New Methods of Expanding T Cells

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Autologous T Cell and Gene Therapies

Service → Product

New tools for T cell expansion

Early clinical trials with autologous T cells:
- focus on safety, efficacy
- complex processes, unreliable methods, exotic reagents

- **Walter et al, Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor, 1995, N Engl J Med 333:1038-1044**
- **Heslop et al, Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes, 1996, Nat Med 2:551-5**
- **Dudley et al, Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes, 2002, Science 298:850-4**
- **Morgan et al, Cancer regression in patients after transfer of genetically engineered lymphocytes, 2006, Science 314: 126-9**

TIL Expansion Strategies

Protocol	TIL Initiation	Rapid Expansion
Selected TIL	Fragments→ Select for tumor reactivity	Rapid expansion T175→Bags
Young TIL	Digest→ No selection for tumor reactivity, CD8+ enrichment	Rapid expansion T175→Bags T175→WAVE GRex flasks

Method

- Initiate multiple independent TIL cultures
- Expand microcultures in 24 well plates
- Screen for tumor recognition (IFNg release)
- Rapid Expansion Protocol
  - Anti-CD3 (OKT3), IL-2, irradiated PBMC feeders
  - 48x T175 Flasks, 50L process volume

Coculture and IFNg ELISA (pg/ml IFNg)
1200 TBI patient; Complete Responder

		Melanoma Cell Line				
		A2-		A2+		Auto TC
	None	888	938	526	624	2831-2
		A1,24	A1,24	A2,3	A2,3	A2
Controls						
None	0	10	0	0	0	0
AK1700-3	266	57	84	53	47	97
JKF6 (A2/MART)	47	16	20	**>21120**	**>23110**	**>2794**
L2D8 (A2/g209)	1	5	6	**>24320**	**>20870**	**>2529**
FRAGMENTS						
F6 d28	27	78	66	**762**	**2330**	**3520**
F8 d28	130	49	26	**667**	**452**	**843**
DIGESTS						
W1 d27	17	19	14	**206**	119	**3450**
W2 d27	25	107	76	6	7	155
W3 d27	0	139	78	146	47	**1115**
W4 d27	5	7	29	76	14	**879**

Complex assay with multiple effector and target controls
Assay demonstrated variation between microculture activities
Recognition of shared and “autologous only” antigens

Coculture and IFNg ELISA (pg/ml IFNg)
1200 TBI patient; Non-responder

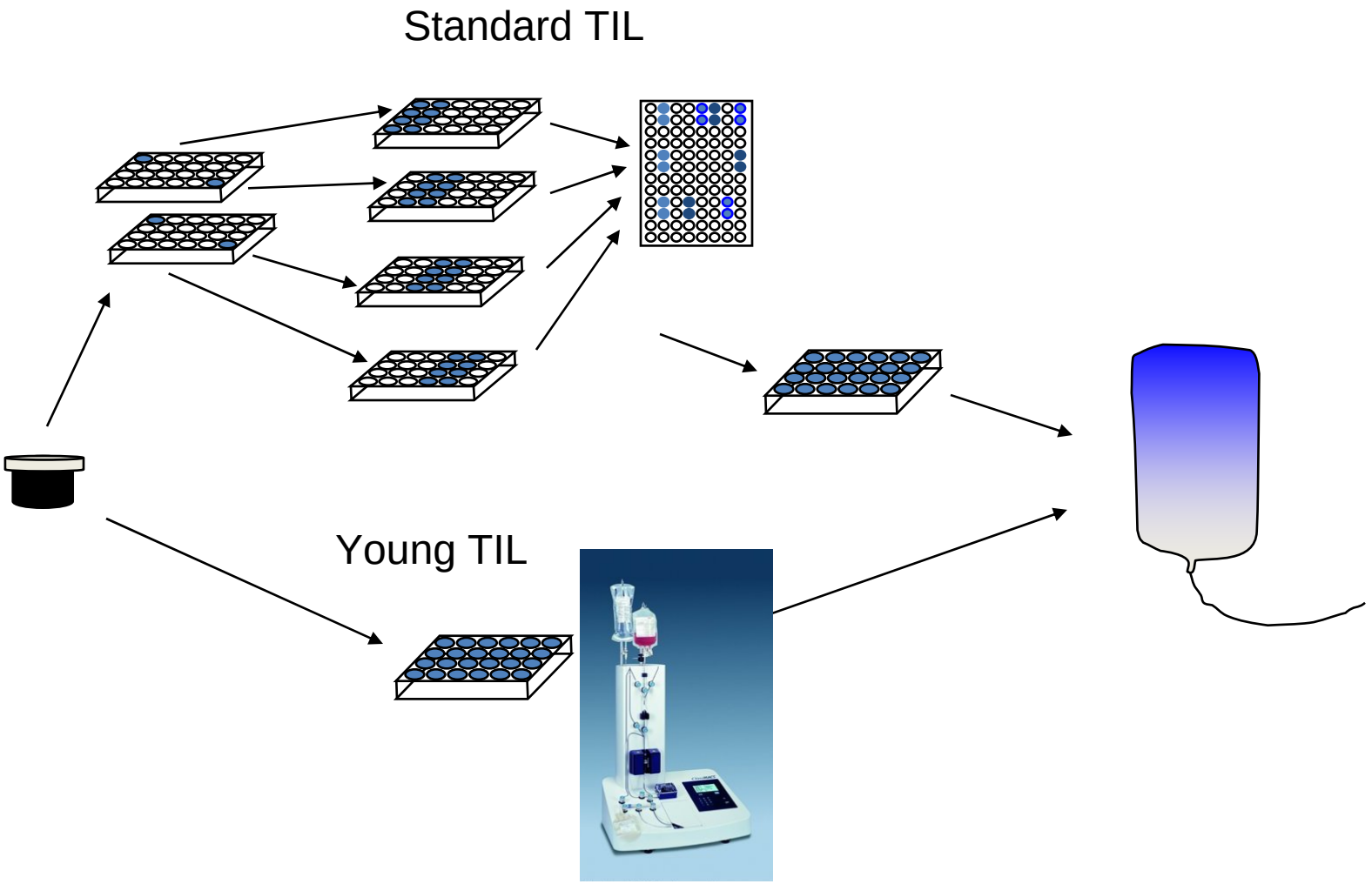
		Melanoma Cell Line				FrTu		
		A2-		A2+		Auto	control1	control2
	None	888	938	526	624	2744	2540	2748
		A1,24	A1,24	A2,3	A2,3	A0201,25	A24	A33,66
Controls								
None	3	0	1	7	11	13	40	33
AK1700-3	105	43	57	57	52	261	103	182
JKF6	18	20	33	**>20690**	**>27790**	**>1858**	36	69
JR6C12	81	13	11	**>15250**	**>18080**	**>1999**	29	54
2744								
W1 d19	16	16	27	**479**	**668**	**1131**	54	74
W2 d19	16	16	1193	454	946	**1910**	47	145
W3 d19	33	24	76	**881**	**2930**	**3120**	33	186
W4 d19	15	11	47	**648**	**1057**	**4380**	27	200
F1 d19	59	103	57	**9250**	**19150**	**2330**	40	101
F2 d19	88	71	62	**416**	**650**	**945**	31	96
F3 d19	47	64	79	**8590**	**14340**	**2730**	62	128
F4 d19	61	96	74	**5590**	**9010**	**2730**	69	161
F5 d19	100	83	42	74	138	**1039**	81	174
F6 d19	69	79	133	**3170**	**3440**	**2080**	108	117
F7 d19	121	100	574	651	951	**1006**	61	66
F8 d19	106	50	121	**3660**	**3770**	**4600**	43	121

Variation between microculture activities
Recognition of shared and “auto only” antigens
Tumor recognition does not predict response

Method

- Enzymatic digestion or mechanical disaggregation to single cell suspension
- Initiate cultures in 24 well plates
- Develop bulk TIL with minimum time in culture, don't screen (CD8+ enrich)
- REP:
  - Flasks → Bags
  - Flasks → WAVE
  - GRex

CD8+ enriched Young TIL Method

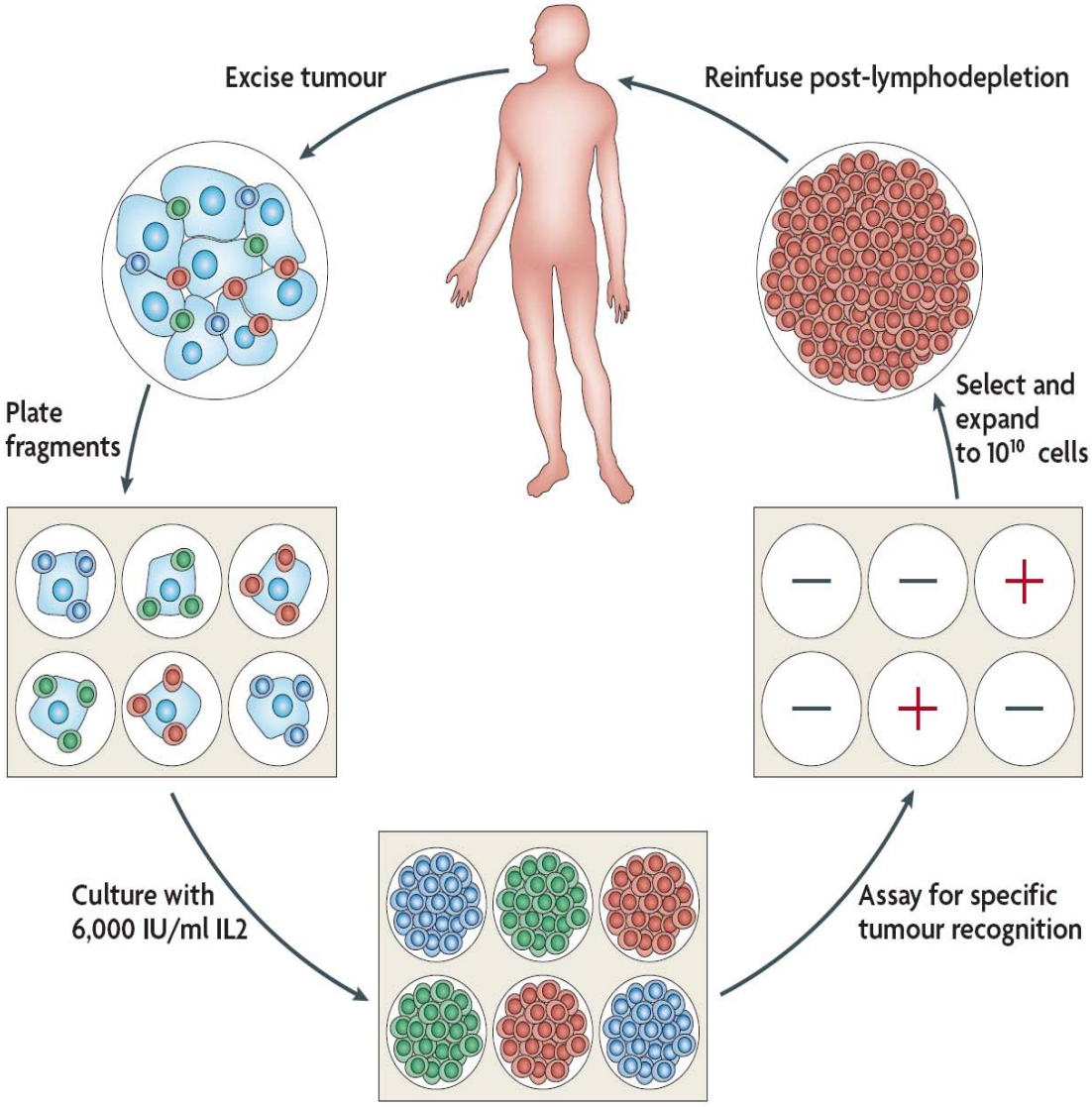


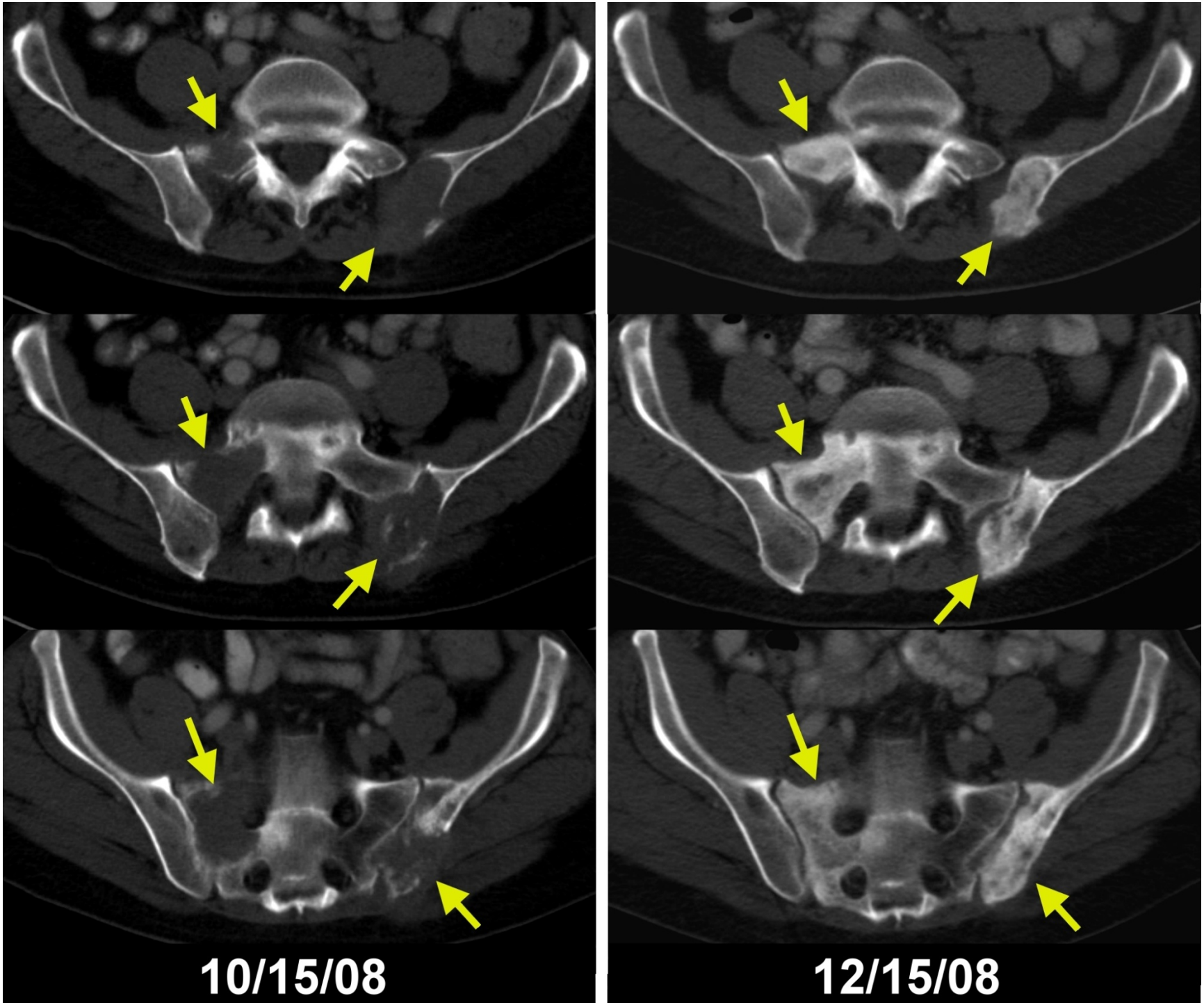
Eliminating tumor selection improves manufacturing reliability and patient accrual

	Selected TIL	Young TIL
Accrual Period	7/2002-7/2007	9/2008-4/2011
Months of accrual	60	30
Accessions to lab	402	272
Excluded (NED, Alt. Prot, etc)	-50	-60
"Eligible" Patients	352	212
No Growth or not active	183 (52%)	51 (24%)
Progressed/clinically excluded	62 (18%)	39 (18%)
Treated	107 (30%)	122 (57%)

Goff SL, et al 2010, J Immunother. 33:840-7
Dudley ME, et al 2010, Clin. Cancer Res. 16:6122-31

Adoptive Cell Therapy for patients with cancer




10/15/08
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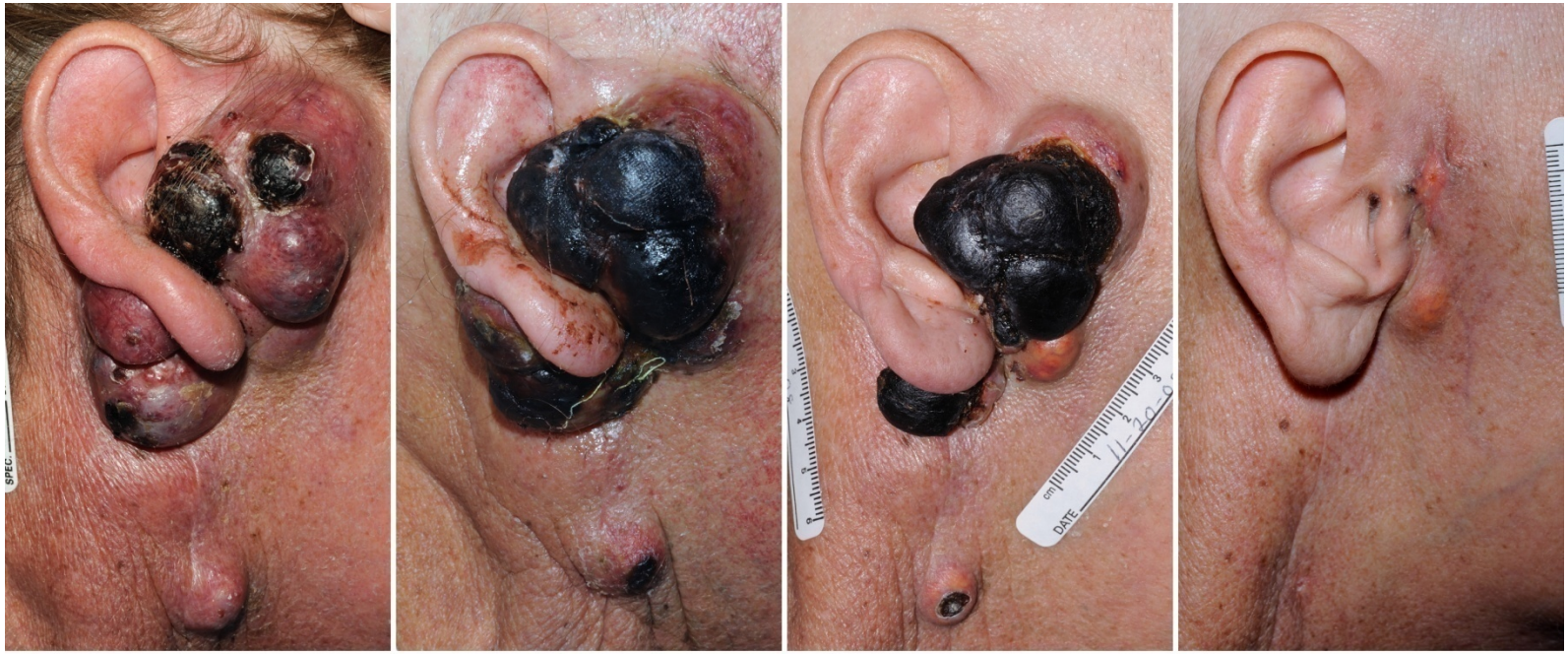
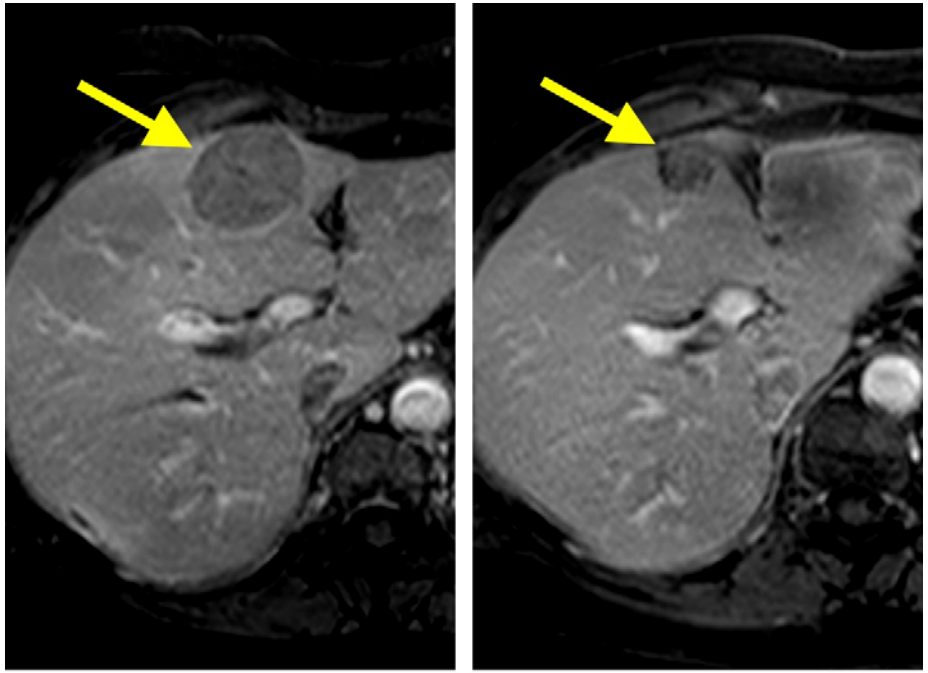


Day -9 Day +11 Day +27 Day +76



Pre-Treatment 3 Months

Treatments using selected TIL vs young TIL

	Selected TIL	Young TIL
Tumor reactivity	100%	-
Objective response (RECIST)	56% (20% CR)	36% (7% CR)
Accrual	30%	57%

Selected TIL: Logistically challenging, lower accrual, exclusion of potential responders, high response rate

Young TIL: Reliable, quantifiable, high accrual, extensive clinical effort, lower response rate

Autologous T Cell and Gene Therapies

Service → Product

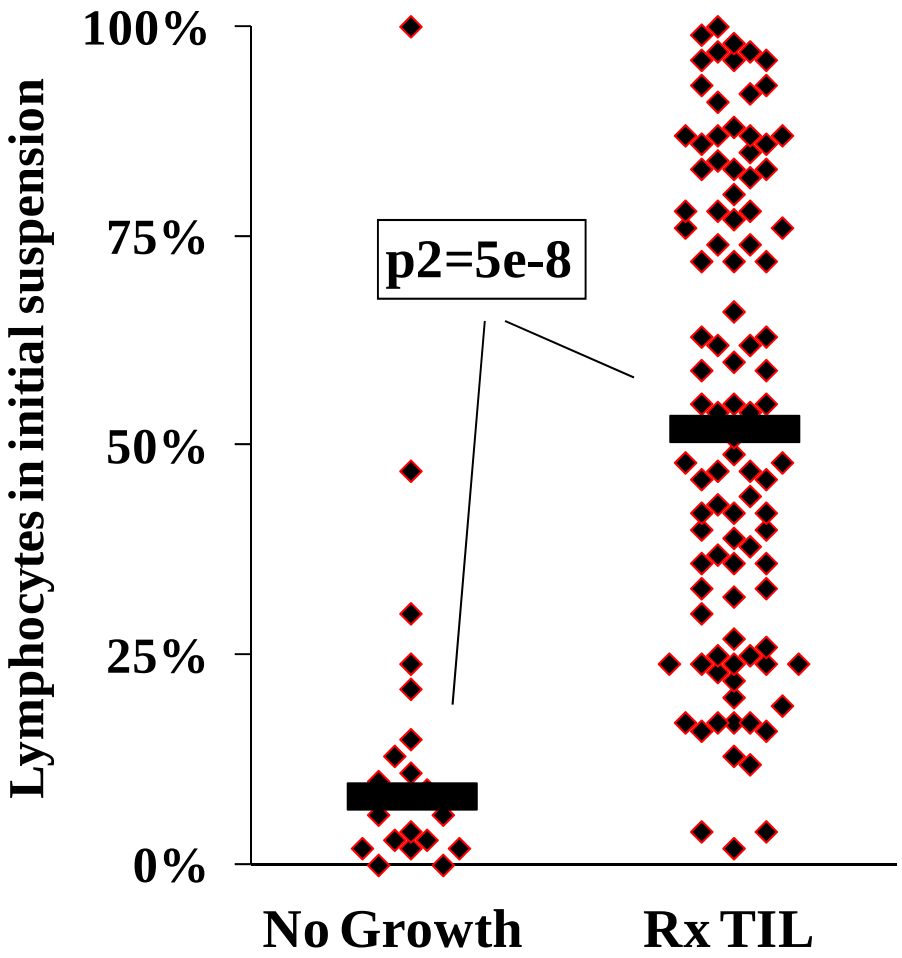
- Two TIL manufacturing strategies emphasize the trade-off between quality and quantity

New tools for T cell expansion

New Tools for T cell expansion

- Biologicals (ECCE)
- Bioreactors

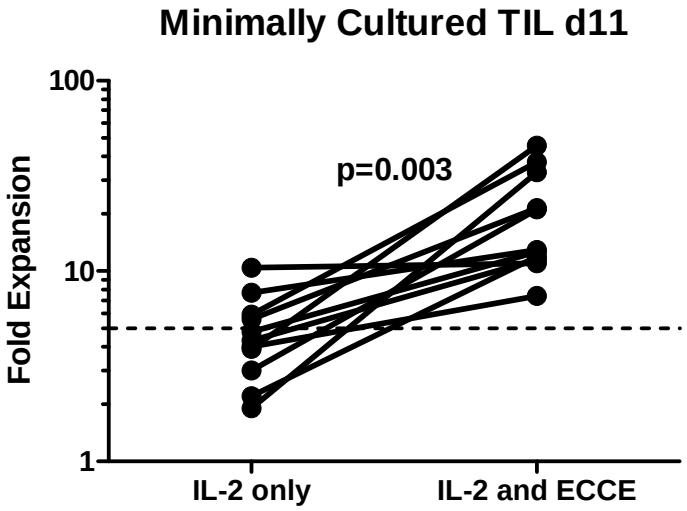
TIL cultures don’t grow from all melanoma suspension digests

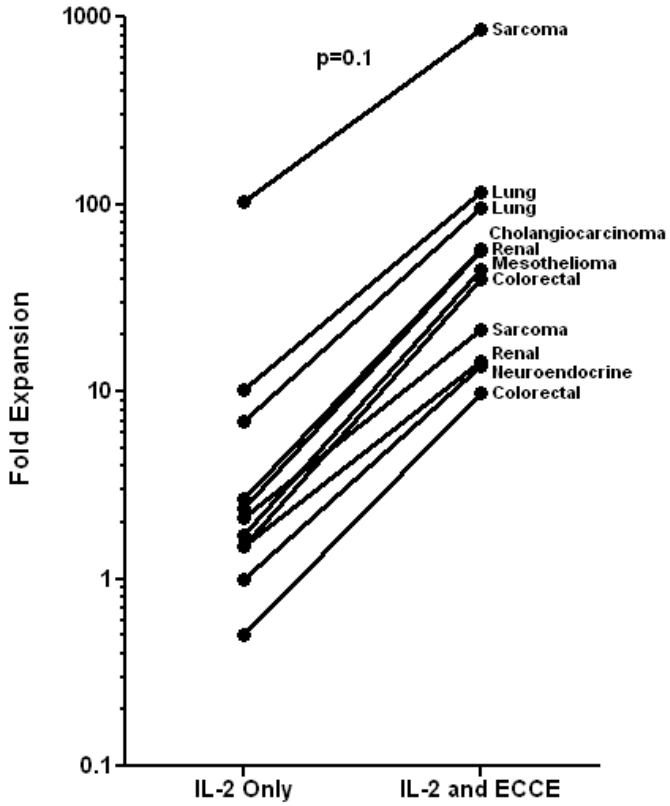


Engineered Cells for Costimulation Enhancement (ECCE)

- T cells require activation for growth
  - TCR stimulation
  - Gamma-chain cytokine
  - Costimulation
- 4-1BBL provides potent costimulation to human CD8+ T cells
  - Function “in trans” of TCR signal
- K562 was transfected to express 4-1BBL
  - A MCB was made under GMP and aliquots prepared for clinical use

ECCE expand human CD8+ TIL in vitro



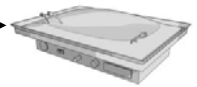
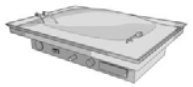
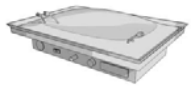
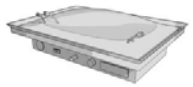


Friedman KM, et al 2011, J Immunother 34:651-61

Biological Tools for T cell expansion

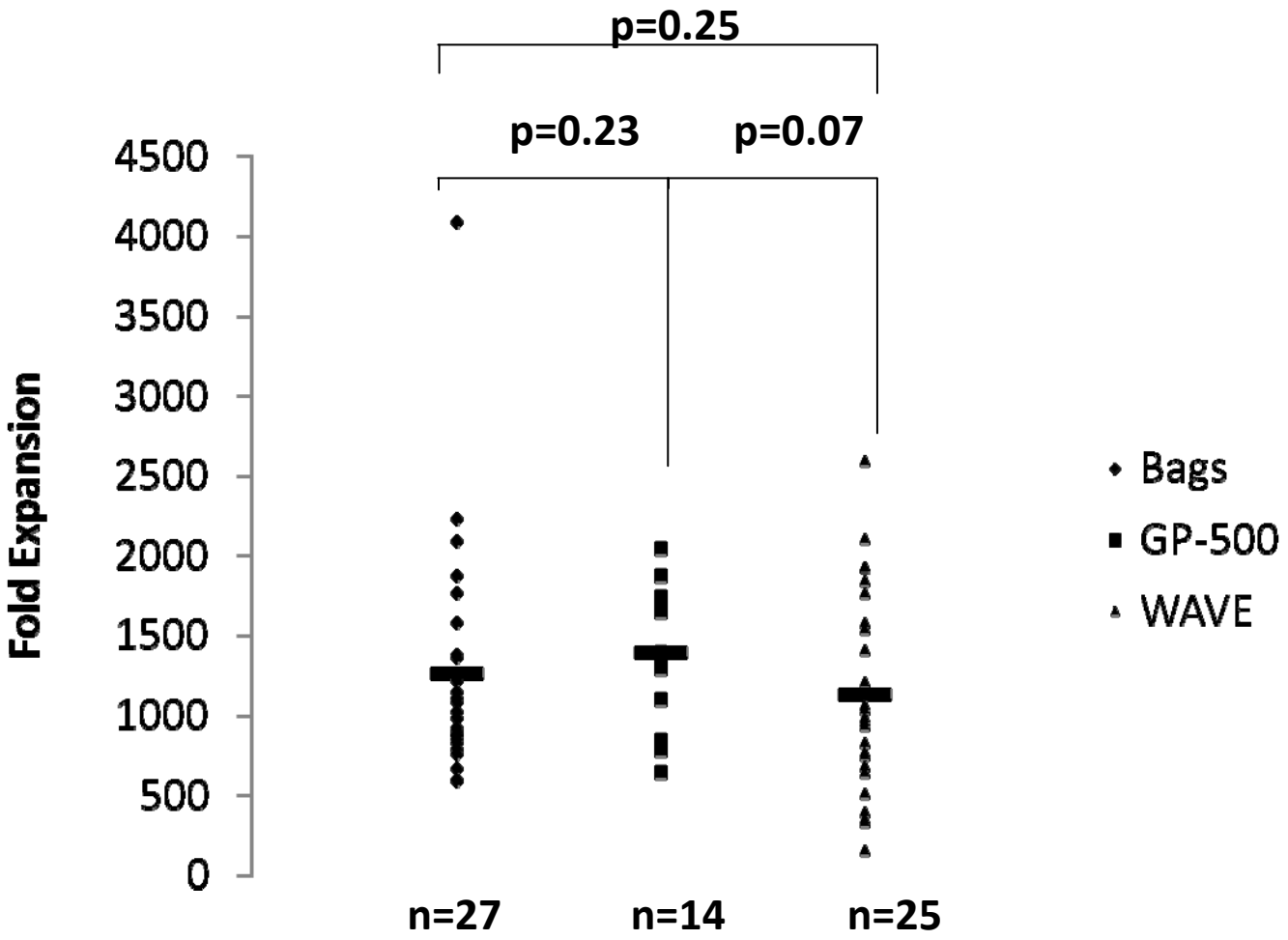
- ECCE
  - Artificial Antigen Presenting Cells
- Soluble costimulatory molecules/blockade
  - CD28, anti-PD-1
- Cytokines
  - IL-7, IL-12, IL-15, IL-21
- Lymphocyte subset selection
- Genetic enhancement

Bioreactor Process Development

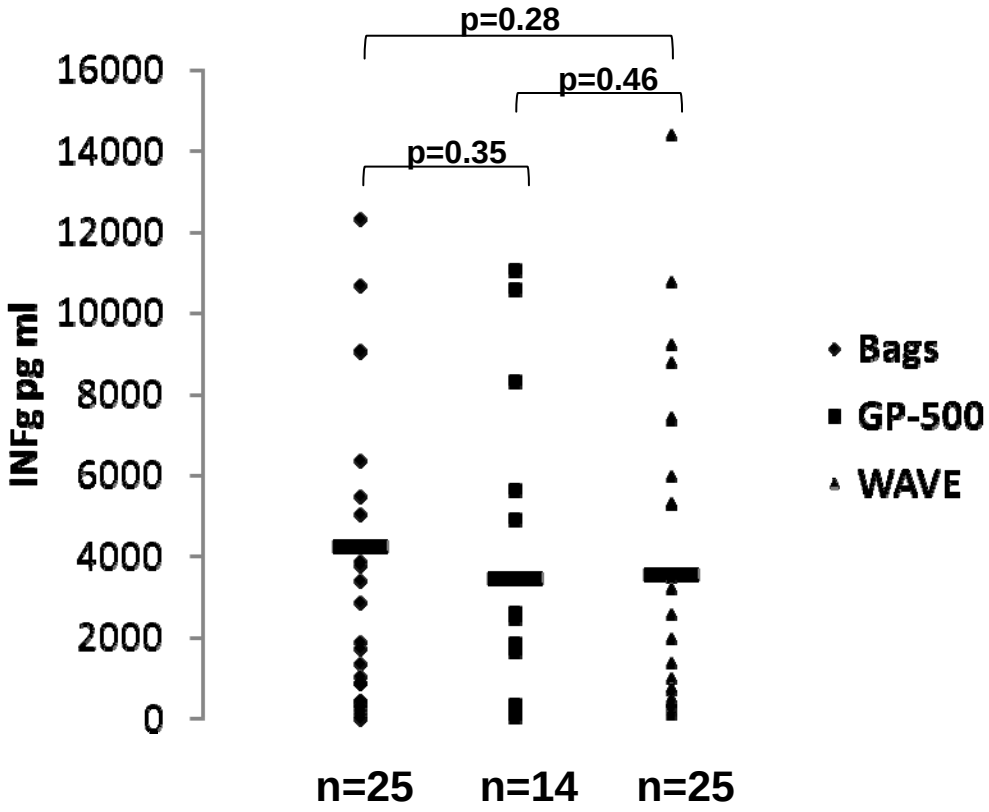
Day 0	Day 7	n	Fold Expansion	FACS	Coculture Data
		>100	>100	>100	>100
	 	5	5	5	5
		25	25	8	25
 	 	4	4	4	
		46	46	5	

Jin J, et al, 2011, submitted
Somerville RPT, et al, 2011, submitted

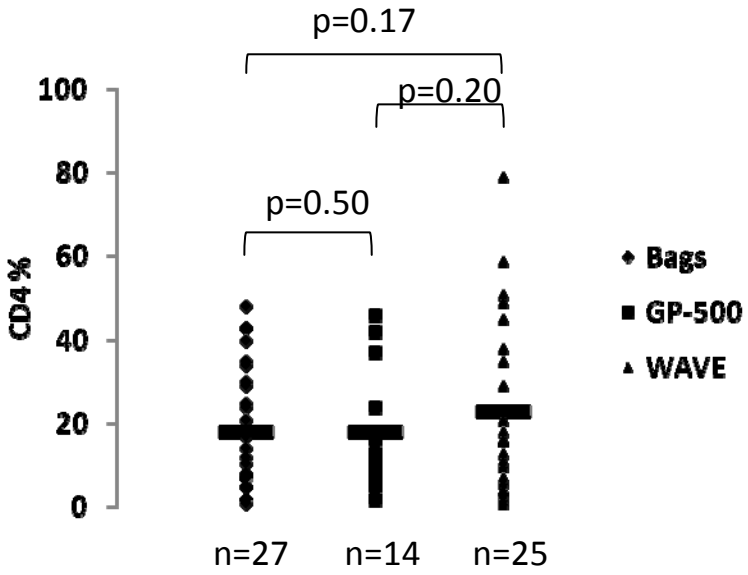
Similar TIL expansion in different bioreactors

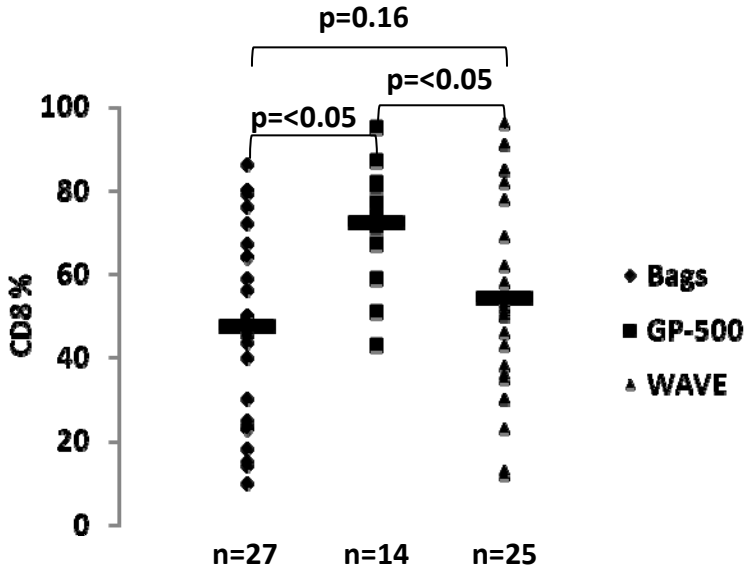


Similar TIL function in different bioreactors

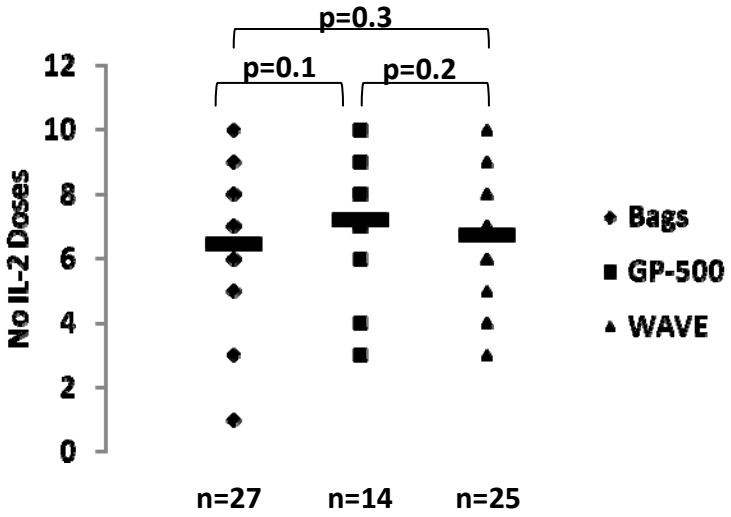


Similar TIL composition in different bioreactors





Different bioreactors produce TIL that mediate similar clinical results



Clinical Responses

	OR	NR
Bags	8 (30%)	19 (79%)
WAVE	6 (24%)	19 (74%)
GRex	4 (29%)	10 (71%)

New Tools for T cell expansion

- Preclinical process/reagent development
- cGMP reagent qualification/production
- Safety data, “first in man” clinical trials
- Efficacy data

Autologous T Cell and Gene Therapies

Service → Product

- Two TIL manufacturing strategies emphasize the trade-off between quality and quantity

New tools for T cell expansion

- Powerful new solutions to technical problems of scale up/scale out

Thank You

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