BIOTHERAPY:
4TH MODALITY OF CANCER TREATMENT

Therapeutic Approaches with Immune Cells

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Adoptive Cellular Therapy

- The administration of cells with antitumor activity as anticancer therapy
- Generally involves *ex vivo* manipulation to enhance activity or enrich cell type
- Most clinical experience has been with lymphocytes

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Clinically tested forms of adoptive cellular therapy

- Lymphokine activated killer cells [LAK]
- Tumor infiltrating lymphocytes [TIL]
- Autolymphocyte Therapy [ALT]
- Dendritic cell therapy [DC]
- Non-myeloablative allogeneic stem cell transplant and donor lymphocyte therapy [DLT]

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How do lymphoctyes kill malignant cells?

- **Granule Exocytosis:** transfer of perforin (cytolysin = pore-forming protein-PFP) in cytoplasmic granules → transfer of serine proteases (gzms) → membrane leaks & apoptosis [NK, LAK, CD8+CTL]
- **Fas Pathway:** binding to Fas (a surface antigen related to TNF receptor → apoptosis [CD4+ & CD8 CTL]

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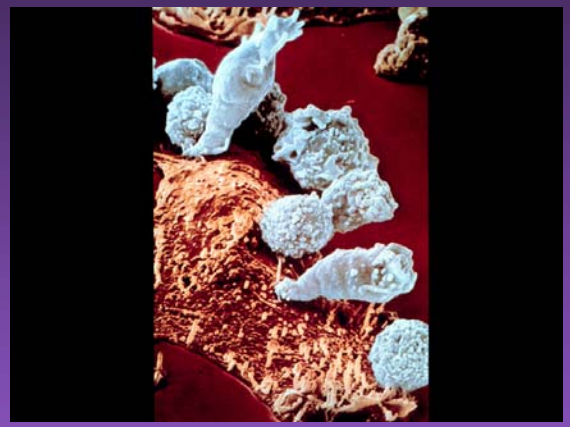
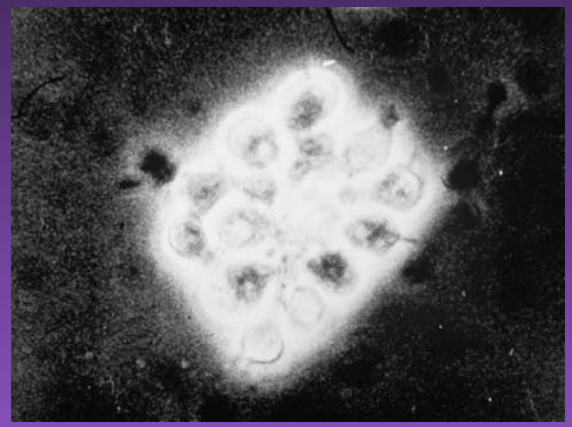
Immune Cells for Adoptive Cellular Therapy





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Lymphokine Activated Killer Cells [LAK]

- A subset of Natural Killer [NK] cells with nonspecific cytotoxicity against tumor cells, whose activity is enhanced by Interleukin-2
- Defined in vitro by the difference in cytotoxicity for PBL vs IL2-stimulated PBL against K562 (NK-sensitive) and Daudi (NK-resistant) cell lines
- CD56+, CD25+, CD20-, CD16+, CD3+/-

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NCI: IL-2 + LAK

DX	# Pts	CR #	PR #	RR %
Renal	72	8	17	35%
Melanoma	48	4	6	21%
Colorectal	30	1	4	17%
Lymphoma	7	1	3	57%
Others	21	0	0	0%
TOTAL	**178**	**14**	**30**	**25%**

Rosenberg SA et al. High Dose Bolus IL-2

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CBRG: IL-2 + LAK

DX	# Pts	CR #	PR #	RR %
Renal	46	2	5	15%
Melanoma	54	3	10	24%
Colorectal	35	0	0	0%
Lymphoma	9	0	2	22%
Other	98	2	8	10%
TOTAL	**242**	**7**	**24**	**13%**

Dillman RO et al. Continuous Infusion IL-2

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Pulsed LAK

- 15-60 min incubation *ex vivo* in pheresis bag with IL2 6000 IU/ml
- enhanced cytotoxicity only if preceded by *in vivo* infusional IL2 to prime LAK cells
- tremendous cost savings compared to 2-4 day LAK incubation *in vitro*

Horton et al: Cancer Res 1990

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Pulsed LAK: clinical trials

- **Oldham et al: Proc ASCO 1991**. Primed with IL2 18 MIU/m^2/day x 5days→10 liter leukapheresis → 6000 IU/ml pulse *ex vivo* → infuse w/CIV IL2. 5/63 responses
- **Yeung et al: Cancer 1993**. Primed with CIV IL2 6 MIU/m^2/day x 4 days → 10-liter leukapheresis → 9 MIU/ml pulse *ex vivo* IL2 → infuse w/ CIV IL2. 6/19 responses

Randomized Trials of IL-2 ± LAK Cell Therapy

1st Author	Year	# of Patients	Treatments*	IL-2/mo in MIU	Result/Comment
Rosenberg Renal Cell & Melanoma	1993 NCI	85/91 79/90	HDB IL-2 + LAK HDB IL-2	1836 1836	**RCC**: 33% vs 24% RR; OS NSD [p=.52] **Melanoma**: 22% vs 27% RR; OS favored LAK [p=.064]
Law Renal Cell	1995 MSK	35 36	MDCIV IL-2 + LAK MDCIV IL-2	120 120	3% vs 9% RR [p=.61] 13 vs 11 mos med OS [p=0.67] LAK more toxic
Koretz Renal Cell & Melanoma	1991 Emory	19 19	LDCIV IL-2 + LAK LDCIV IL-2	24 24	No responses in either arm

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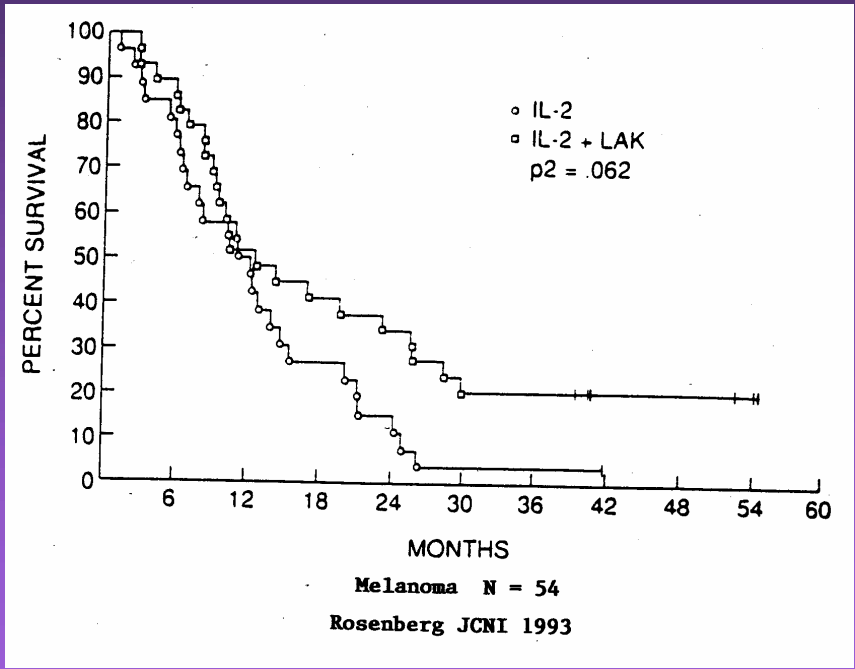
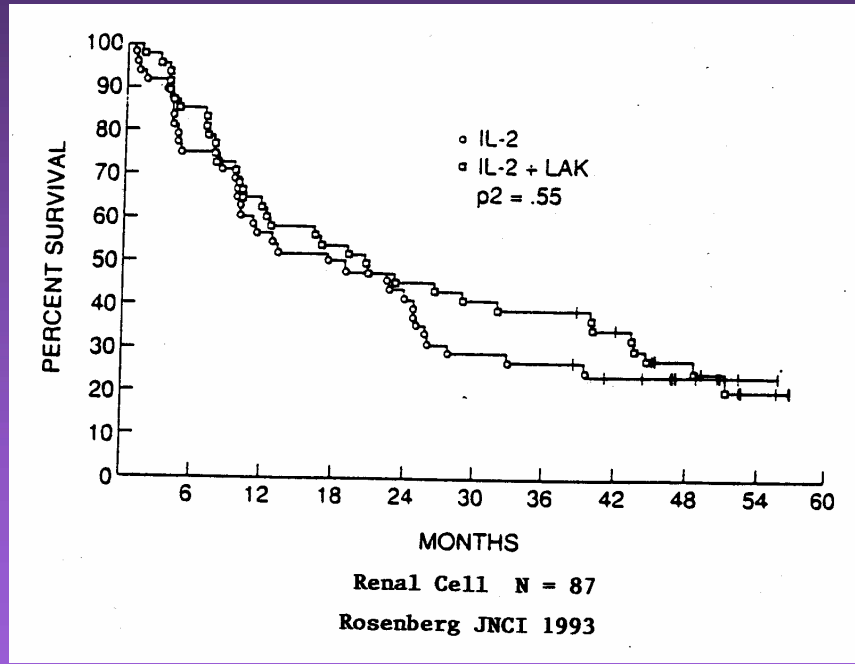
NCI: IL-2 ± LAK





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Published Experience with Brain LAK in Recurrent GBM

Citation		Method of LAK	# pts	60 day mortality	Median survival
Barba 1989	J Neurosurg	Stereotactic + IL-2	9	33%	<4 mos
Lillehei 1991	Neurosurg	Plasma clot →IL-2/LAK via catheter	20	--	< 5 mos
Merchant 1988	Cancer	Surgical + IL-2	13	16%	< 6 mos
Jeffes 1993	J Neuro-Oncol	Surgical	19	--	7.5 mos
Dillman	J Immunother	Surgical ± IL-2	40	2%	9.0 mos
Hayes 1995	Cancer	+ IL-2 via Ommaya	15	--	12.2 mos

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Survival After Resection of Recurrent GBM

Sipos	Neurochir	1997	60 pts	4.3 mos
Dirks	Can J Surg	1993	43 pts	4.4 mos
Brem	Lancet	1995	112 pts	5.3 mos
Brem	Lancet	1995	110 pts*	7.2 mos
Ammirati	Neurosurg	1987	35 pts	7.2 mos
Harsh	Neurosurg	1987	39 pts	8.4 mos
Salc	Neurosurg	1994	40 pts	8.6 mos
Dillman	J Immunother	2004	40 pts	9.0 mos

Cytotoxic T-Lymphocytes

- CD8+ T-Lymphocytes
- Cytotoxicity restricted to recognition of foreign or tumor antigen in combination with HLA Class I histocompatibility antigens of self
- Highly specific, self-restricted activity
- 10- to 100-fold more cytotoxic than LAK

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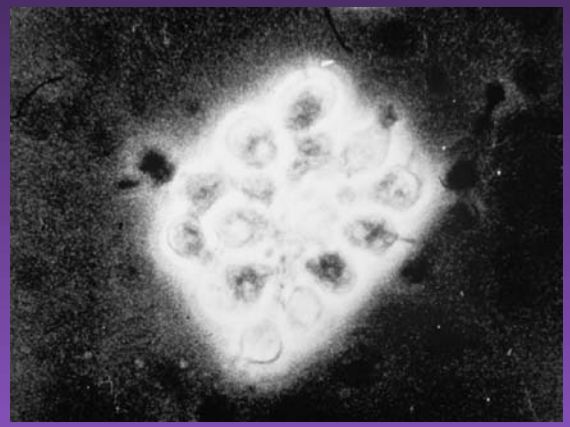
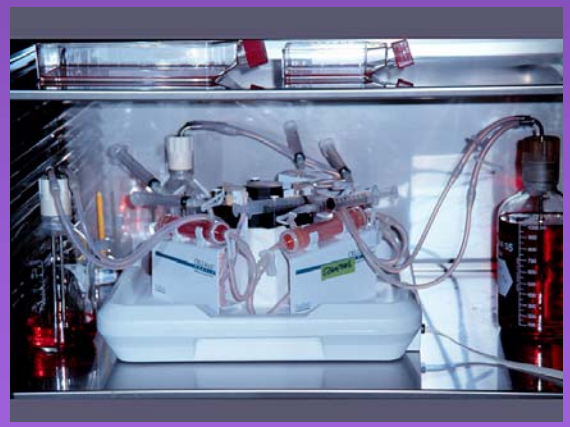
Tumor Infiltrating Lymphocytes [TIL]

- Presumption that some lymphocytes which infiltrate tumor masses *in vivo* are antigen-specific, HLA- restricted CTL
- Cultures of single cell suspensions of fresh tumors in IL-2 results in selection of T-lymphocytes with varying degrees of tumor-specific and patient-specific cytotoxicity
- May be predominantly CD8+ or CD4+, may be cytotoxic or non-cytotoxic

Hollow Fiber Bioreactor to Grow TIL





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Success rates in growing TIL

First Author	Site	years work	# TIL	% TIL
Lewko	CTI	91-94	90/113	80%
Malone	Hoag	96-99	26/34	76%
Dillman	BTX	87-90	56/82	72%
Schiltz	Hoag	91-95	67/94	71%
Oldham	BTX	87-90	129/196	66%
Yanelli	NCI	89-93	160/255	63%
TOTAL			**502/740**	**68%**

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Localization of Indium-111 Labeled TIL

- Fisher B et al (J Clin Oncol 1989)
  - tumor sites imaged in 6/6 melanoma patients within 24-144 hrs
  - initial localization to lung, liver, and spleen during first two hours
- Pockam BA et al (Cancer 1994)
  - tumor uptake in 26/38 melanoma patients
  - more likely to localize if CTX pre TIL: 20/26 (81%) vs 5/12 (42%) (p=.026)
  - those who imaged received more TIL (p=.005)
  - response 10/26 who imaged vs 0/12 who did not (p=.022)
- Dillman et al (Cancer Biother Radiopharm 1997)
  - Tumor uptake in 8/8 patients (5 RCC, 2 melanoma, 1 colon)
  - Sites imaged included: bone, brain, lung, liver, lymph nodes and soft tissue mass
  - No objective responses

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TIL Therapy: phase II trials

Auth	Org	Dx	IL2 Rx	#Pts	RR
Rosenberg	NCI	Mel	HB+CTX	86	34%
Pierce	UCLA	RCC	LCIV +IFN	48	33%
Dillman	CBRG	Mel	HCIV+CTX	21	24%
Kradin	MGH	RCC, MEL, Lung	LCIV	28	21%
Goedegeburre	Brigham	Mel, RCC	LB	26	11%
Oldham	CBRG	Not Mel	HCIV +CTX	30	3%
Bukowski	Cleve	RCC	CIV	18	0%
TOTAL				**257**	**24%**

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Randomized Trials of TIL Cell Therapy

1st Author	Year	# of Patients	Treatments*	IL-2/mo in MIU	Results
Figlin Metastatic Renal Cell	1999 consortium	39/72/81 68/79	MDCIV IL-2 + TIL MDCIV IL-2 + placebo	160 160	NSD RR or OS by intent-to-treat analysis, but 41% did not receive intended TIL
Ratto Stage II-III NSCLC	1996 Italy	113/131 113/113	SC IL-2 + TIL→ VBL/CSP + RT VBL/CSP + RT	2-16 MIU/m^2 qd x 14 as tolerated	22.4 vs 14.1 mos OS p.05
Dreno Stage III Melanoma	2002 France	44 44	SC IL-2 + TIL SC IL-2	IL-2 x 2 mos	NSD median F/U 4 yrs ↓ relapse if only 1 + node

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Correlates of TIL antitumor effects in vivo

- number of cells: $\geq 10^{11}$ cells
- cytotoxicity against allogeneic or autologous targets
- ease and rapidity of cell expansion
- not clear that HLA-restricted TIL [CTL] any better than nonspecific TIL [T-LAK]

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Autolymphocyte Therapy

- **Non-cytotoxic T cells, of memory-helper phenotype, derived from peripheral blood mononuclear cells by cell culture in the presence of autologous lymphokines (ALK) secreted by autologous mononuclear cells after they have been stimulated with anti-CD3 monoclonal antibody in the presence of putative inhibitors of suppressor T cells; there is no incubation with IL-2**

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AutoLymphocyte Therapy

- **Autolymphokine (ALK) Media: About 5 x 10^9 autologous lymphocytes stimulated *in vitro* for 3 days with anti-CD3 monoclonal antibody in the presence of indomethicin and cis-retinoic acid to obtain media containing significant amounts of TNF-α, IL-1β, interferon-γ, and IL-6, but no IL-2**
- **ALT: for 6 months, 3-5 x 10^9 PBMC incubated with aliquot of supplemented ALK media for 6 days $\rightarrow$ CD3+, CD4+, CD29+ (4B4) "helper" lymphocytes, not cytotoxic**
- **Infused i.v. monthly while patient on cimetidine 600 mg po q6h**

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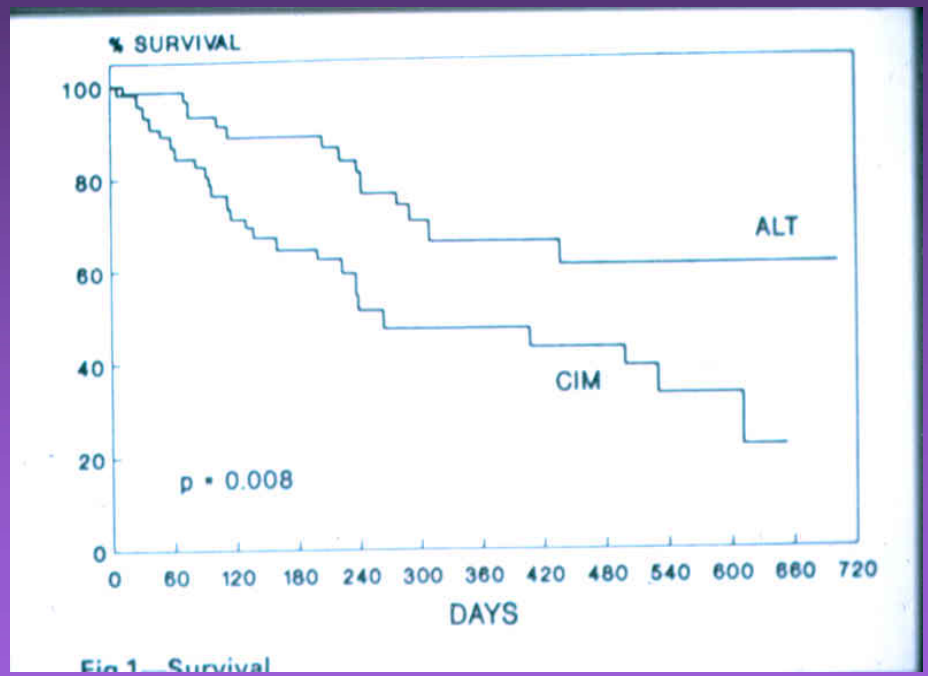
Osband ME et al, Lancet 1990; Nayak Proc AACR 1995; Schwartz J Immunother 1998; Cornforth J Immunother 1998

Auto-Lymphocyte Therapy

- 90 patient randomized trial in metastatic kidney cancer
- Cimetidine + ALT vs Cimetidine
- 21% vs 5% OR
- Better OS p=.008



Osband ME et al, Lancet 335:994, 1990

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Auto-Lymphocyte Therapy

- **Lavin PT et al. Transplant Proc 24:3059-3064, 1992**
  - **335 patients, 3 sites, experience in metastatic renal cell carcinoma**
  - **10/259 (4%) response rate**
- **Dillman RO et al. Cancer Biother Radiopharm 18:727-733, 2003**
  - **47 patients, metastatic solid tumors, response rate of 4%**
  - **1/13 renal cell**
  - **0/13 colon**
  - **0/6 breast**
  - **0/5 lung**

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Auto-Lymphocyte Therapy

- **Randomized trial vs observation in node + or T4 regionally advanced disease stopped with 20 patients in each arm**
- **180 patient randomized trial vs interferon-alpha in metastatic disease—NSD**

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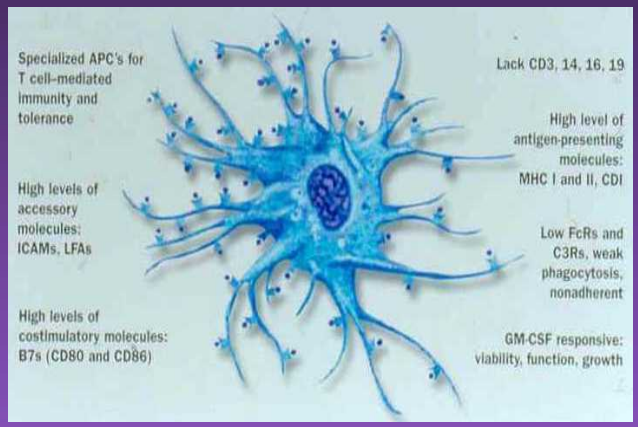
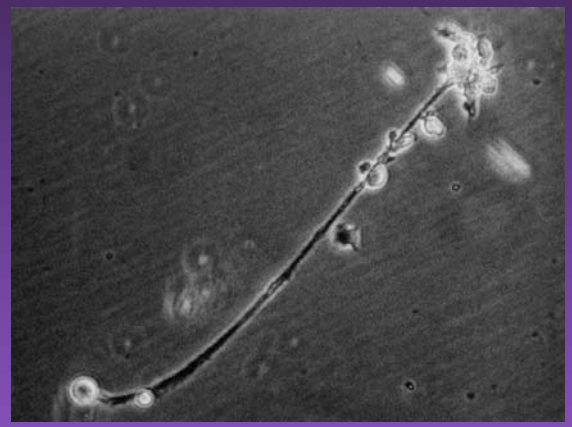
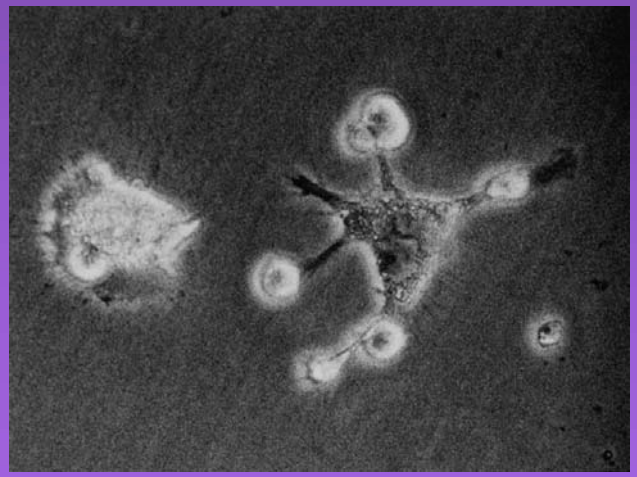
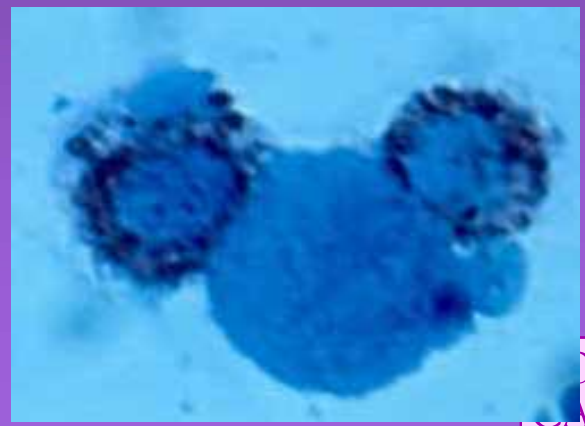
Dendritic Cells









Dendritic Cells

- Derived from peripheral blood mononuclear cells or stem cells by incubation with GM-CSF, IL-4, ± TNF-α
- Usually applied in vaccine strategy
- Some trials involving iv infusion

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Intravenous Dendritic Cells

- DC loaded with Prostate membrane specific antigen, responses alleged in 5 patients in phase I trial—Tjoa, Prostate 1997
- In 5 patients with melanoma, i.v. infusion of radiolabeled DC resulted in transient localization in lung followed by retention in liver, and spleen while intralymphatic injected resulted in uptake and retention in lymph nodes for more than 24 hrs– Mackensen, Cancer Immunol Immunother 1999
- DC loaded with melanoma peptides, one CR in 16 patients, infusion well-tolerated—Lau R, J Immunother 2001
- Colon and Pancreas cancer: 6 pts received DC from CD34+ cells; 6 pts received DC from PBMC—Triozzi J Hematother Stem Cell Res 2003

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Donor Lymphocyte Infusions or Mini-Allogeneic Transplants

- Allogeneic transplant but using marrow suppressive rather than ablative doses
- HLA matched donor
- Immunosuppression to decrease GVHD
- Relying on Graft vs Tumor Effect
- Can give infusions of donor lymphocytes (DLI) to supplement anti-tumor effect or to eliminate/reduce chimerism

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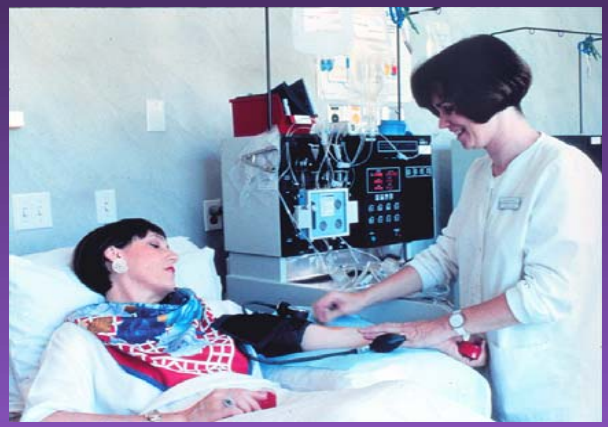
Hematopoietic Stem Cells



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Allogeneic Lymphocytes

- 19 patients, refractory metastatic renal-cell cancer
  - HLA-identical sibling or a sibling with a mismatch of a single HLA antigen suitable donors
- Regimen
  - FluCy to temporarily ablate hematopoiesis and lymphocytes
  - IV infusion of allogeneic PBSC
  - Cyclosporine to decrease GVHD, withdrawn early in patients with mixed T-cell chimerism or PD
  - Patients with no response received up to three infusions of donor lymphocytes
- Results
  - 3 CR, 10 PR, 2 transplant related deaths
  - Regression of lesions was delayed (median 4 mos) suggesting benefit from immune therapy rather than chemotherapy

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Childs R et al. N Engl J Med 343:750-8, 2000 NIH

Allogeneic Lymphocytes

- 20 patients, with B-cell lymphoma, recurred after autologous transplant, but still responsive to chemotherapy
  - HLA-identical sibling or a sibling with a mismatch of a single HLA antigen suitable donors
- Regimen
  - FluCy + rituximab, FluCy+AraC to temporarily ablate host hematopoiesis and lymphocytes
  - IV infusion of allogeneic PBSC
  - Tacrolimus and methotrexate to decrease GVHD, adjusted or stopped if mixed T-cell chimerism or PD
  - Patients with no response received up to three infusions of donor lymphocytes
- Results
  - 95% PFS at median F/U of 2 years

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Escalon MP et al. J Clin Oncol 22:2419-23, 2004 MD Anderson

Allogeneic Lymphocytes

- 53 pts post stem cell transplants from HLA-matched related or unrelated donors
  - DX: 10 MDS, 10 AML/ALL, 11 CLL, 9 myeloma 9 lymphoma, 4 solid tumors
- Regimen
  - 2 Gy TBI ± fludarabine to temporarily ablate host hematopoiesis and lymphocytes
  - Mycophenolate mofetil and cyclosporine to decrease GVHD
  - Donor lymphocyte infusion (DLI) with a median CD3 dose of 10 million cells/kg, for persistent disease (n = 8), disease relapse (n = 17), progressive disease (n = 12), low donor chimerism with disease (n = 11), or low chimerism with remission (n = 5)
- Results
  - 25% (7/48) response rate, 32% OS at median 30 mos F/U
  - 17% grade II-IV GVHD

Bethge WA et al. Blood 103:790-5,2004 Fred Hutchison

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Strategies to improve DLI or Mini Allo Transplants

- T cell reduction to reduce GVHD
- Variations in preparative regimens: monoclonal antibodies, chemotherapy, radiation therapy, radioimmunotherapy
- Variations in immunosuppressive agents
- Subset selection of donor lymphocytes

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Obstacles to Adoptive Cellular Therapy

- Need for cell biology support system
- Autologous cells: cost and inefficiency-- must make a new product for each individual patient
- Allogeneic cells: compatibility matching and morbidity of GVHD

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Conclusions

- Adoptive cellular therapy with autologous lymphocytes has been associated with significant tumor responses in clinical trials, but there is no FDA approved product (process)
- Donor Lymphocyte Infusions have had limited benefit in treatment of refractory solid tumors, but activity in hematopoietic malignancies
- Cost:Benefit of technology remains a formidable obstacle to use and investigation

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