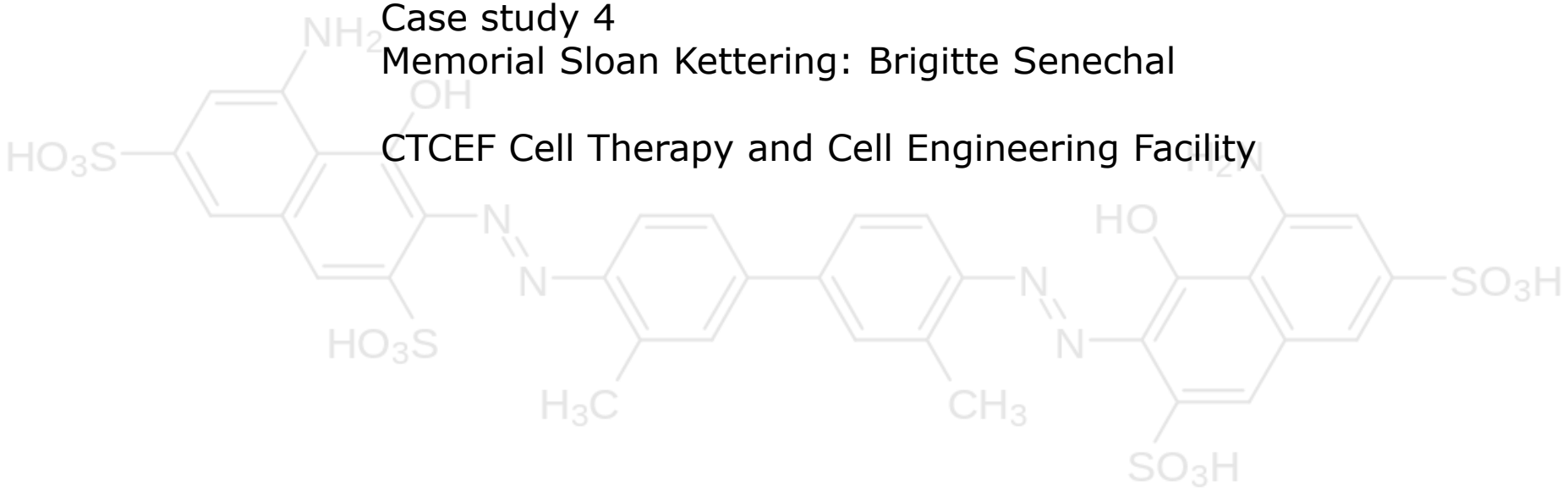


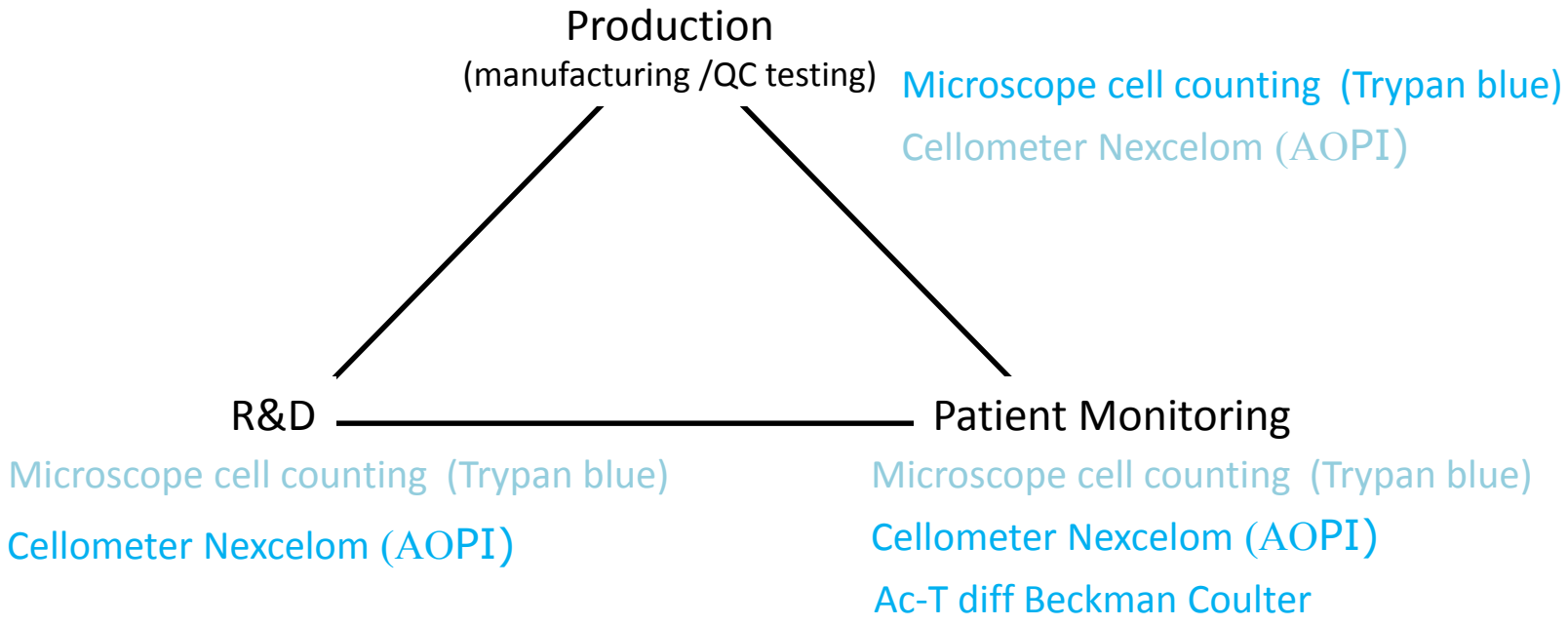
Case study 4
Memorial Sloan Kettering: Brigitte Senechal

CTCEF Cell Therapy and Cell Engineering Facility

Memorial Sloan Kettering Cancer Center

CTCEF / Isabelle Riviere
Cell Therapy and Cell Engineering Facility (2015)

Production of CAR T cells from apheresis product



GTF Gene Transfer and Somatic Cell Engineering Facility

Research lab

Multiple factors that may affect cell counting measurements

Lab space/equipment
Different lab
Multiple pieces of the same equipment
Different methods (optical/diffraction, impedence, fluorescence-AOPI, TB…)

Operators
15 operators

Cell sample properties :

- Fresh v frozen
- Cell lines v patient isolated
- Manipulated v un-manipulated
- Homogeneity / over time / over source
- (species)

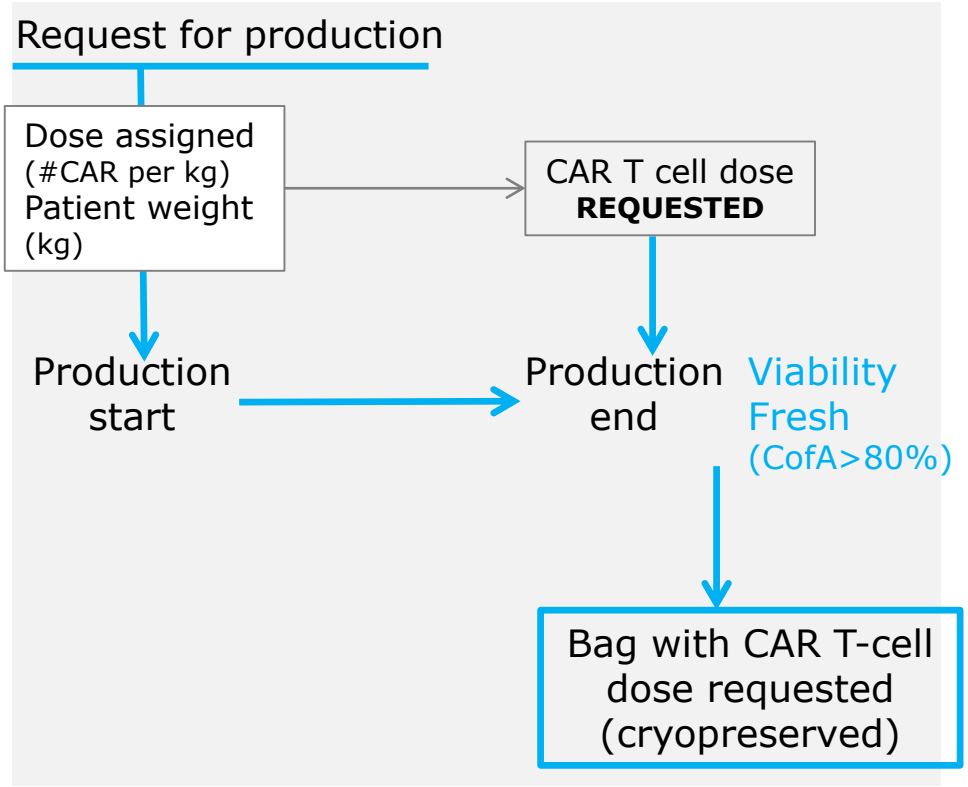
…

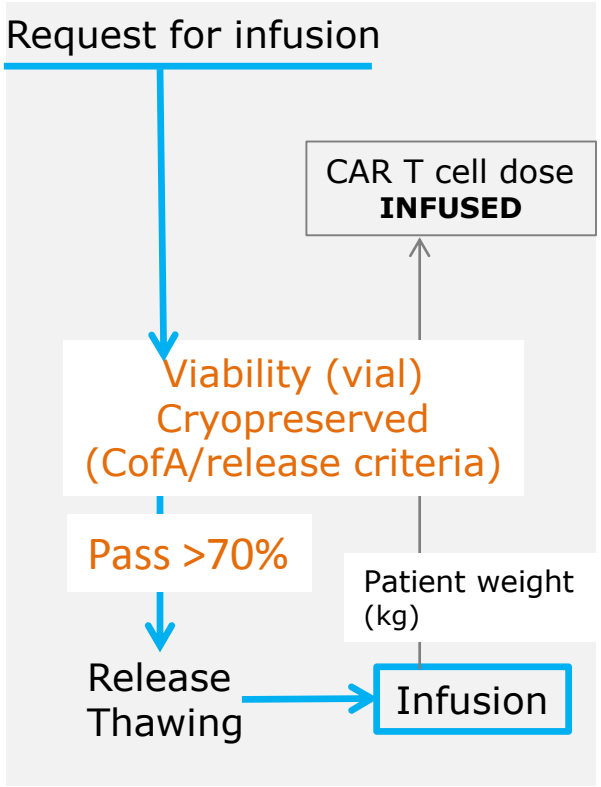
Verification
SOP
training

Production
(manufacturing /QC testing)

→CAR cell dose preparation and release: process

current method: trypan blue





→CAR cell dose preparation and release

Microscope cell counting (Trypan blue) / our reference

Counting Instructions (SOP-6010):

Trypan blue
1:1 cell:tb
Read within 3 minutes
2 chambers
Average %

AOPI (Nexcelom)
1:1
1 reading
Dilution if >10e7

Release Instructions (SOP-5008):

Cryopreserved Viability-trypan blue

>70% pass (CofA)

<70% / 2nd operator
<70% / 2nd vial thawed
<70% / 2nd operator

QA report to PI
PI requests deviation

IRB approval

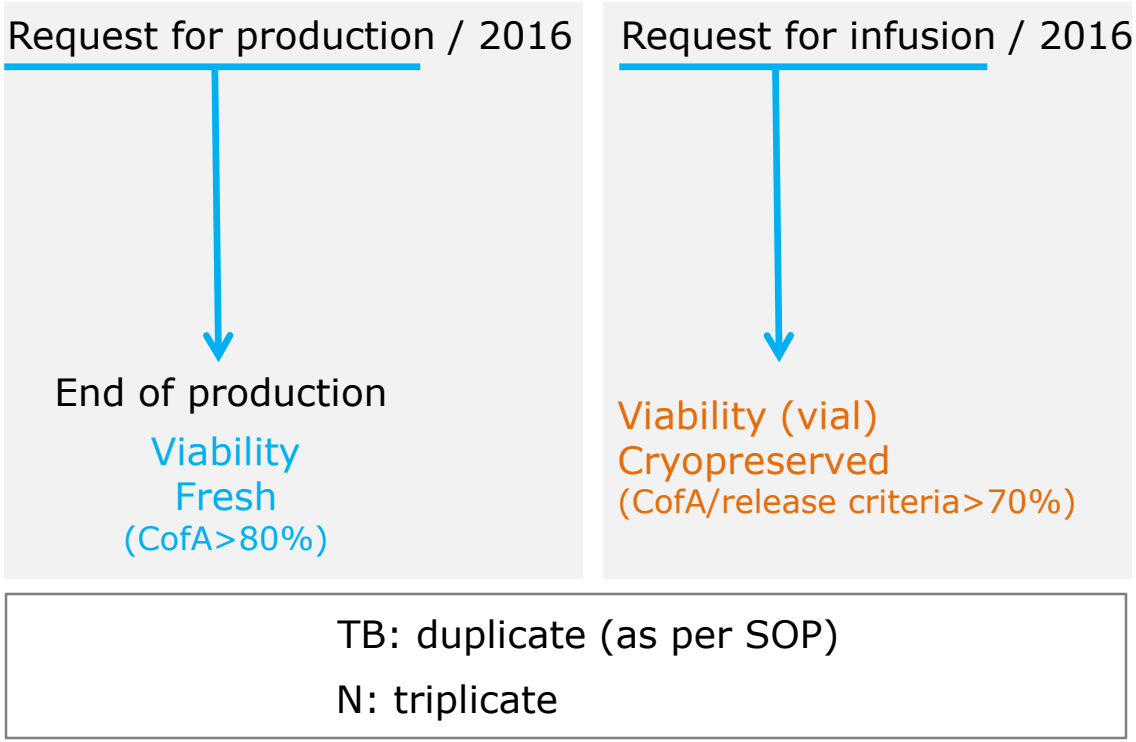
→ Cellometer Nexcelom (AOPI)?

Microscope cell counting (Trypan blue)

- •Subjective -> not subjective
- •Great inter-operator variability -> minimal inter run variability
- •Fully manual -> automated
- •Time consuming -> rapid

Health & safety: TB v PI

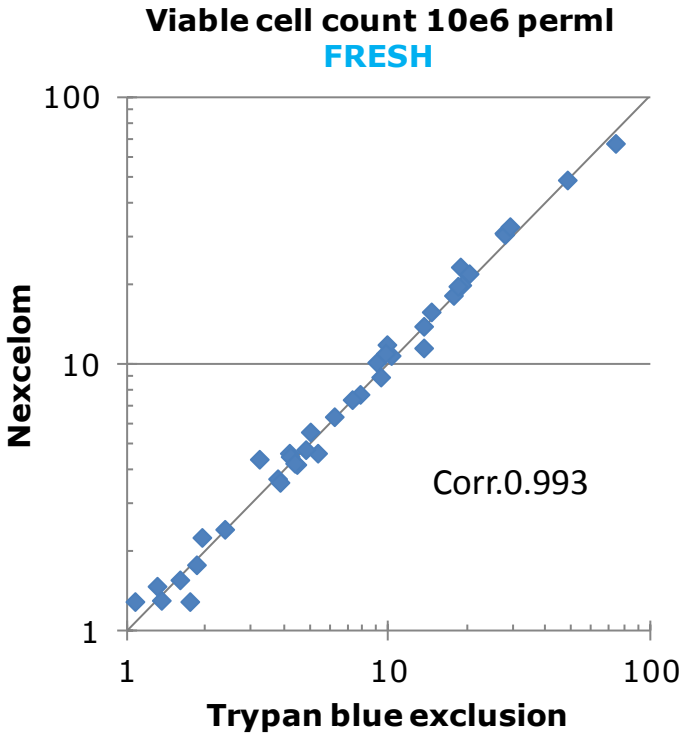
Microscope cell counting (Trypan blue) v Nexcelom (AOPI):study design



- *Data for 39 CAR products / fresh*
- *Data for 23 CAR products / cryopreserved*
- *Data paired for 23 products*

Data for 39 fresh CAR T cells products

Comparison of viable cell counts with two methods



	TB	N
median	7,250,000	7,386,667
average	12,007,179	12,481,111
min	1,070,000	1,296,667
max	73,750,000	67,200,000

n	39
ttest	0.044
median of difference	2.9%

Absolute viable cell counts are comparable for 39 products assessed in parallel

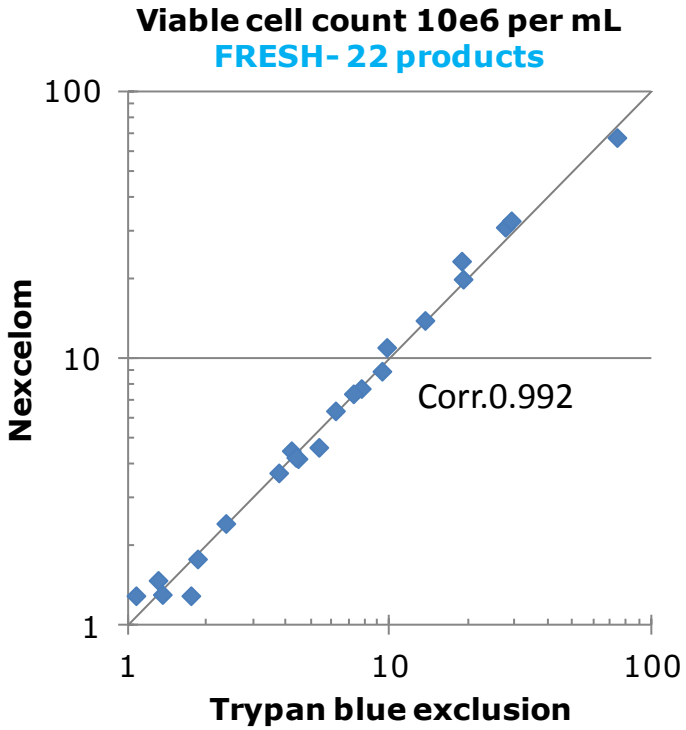
(the small difference of 2.9% is limit significant p=0.044)

Data for 39 fresh CAR T cells products
Comparison of viable cell counts with two methods
(continued)

Out of the 39 products tested, 17 were tested without respecting dilution instructions for the Nexcelom

Same comparison was repeated for 22 products tested with respect to dilution instructions

- *Again absolute viable cell counts are comparable for 22 products*
  The small difference of 2 % is not significant (p=0.256)

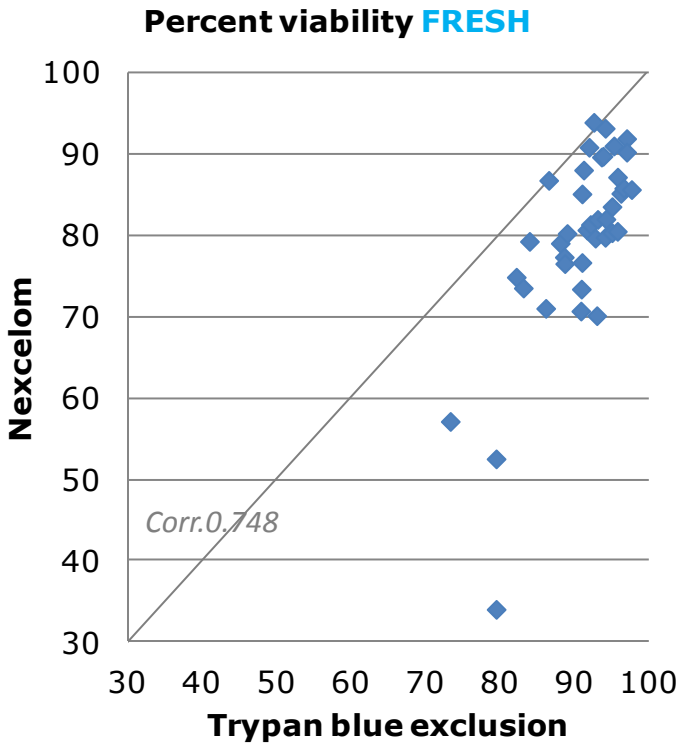


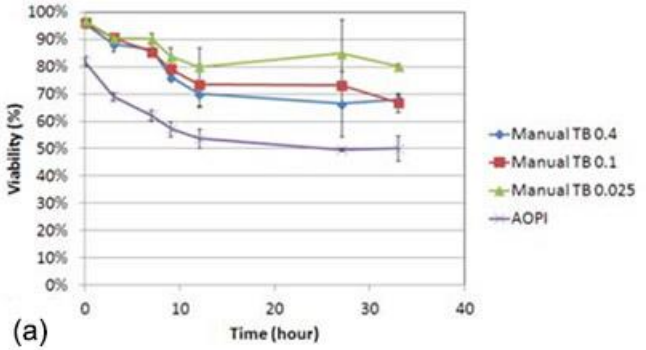
	TB	N
median	5,772,500	5,516,667
average	11,544,318	11,836,515
max	1,070,000	1,296,667
min	73,750,000	67,200,000
n		22
ttest		0.256
median of difference		2.0%

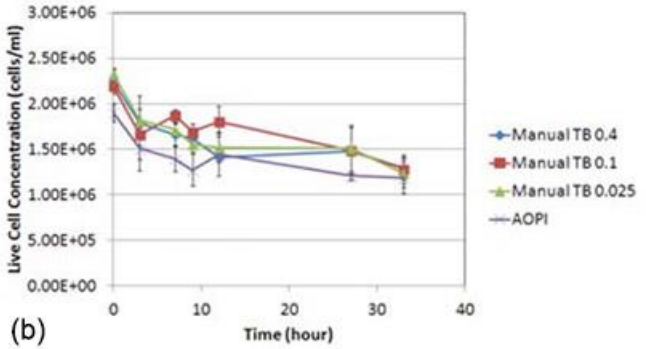
viable cell counts condition the formulation, therefore the two counting methods will allow the preparation of the same cell dose

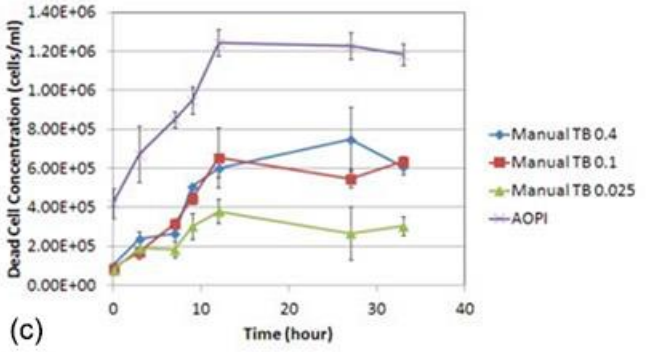
Data for 39 fresh CAR T cells products

Comparison of % viability with two methods









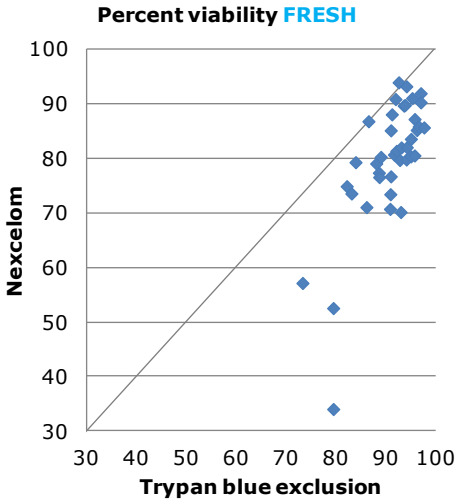
Percent viable cells are not comparable due to Nexcelom detecting more dead cells in fresh CAR T cells products

http://www.nexcelom.com/Applications/cell-viability-3-comparing-trypan-blue-and-aopi-staining-methods.php

Memorial Sloan Kettering Cancer Center

Data for 39 fresh CAR T cells products

Comparison of % viability with two methods (continued)



	TB	N
median	92.19	80.67
average	90.87	79.75
max	73.34	34.03
min	97.71	93.90

	fresh	frozen
release criteria	>80%	>70%
TB	92%	100%
N	92%	92%
concordance	100%	92%

ttest 2.09E-10

median of difference - 11.0%

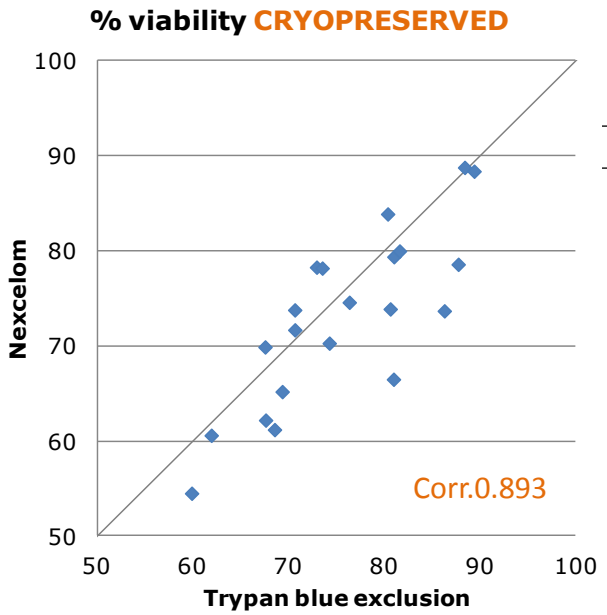
TB	NEXCELOM
73.34	57.13
79.50	52.53
79.51	34.03

→ *Three products did not pass the 80% criteria with both methods*

Despite the low correlation between % viability determined by the 2 methods,
The same three products will not pass the cut-off of 80%, thus 100% agreement

Data for 23 cryopreserved CAR T cells products

Comparison of % viability with two methods after thawing 23 cryopreserved products



	TB	N
median	73.48	73.70
average	73.57	70.29
min	45.00	38.20
max	89.35	88.80

ttest	0.006
median of difference	- 1.7%

release criteria	>70%
TB	65%
N	56%

concordance 96%

Thawed product		Fresh product	
TB	N	TB	N
45.00	38.20	79.50	52.53
58.37	44.75	73.34	57.13
59.84	54.50	88.68	77.33
61.89	60.60	91.71	80.67
67.51	69.90	86.58	86.77
67.57	62.20	90.90	70.70
68.50	61.20	91.05	76.67
69.30	65.20	90.97	73.40
70.61	73.80	94.28	82.00
70.62	71.70	93.15	81.97
72.89	78.30	92.64	93.90
73.48	78.20	93.89	89.73
74.22	70.30	88.14	79.03
76.31	74.60	92.00	90.83
80.32	83.90	94.18	93.17
80.59	73.90	89.05	80.23
80.93	66.50	93.04	70.13
80.97	79.40	95.34	91.00
81.56	80.00	93.67	89.63
86.24	73.70	95.81	80.50
87.69	78.60	91.27	88.03
88.36	88.80	97.06	90.23
89.35	88.40	95.84	87.17

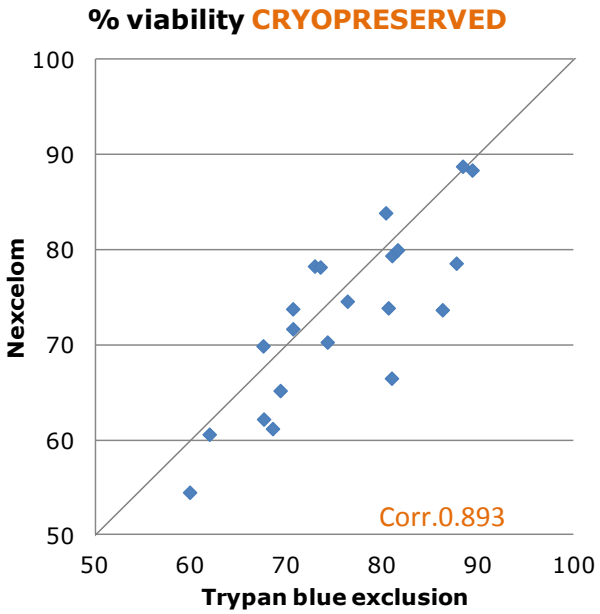
% viabilities are highly correlated between the two methods after thawing Products

The same 8 products will not pass the 70% release criteria

Only one product was below 70% with the Nexcelom but passed the release criteria for the trypan blue, bringing the concordance to 96%

Data for 23 cryopreserved CAR T cells products

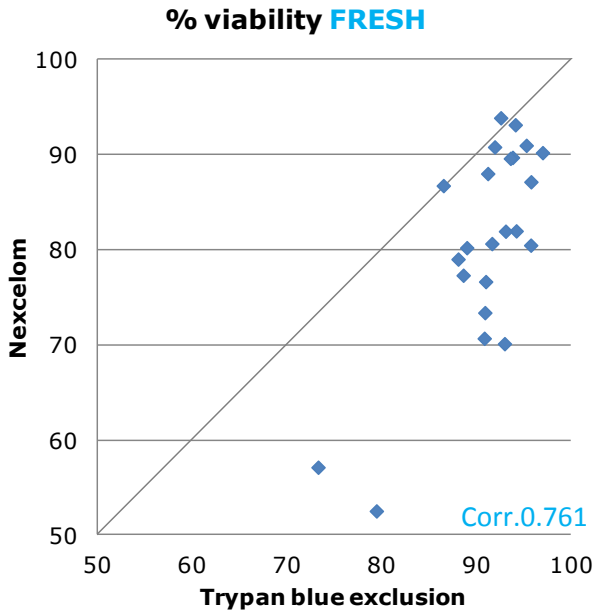
% viability with two methods correlate well on thawed products but not on fresh products



	TB	N
median	73.48	73.70
average	73.57	70.29
min	45.00	38.20
max	89.35	88.80

ttest 0.006

median of difference **- 1.7%**



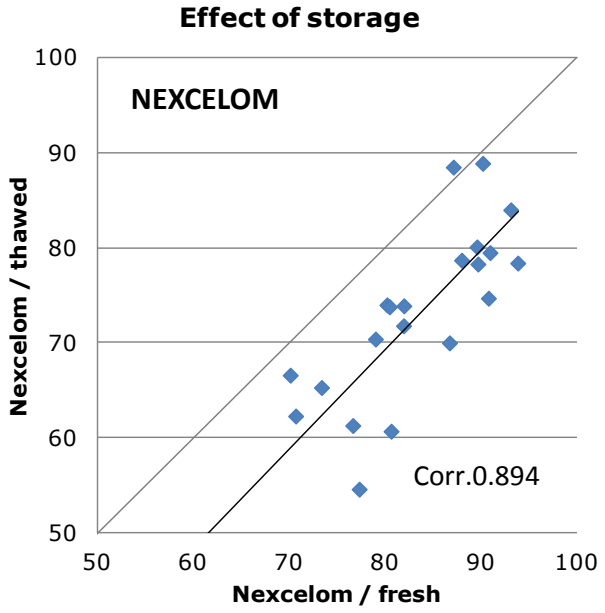
	TB	N
median	92.00	81.97
average	90.96	80.99
min	73.34	52.53
max	97.06	93.90

ttest 1.18E-06

median of difference - 10%

Fresh-cryopreserved data for 23 CAR T cells products

Comparison of viability before and after cryopreservation with the same assay



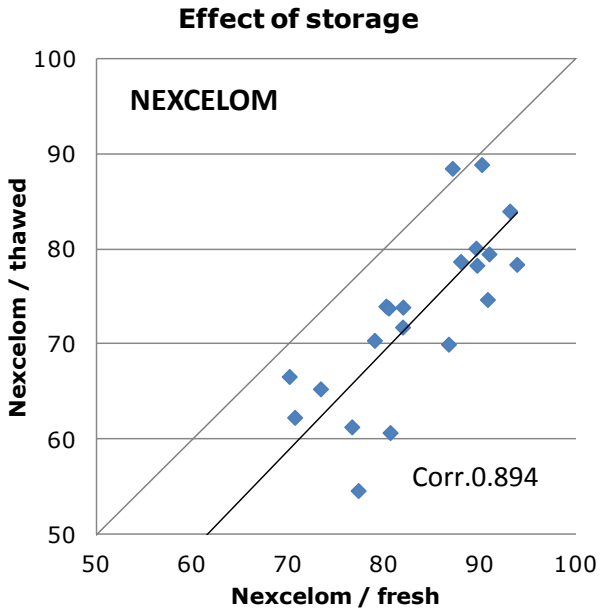
ttest 3.55E-09

median of difference -10%

Fresh	thawed
52.53	38.20
57.13	44.75
70.13	66.50
70.70	62.20
73.40	65.20
76.67	61.20
77.33	54.50
79.03	70.30
80.23	73.90
80.50	73.70
80.67	60.60
81.97	71.70
82.00	73.80
86.77	69.90
87.17	88.40
88.03	78.60
89.63	80.00
89.73	78.20
90.23	88.80
90.83	74.60
91.00	79.40
93.17	83.90
93.90	78.30

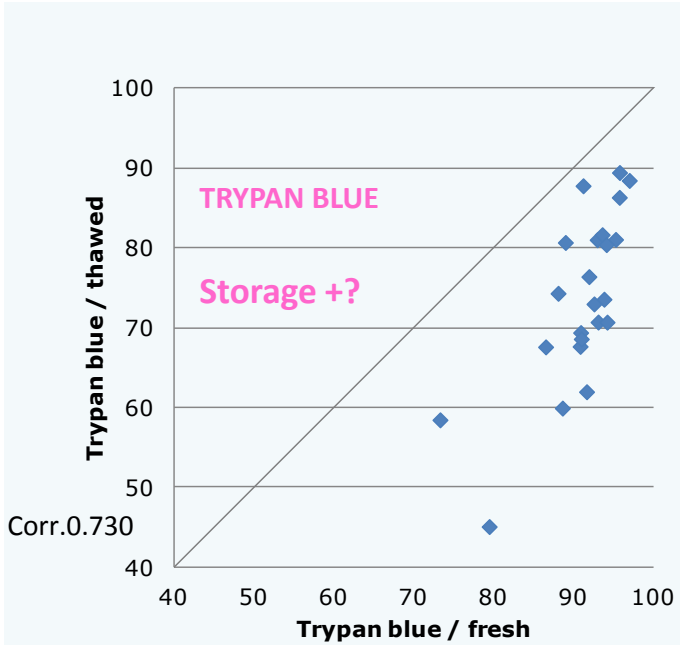
Upon thawing there is on average a 10% decrease of the viability when measured with Nexcelom

Fresh-cryopreserved data for 23 CAR T cells products



ttest 3.55E-09

median of difference -10%



ttest 2.13E-10

median of difference -17%

the viability measured on thawed products by trypan blue is not well correlated to the viability before thawing

decrease of viability after thawing seems higher with the Trypan blue (-17%) than with the Nexcelom (-10%)

! the viability of fresh products determined by Trypan blue is higher than with Nexcelom (~+10%) [slide 10]

Hypothesis:

PI more sensitive than TB to detect dead cells ?

PI is less specific than TB?

1 F/T cycle increases correlation between the 2 assays: the discrepant signal wiped out?

Preliminary Conclusions

Trypan blue to Nexcelom / formulation and release of cryopreserved CAR T cells

Viable cell count is not affected -> formulation of the same CAR T cell dose

% viability fresh significantly different with nexcelom detecting more dead cells (-11%, $p<0.001$)
But 100% agreement for a provisional release criteria of 80% on fresh products

% viability cryopreserved slightly different between the two methods
(-1.7%, $p=0.006$)
With excellent agreement of 96% for a release criteria of 70% (only 1 discrepant)

..to confirm with more products

…to gain confidence and understanding of discrepant data observed on fresh products: AOPI detected more dead cells than TB in T cells expanded in vitro