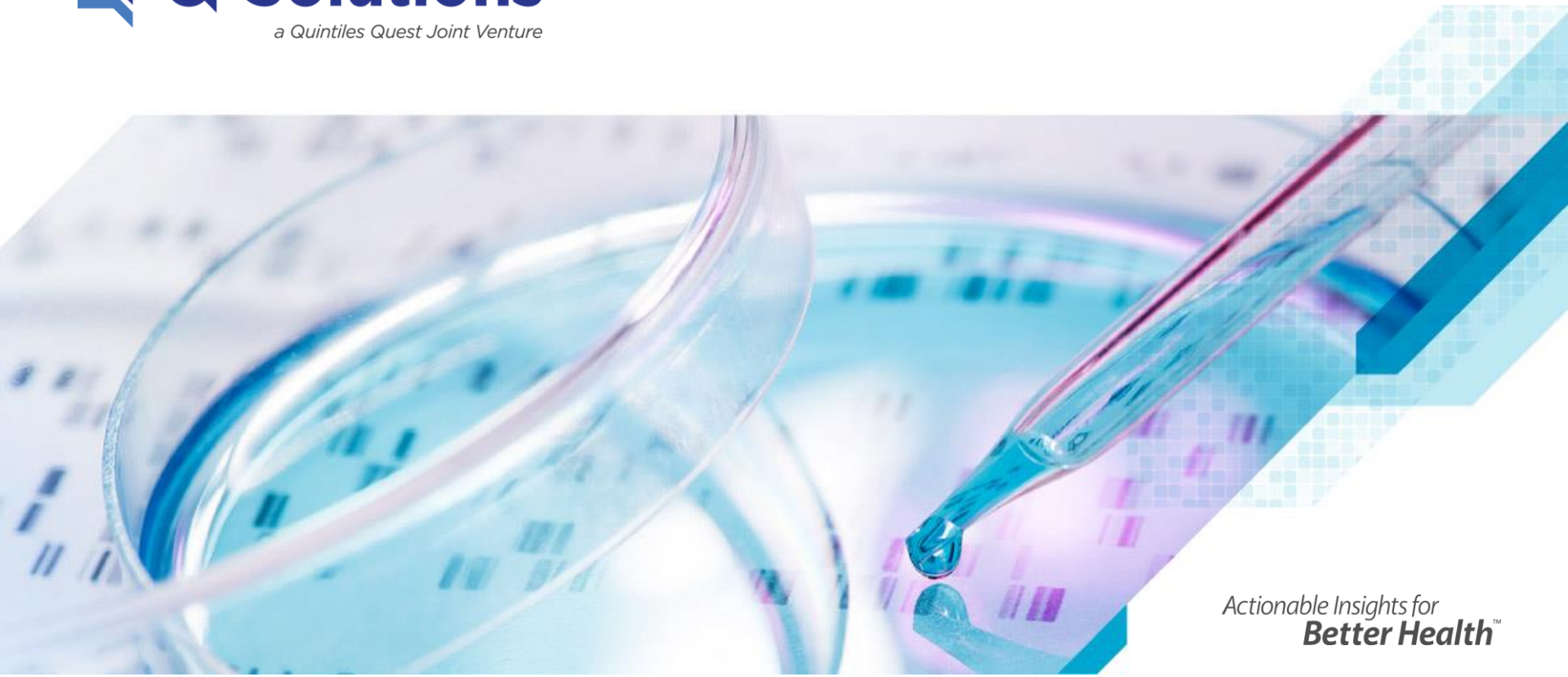
Q² Solutions
a Quintiles Quest Joint Venture



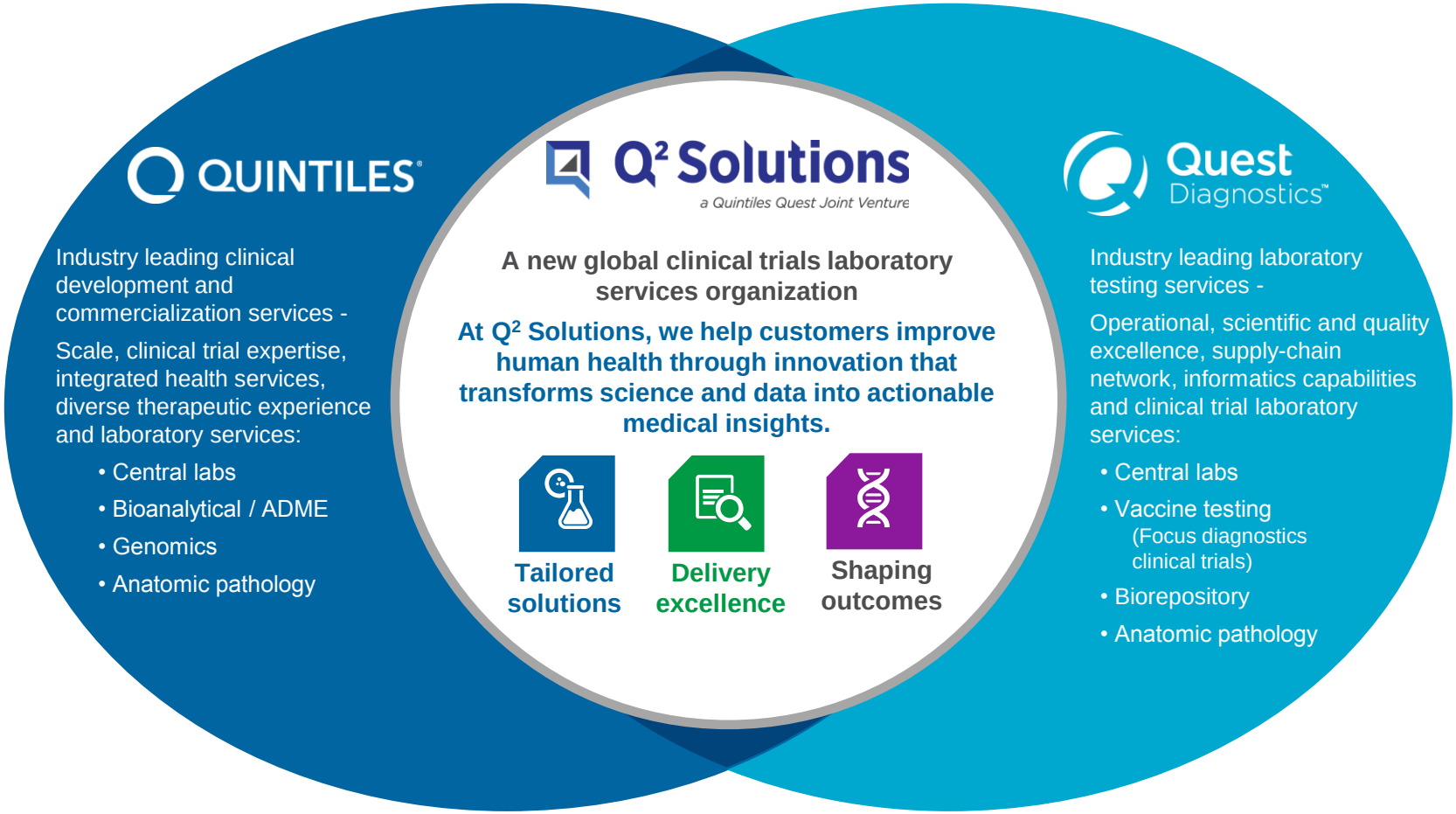
Validating Flow Cytometry Assays for Immuno-Oncology Trials in the Central Lab

Alistair Watt, Ph.D. Director, Translational Science Laboratory

Molecular Medicine Tri Conference | February 2017

Q² Solutions - a global clinical trials laboratory services joint venture formed to provide greater innovation, quality and value for our customers

This joint venture was born out of a shared commitment to quality, customer service and – above all – helping bring new treatments to patients



Q² Solutions Global Delivery Network

Global Presence, Local Knowledge

Supporting investigative sites worldwide

LEGEND

- Central Lab
- Affiliate / Partner Labs
- BioAnalytical / ADME Labs
- Genomics Lab
- Project Delivery & Support Hub
- Specialty Centers of Excellence

* **Translational Science Lab**

Q² Solutions
a Quintiles Quest Joint Venture

State of the Art Flow Cytometry

Assay Development, Validation and Deployment Globally

- **8 & 10 Color Instruments**
- **Whole Blood, PBMC and Cell Culture**
- **Cell Surface, Intracellular**
- **%, Absolute, fluorescent intensity**

Q² Solutions
a Quintiles Quest Joint Venture

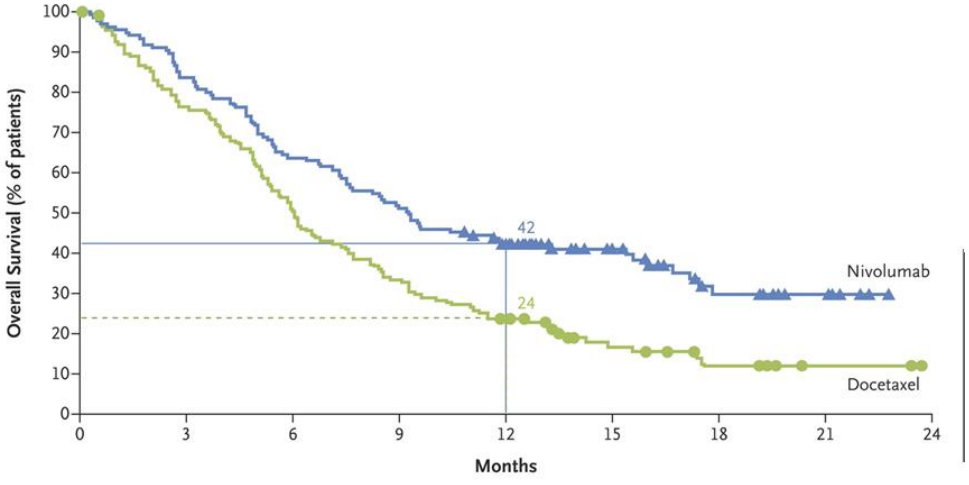
State of the Art Flow Cytometry

Assay Development, Validation and Deployment Globally

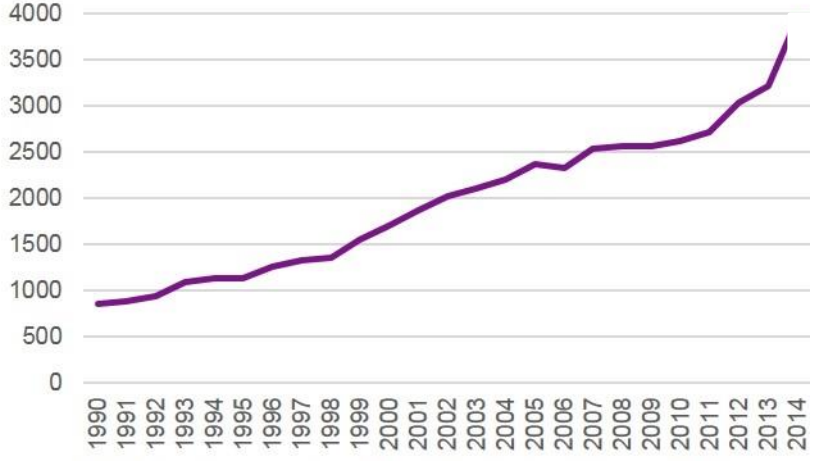
- **Apoptosis Assays** (Annexin V)
- **Basophil Activation** (CD63, CD203c)
- **B Cell Subset Assays** (activation, memory, naïve, transitional, plasma, plasma blasts
- **Cell Culture/Predictive Bioassays**
- **Dendritic Cell Assays** (pDC, mDC)
- **Leukemia/Lymphoma Assays** (EuroFlow)
- **Magnetic Bead Separation** (RNA, DNA)
- **Minimal Residual Disease** (CLL, AML)
- **Monocyte/Macrophage Subset Assays** (classical, non-classical, intermediate, & pro-inflammatory monocytes)
- **Myeloid Derived Suppressor Cell Assays**
- **NK & Innate Lymphoid Cell Assays**
- **Phosphorylated Protein Assays** (pSMAD, pCCR5, pSTAT5 WB & PBMC)
- **Platelet Assays** (VASP, PLA-TRAP)
- **Receptor Occupancy Assays**
- **Stem Cell Assays** (viability, enumeration)
- **TBN (M) K**
- **Th1, Th2, Th17, Tmem, Tnaive**
- **Th17** (intracellular IL-17)
- **T Cell Activation** (ICOS, CD38, HLA-DR, CD28, CD25)
- **T Cell Checkpoint assays** (CTLA-4, PD-1)
- **T Cell Proliferation** (Ki67)
- **T Regulatory Cell Assays** (surface, FoxP3)

The Opportunity: Immuno-oncology As The Future Of Cancer Treatment

- Over 45 immuno-oncology drugs approved (US)*
- 57 immuno-oncology drugs in development*
- Over 250 studies registered/ongoing*
- Increasing number of relevant publications annually**



Brahmer et al, N Engl J Med 2015; 373:123-135

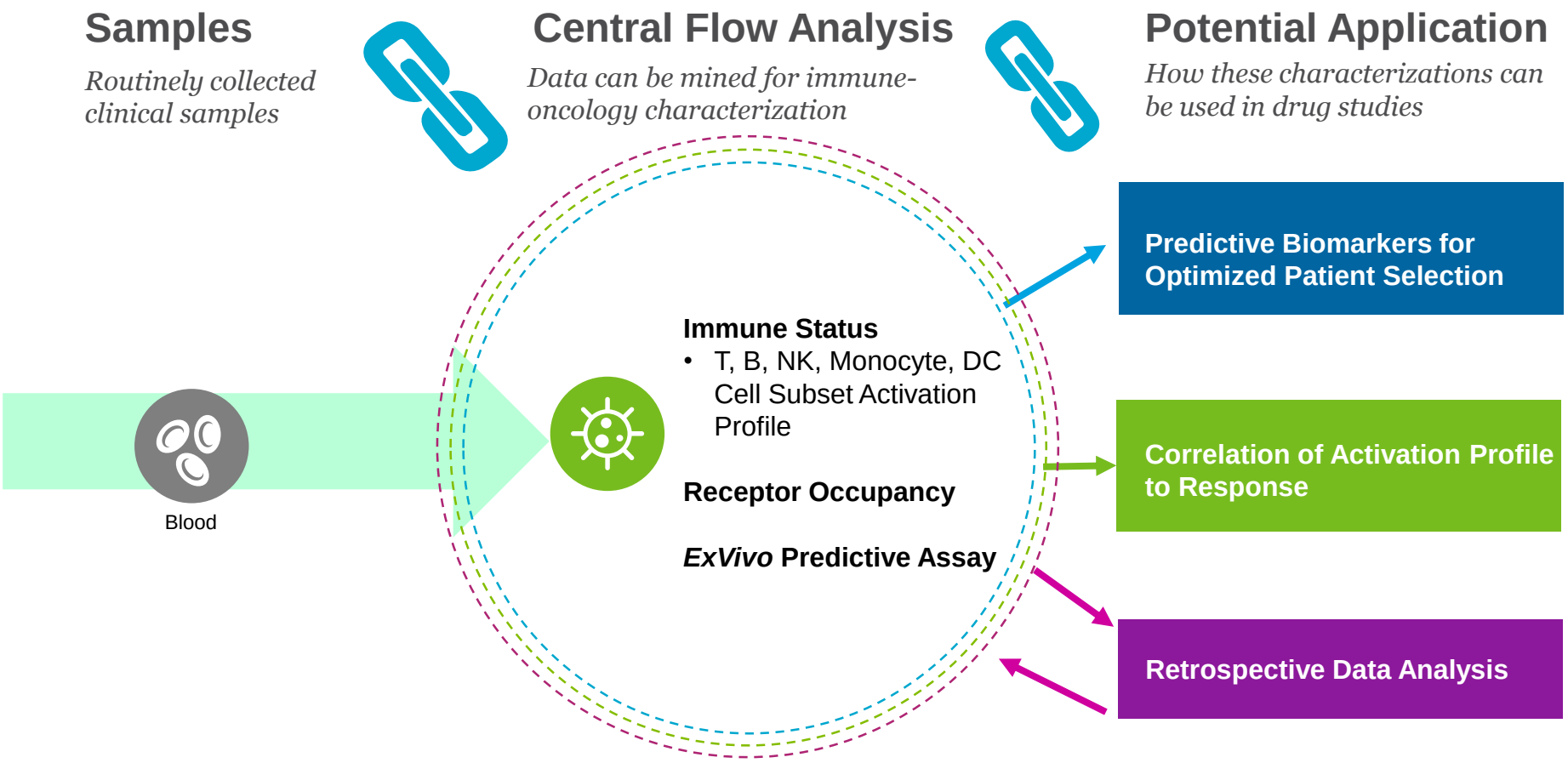


*UBS Immuno-oncology Monthly Handbook (Jan 2015)
**PubMed; keywords 'cancer immunotherapy'

Q² Solutions
a Quintiles Quest Joint Venture

Flow Cytometry in Immuno-Oncology

New opportunities for immune characterization



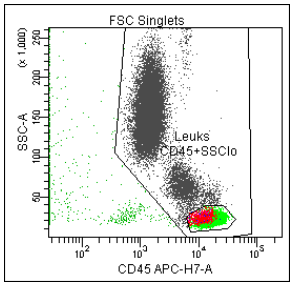
Q² Solutions
a Quintiles Quest Joint Venture

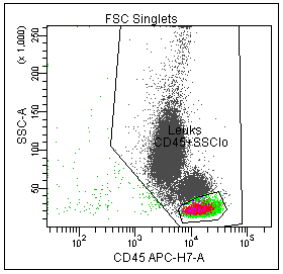
Flow Cytometry Assay Development

> ***Panel design***
  - Antibody / fluorochrome selection
  - Isotype / empty channel
  - %, Absolute cell count, fluorescent intensity

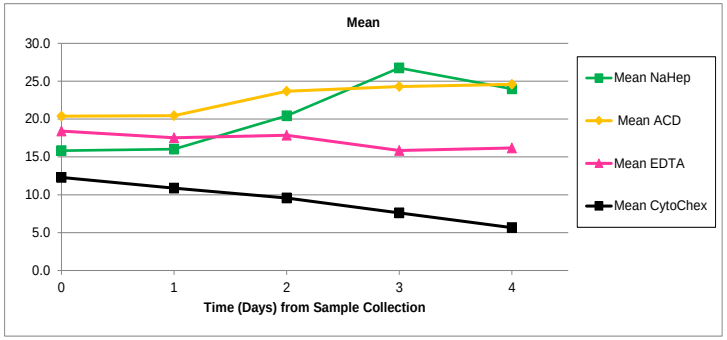
Tube	Fluorochrome							
	BV421	*BV510*	*AF488*	*PE*	*PerCP-Cy5.5*	*PE-Cy7*	*APC*	*APC-H7*
1	CD197	CD45	IgG1	CD3	CD45RA	/	CD8	CD4
2	CD197	CD45	Ki67	CD3	CD45RA	/	CD8	CD4

> ***QC material selection***
  - Immunotrol, CD Chex, PBMCs, Stimulated Cells, Lyophilised cells





> ***Sample type***
  - Cytochex BCT, EDTA, Heparin, ACD, PBMC, Lyse/Fixed Whole Blood
    - Comparative stability analysis



> ***Gating Strategy***
  - Fixed gates
  - Gating of full patient data sets (eg. MRD)
  - Central gating

Q² Solutions
a Quintiles Quest Joint Venture

Flow Cytometry Validation

> ***QC Precision*** *(≤ 20%CV)*
  - replicates of QC material analysed over 5 independent analytical runs (mean, SD and %CV)
  - Sets acceptance criteria for daily QC run

> ***Sample Precision*** *(≤ 20%CV)*
  - Minimum 6 samples in triplicate (mean, %CV)

> ***Stability***
  - Minimum of 6 samples (7d Cytochex, 4d Heparin, EDTA , long term PBMC)

> ***Custom***
  - Stability/Precision in Spiked or Diseased Samples
  - Stability/Precision for Receptor Occupancy
  - 'Fit for Purpose'
  - Interference
  - Linearity
  - Sensitivity

Flow Cytometry Validation

> ***QC Precision*** *(≤ 20%CV)*
  - replicates of QC material analysed over 5 independent analytical runs (mean, SD and %CV)
  - Sets acceptance criteria for daily QC run

> ***Sample Precision*** *(≤ 20%CV)*
  - Minimum 6 samples in triplicate (mean, %CV)

> ***Stability***
  - Minimum of 6 samples (7d Cytochex, 4d Heparin, EDTA , long term PBMC)

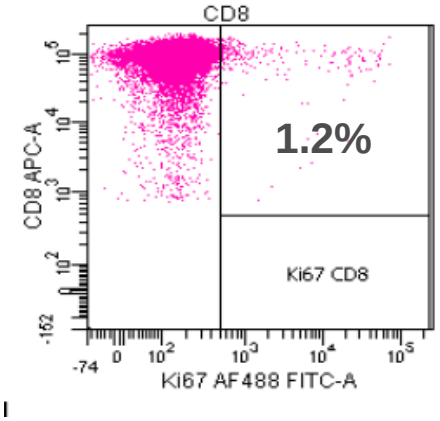
> ***Custom***
  - Stability/Precision in Spiked or Diseased Samples
  - Stability/Precision for Receptor Occupancy
  - 'Fit for Purpose'
  - Interference
  - Linearity
  - Sensitivity

Q² Solutions
a Quintiles Quest Joint Venture

Stability/Precision in Spiked Samples

A263 T Cell Subset Proliferation (Ki67)

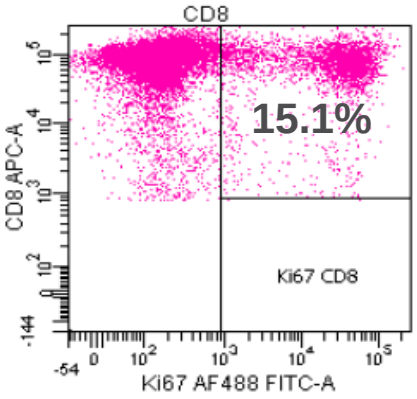
CD8+Ki67+ Normal Donor Samples



Precision

Average SD	0.3
Average %CV	22.9

CD8+Ki67+ Spiked Donor Samples

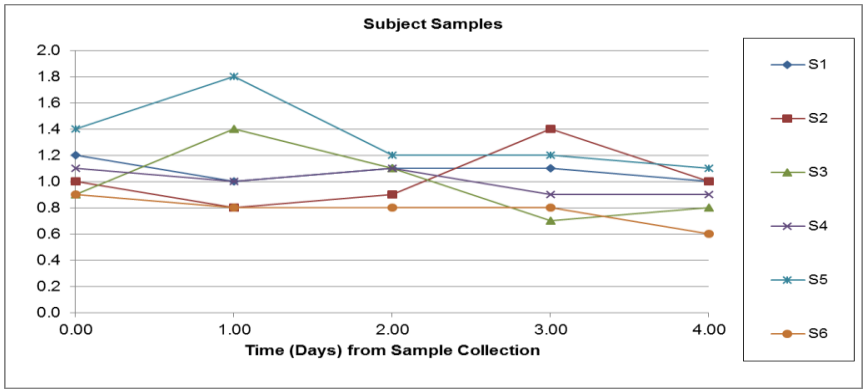


Precision

Average SD	1.4
Average %CV	6.4

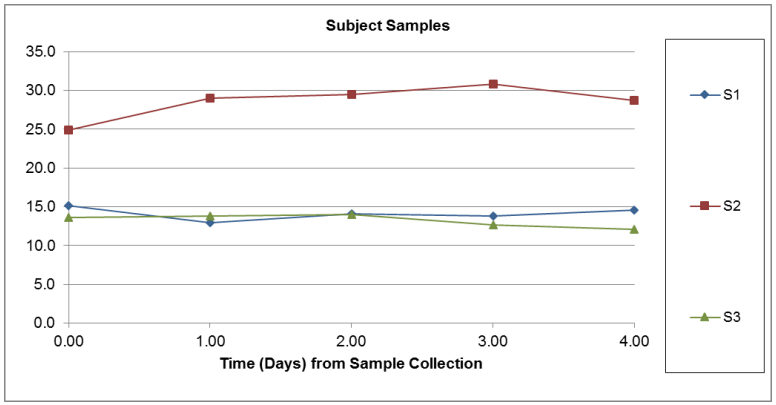
Stability

Mean	1.1	1.1	1.0	1.0	0.9
% Change from Day 0		23.5	11.0	19.0	16.8



Stability

Mean	17.9	18.6	19.2	19.1	18.5
% Change from Day 0		10.8	9.3	13.0	9.9

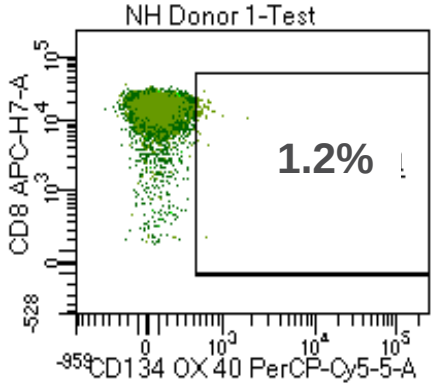


Q² Solutions
a Quintiles Quest Joint Venture

Stability /Precision in Spiked Samples

A227 Activated TBNK (Tim3/OX40/CTLA-4/CD137/ICOS)

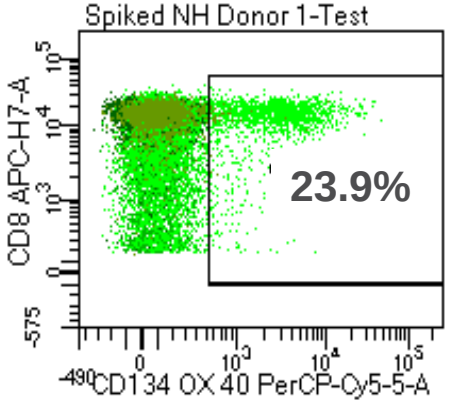
CD8+OX40+ Normal Donor Samples



Precision

Average SD	0.98
Average %CV	44.1

CD8+OX40+ Spiked Donor Samples

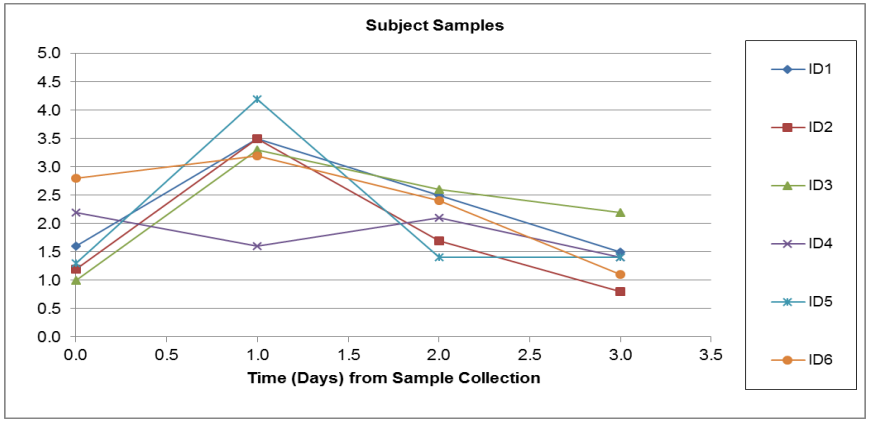


Precision

Average SD	1.35
Average %CV	7.3

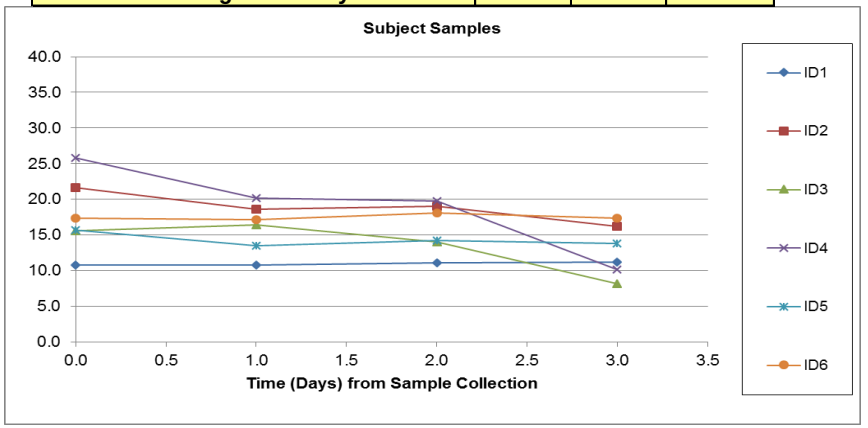
Stability

Mean	1.7	3.2	2.1	1.4
% Change from Day 0		134.2	47.4	44.1



Stability

Mean	17.8	16.1	16.0	12.8
% Change from Day 0		9.3	10.3	24.8



Q² Solutions
a Quintiles Quest Joint Venture

Stability /Precision for Receptor Occupancy

PD1 Receptor Occupancy Assay – CD3+CD4+ PD1 RO%

PD-1 RO % = 100 × [Frequency of cells bound with anti-PD1 (Tube 2) ÷ Frequency of total PD1+ cells (Tube 1)]

Precision

	30µg/mL			1µg/mL			0µg/mL		
Sample	1	2	3	1	2	3	1	2	3
Rep 1 Day 1 Run 1	81.9	81.0	79.8	66.2	66.7	63.7	12.7	11.8	6.7
Rep 2 Day 1 Run 2	79.3	86.0	85.3	63.6	66.3	65.9	9.1	7.6	7.6
Rep 3 Day 2	74.3	79.5	79.4	56.1	64.7	58.1	9.7	10.6	8.2
SD	3.86	3.40	3.30	5.24	1.06	4.02	1.93	2.16	0.75
%CV	4.92	4.14	4.05	8.46	1.61	6.43	18.37	21.63	10.07
Mean	78.50	82.17	81.50	61.97	65.90	62.57	10.50	10.00	7.50

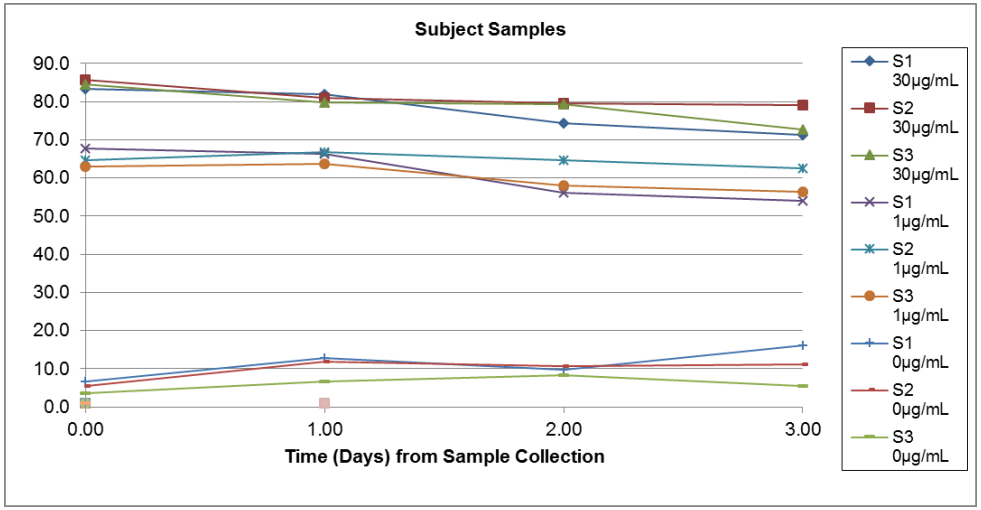
Average SD 30µg/mL	**3.52**
Average %CV 30µg/mL	**4.37**
Average SD 1µg/mL	**3.44**
Average %CV 1µg/mL	**5.50**
Average SD 0µg/mL	**1.62**
Average %CV 0µg/mL	**16.69**

Average SD Overall	**2.86**
Average %CV Overall	**8.85**

Stability

Mean 30µg/mL	**84.5**	**80.9**	**77.7**	**74.4**
% Change from Day 0 30µg/mL		**4.2**	**8.0**	**12.0**
Mean 1µg/mL	**65.1**	**65.5**	**59.6**	**57.6**
% Change from Day 0 1µg/mL		**2.2**	**8.3**	**11.5**
Mean 0µg/mL	**5.2**	**10.4**	**9.5**	**10.9**
% Change from Day 0 0µg/mL		**98.1**	**89.6**	**98.6**

Mean Overall	**51.6**	**52.3**	**49.0**	**47.6**
% Change from Day 0 Overall		**34.8**	**35.3**	**40.7**



Q² Solutions
a Quintiles Quest Joint Venture

Fit For Purpose Validation

A264 T Cell Assay (OX40, GITR, PD-1,CTLA4)

Cell Subsets	*Reportable*	*Unit*
T-Cells	**CD3+CD4+ (%CD45+)**	**%**
	CD3+ ABS	Cells/uL
	CD3+CD8+ (%CD45+)	**%**
	CD3+CD8+ ABS	Cells/uL
CD4+ T Cell Subsets	**CD3+CD4+CD45RA+CD197+(%CD4+)**	**%**
	CD3+CD4+CD45RA+CD197+ ABS	Cells/uL
	CD3+CD4+Total Mem (%CD4+)	**%**
	CD3+CD4+Total Mem ABS	Cells/uL
	CD3+CD4+CD45RA-CD197+(%CD4+)	**%**
	CD3+CD4+CD45RA-CD197+ ABS	Cells/uL
	CD3+CD4+CD45RA-CD197-(%CD4+)	**%**
	CD3+CD4+CD45RA-CD197- ABS	Cells/uL
	CD3+CD4+CD45RA+CD197-(%CD4+)	**%**
	CD3+CD4+CD45RA+CD197- ABS	Cells/uL
CD8+ T Cell Subsets	**CD3+CD8+CD45RA+CD197+(%CD8+)**	**%**
	CD3+CD8+CD45RA+CD197+ ABS	Cells/uL
	CD3+CD8+Total Mem (%CD8+)	**%**
	CD3+CD8+Total Mem ABS	Cells/uL
	CD3+CD8+CD45RA-CD197+(%CD8+)	**%**
	CD3+CD8+CD45RA-CD197+ ABS	Cells/uL
	CD3+CD8+CD45RA-CD197-(%CD8+)	**%**
	CD3+CD8+CD45RA-CD197- ABS	Cells/uL
	CD3+CD8+CD45RA+CD197-(%CD8+)	**%**
	CD3+CD8+CD45RA+CD197- ABS	Cells/uL
GITR+ CD4+T Cell Subsets	CD3+CD4+CD45RA+CD197+GITR+(%CD4 Naive)	%
	CD3+CD4+CD45RA+CD197+GITR+ ABS	Cells/uL
	CD3+CD4+Total Mem GITR+(%CD4 Total Mem)	%
	CD3+CD4+Total Mem GITR+ ABS	Cells/uL
	CD3+CD4+CD45RA-CD197+GITR+(%CD4 CM)	%
	CD3+CD4+CD45RA-CD197+GITR+ ABS	Cells/uL
	CD3+CD4+CD45RA-CD197-GITR+(%CD4 EM)	**%**
	CD3+CD4+CD45RA-CD197-GITR+ ABS	Cells/uL
	CD3+CD4+CD45RA+CD197-GITR+(%CD4 TEMRA)	%
	CD3+CD4+CD45RA+CD197-GITR+ ABS	Cells/uL
GITR+ CD8+T Cell Subsets	CD3+CD8+CD45RA+CD197+GITR+(%CD8 Naive)	%
	CD3+CD8+CD45RA+CD197+GITR+ ABS	Cells/uL
	CD3+CD8+Total Mem GITR+(%CD8 Total Mem)	%
	CD3+CD8+Total Mem GITR+ ABS	Cells/uL
	CD3+CD8+CD45RA-CD197+GITR+(%CD8 CM)	%
	CD3+CD8+CD45RA-CD197+GITR+ ABS	Cells/uL
	CD3+CD8+CD45RA-CD197-GITR+(%CD8 EM)	**%**
	CD3+CD8+CD45RA-CD197-GITR+ ABS	Cells/uL
	CD3+CD8+CD45RA+CD197-GITR+(%CD8 TEMRA)	%
	CD3+CD8+CD45RA+CD197-GITR+ ABS	Cells/uL
GITR Expression CD4+T Cell Subsets	CD3+CD4+CD45RA+CD197+ GITR MESF	MESF
	CD3+CD4+Total Mem GITR MESF	MESF
	CD3+CD4+CD45RA-CD197+GITR MESF	MESF
	CD3+CD4+CD45RA-CD197-GITR MESF	**MESF**
	CD3+CD4+CD45RA+CD197-GITR MESF	MESF

PD1+ CD8+T Cell Subsets	CD3+CD8+CD45RA+CD197+PD1+(%CD8 Naïve)	%
	CD3+CD8+CD45RA+CD197+PD1+ ABS	Cells/uL
	CD3+CD8+Total Mem PD1+(%CD8 Total Mem)	%
	CD3+CD8+Total Mem PD1+ ABS	Cells/uL
	CD3+CD8+CD45RA-CD197+PD1+(%CD8 CM)	%
	CD3+CD8+CD45RA-CD197+PD1+ ABS	Cells/uL
	CD3+CD8+CD45RA-CD197-PD1+(%CD8 EM)	**%**
	CD3+CD8+CD45RA-CD197-PD1+ ABS	Cells/uL
	CD3+CD8+CD45RA+CD197-PD1+(%CD8 TEMRA)	%
	CD3+CD8+CD45RA+CD197-PD1+ ABS	Cells/uL
PD1 Expression CD4+T Cell Subsets	CD3+CD4+CD45RA+CD197+ PD1 MESF	MESF
	CD3+CD4+Total Mem PD1 MESF	MESF
	CD3+CD4+CD45RA-CD197+PD1 MESF	MESF
	CD3+CD4+CD45RA-CD197-PD1 MESF	**MESF**
	CD3+CD4+CD45RA+CD197-PD1 MESF	MESF
PD1 Expression CD8+T Cell Subsets	CD3+CD8+CD45RA+CD197+PD1 MESF	MESF
	CD3+CD8+Total Mem PD1 MESF	MESF
	CD3+CD8+CD45RA-CD197+PD1 MESF	MESF
	CD3+CD8+CD45RA-CD197-PD1 MESF	**MESF**
	CD3+CD8+CD45RA+CD197- PD1 MESF	MESF
CTLA4+ CD4+T Cell Subsets	CD3+CD4+CD45RA+CD197+CTLA4+(%CD4 Naive)	%
	CD3+CD4+CD45RA+CD197+CTLA4+ ABS	Cells/uL
	CD3+CD4+Total Mem CTLA4+(%CD4 Total Mem)	%
	CD3+CD4+Total Mem CTLA4+ ABS	Cells/uL
	CD3+CD4+CD45RA-CD197+CTLA4+(%CD4 CM)	%
	CD3+CD4+CD45RA-CD197+CTLA4+ ABS	Cells/uL
	CD3+CD4+CD45RA-CD197-CTLA4+(%CD4 EM)	**%**
	CD3+CD4+CD45RA-CD197-CTLA4+ ABS	Cells/uL
	CD3+CD4+CD45RA+CD197-CTLA4+(%CD4 TEMRA)	%
	CD3+CD4+CD45RA+CD197-CTLA4+ ABS	Cells/uL
CTLA4+ CD8+T Cell Subsets	CD3+CD8+CD45RA+CD197+CTLA4+(%CD8 Naive)	%
	CD3+CD8+CD45RA+CD197+CTLA4+ ABS	Cells/uL
	CD3+CD8+Total Mem CTLA4+(%CD8 Total Mem)	%
	CD3+CD8+Total Mem CTLA4+ ABS	Cells/uL
	CD3+CD8+CD45RA-CD197+CTLA4+(%CD8 CM)	%
	CD3+CD8+CD45RA-CD197+CTLA4+ ABS	Cells/uL
	CD3+CD8+CD45RA-CD197-CTLA4+(%CD8 EM)	**%**
	CD3+CD8+CD45RA-CD197-CTLA4+ ABS	Cells/uL
	CD3+CD8+CD45RA-CD197-CTLA4+(%CD8 TEMRA)	%
	CD3+CD8+CD45RA-CD197-CTLA4+ ABS	Cells/uL
CTLA4 Expression CD4+T Cell Subsets	CD3+CD4+CD45RA+CD197+ CTLA4 MESF	MESF
	CD3+CD4+Total Mem CTLA4 MESF	MESF
	CD3+CD4+CD45RA-CD197+CTLA4 MESF	MESF
	CD3+CD4+CD45RA-CD197-CTLA4 MESF	**MESF**
	CD3+CD4+CD45RA+CD197-CTLA4 MESF	MESF
CTLA4 Expression CD8+T Cell Subsets	CD3+CD8+CD45RA+CD197+CTLA4 MESF	MESF
	CD3+CD8+Total Mem CTLA4 MESF	MESF
	CD3+CD8+CD45RA-CD197+CTLA4 MESF	MESF
	CD3+CD8+CD45RA-CD197-CTLA4 MESF	**MESF**
	CD3+CD8+CD45RA+CD197- CTLA4 MESF	MESF

GITR Expression CD8+T Cell Subsets	CD3+CD8+CD45RA+CD197+GITR MESF	MESF
	CD3+CD8+Total Mem GITR MESF	MESF
	CD3+CD8+CD45RA-CD197+GITR MESF	MESF
	CD3+CD8+CD45RA-CD197-GITR MESF	**MESF**
	CD3+CD8+CD45RA+CD197- GITR MESF	MESF
OX40+ CD4+T Cell Subsets	CD3+CD4+CD45RA+CD197+OX40+(%CD4 Naive)	%
	CD3+CD4+CD45RA+CD197+OX40+ ABS	Cells/uL
	CD3+CD4+Total Mem OX40+(%CD4 Total Mem)	%
	CD3+CD4+Total Mem OX40+ ABS	Cells/uL
	CD3+CD4+CD45RA-CD197+OX40+(%CD4 CM)	%
	CD3+CD4+CD45RA-CD197+OX40+ ABS	Cells/uL
	CD3+CD4+CD45RA-CD197-OX40+(%CD4 EM)	**%**
	CD3+CD4+CD45RA-CD197-OX40+ ABS	Cells/uL
	CD3+CD4+CD45RA+CD197-OX40+(%CD4 TEMRA)	%
	CD3+CD4+CD45RA+CD197-OX40+ ABS	Cells/uL
OX40+ CD8+T Cell Subsets	CD3+CD8+CD45RA+CD197+OX40+(%CD8 Naive)	%
	CD3+CD8+CD45RA+CD197+OX40+ ABS	Cells/uL
	CD3+CD8+Total Mem OX40+(%CD8 Total Mem)	%
	CD3+CD8+Total Mem OX40+ ABS	Cells/uL
	CD3+CD8+CD45RA-CD197+OX40+(%CD8 CM)	%
	CD3+CD8+CD45RA-CD197+OX40+ ABS	Cells/uL
	CD3+CD8+CD45RA-CD197-OX40+(%CD8 EM)	**%**
	CD3+CD8+CD45RA-CD197-OX40+ ABS	Cells/uL
	CD3+CD8+CD45RA+CD197-OX40+(%CD8 TEMRA)	%
	CD3+CD8+CD45RA+CD197-OX40+ ABS	Cells/uL
OX40 Expression CD4+T Cell Subsets	CD3+CD4+CD45RA+CD197+ OX40 MESF	MESF
	CD3+CD4+Total Mem OX40 MESF	MESF
	CD3+CD4+CD45RA-CD197+OX40 MESF	MESF
	CD3+CD4+CD45RA-CD197-OX40 MESF	**MESF**
	CD3+CD4+CD45RA+CD197-OX40 MESF	MESF
OX40 Expression CD8+T Cell Subsets	CD3+CD8+CD45RA+CD197+OX40 MESF	MESF
	CD3+CD8+Total Mem OX40 MESF	MESF
	CD3+CD8+CD45RA-CD197+OX40 MESF	MESF
	CD3+CD8+CD45RA-CD197-OX40 MESF	**MESF**
	CD3+CD8+CD45RA+CD197- OX40 MESF	MESF
PD1+ CD4+T Cell Subsets	CD3+CD4+CD45RA+CD197+PD1+(%CD4 Naive)	%
	CD3+CD4+CD45RA+CD197+PD1+ ABS	Cells/uL
	CD3+CD4+Total Mem PD1+(%CD4 Total Mem)	%
	CD3+CD4+Total Mem PD1+ ABS	Cells/uL
	CD3+CD4+CD45RA-CD197+PD1+(%CD4 CM)	%
	CD3+CD4+CD45RA-CD197+PD1+ ABS	Cells/uL
	CD3+CD4+CD45RA-CD197-PD1+(%CD4 EM)	**%**
	CD3+CD4+CD45RA-CD197-PD1+ ABS	Cells/uL
	CD3+CD4+CD45RA-CD197+PD1+(%CD4 TEMRA)	%
	CD3+CD4+CD45RA+CD197-PD1+ ABS	Cells/uL

144 Reportable Parameters

Q² Solutions
a Quintiles Quest Joint Venture

Fit For Purpose Validation

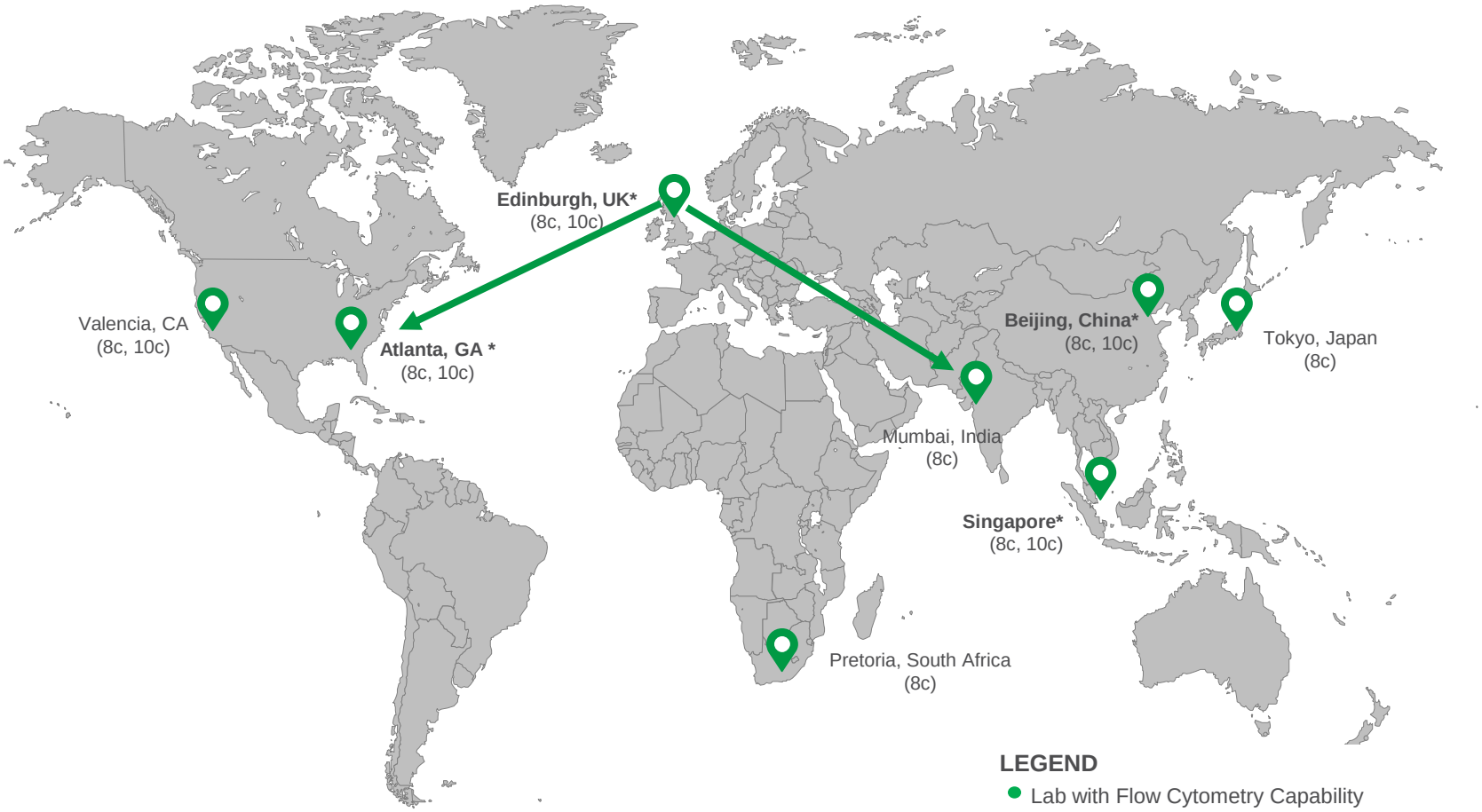
A264 T Cell Assay (OX40, GITR, PD-1,CTLA4)

Cell Subsets	*Reportable*	*Unit*
T-Cells	CD3+CD4+ (%CD45+)	%
	CD3+CD8+ (%CD45+)	%
CD4+ T Cell Subsets	CD3+CD4+CD45RA+CD197+(%CD4+)	%
	CD3+CD4+Total Mem (%CD4+)	%
	CD3+CD4+CD45RA-CD197+(%CD4+)	%
	CD3+CD4+CD45RA-CD197-(%CD4+)	%
	CD3+CD4+CD45RA+CD197-(%CD4+)	%
CD8+ T Cell Subsets	CD3+CD8+CD45RA+CD197+(%CD8+)	%
	CD3+CD8+Total Mem (%CD8+)	%
	CD3+CD8+CD45RA-CD197+(%CD8+)	%
	CD3+CD8+CD45RA-CD197-(%CD8+)	%
	CD3+CD8+CD45RA+CD197-(%CD8+)	%
GITR+ CD4+T Cell Subsets	CD3+CD4+CD45RA-CD197-GITR+(%CD4 EM)	%
GITR+ CD8+T Cell Subsets	CD3+CD8+CD45RA-CD197-GITR+(%CD8 EM)	%
GITR Expression CD4+T Cell Subsets	CD3+CD4+CD45RA-CD197-GITR MESF	MESF
GITR Expression CD8+T Cell Subsets	CD3+CD8+CD45RA-CD197-GITR MESF	MESF
OX40+ CD4+T Cell Subsets	CD3+CD4+CD45RA-CD197-OX40+(%CD4 EM)	%
OX40+ CD8+T Cell Subsets	CD3+CD8+CD45RA-CD197-OX40+(%CD8 EM)	%
OX40 Expression CD4+T Cell Subsets	CD3+CD4+CD45RA-CD197-OX40 MESF	MESF
OX40 Expression CD8+T Cell Subsets	CD3+CD8+CD45RA-CD197-OX40 MESF	MESF
PD1+ CD4+T Cell Subsets	CD3+CD4+CD45RA-CD197-PD1+(%CD4 EM)	%
PD1+ CD8+T Cell Subsets	CD3+CD8+CD45RA-CD197-PD1+(%CD8 EM)	%
PD1 Expression CD4+T Cell Subsets	CD3+CD4+CD45RA-CD197-PD1 MESF	MESF
PD1 Expression CD8+T Cell Subsets	CD3+CD8+CD45RA-CD197-PD1 MESF	MESF
CTLA4+ CD4+T Cell Subsets	CD3+CD4+CD45RA-CD197-CTLA4+(%CD4 EM)	%
CTLA4+ CD8+T Cell Subsets	CD3+CD8+CD45RA-CD197-CTLA4+(%CD8 EM)	%
CTLA4 Expression CD4+T Cell Subsets	CD3+CD4+CD45RA-CD197-CTLA4 MESF	MESF
CTLA4 Expression CD8+T Cell Subsets	CD3+CD8+CD45RA-CD197-CTLA4 MESF	MESF

- 30 Validation Parameters
- Coverage of all markers on EffMem Subsets
- Parameters not included in validation data deemed '*Exploratory Biomarker*'
  - Performance adopted from equivalent EffMem population
  - Reported in database with comment
- *Exploratory Biomarker* validated retrospectively if clinical trial data needed for submission

Q² Solutions
a Quintiles Quest Joint Venture

Q² Solutions Flow Cytometry Network



Q² Solutions
a Quintiles Quest Joint Venture

Flow Cytometry Technology Transfer

- ***Split sample method comparison*** *(≤ 20% bias)*
  - Minimum of 12 samples over 2 independent analytical runs.
  - Dependent on stability of assay

- ***QC Precision***
  - Using Globally harmonised QC lots (CD Chex/PBMC)
  - Range confirmation against ADL

- ***Sample Precision*** *(comparable precision to ADL validation)*
  - Minimum 5 samples in triplicate (mean %CV)

- ***Gating Assessment*** *(≤ 10% bias)*
  - 12 *.fcs* files blinded

Flow Cytometry Assay Standardization

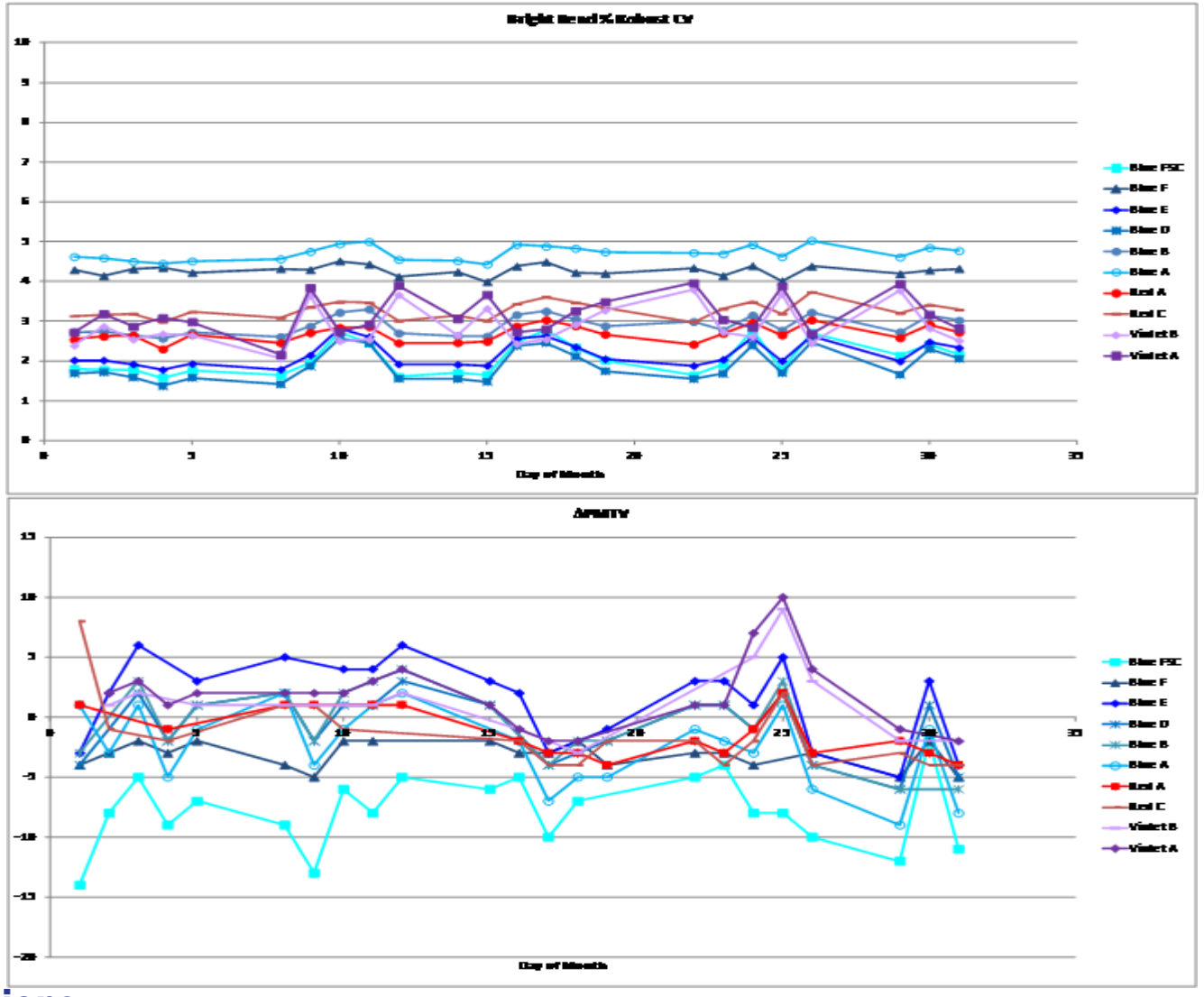
- ***Assay SOPs***
- ***Assay gating guides***
- ***Assay presentation***
- ***Assay QC***
  - Defined during assay validation
  - Global QC ranges
    - » Globally harmonized QC lots
    - » Ranges defined centrally based on global data-set
  - QC data tracked and analyzed by the Global Cytometry Group.
- ***Analyst Proficiency Assessment***
  - Gating Assessment (versus validating scientist)
  - Proficiency Testing every 6 months (3 samples)
- ***Central Gating***
  - Improve capacity
  - Improve consistency of highly variable assays (MRD CLL) using senior analyst

Flow Cytometry Instrument Standardization

- **Individual Instrument Performance Completely Characterized & Tracked Daily**
  - Data generated with and exported from BD CS&T Beads & Software
- **Standardized Quantitative Instrument Setup**
  - All global instruments are standardized against a composite predicate instrument
    - Settings Derived From Cell-based Optimization Using Characterization Data
    - Settings Transferred Between Instruments Using Standardized Documents with FCB targets
    - This provides for both qualitative and quantitative standardization of assays.
- **Applications Settings & Embedded Reusable Spillover**
  - Instrument standardization is maintained daily through the use of updatable application settings and embedded reusable spillover at the tube level of each panel.
- **Defined Intra/Inter Instrument Performance Tracking**
  - Instrument characterization data is transmitted to Global Harmonization in Edinburgh
  - Trends are tracked daily to indentify potential problems and prevent downtime
- **Q^2 Solutions Instrument Performance Specifications**
  - Developed in conjunction with BD. These are superior to BD manufacturing & service specs.
  - Provides for daily assurance of superior/optimal instrument alignment and performance

Global Harmonization Data

Examples: Bright Bead CV & Δ PMTV from baseline



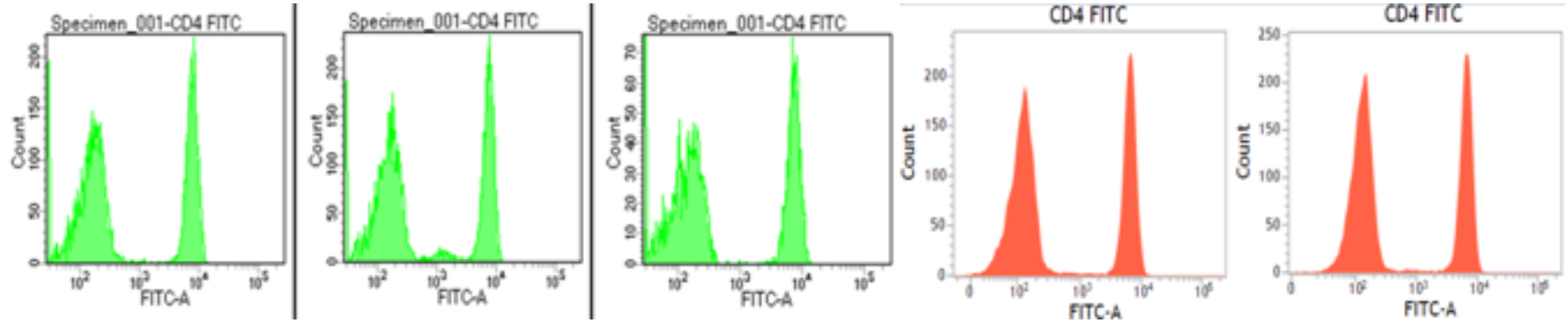
Flow Cytometry Quantitative Standardization

Innovative solution and harmonization
Reproducible quality data, instrument to instrument, site to site

B221

A comparison of stable fluorochrome-specific beads and hard-dyed beads for standardized quantitative flow cytometer setup

Ming Yan, Linda Zhu, Eric Crowther, Marybeth Sharkey, and Maria C. Jaimes
BD Biosciences, San Jose, CA 95131, USA
Mark G. Edinger and Todd Rogers, Quintiles Laboratories, USA



Canto II | Canto II | LSR II | Verse | Verse

Q² Solutions
a Quintiles Quest Joint Venture

Flow Cytometry Quantitative Standardization

Reproducible Quantitative Setup

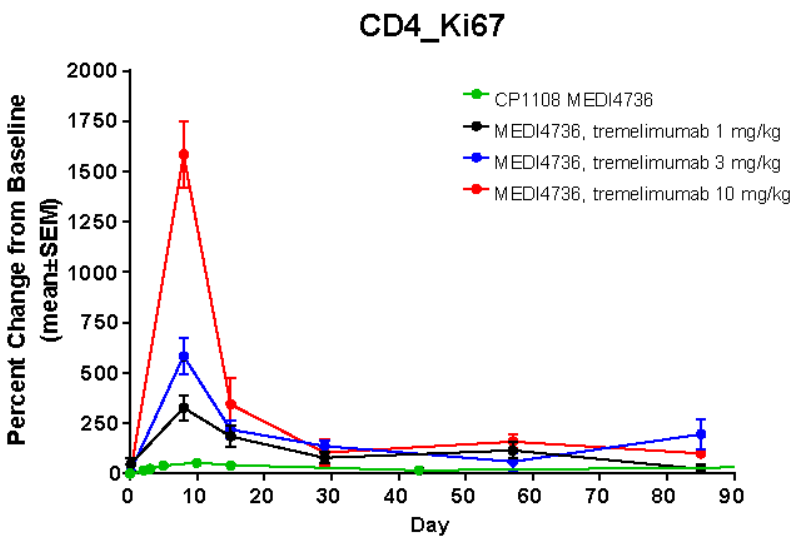
- Fluorochrome specific beads rather than beads hard-dyed with fluorochrome surrogates provide a better basis for quantitative setup
- Fluorochrome specific beads to be of practical use over a long period of time must be provide exhibit stabile levels of fluorescent intensity.
- Covalently linked fluorochrome beads that are dried and refrigerated for long term storage are the best candidates for quantitative standardization for this reason.
- FCB (fluorescent control beads) were custom ordered from BD Biosciences for this purpose.
- The existing qualitative standardization of all flow cytometers globally at Q^2 Solutions was modified to utilize these new beads.
- New SOPs were written and distributed globally for routine flow cytometry practice.

Q² Solutions Flow Panels Identify Dose Dependent PD Effect of Checkpoint Inhibitor Combination Therapy

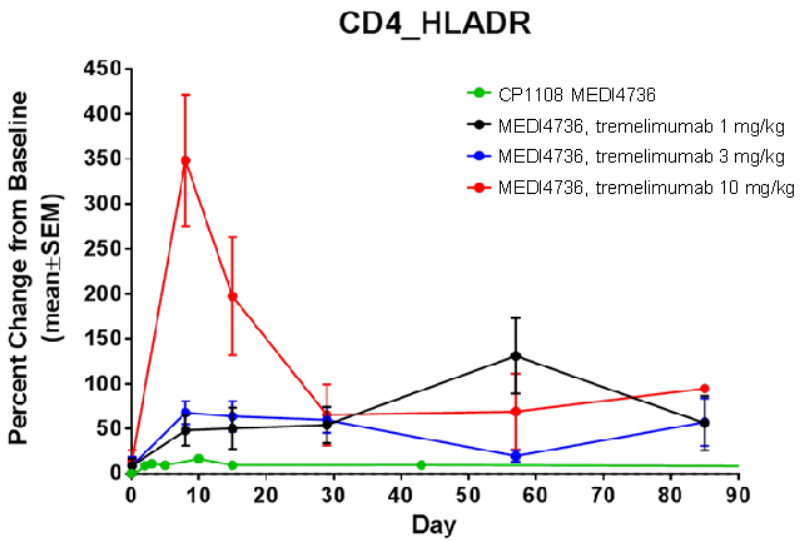
MEDI4736 (anti-PD-L1) & Tremelimumab (anti-CTLA-4)

Combined data from A115 & A129 Flow Panels running in US (Atlanta), Europe and Asia (Singapore)

A129 Proliferating T Cell Panel



A115 Activation & Memory T Cell Panel



Q² Solutions
a Quintiles Quest Joint Venture

Safety and antitumour activity of durvalumab plus Tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study
Scott Antonia, Sarah B Goldberg, Ani Balmanoukian, Jamie E Chaft, Rachel E Sanborn, Ashok Gupta, Rajesh Narwal, Keith Steele, Yu Gu, Joyson J Karakunnel, Naiyer A Rizvi. Lancet Oncology 2016. Published February 5, 2016

Flow Cytometry Panel Development

Considerations

- Early engagement
- Focussed parameter list
- Custom panel design
- Tube type selection for maximum sample stability
- Ongoing data review

Thank You! / Q&A

Q² Solutions
a Quintiles Quest Joint Venture