

Choosing Reagents for T Cell Therapy

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Production Assistance for Cellular Therapies
National Heart Lung and Blood Program




Methodist The Methodist Hospital
Research Institute

Medium

- CTL Medium 1981 to ~2003
 - RPMI 1640
 - 10% FBS
 - L-glutamine
 - IL-2 from day 14
- DC medium
 - CellGenix (GMP grade)
 - IL-4, GM-CSF, TNF- α , IL-1 β , PGE1 and IL-6

Serum or Serum-Free?



- Ideally serum-free
 - So far not possible for T cell expansion
- FBS, Human AB or autologous serum
- FBS
 - Well characterized, designated herds
 - Many viruses are species-specific
 - Can reactivate “FBS”-specific T cells
- Human AB
 - Less antigenicity
 - No longer considered superior to FBS
 - More likely to have compatible microorganisms
- Autologous serum
 - Cannot standardize
 - May contain inhibitory factors (from tumors)
 - May contain drugs

Screening of Donors for Human Serum

- No more than 30 days before collection
 - Hepatitis B Surface Antigen
 - Anti-Hepatitis B Core Antigen
 - Anti-Hepatitis C
 - Anti HTLV I/II
 - Anti HIV 1 & 2
 - Syphilis
 - West Nile
 - Chagas (Trypanosoma cruzi)
 - Parvovirus B19

Testing Lots –RPMI and Serum

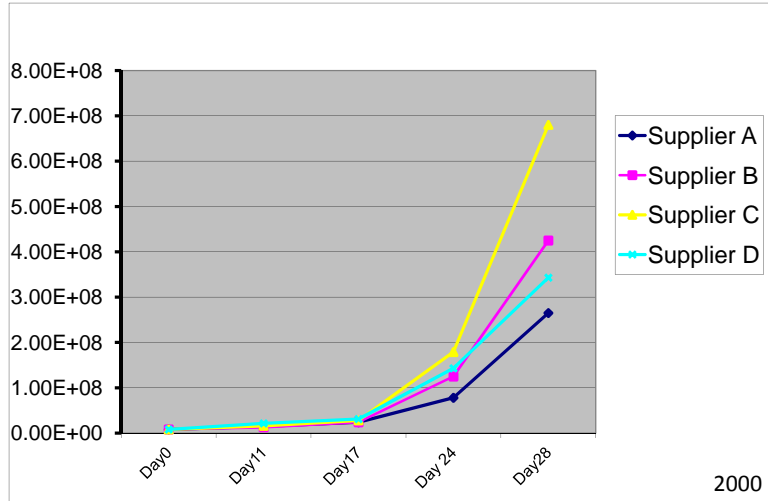
- Test lots from different suppliers
 - Reputable supplier
- Ensure they have a large stock of lots to be tested
- Compare with known “good” lot.
- Test using cell type to be grown in it over several weeks
 - Expansion, phenotype and function

So You Think RPMI is RPMI?

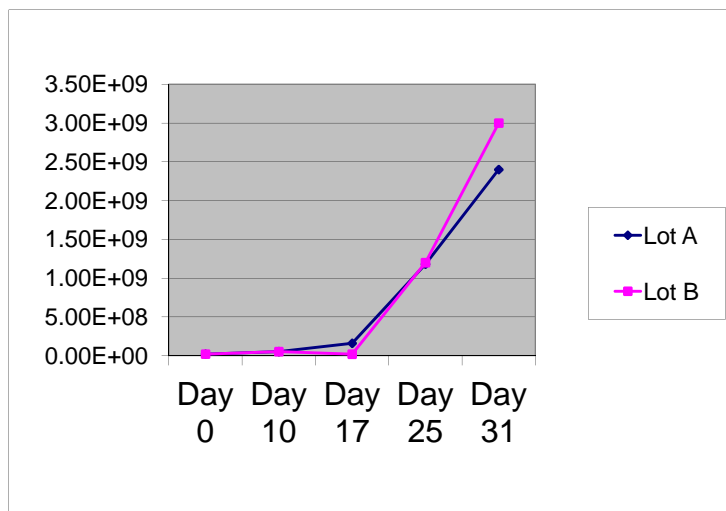


- Same formulation
- Staggeringly different effects on T cells
- Failure to thrive in one lot from one supplier

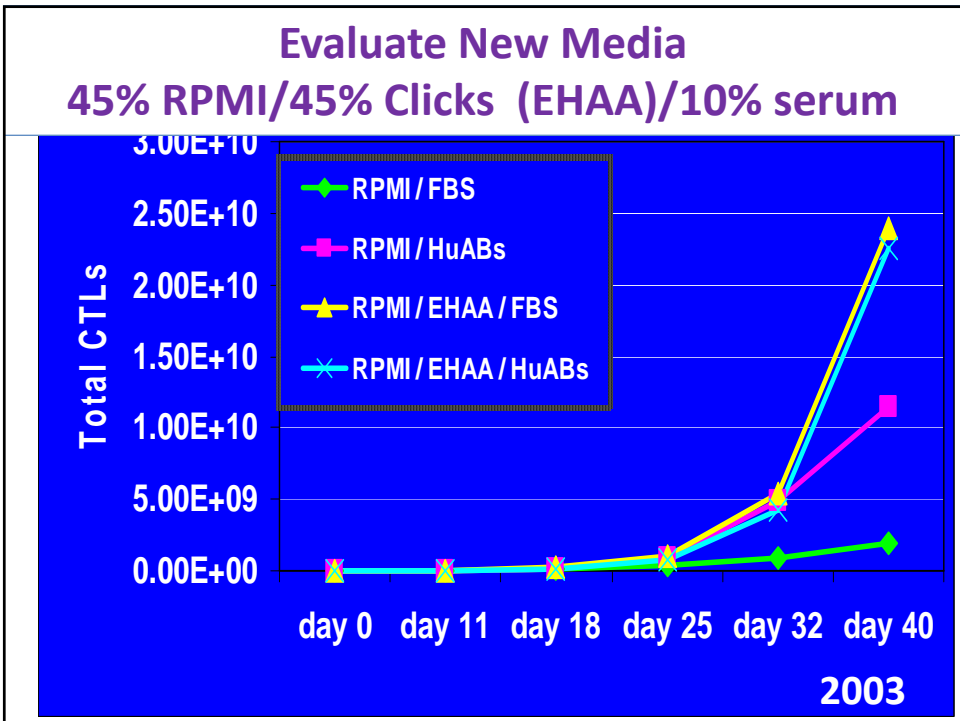
Rates of CTL Expansion with RPMI from Different Suppliers



Little Lot to Lot Variation Within a Good Supplier



Cells Can Always Grow Better



Importance of Zinc in TC

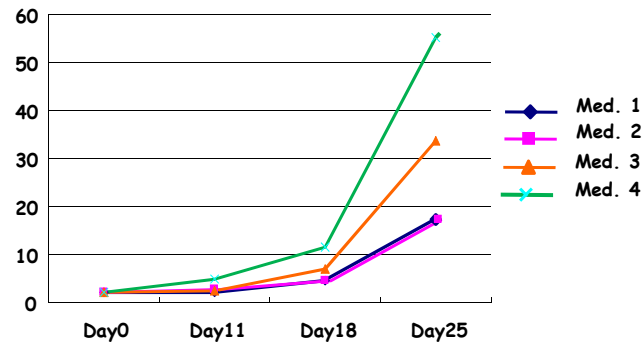
- Essential part of > 300 enzymes
 - Cellular signaling, cell growth, transcription, apoptosis, antioxidant
 - TH1 responses
 - Prevents Oxidative stress

- Zinc in TC medium
 - Not present in RPMI 1540 or Clicks
 - 0.874 mg/L in Adv RPMI
 - 0.89 mg/L human serum (0.73 μ M)
 - 0.089 mg/L in medium (10%) (0.073 μ M)

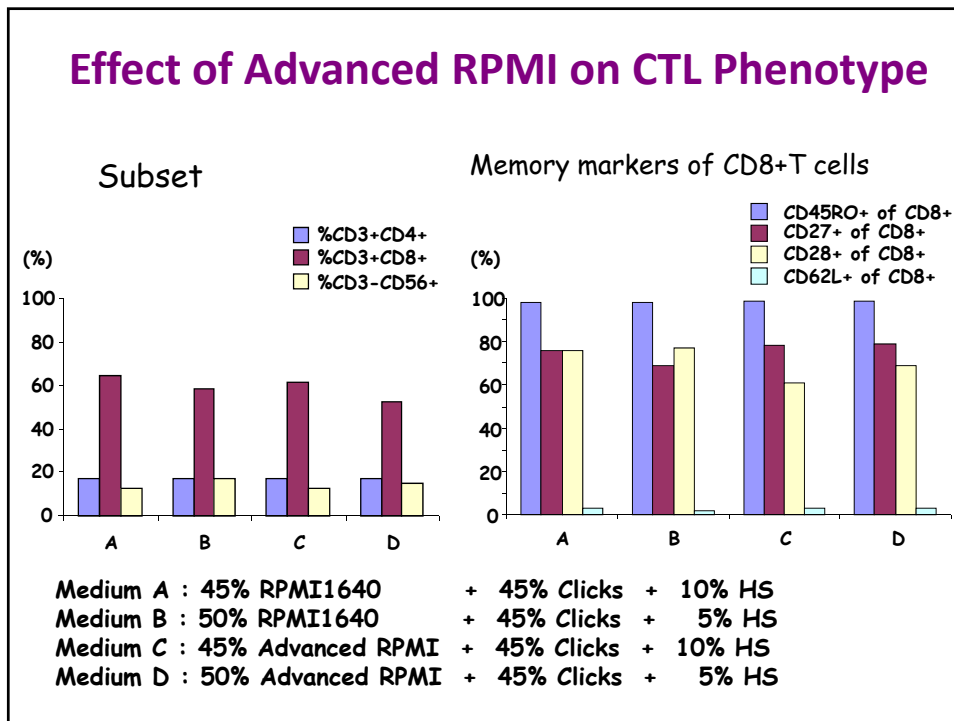
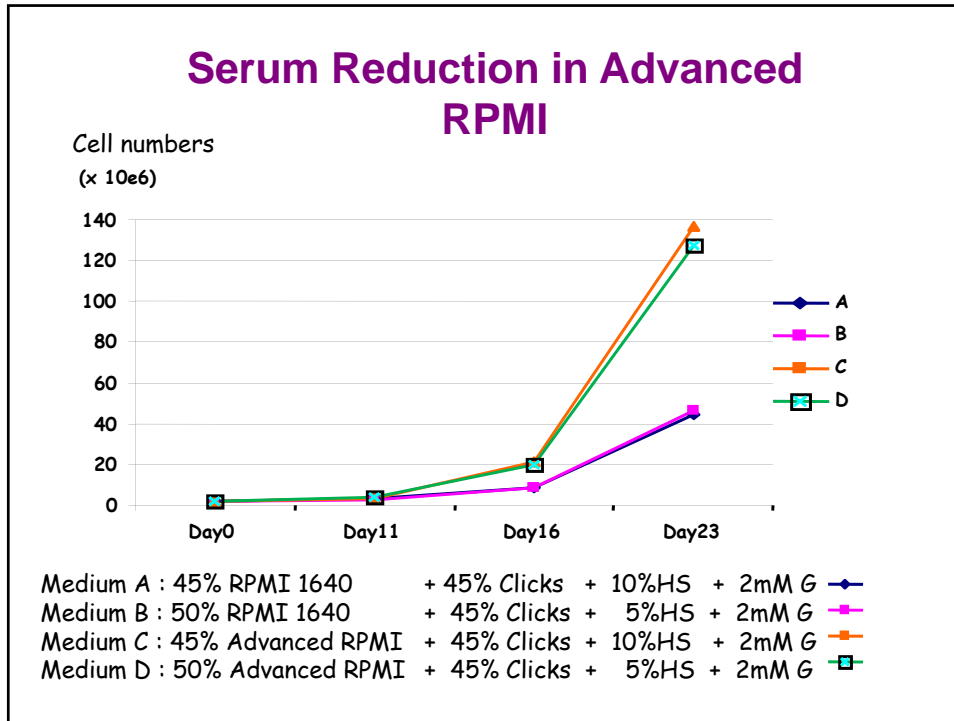
Effect of Advanced RPMI on CTL Expansion

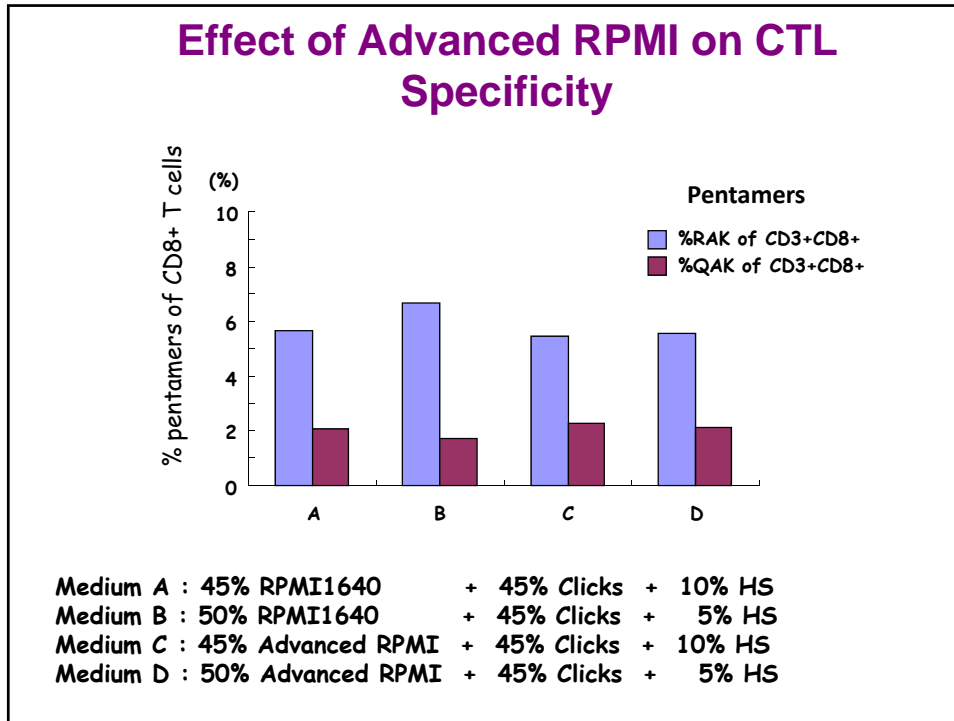
Cell numbers
($\times 10^6$)

2008



Medium 1 : 90% RPMI1640 + 10%HS + 2mM G
Medium 2 : 45% RPMI1640 + 45% Clicks + 10%HS + 2mM G
Medium 3 : 90% Advanced RPMI + 10%HS + 2mM G
Medium 4 : 45% Advanced RPMI + 45% Clicks + 10%HS + 2mM G





Component	RPMI 1640	Advanced RPMI	Click's
Amino Acids	mg/L	mg/L	mg/L
Glycine	10	10	30
L-Alanine	0	8.9	35
L-Arginine	200	200	317
L-Asparagine	50	50	60
L-Aspartic acid	20	20	53
L-Cystine 2HCl	65	65	78
L-Glutamic Acid	20	20	59
L-Histidine	15	15	105
L-Hydroxyproline	20	20	0
L-Isoleucine	50	50	130
L-Leucine	50	50	130
L-Lysine hydrochloride	40	40	181
L-Methionine	15	15	37
L-Phenylalanine	15	15	80
L-Proline	20	20	46
L-Serine	30	30	42
L-Threonine	20	20	120
L-Tryptophan	5	5	25
L-Tyrosine disodium salt	29	29	119
L-Valine	20	20	115

Vitamins	RPMI	Adv RPMI	Click's
Ascorbic Acid phosphate	0	2.5	0
Biotin	0.2	0.2	0
Choline chloride	3	3	2
D-Calcium pantothenate	.25	.25	0
Folic Acid	1	1	2
i-Inositol	35	35	0
Niacinamide	1	1	2
Para-Aminobenzoic Acid	1	1	0
Pyridoxine hydrochloride	1	1	2
Riboflavin	0.2	0.2	0.2
Thiamine hydrochloride	1	1	2
Vitamin B12	0.005	0.005	0

Inorganic Salts	RPMI	Adv RPMI	Click's
Calcium nitrate	100	100	0
Magnesium Sulfate	48.84	48.84	0
Potassium Chloride	400	400	400
Sodium Bicarbonate	2000	2000	1350
Sodium Chloride	6000	6000	8000
Sodium Phosphate dibasic anhydrous	800	800	479
Zinc Sulfate	0	0.874	0

Proteins	RPMI	Adv RPMI	Click's
AlbuMAX II	0	400	0
Human Transferrin (Holo)	0	7.5	0
Insulin Recombinant full Chain	0	10	0
Reducing Agents			
Glutathione, monosodium	1	1	0
Sodium Pyruvate	0	110	275
Trace Elements			
Ammonium Metavanadate	0	0.0003	0
Cupric Sulfate	0	0.00125	0
Manganous Chloride	0	5	0
Sodium Selenite	0	0.005	0
Other Components			
D-Glucose (Dextrose)	2000	2000	1000
Ethanolamine	0	1.9	0
Glutathione (reduced)	1	1	0
Phenol Red	5	5	10

Cost of Improved Media

Component	Cost per Liter
Human AB serum	\$850
FBS	\$360
RPMI	\$34
Advanced RPMI	\$320
Click's	\$64
1L RPMI/Clicks with 10% HABs	~ \$134
1L Adv RPMI/Clicks with 5% HABs	~ \$254

Choice

- GMP compliance
 - Phase I/II <<< Phase III
- Efficacy
 - Cell growth and potency
- Cost

Sourcing Cytokines

- From the pharmacy
 - IL-2 (Proleukin, Chiron)
 - GM-CSF (Sargramostim, Bayer Healthcare)
 - PGE-1 (Cytotec[®], Pharmacia Ltd)
- From Cell Genix
 - GMP grade; IL-6 and IL-15
 - FDA drug master file cross reference available
- From R & D Systems
 - Animal free facility
 - Made in E.Coli; IL-1 β , IL-4, IL-7, TNF- α
 - Two virus exclusions steps
- From the NIH
 - Clinical grade IL-12
- Miltenyi also make clinical grade cytokines

Testing Cytokines

- If changing sources, test against known good lot
- Check storage conditions
 - Lyophilized
 - After reconstitution
 - Make many small aliquots that can be discarded after use

Peptide Sourcing (For T cell Stimulation)

- Miltenyi
- JPT Technologies
 - HPLC purified > 90%*
 - Hydrophobic peptides >70%
 - Impurities are mostly shorter versions
 - Each present at lower concentration

Questions Regarding from FDA

- Include data regarding residual class II or III solvents in your peptide C of A.
- Refer to the International Conference on Harmonization document "Q3C (R4): Impurities: Guideline for Residual Solvents."
 - www.ich.org.
- Contaminants after lyophilization
 - DMSO (A class III solvent, used clinically therefore not a problem at volumes used)
 - Acetonitrile

Acetonitrile

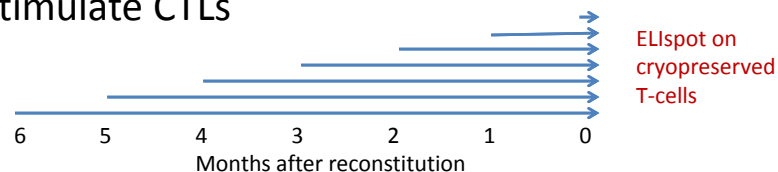
- Class 2 solvent,
- Permitted daily exposure is 4.1 mg per day
- Lyophilized peptides contain ~ 30ppm of acetonitrile
- At each *ex vivo* stimulation, CTLs receive max 18.5 ug combined peptides containing a max of 0.555 ng of acetonitrile.
- After 3 stimulations, CTL are exposed to less than 2.7 ng acetonitrile.
- 4 washes after the last stimulation. Each removes ~ 95% reducing dose by >5 logs.
- Hence patients will be exposed to almost 10 logs less acetonitrile per CTL dose than the daily PDE.

Peptide Storage

- Order in small lyophilized lots
- Reconstitute in DMSO
 - Half life ~3 months at -80°C
 - Stability testing on going
 - Cysteine-containing peptides (dimerize)

Stability testing

- Select cysteine-containing peptides
 - Known epitopes from common viral proteins
- Order multiple lyophilized vials
- Identify 2 donors that recognize epitopes
 - Generate CTL lines by stimulation with peptides
 - Cryopreserve multiple aliquots of CTLs
- Reconstitute 1 vial per month and use to stimulate CTLs



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