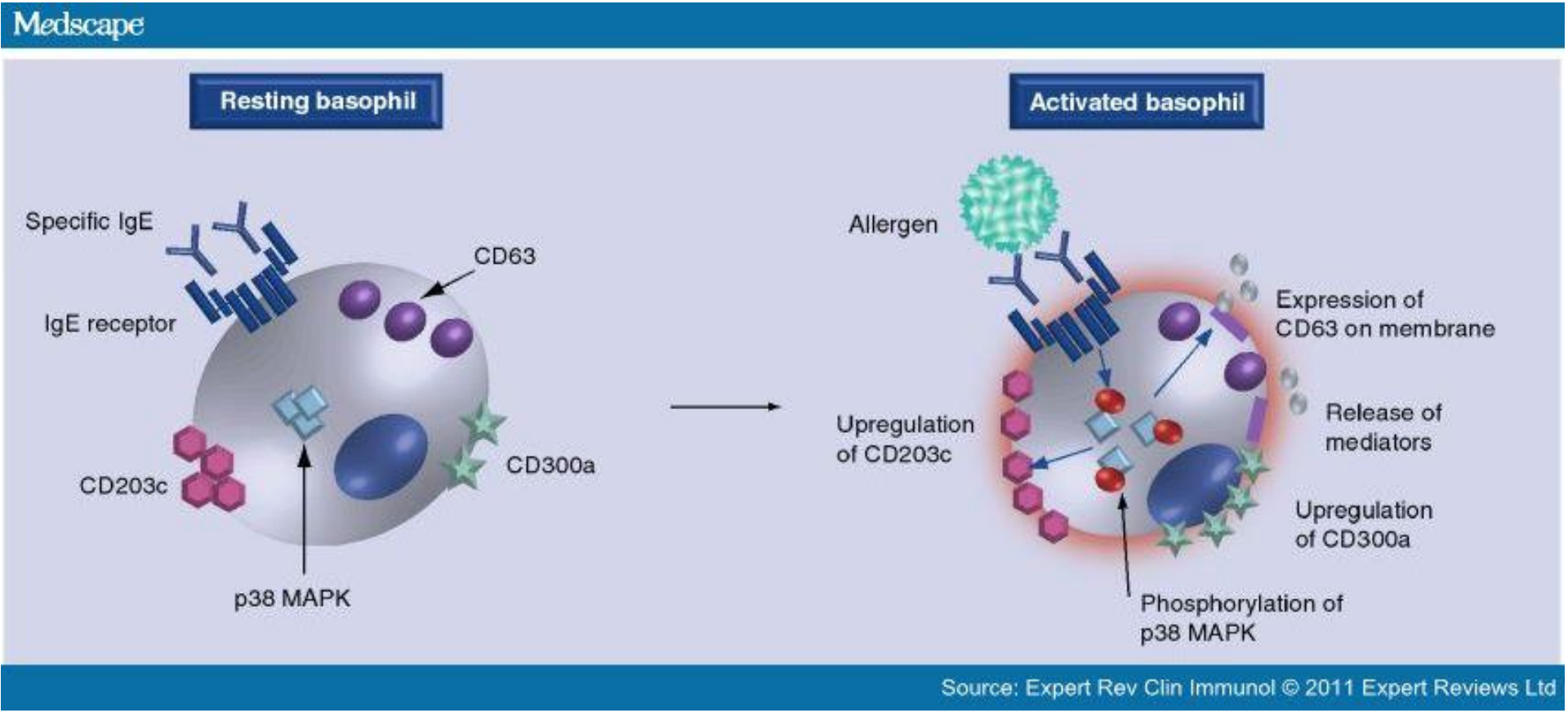
CD4+ cells are less responsive to TARC



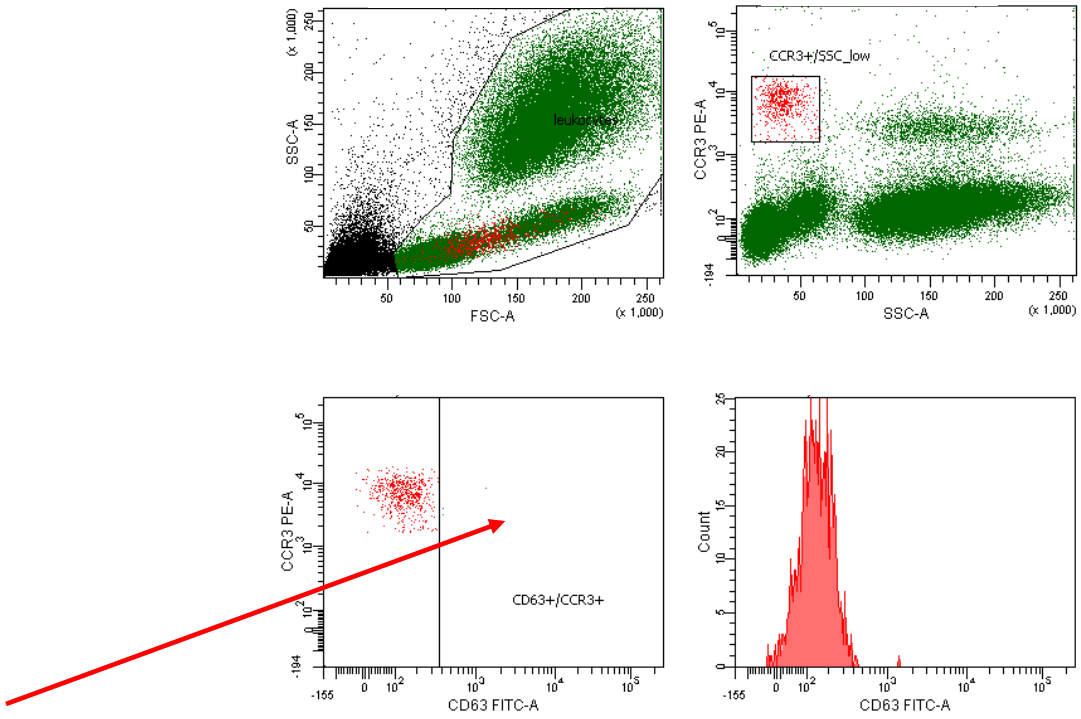
CD4+ stimulation is attenuated ex vivo



Principle of Basophil activation



CD63 expression in basophils: PBS



Experiment Name:
Plate Name:
Specimen Name:
Well Name:
Record Date:
$OP:

Population	#Events	%Parent	%Total	CD63 FITC-A Mean
All Events	357,177	####	####	74
leukocytes	53,416	14.96	14.96	243
CCR3+/SSC_low	633	1.19	0.18	145
CD63+/CCR3+	3	0.47	0.00	718

CD63 expression in basophils: FcεR1

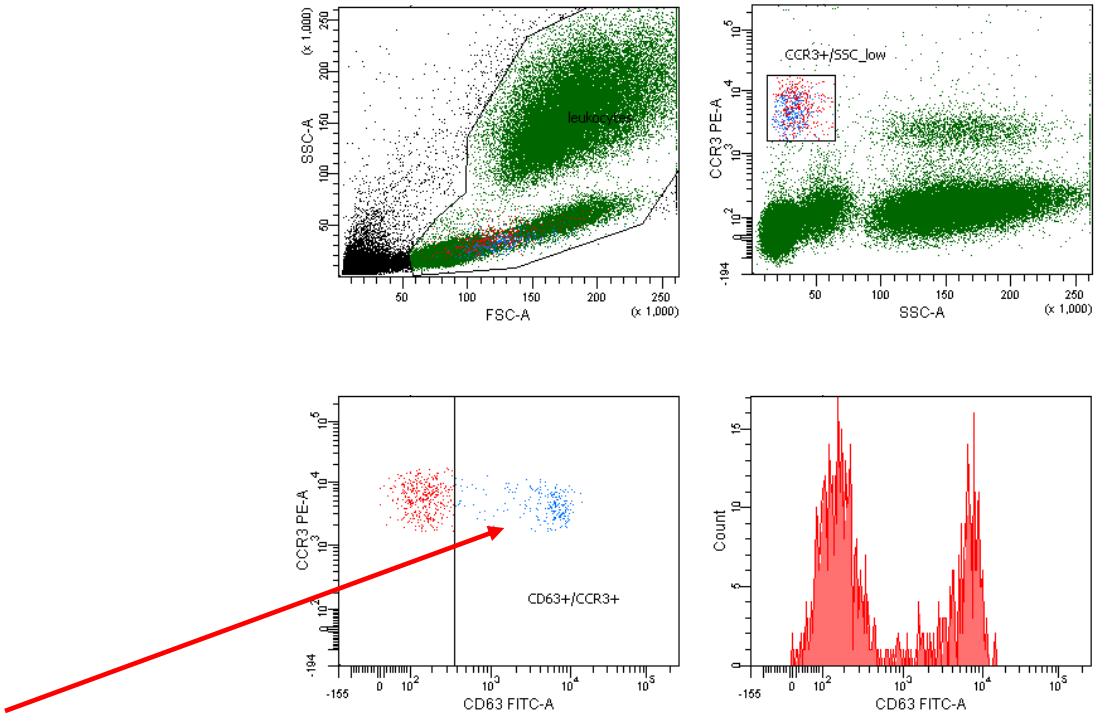


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Plate Name:
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Well Name:
Record Date:
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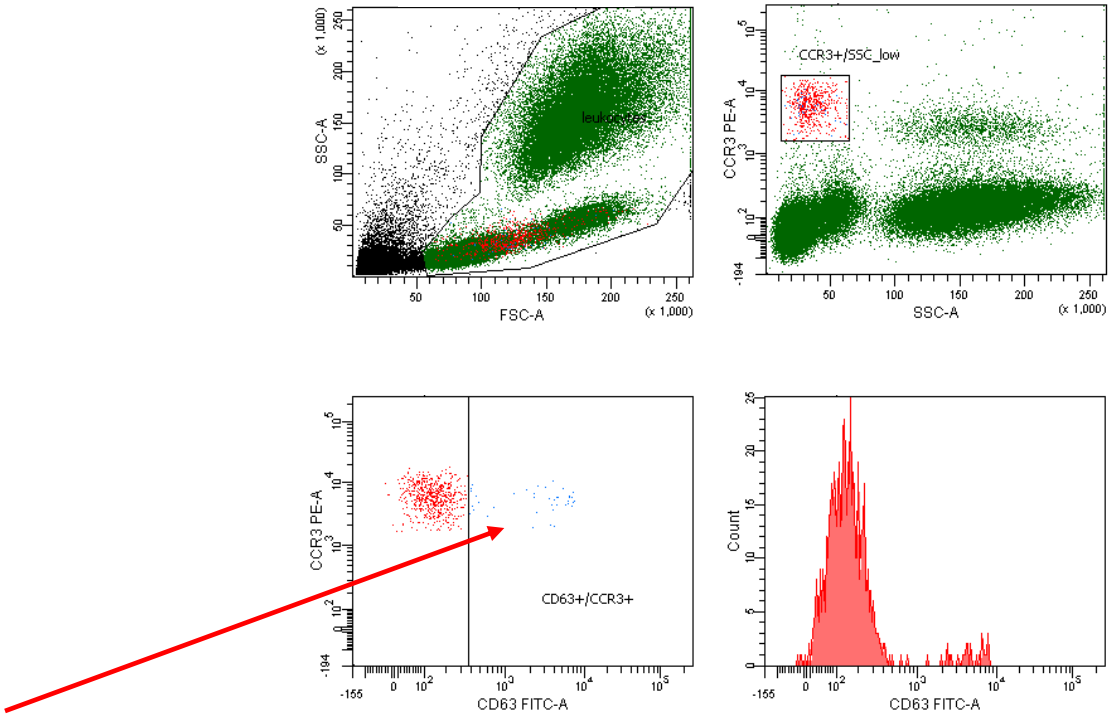
Population	#Events	%Parent	%Total	CD63 FITC-A Mean
All Events	206,904	####	####	114
leukocytes	55,731	26.94	26.94	263
CCR3+/SSC_low	619	1.11	0.30	2,202
CD63+/CCR3+	236	38.13	0.11	5,508

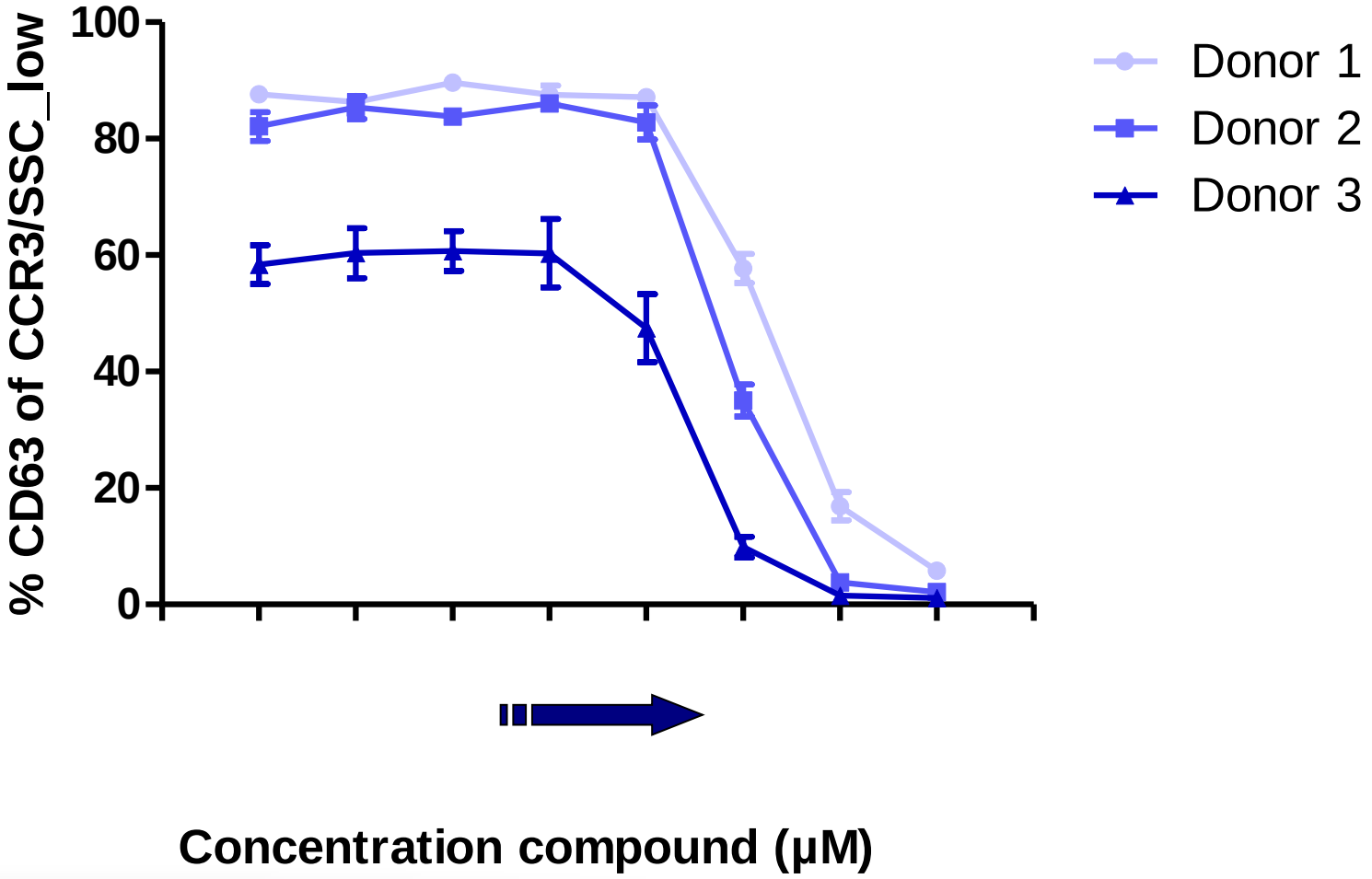
CD63 expression in basophils: FcεR1+compound



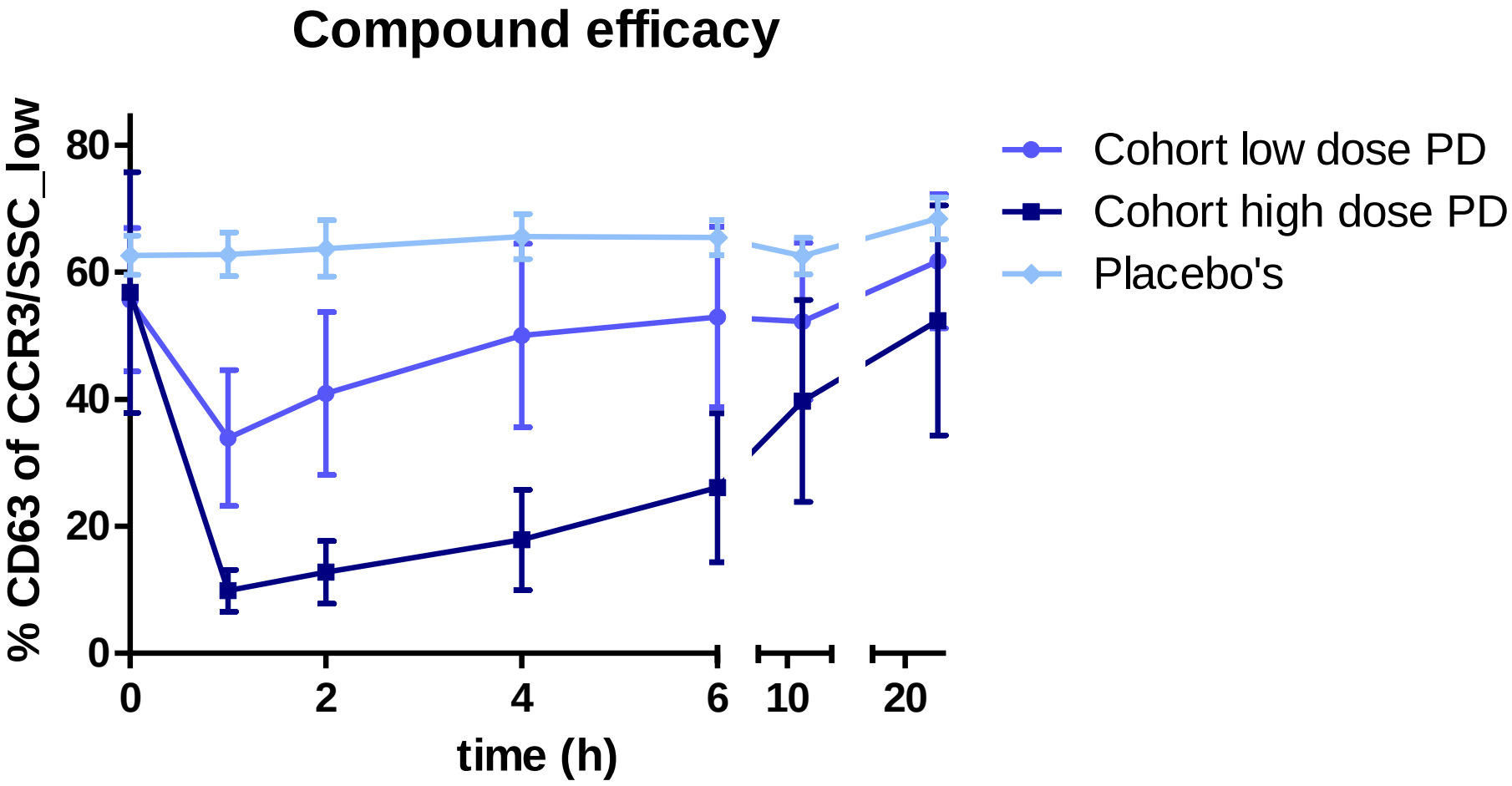
Experiment Name:
Plate Name:
Specimen Name:
Well Name:
Record Date:
$OP:

Population	#Events	%Parent	%Total	CD63 FITC-A Mean
All Events	218,818	####	####	97
leukocytes	53,345	24.38	24.38	225
CCR3+/SSC_low	621	1.16	0.28	365
CD63+/CCR3+	40	6.44	0.02	3,610

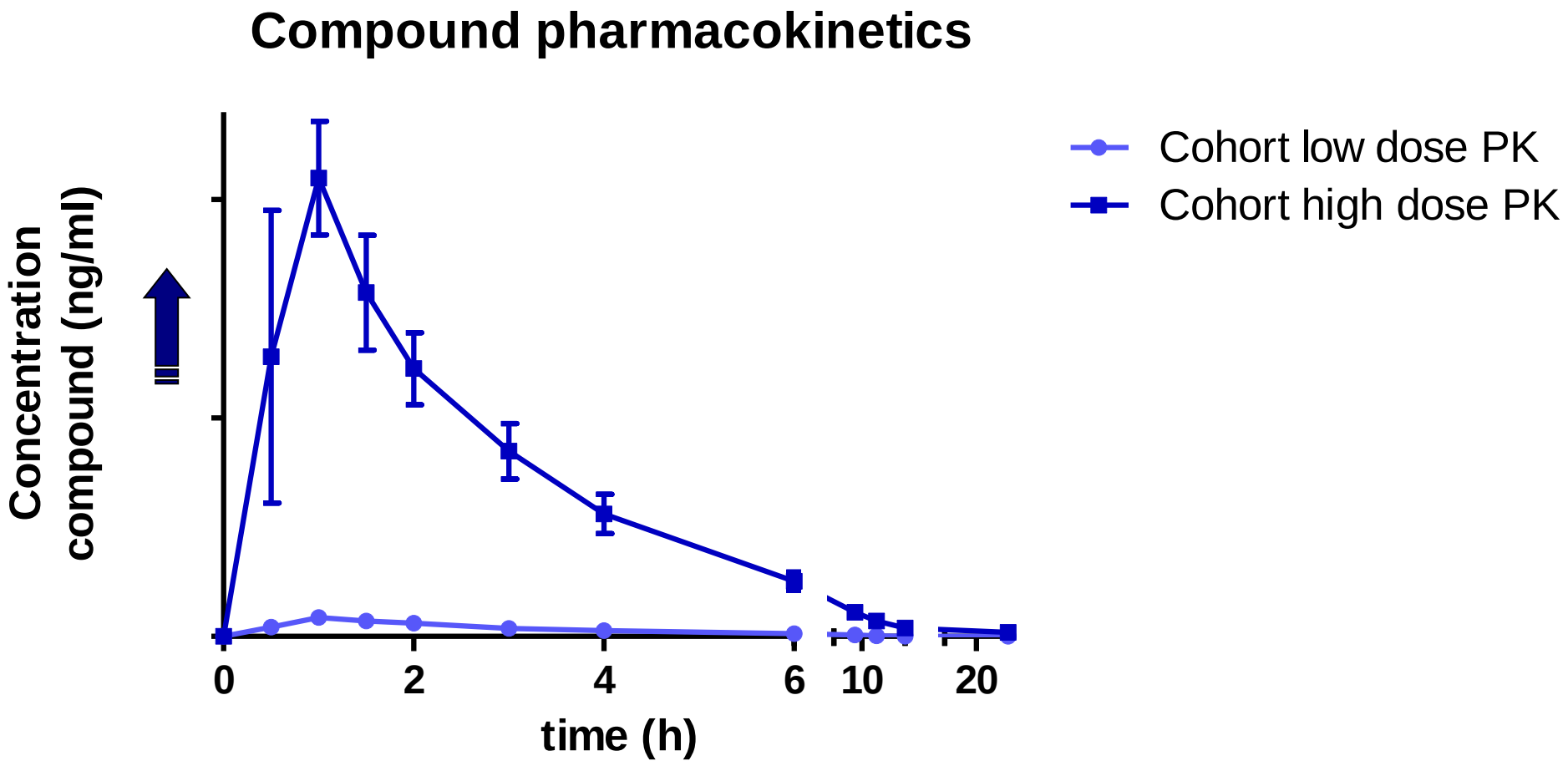
Inhibition of CD63 is dose-dependent



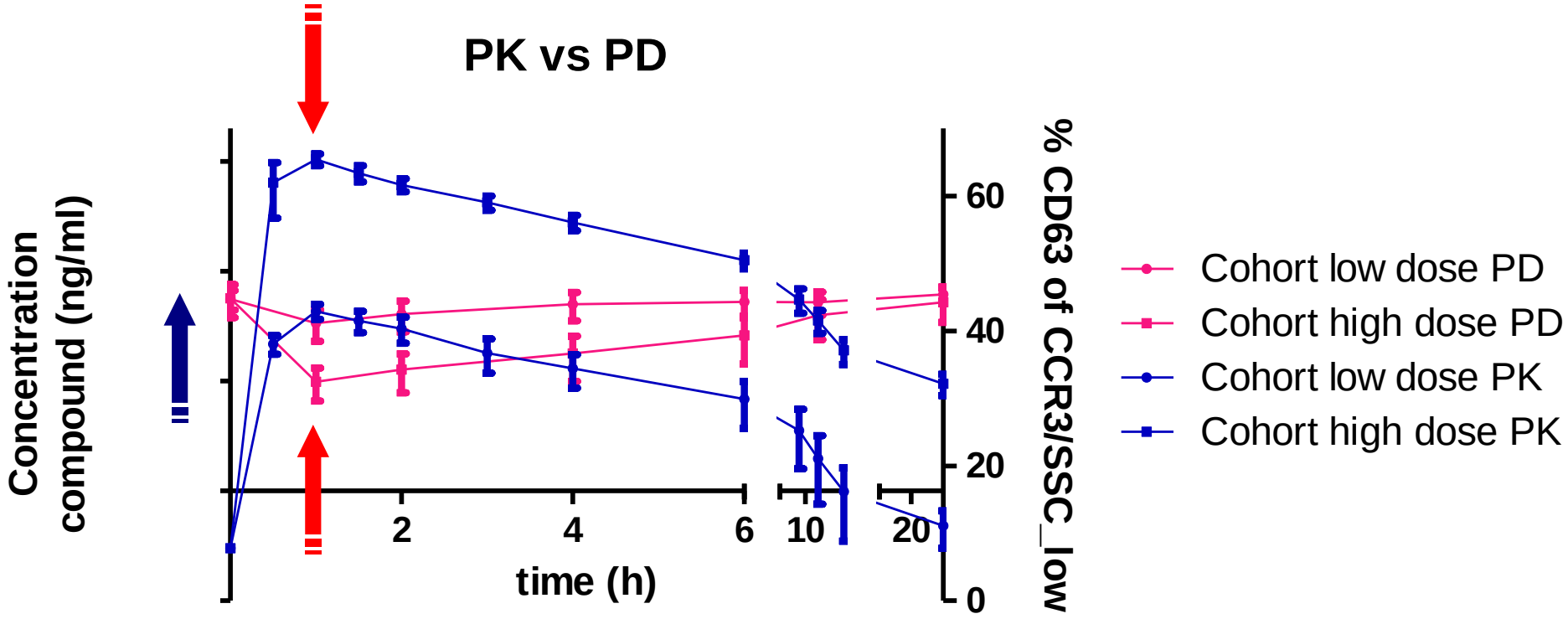
CD63 expression in dosed volunteers



Pharmacokinetic profiles

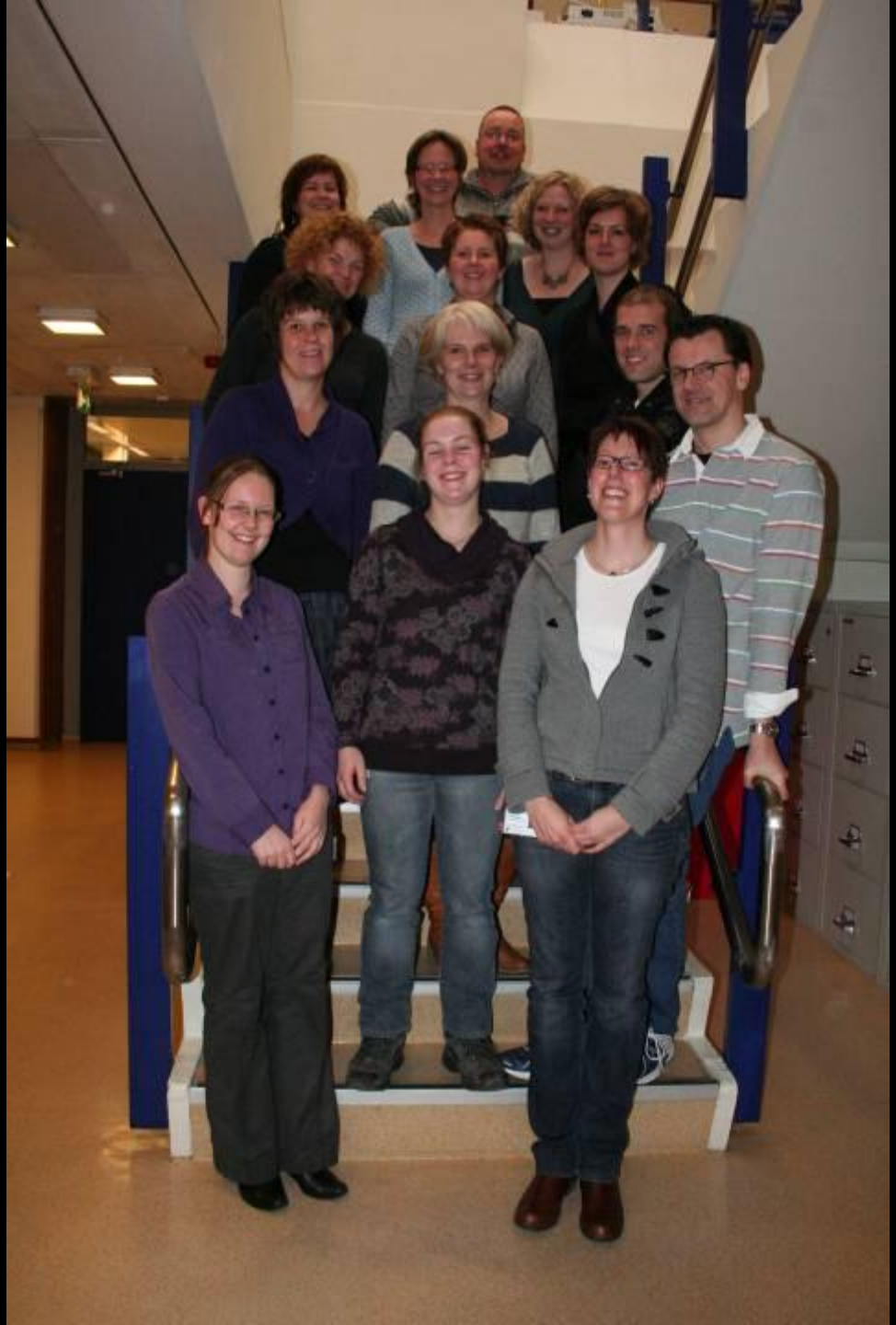


Integration of PK/PD results:



Conclusions:

- Use of good biomarkers is required for proper decision making during Drug Development
- Flow Cytometry is a powerful tool to demonstrate efficacy of Drugs as early as Phase I




PRA
A Clear Difference