Using existing LabKey modules to build a platform for immunotherapy clinical trials: *an out-of-the-box approach*

Hannah Smithers

Correlative Studies Laboratory

Ben Towne Center for Childhood Cancer Research

Seattle Children's Research Institute

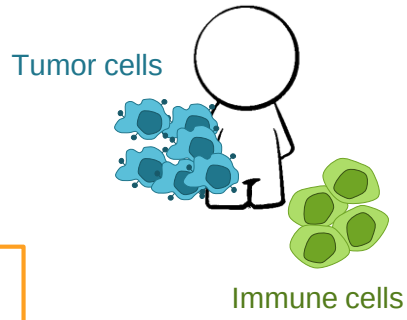
Seattle Children's

HOSPITAL • RESEARCH • FOUNDATION

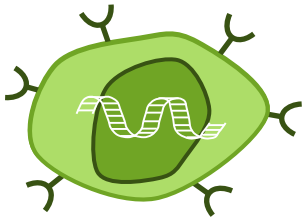
Outline

- Our T cell immunotherapy trials
- Merging cross-laboratory data into LabKey
- Looking back and moving forward

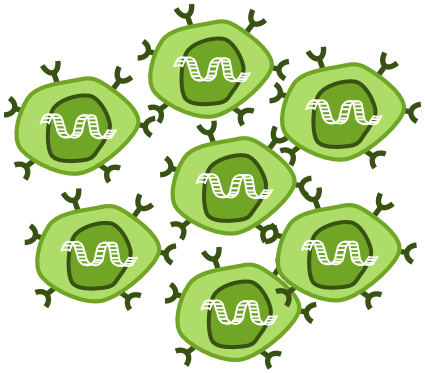
Cellular immunotherapy in cancer



Immune cells are isolated from the blood of a patient with cancer

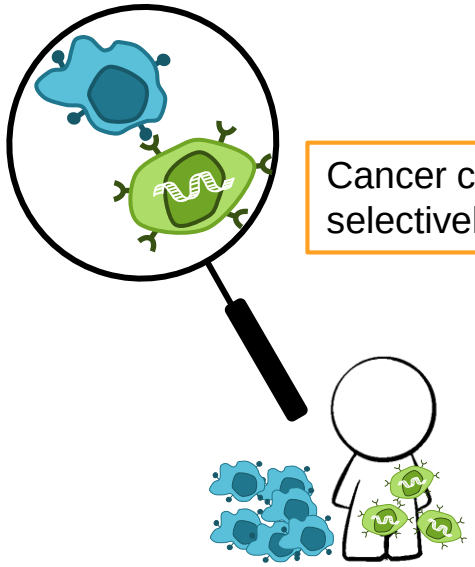


T cells that recognize CD19-expressing tumor cells



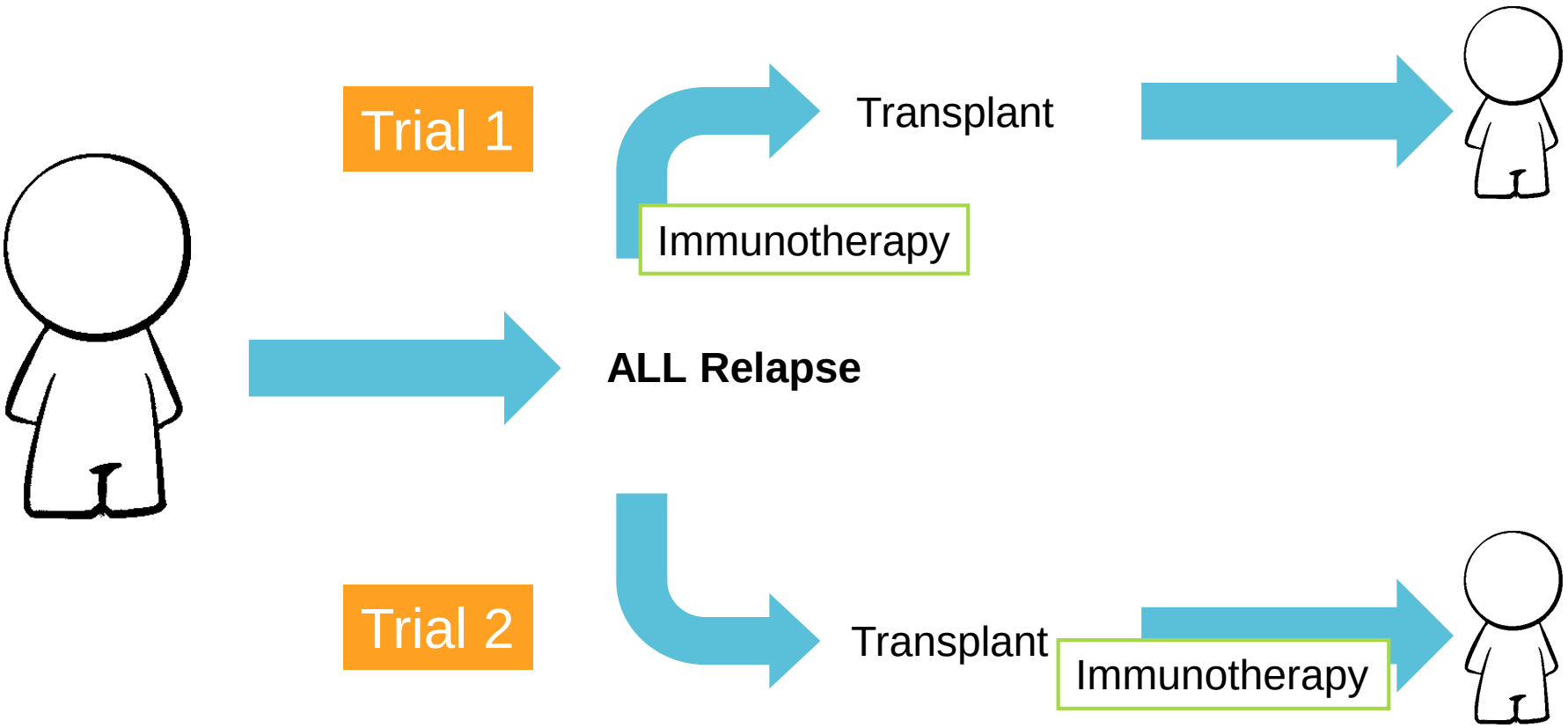
Activated and expanded in culture

Transfused back into patient



Cancer cells are selectively killed

Immunotherapy trials targeting pediatric ALL



ALL= Acute lymphoid leukemia

What makes a successful engineered T cell

- Cell characteristics pre-modification
  - Last chemotherapy treatment
  - Type of drug
  - Cells before and after transplant

- Characteristics of genetically modified cells
  - Cell markers that make them better “killers”
    - Differentiation
    - Memory phenotype
    - Proliferative potential
    - Function

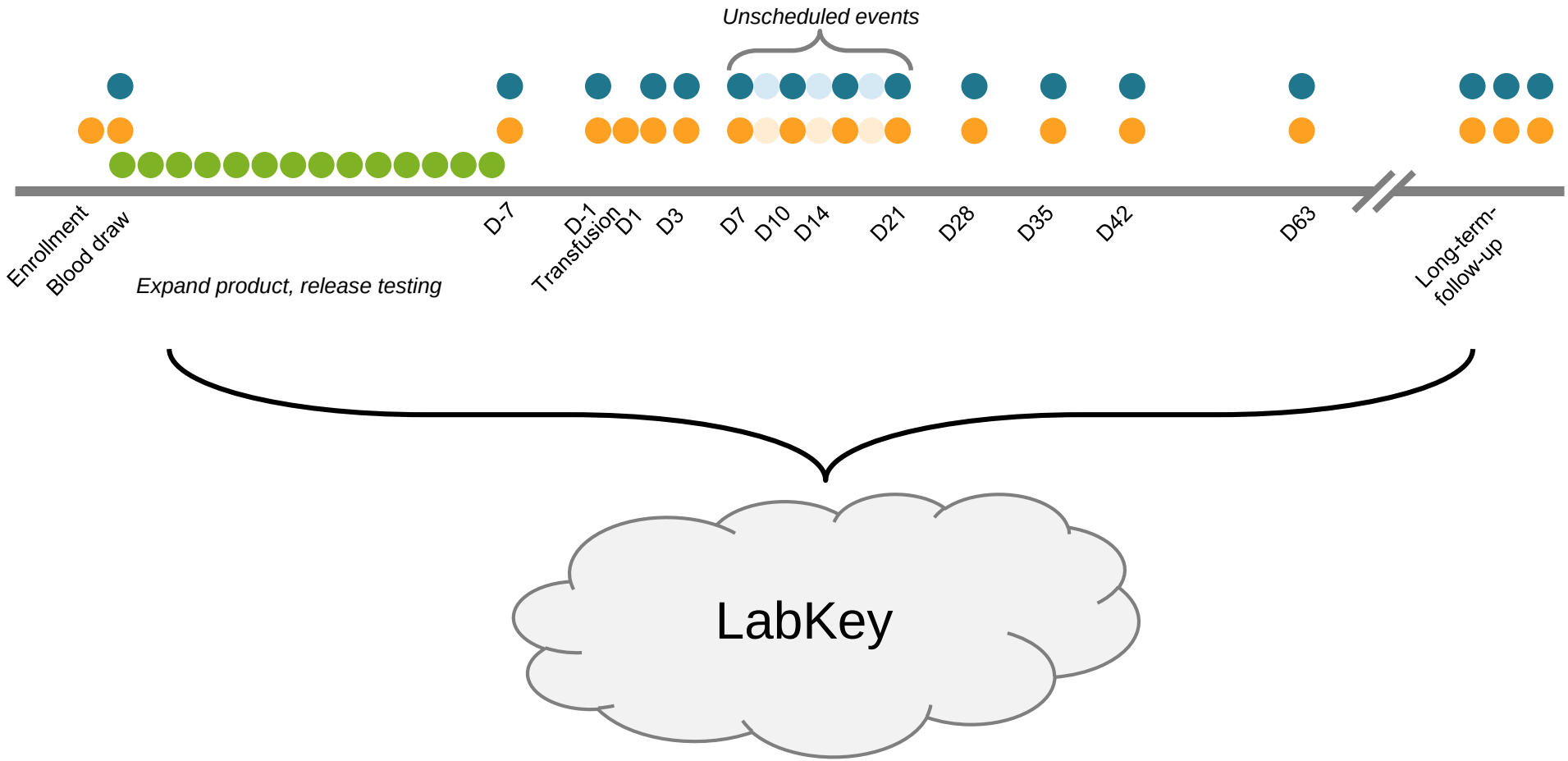
- Persistence of engineered T cells
  - How long is long enough to gain complete long term remission?

Multidisciplinary data collection

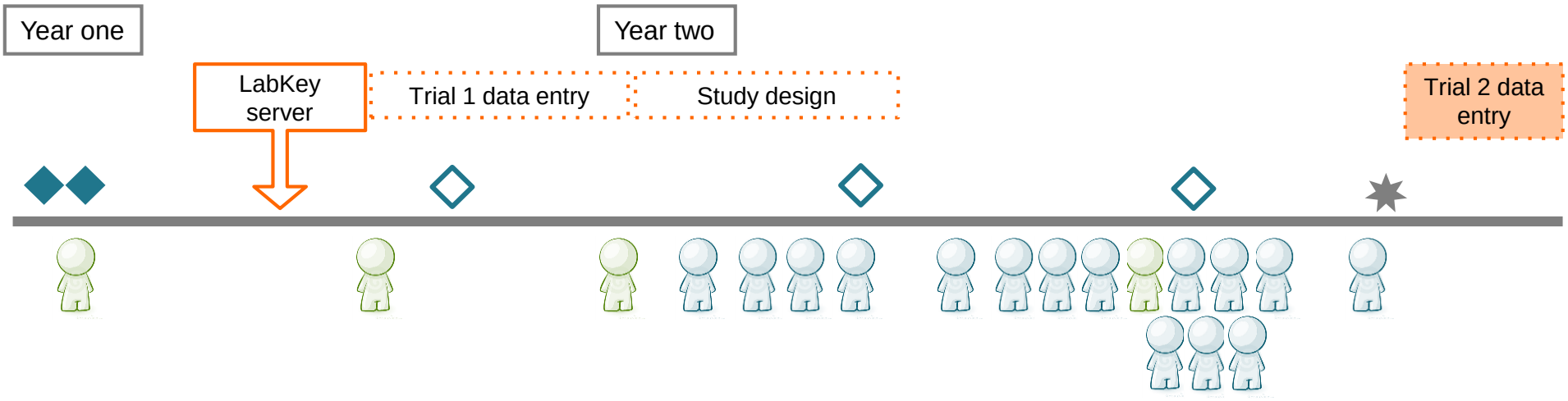
Correlative studies laboratory
- Flow
- qPCR
- Cellular assays
- Luminex
- Deep sequencing

Clinical team
- Clinical report forms

Cell production core
- Manufacturing
- Cell growth curves
- Release testing assays



Demand for LabKey continues to grow



Trial 1

16 samples over 11 visits:

- Flow cytometry (5 panels)
- qPCR assay
- Luminex assays
- Deep sequencing analysis

128 data sets/patient

Trial 2

23 samples over 12 visits:

- Flow cytometry (5 panels)
- qPCR assay
- Luminex assays
- Deep sequencing analysis

184 data sets/patient

CSL staff, LabKey administrator and user

CSL staff

Outline

- Our T cell immunotherapy trials
- Merging cross-laboratory data into LabKey
  - Data management
  - Non-assay data
  - Assay data
  - Push to study
- Looking back and moving forward

Data management

- Clinical data
  - Clinical report forms (CRFs) entered into software designed to capture patient information (DatStat)
- Cell product data
  - Paper based
- Assay data
  - Shared drives (tabular data, flow files, luminex files)
- Specimen data
  - Specimen repository software (FreezerPro)

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Subject information

CRFs completed by clinical staff

Data entered by CRAs

DATSTAT

Trial 1

Loaded manually

Push to study

Trial 2

Automatic integration

CRF data

- Clinical BM Results
- Physical
- AE Log
- Chemistry
- Concomitant Medication Log
- CSL Blood
- CSL BM
- CSL Other
- Demography
- Dose Administration
- Eligibility_BloodDraw
- Eligibility_Cytoxan
- Eligibility_Tcell
- EndTreatment
- Enrollment
- Exclusion
- Haematology_Recovery
- Hematology
- History_ALL
- Inclusion
- MedicalHistory
- PriorRadiotherapy
- PriorSurgery
- Reinduction1
- Reinduction2

CRF tables can be challenging to work with

Good structure: one row per patient per visit, information easy to access

HE WBC VALUE	HE RBC VALUE	HE HEMO VALUE	HE HEMA VALUE	HE PLATE VALUE	HE ANC VALUE	HE POLYS VALUE	HE LYMPHO VALUE	HE MONO VALUE	HE EOSIN VALUE	HE BASO VALUE	HE BLASTS VALUE	HE OTHER VALUE
2.5	3.48	11.4	33.7	74	875	600	1575	0	50	0	0	275
0.6	3.8	10.9	34.4	82	168	138	360	48	12	0	0	30
1.2	3.73	10.9	33.8	139	520	360	660	0	0	0	0	180
1.8	3.61	10.6	34.1	149	792	576	774	234	0	0	0	216
3.3	3.97	12.2	38.4	104	1172	1172	1030	970	50	30	0	50
2.2	3.12	10.2	30.5	77	689	689	1379	51	20	31	0	
1.7	3.74	10.4	32.7	116	1564	1224	68	17	0	17	0	340
1.4	3.4	9.7	29.5	144	1232	1162	70	28	70	0	0	70

Bad structure: 900 column table, multiple visits per row

AE DESCRIPTION 1	AE SAE 1	AE START DATE 1	AE STOP DATE 1	AE CON MED 1	AE RELATION TCELL 1	AE GRADE 1	AE OUTCOME 1	AE OUTCOME SPECIFY 1	AE DESCRIPTION 2	AE SAE 2	AE START DATE 2	...
Anemia	0	2013-06-19	2013-06-20	1	1	3	1		Anemia	0	2013-06-2	
Fever	0	2013-09-25	2013-09-25	1	1	3	1		White blood cell decreased	0	2013-09-2	

Solutions:

- Transform tables to one row per visit
  - Trial 1: after import
  - Trial 2: before import

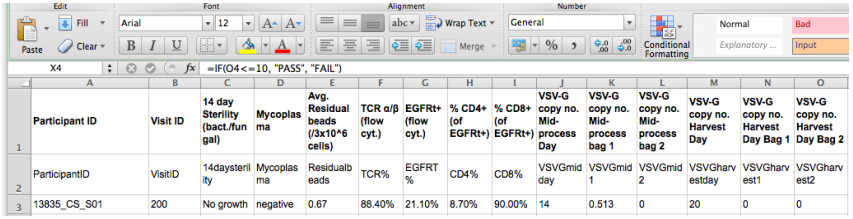
Remaining problems:

- Repetitive data
- Comments section includes valuable data
- Design CRFs with LabKey in mind

Pre- and post-clinical cellular information

Cell production core:
- GMP facility
- Auditable by FDA
  - Maintain paper records

Data entered into excel



	Participant ID	Visit ID	14 day Sterility (bact./fungal)	Mycoplasma	Avg. Residual beads (/3x10^6 cells)	TCR α/β (flow cyt.)	EGFRt+ (flow cyt.)	% CD4+ (of EGFRt+)	% CD8+ (of EGFRt+)	VSV-G copy no. Mid-process Day	VSV-G copy no. Mid-process bag 1	VSV-G copy no. Mid-process bag 2	VSV-G copy no. Harvest Day	VSV-G copy no. Harvest Day Bag 1	VSV-G copy no. Harvest Day Bag 2
1															
2	ParticipantID	VisitID	14daysterility	Mycoplasma	Residualbeads	TCR%	EGFRT%	CD4%	CD8%	VSVGmidday	VSVGmid1	VSVGmid2	VSVGharvestday	VSVGharvest1	VSVGharvest2
3	13835_CS_S01	200	No growth	negative	0.67	88.40%	21.10%	8.70%	90.00%	14	0.513	0	20	0	0

Upload to study

Correlative studies:
- Cell processing recorded real-time
  - Maintain paper records

Participant ID	Sequence Num	14 Day Sterility	Mycoplasma	Av. Residual Beads (/3x10^6cells)	TCRa/b %	EGFRt %	CD4 %	CD8 %	VSV-G Mid-Process Day	VSV-G Mid-Process Bag1 (copies/50ngDNA)	VSV-G Mid-Process Bag2 (copies/50ngDNA)	VSV-G Harvest Day	VSV-G Harvest Bag1 (copies/50ngDNA)	VS Ba (co
13835_CS_S01	200.0	No growth	negative	0.67	0.884	0.211	0.087	0.9	14	0.513	0.0	20	0.0	

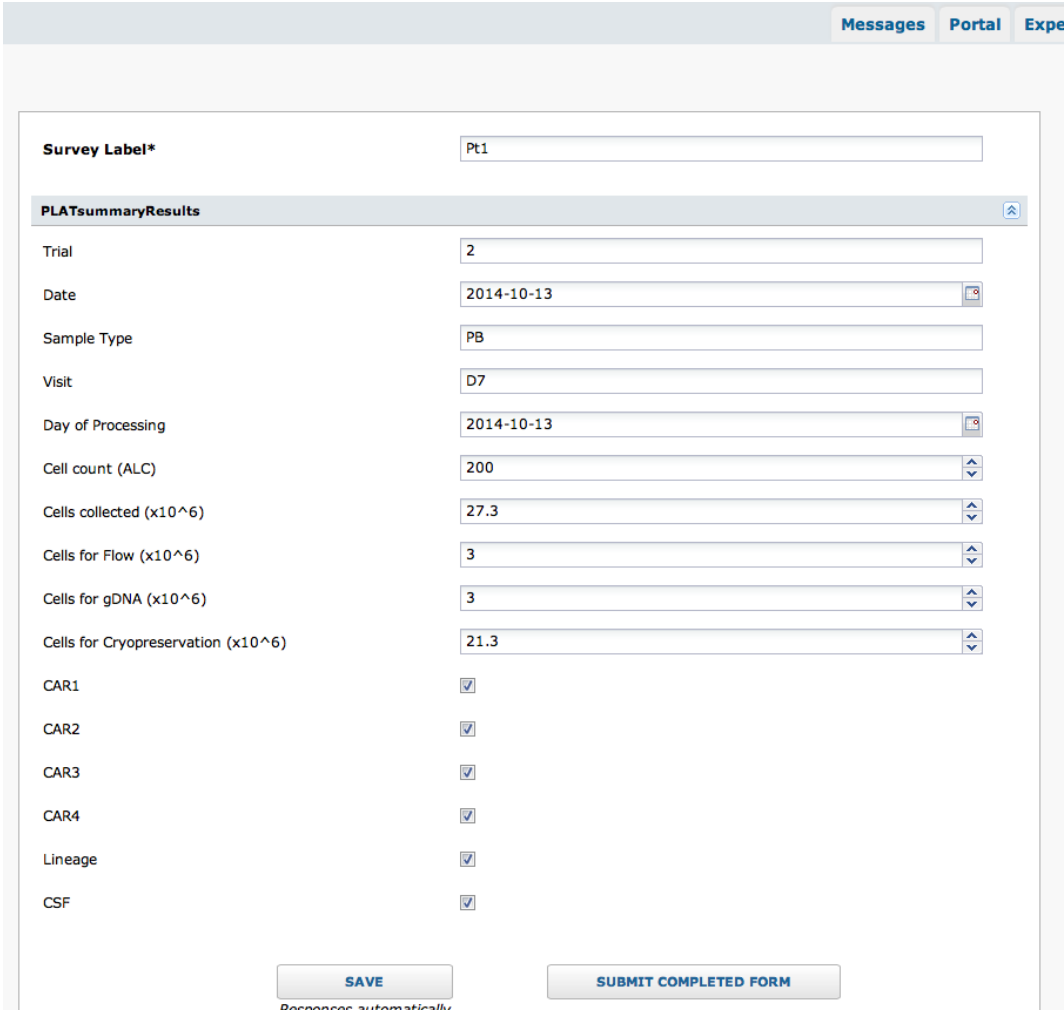
Coping with paper-based systems

Problems:

- Not ideal for storage
- Error prone
- Not systematic

Solution:

- Create surveys
  - Pre-clinical cell manufacturing
  - Post-clinical cell processing



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Assay data: qPCR

```
File Name       2014-04-30 WPRE 13835_CS_S03.pcrd

Created By Useradmin
Notes
ID
Run Started     4/30/2014 11:51:37 AM UTC -07:00

Run Ended       4/30/2014 2:02:08 PM UTC -07:00

Sample Vol      50
Lid Temp        105
Protocol File Name      WPRE Persistence Protocol.prcl

Plate Setup File Name   2014-04-26 WPRE 13835_CS_S03.pltd

Base Serial Number      CC015902
Optical Head Serial Number      785BR6560
CFX Manager Version     3.0.1216.0723.

Well group      All Wells
Amplification step      4
Melt step       7


Well    Fluor   Target  Content Sample  Cq      Starting Quantity (SQ)
A01     SYBR    WPRE    Std-01  Tube5   18.88   1.000E+06
A02     SYBR    WPRE    Std-01  Tube5   18.95   1.000E+06
A03     SYBR    WPRE    Std-01  Tube5   18.52   1.000E+06
A04     SYBR    ALB     Std-07  Tube5   20.30   1.000E+06
A05     SYBR    ALB     Std-07  Tube5   20.49   1.000E+06
A06     SYBR    ALB     Std-07  Tube5   20.32   1.000E+06
A07     SYBR    WPRE    Unkn    PreP PB 33.18   6.629E+01
A08     SYBR    WPRE    Unkn    PreP PB 32.86   8.200E+01
A09     SYBR    WPRE    Unkn    PreP PB 32.78   8.654E+01
A10     SYBR    ALB     Unkn    PreP PB 26.00   2.697E+04
A11     SYBR    ALB     Unkn    PreP PB 25.89   2.902E+04
A12     SYBR    ALB     Unkn    PreP PB 25.81   3.046E+04
B01     SYBR    WPRE    Std-02  Tube6   22.29   1.000E+05
B02     SYBR    WPRE    Std-02  Tube6   22.06   1.000E+05
```

.txt

General assay

Persistence in vivo

Tube name		Visit ID	Visit description	Date of draw	Sample Type	DNA input	WPRE SQ Rep1	WPRE SQ Rep2	WPRE SQ Rep3	WPRE StDev	WPRE SQ Mean	Albumin SQ Rep1	Albumin SQ Rep2	Albumin SQ Rep3	Albumin StDev	Albumin Mean	TRANSDUCED CELLS/100ug	% PERSISTENCE
TubeName	ParticipantID	VisitID	Visitdescription	Date	SampleType	DNAinput	WPRESQRep1	WPRESQRep2	WPRESQRep3	WPRESTDev	WPRESQMean	AlbuminSQRep1	AlbuminSQRep2	AlbuminSQRep3	AlbuminStDev	AlbuminMean	TransducedCellsPer:	PercentPersistence
Sample 1	13835_CS_S03	oP	200	30-Dec-13	PB	250	66.29000	82.00000	86.54000	10.63	78.28	26970.00000	29020.00000	30460.00000	1753.86	28816.67	2.92	0.02
Sample 2	13835_CS_S03	oC	300	24-Jan-14	PB	250	16.22000	14.32000	22.11000	4.06	17.55	23890.00000	34520.00000	33920.00000	5971.57	30776.67	0.65	0.00
Sample 3	13835_CS_S03	oC	300	24-Jan-14	BM	250	17.98000	19.02000	21.78000	1.96	19.59	23880.00000	21280.00000	30250.00000	4615.15	25136.67	0.73	0.01
Sample 4	13835_CS_S03	0	900	10-Feb-14	PB	250	1793.00000	1801.00000	1884.00000	50.39	1826.00	34630.00000	N/A	N/A	#DIV/0!	34630.00	68.13	0.39
Sample 5	13835_CS_S03	4	1000	13-Feb-14	PB	250	57.47000	53.66000	44.91000	6.44	52.01	24090.00000	18340.00000	30510.00000	6088.07	24313.33	1.94	0.02
Sample 6	13835_CS_S03	1	1100	18-Feb-14	PB	250	17.85000	22.01000	22.32000	2.50	20.73	20430.00000	29100.00000	31700.00000	5901.16	27076.67	0.77	0.01
Sample 7	13835_CS_S03	1	1100	14-Feb-14	BM	250	11.27000	11.07000	10.63000	0.33	10.99	31280.00000	42810.00000	32200.00000	6407.80	35430.00	0.41	0.00
Sample 8	13835_CS_S03	TD7	800.2	19-Mar-14	BM	250	20.28000	25.80000	27.06000	3.61	24.38	17180.00000	17040.00000	19510.00000	1387.41	17910.00	0.91	0.01
Sample 9	N/A	A	N/A	N/A	N/A	250	N/A	N/A	N/A	#DIV/0!	#DIV/0!	N/A	N/A	N/A	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

.xls

Tube Name	Participant ID	Visit ID	Visit Description	Date	Sample Type	DNA Input	WPRE SQ Rep1	WPRE SQ Rep2	WPRE SQ Rep3	WPRE StDev	WPRE SQ Mean	Albumin SQ Rep1	Albumin SQ Rep2	Albumin SQ Rep3	Albumin StDev
Sample 1	13835_CS_S01	500	D1	2013-06-19	PB	250.0	1.72398	2.21507	3.88583	1.13	2.61	749.43076	652.65947	751.76602	56.56
Sample 2	13835_CS_S01	800	D7	2013-06-25	PB	250.0	178.79287	171.87734	164.65202	7.07	171.77	585.37836	477.86787	706.67871	114.47
Sample 3	13835_CS_S01	800	D7	2013-06-27	BM	250.0	2832.70386	3000.91663	2715.98686	143.24	2849.87	6406.82864	5669.66889	5764.13286	401.12
Sample 4	13835_CS_S01	1000	D14	2013-07-01	PB	250.0	824.13911	770.18381	842.04087	37.41	812.12	2602.52382	3224.65602	2176.10473	527.31

Push to study

- Problems:
  - Time consuming
  - Error prone
- Solutions:
  - Develop process to properly read .txt data and queries to perform calculations

Assay data: luminex

Luminex module

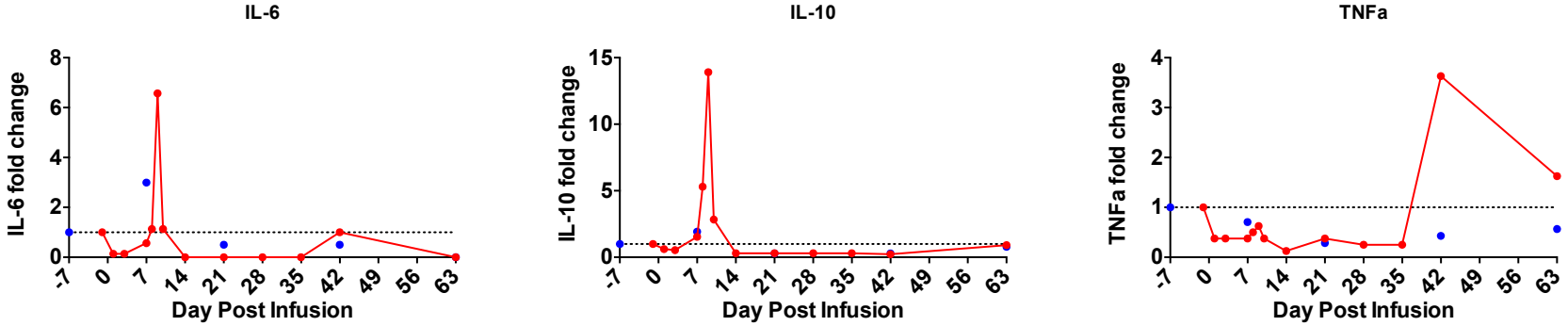
- Straightforward entry
  - Files exported from machine → LabKey
- Averages or replicates
- Can add keywords

Analyte	Well Role	Type	Well	Description	Flagged As Excluded	Specimen ID	Participant ID	Visit ID	Extra Specimen Info	Date	FI	FI-Bkgd	Std Dev	Obs Conc BioPlex 5PL	Exp Conc
GM-CSF (20)	Unknown	X2	D3,D4	14602_CS_S01, 400, 2014-03-17, PB	no		14602_CS_S01	400.0	PB	2014-03-17	84.5	75.0	71.42	0.05	
GM-CSF (20)	Unknown	X3	E3,E4	14602_CS_S01, 500, 2014-03-19, PB	no		14602_CS_S01	500.0	PB	2014-03-19	37.5	28.0	0.71	0.02	

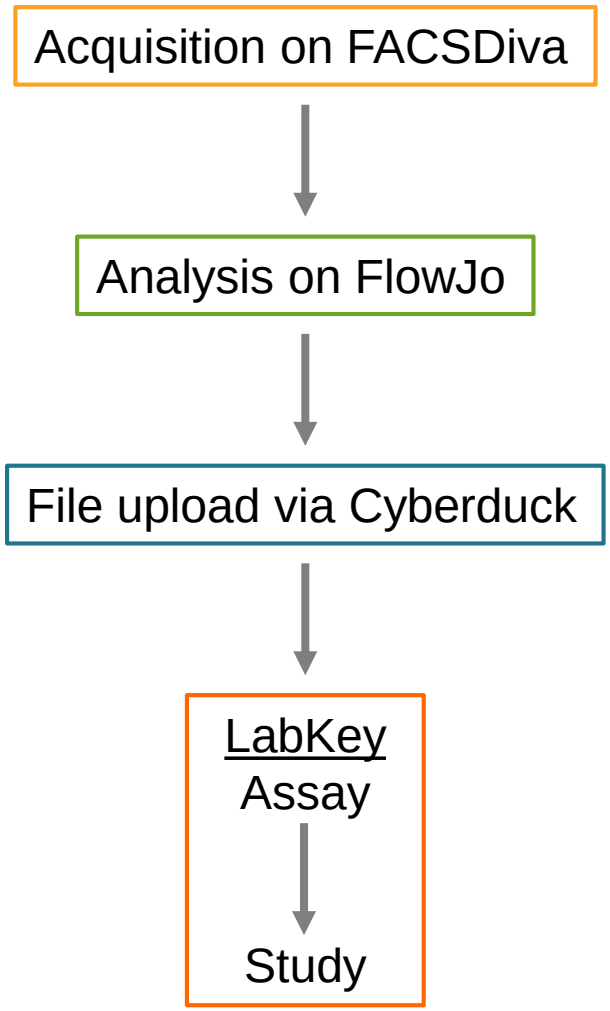
Graphing luminex data

Present normalized data instead of raw concentration

Normalize



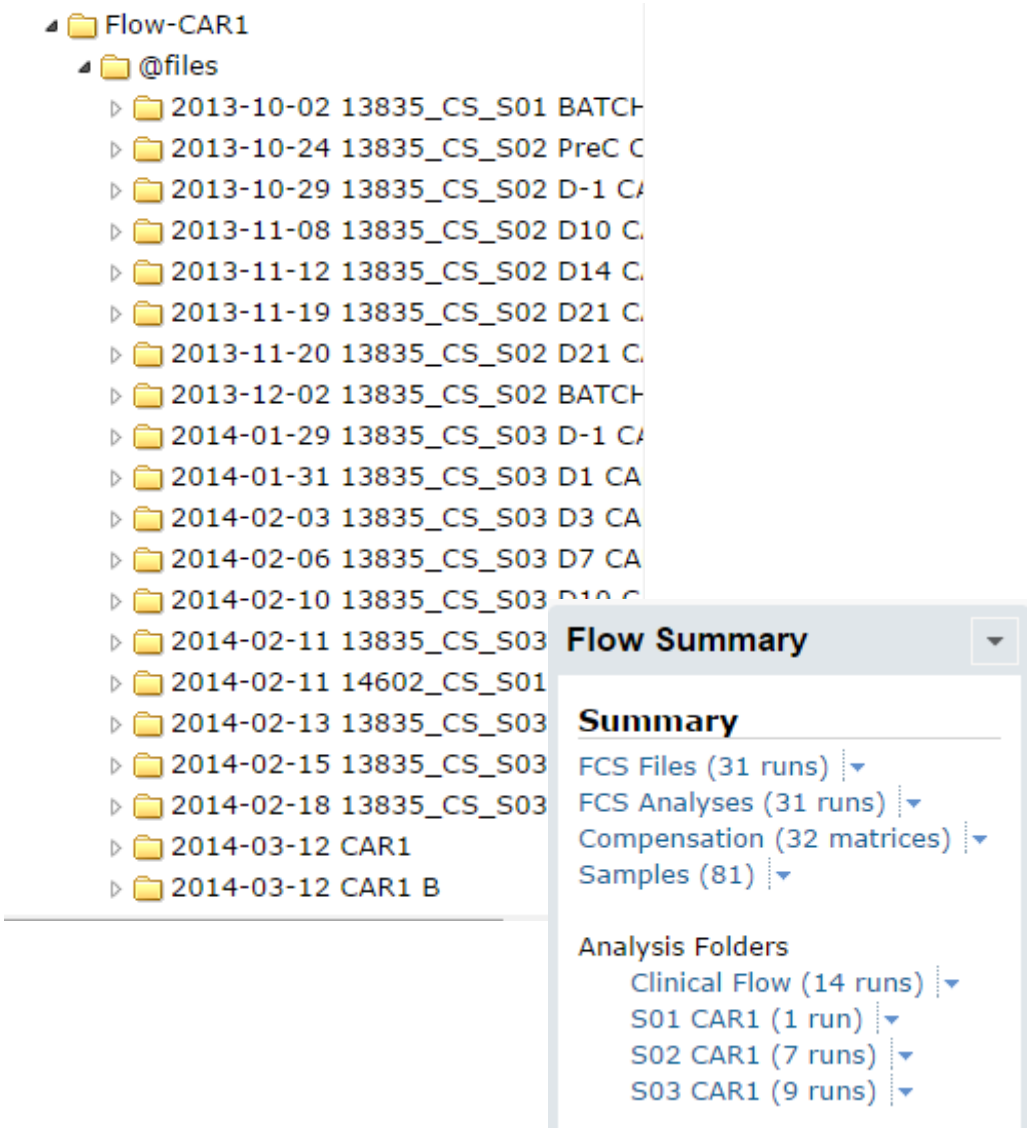
Assay data: flow cytometry



Trial 1	Trial 2
- Embed metadata in .fcs files (~30% files)	- Compensate sample .fcs files
- Compensate sample .fcs files - 115 .wsp files per patient	- 5 .wsp files per patient
- FTI client used to automate file upload	
- Manually direct files to appropriate folder - Provide metadata for .fcs files - Quality control - Push to study	

Organizing .fcs files is a bottleneck

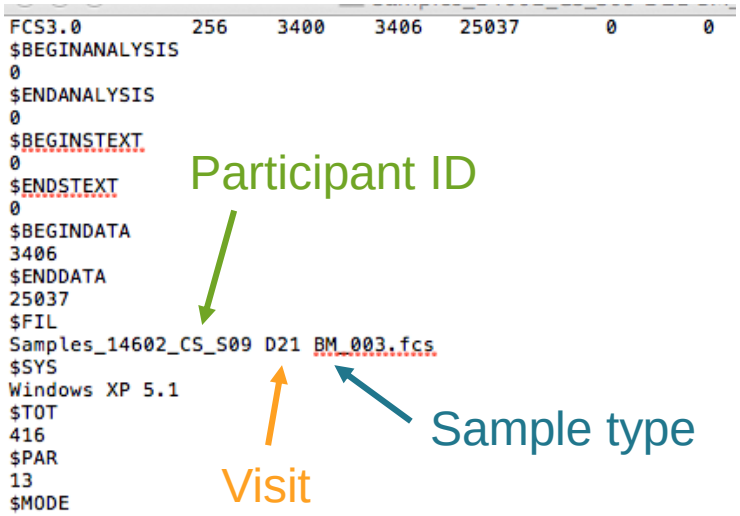
- Import data to individual folders
  - One panel per folder
    - Trial 1: 300+ .wsp files
    - Trial 2: 75+ .wsp files
- Create metadata
- Quality control



Managing .fcs metadata

- Link .fcs files to keywords

- Ways to link files
  - Embed directly into .fcs files
  - Manually enter metadata
  - Automate file parsing

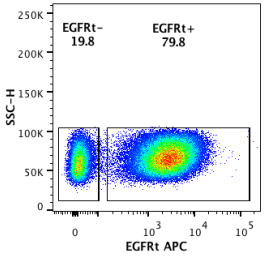


Linked metadata

EDIT	DETAILS	Samples_S01 D-1 PB_003.fcs	Samples		13835_CS_S01	400	PB	2013-06-17	CAR1	D-1
EDIT	DETAILS	Samples_S01 D1 PB_004.fcs	Samples		13835_CS_S01	500	PB	2013-06-19	CAR1	D1
EDIT	DETAILS	Samples_S01 D7 PB_005.fcs	Samples		13835_CS_S01	800	PB	2013-06-25	CAR1	D7
EDIT	DETAILS	Samples_S01 D14 PB_007.fcs	Samples		13835_CS_S01	1000	PB	2013-07-01	CAR1	D14
EDIT	DETAILS	Samples_S01 D21 PB_008.fcs	Samples		13835_CS_S01	1100	PB	2013-07-09	CAR1	D21
EDIT	DETAILS	Samples_S01 D28 PB_010.fcs	Samples		13835_CS_S01	1200	PB	2013-07-15	CAR1	D28
EDIT	DETAILS	Samples_S01 D42 PB_011.fcs	Samples		13835_CS_S01	1400	PB	2013-07-29	CAR1	D42

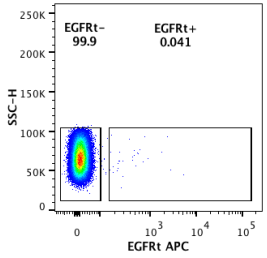
Adapting ICS metadata for FMO controls

All stain



subtract

FMO control



= *true T cell population*

Background and Foreground Match Columns

Select the columns that match between both the foreground and background wells.

For example, you usually want to match wells from the same FCSAnalysis Run and from the sample draw.

FCSAnalysis Run
and Sample ParticipantID
and Sample VisitID
and Sample SampleType

Background Column and Value

Specify the column and value filter(s) which uniquely identify the background wells from the foreground wells.
If multiple wells match the background criteria, the background value will be the average of all the matched wells.

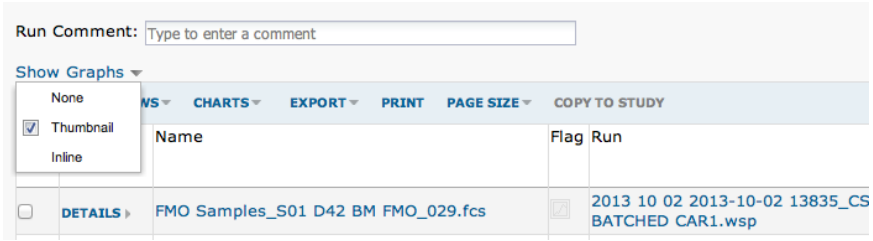
For example, if your background wells may have either "neg" or "NegControl" for the "Peptide" keyword then select column "Keyword Peptide", select operator "Equals One Of", and set the value to "neg;NegControl". Multiple values are combined with ';' when using the "Equals One Of" operator.

Background Column	Operator	Value
Keyword TUBE NAME	Contains	FMO

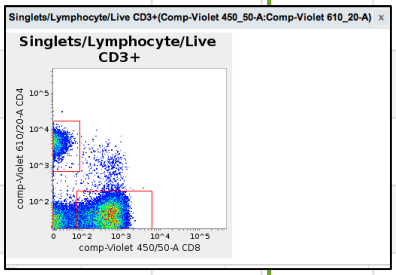
Name	Participant ID	Visit ID	Sample Type	Count	Lymphocyte/Live CD3+:%P	Lymphocyte/Live CD3+/EGFRt+:%P	BG Lymphocyte/Live CD3+/EGFRt+:%P	Lymphocyte/Live CD3+/EGFRt+/CD8+:%P
Samples_S01 D21 BM_009.fcs	13835_CS_S01	1100	BM	188,262	30.113	1.281	0.031	28.333

QC, QC, QC!

- Quality control is a requirement
  - FlowJo user errors
  - FlowJo bugs- 10.0.6
  - Erroneous file transfer
- Thumbnail view is a helpful tool

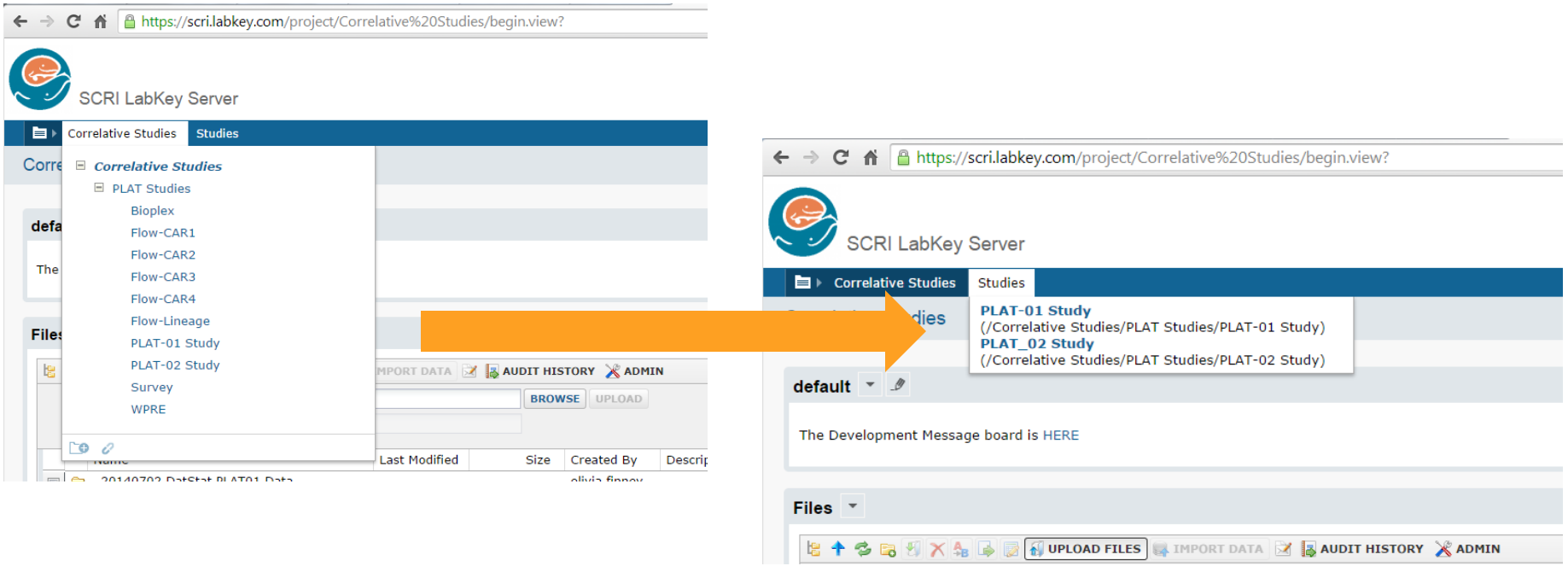


Name	Participant ID	Visit Description	Visit ID	Sample Type	Lymphocytes:%P	Lymphocytes(Comp-Blue 530_30-A:Comp-Blue 695_40-A)	CD3:%P	B cells:%P	B cells:Median(Comp-Blue 530_30-A)	NKT cells:%G
Samples_S01 D-1 PB_003.fcs	13835_CS_S01	D-1	400	PB	92.691				220.446	2.703
Samples_S01 D21 BM_008.fcs	13835_CS_S01	D21	1100	BM	86.659				240.843	6.002
Samples_S01 D7 PB_004.fcs	13835_CS_S01	D7	800	PB	95.166				291.497	14.845
Samples_S01 D42 BM_011.fcs	13835_CS_S01	D42	1400	BM	94.536				438.268	1.856
Samples_S01 cCS0001_012.fcs	13835_CS_S01	cCS0001			73.866		77.43	4.198	580.397	3.918

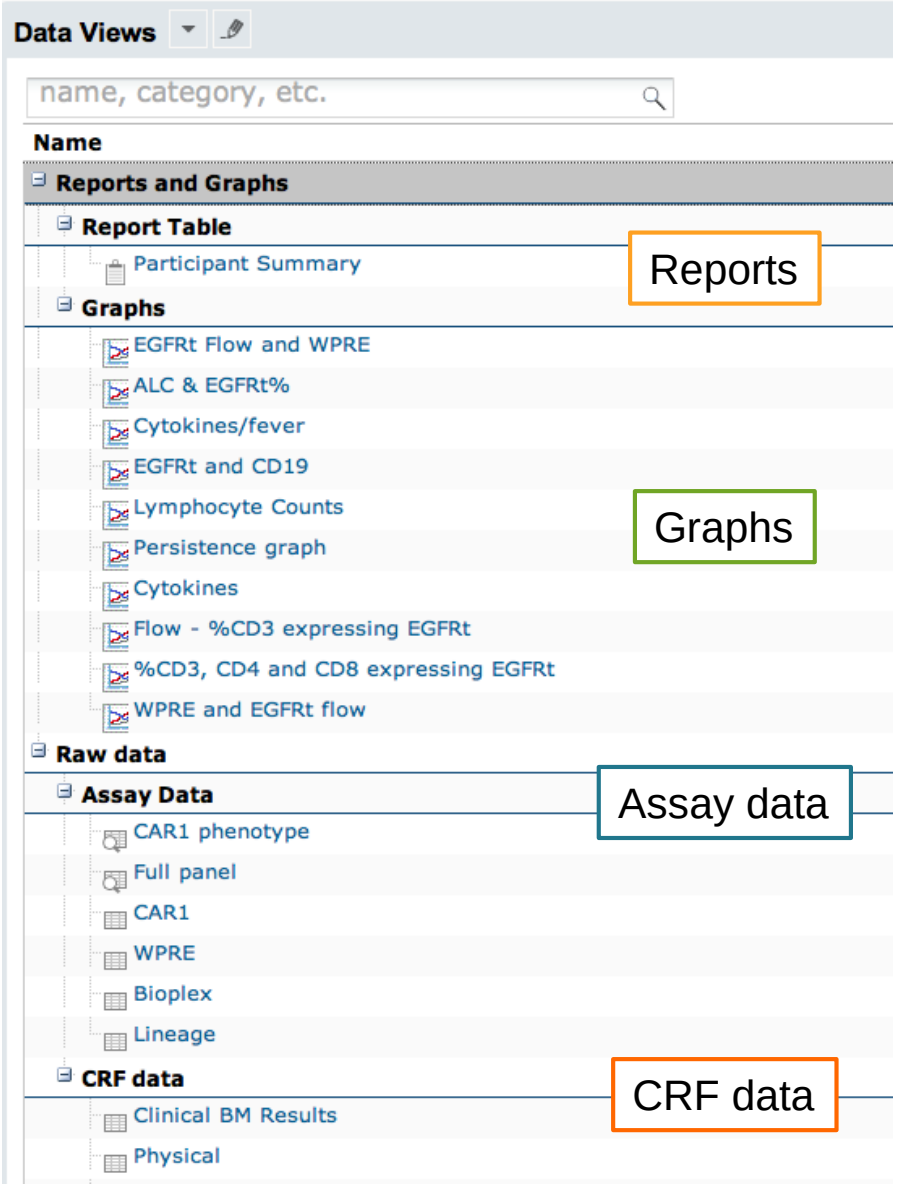


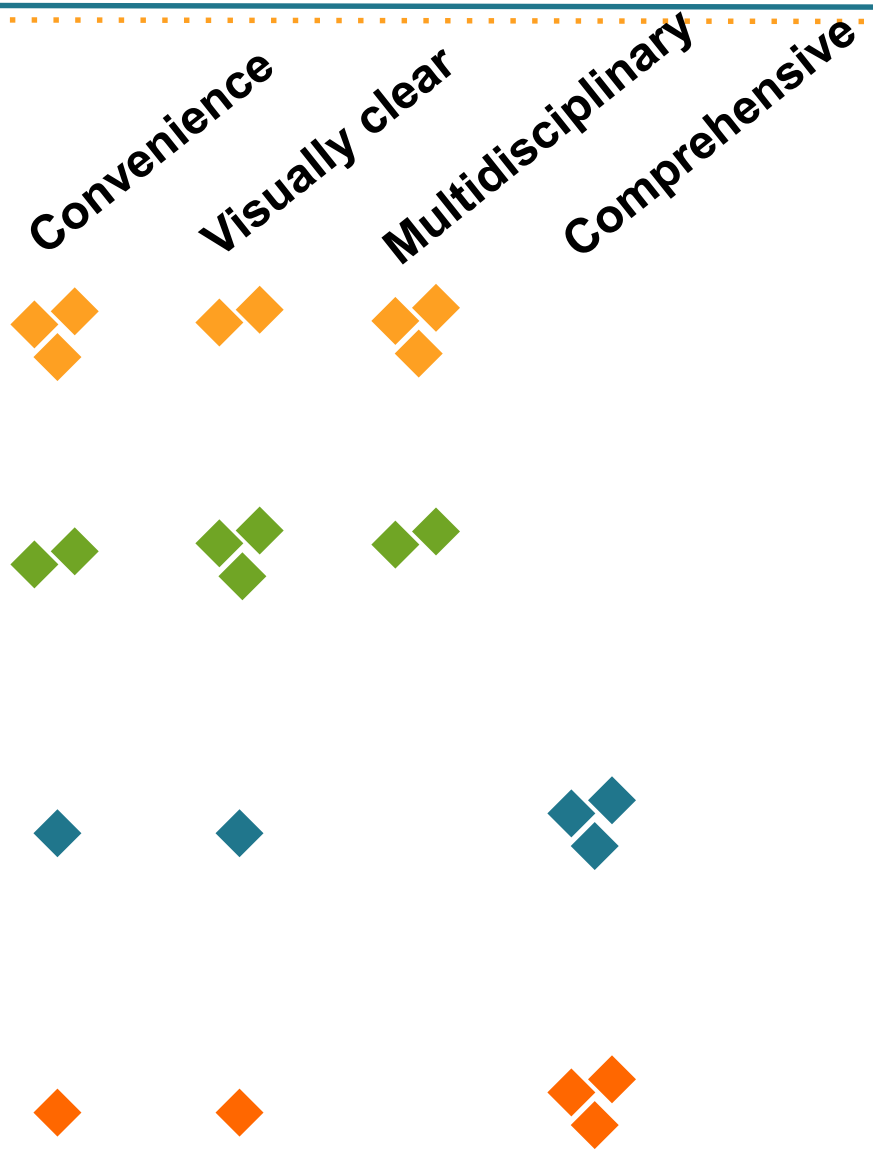
The meat-and-potatoes: *push to study*

- Data
  - Create → Import → QC
- Study design
  - Define properties, visits, cohorts, etc.



Study: clinical and assay data





Study: reports

- Table format
  - Any variable that is a measure
  - No graphs
- Very accessible for general LabKey user
- Multi-patient or cohort view

Participant Summary

Participant Summary

EXPORT TRANSPOSE

Filter Report

13835_CS_S01

Cohort: 1

Groups:

Visit Label	Visit Date	HE LYMPHO VALUE	HE BLASTS VALUE	BM PERCENT ABNORMAL	Lymphocyte/Live CD3+/EGFRt+:%P	B cells:%P	B cells:Median(Comp-Blue 530_30-A)
Screening		1575	0	19			
PreP					25.705	0.075	240.843
		1379	0				
PreC		68	0				
					0.367	15.206	1,175.046
D-1		70	0				
					0.031	0.032	220.446
D1		68	0				
					0.101		
D2		4	0				
D7		0	0	0	16.557	0.144	236.756
		0	0	0	4.613	0.006	291.497
		0	0	0	4.613	0.144	236.756
		0	0	0	16.557	0.006	291.497
D14		360	0				
					3.662	0.002	246.186
D21		660	0	0	0.195	0.014	239.669
		660	0	0	0.195	0.125	240.843
		660	0	0	1.281	0.125	240.843
		660	0	0	1.281	0.014	239.669
D29		774	0				
					0.073	0.028	243.799
D42		1030	0	0	0.112	17.603	438.268
		1030	0	0	0.000	17.603	438.268
		1030	0	0	0.112	0.039	252.858
		1030	0	0	0.000	0.039	252.858

13835_CS_S02

Study: standard graphing feature

Advantages

- Fast and easy creation
- Multidisciplinary data integration
- Can choose multiple measures
- Available to many users without the need for graphing software

Disadvantages

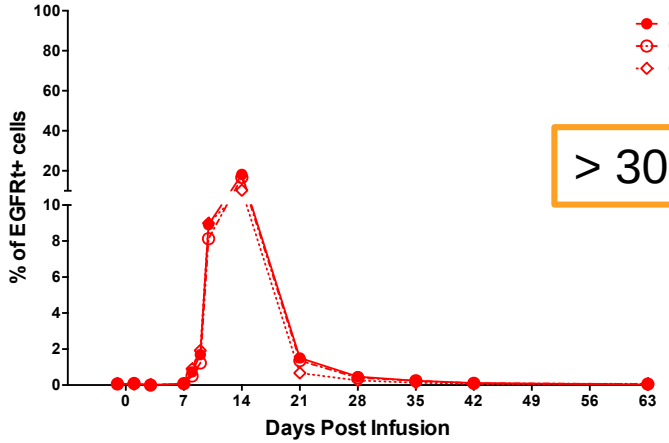
- Not as customizable as graphing softwares
- Not ideal for publications
- Time-consuming to find measures

Study: flow graphs

Graph depicting our modified T cell expression over:
All CD3+, CD3+/CD8+ and CD3+/CD4+ cells

Classical workflow:

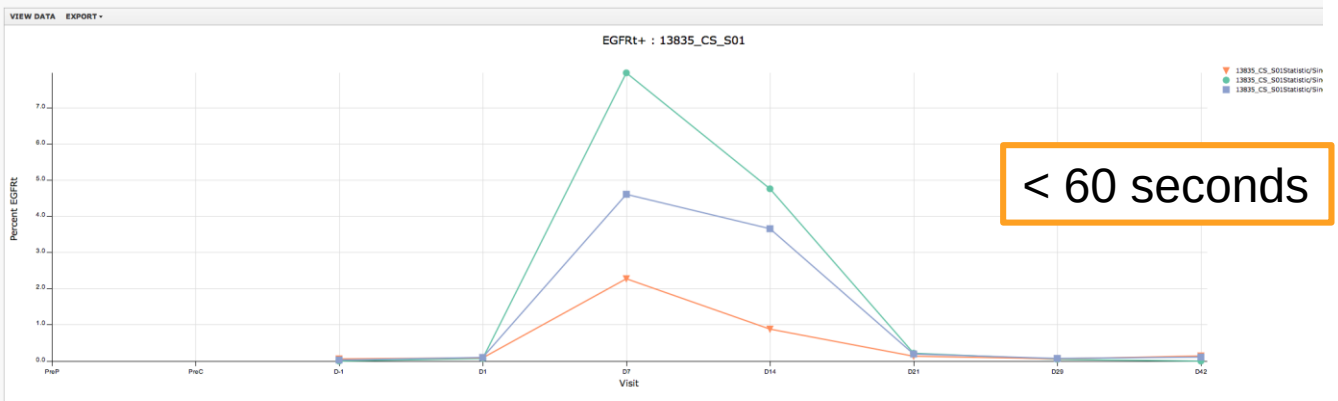
- Export data from FlowJo to excel
- Organize excel sheet
- Copy/paste data into Prism
- Modify graph



> 30 minutes

Using LabKey:

- New Time Chart
- Choose measure(s)
- Modify graph



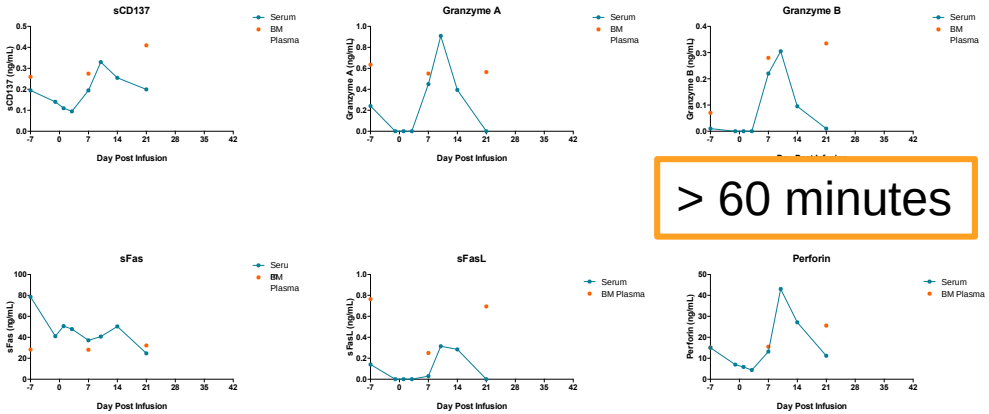
< 60 seconds

Study: luminex graphs

Luminex data: Multiple analyte view

Classical workflow:

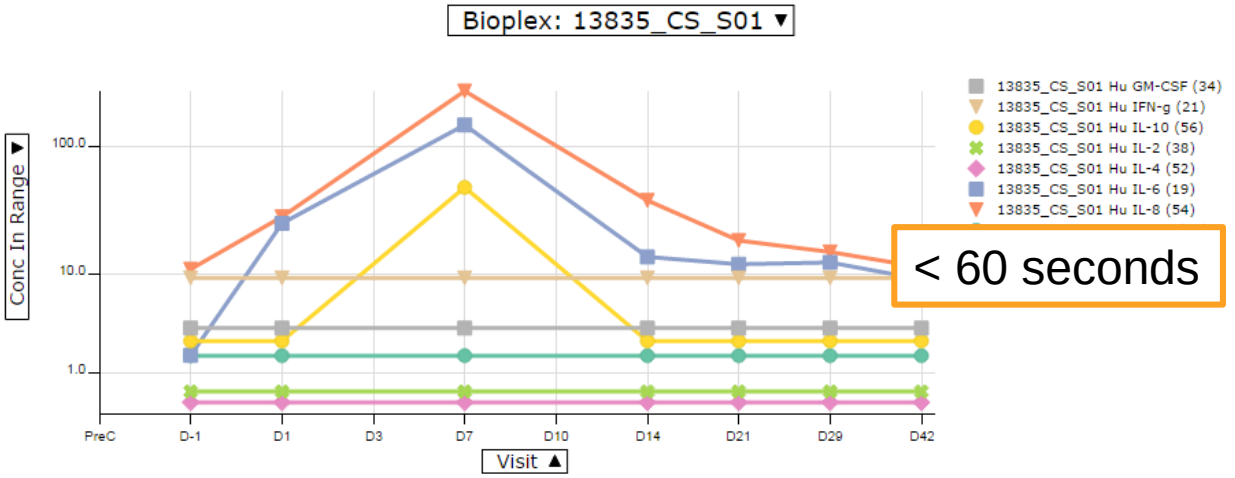
- Transfer data from luminex excel to working spreadsheet
- Organize excel sheet
- Copy/paste data into Prism
- Modify graph(s)



> 60 minutes

Using LabKey:

- New Time Chart
- Choose measure(s)
- Modify graph



< 60 seconds

Study: integrated graphs

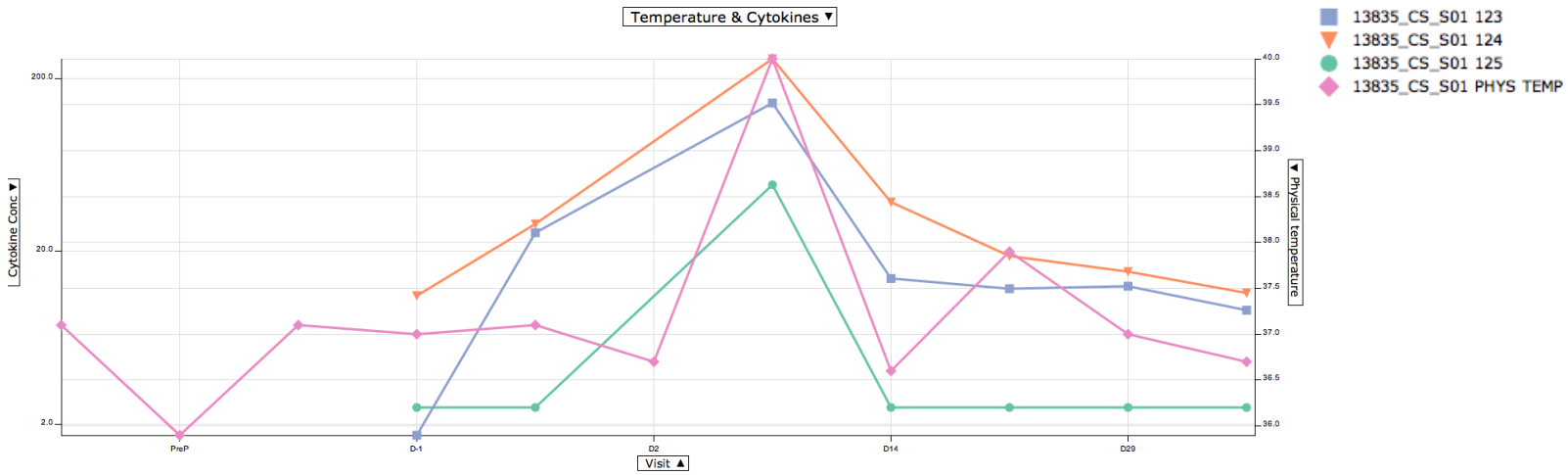
CRF data

Patient temperature

Assay data

Luminex analytes

Graphs



Temperature and cytokines spike concurrently:
indicative of cytokine release syndrome

Study: integrated graphs

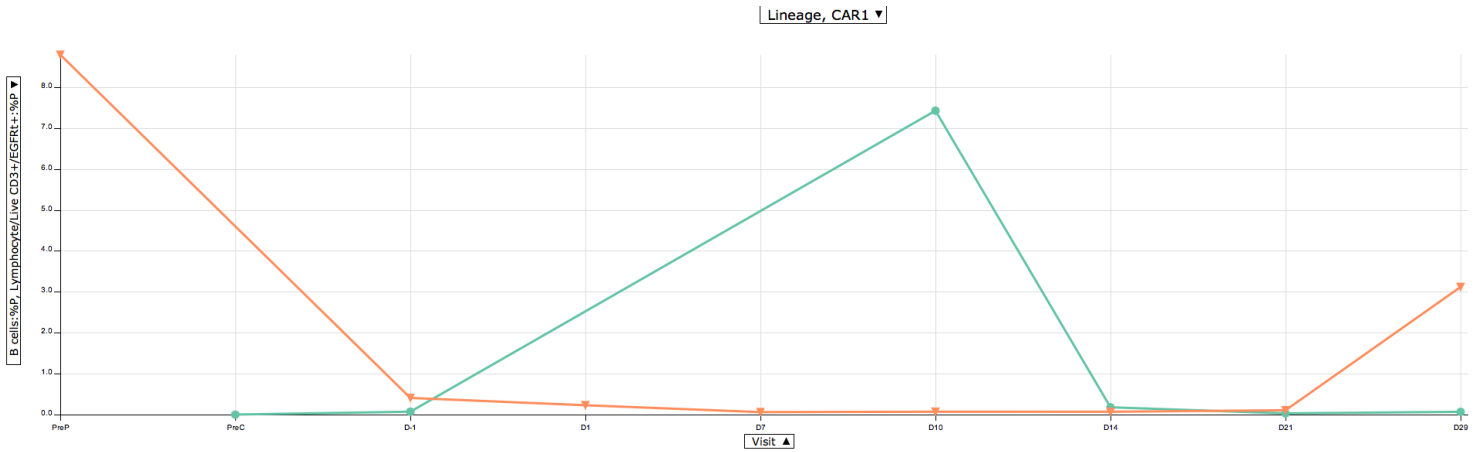
CRF data

Assay data

Flow panel 1:
Modified T cells

Flow panel 2:
B cells

Graphs



As T cells increase, B cells are depleted: Our modified T cells are capable of killing tumor cells

Outline

- Our T cell immunotherapy trials
- Merging cross-laboratory data into LabKey
- Looking back and moving forward

What we've learned

- Design data with LabKey in mind
  - Keywords
  - Data collection and storage
- Simpler system ≠ no quality control
- Out-of-the-box does not mean ready to use
- You get out what you put in

Goals

- Import data from Trial 2
- Create surveys for pre- and post-clinical data
- Write SQL queries to customize tables
- Create user-friendly space
  - Readily accessible and available data summaries
  - Enhance data sharing
- Make report generation easy
  - Milestones, FDA reports, etc.

Thank you!

CSL Team
Olivia Finney
Juliana Williams
Roxana Hicks
Ben Futrell

Clinical Team
Rebecca Gardner
Dione Froman
PIRC team

Ben Towne Center
Jensen Lab
Michael Jensen
Doug Kisker
Kean Lab
Crane Lab
TCPC

LabKey
Avital Sadot
Greg Taylor
Kevin Krouse
Karl Lum
Matt Bellew

FHCRC
Riddell Lab
Cam Turtle
Paul Litwin

Volunteers
Ryan James

Seattle Children's
HOSPITAL • RESEARCH • FOUNDATION

ben towne
pediatric cancer research foundation

Questions?