Artificial Antigen Presenting Cells as a Standardized Platform for Tumor Infiltrating Lymphocyte (TIL) expansion

Concurrent Session 404: T cell Manufacturing and Potency

27th Annual Meeting of the Society for Immunotherapy of Cancer

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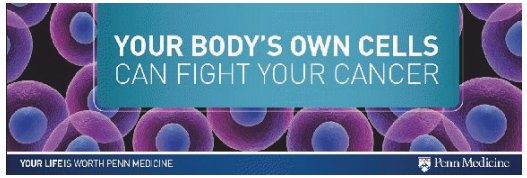
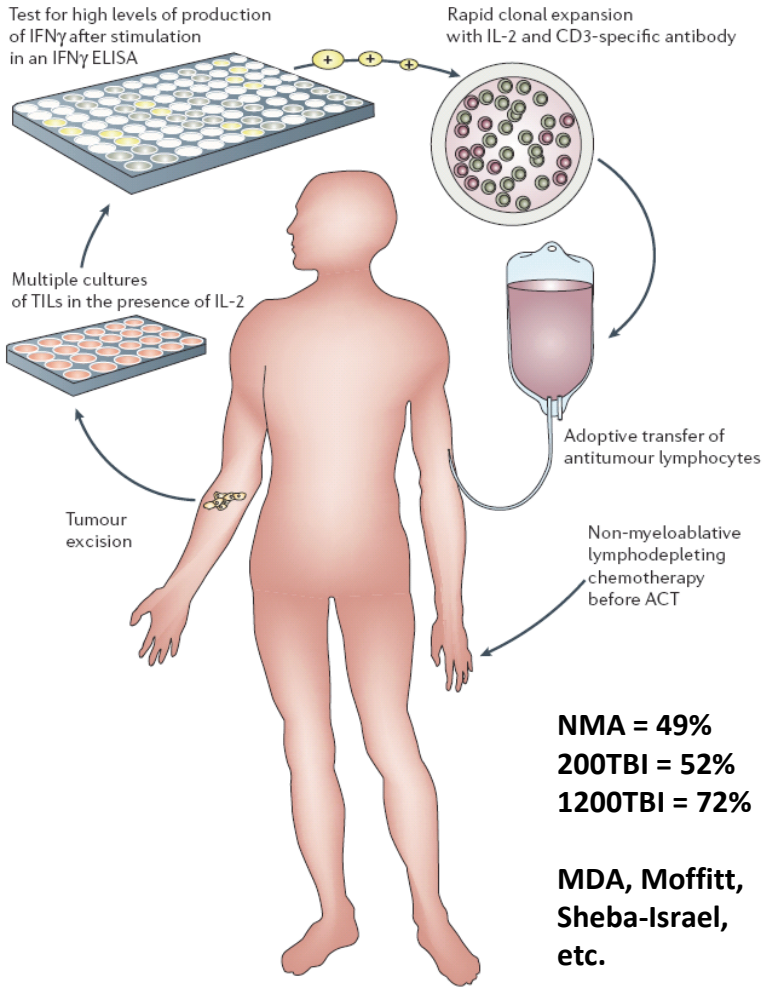


Disclosures

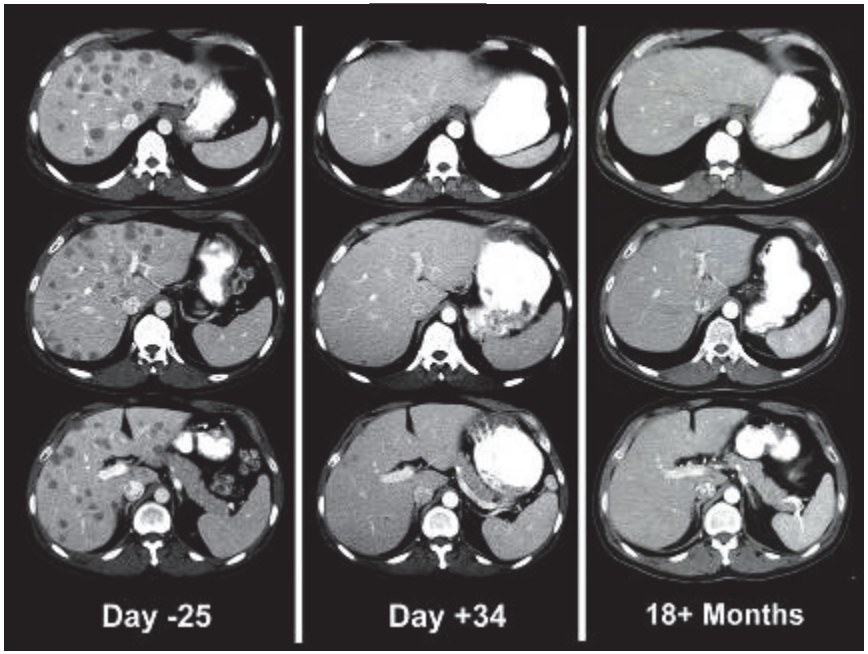
- Scientific Advisory Board Member; Genesis Biopharma, Inc.
  - Fees for Consultation

Tumor regression after administration of endogenous tumor-reactive T cells



NMA = 49%
200TBI = 52%
1200TBI = 72%

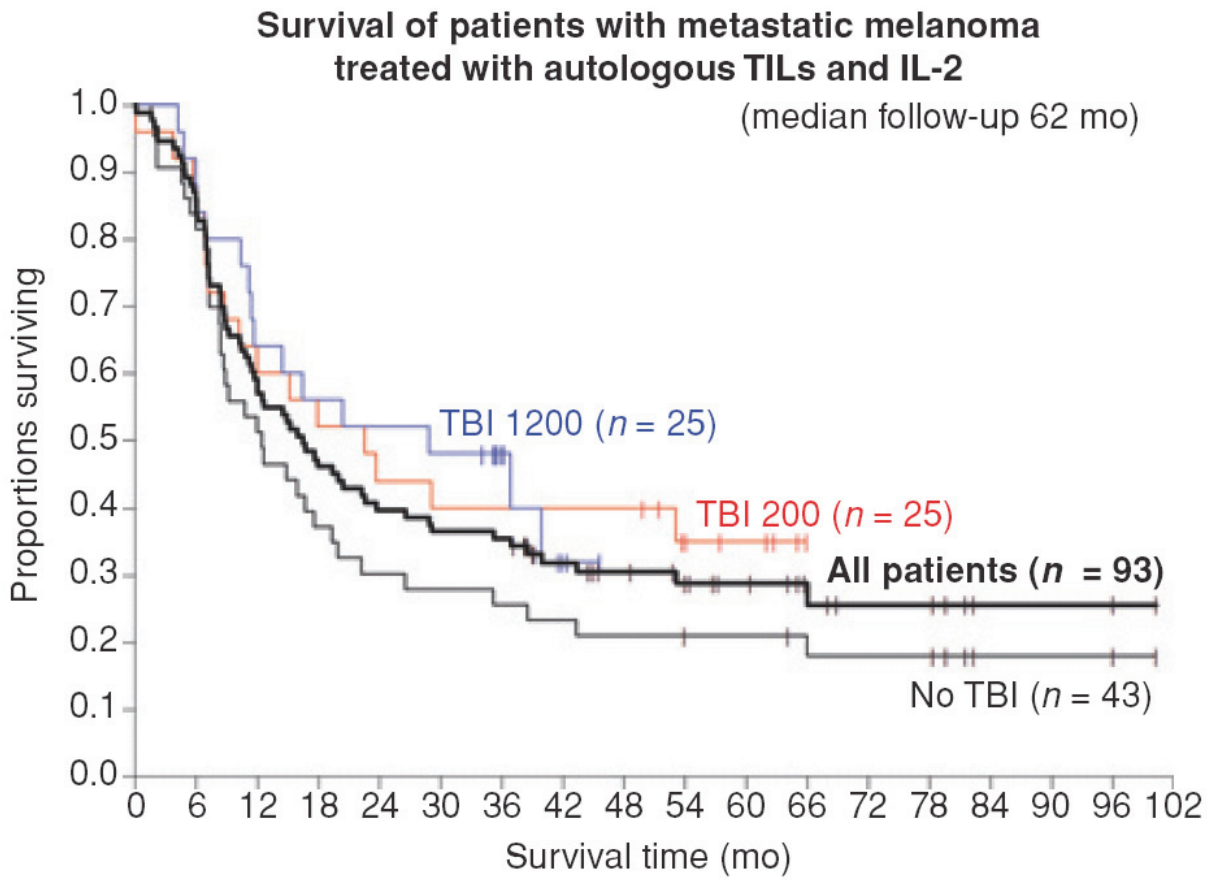
MDA, Moffitt, Sheba-Israel, etc.



(*Right*) Adapted from Rosenberg SA and Dudley ME, Curr Opin Immunol. 2009 Apr;21(2):233-40.

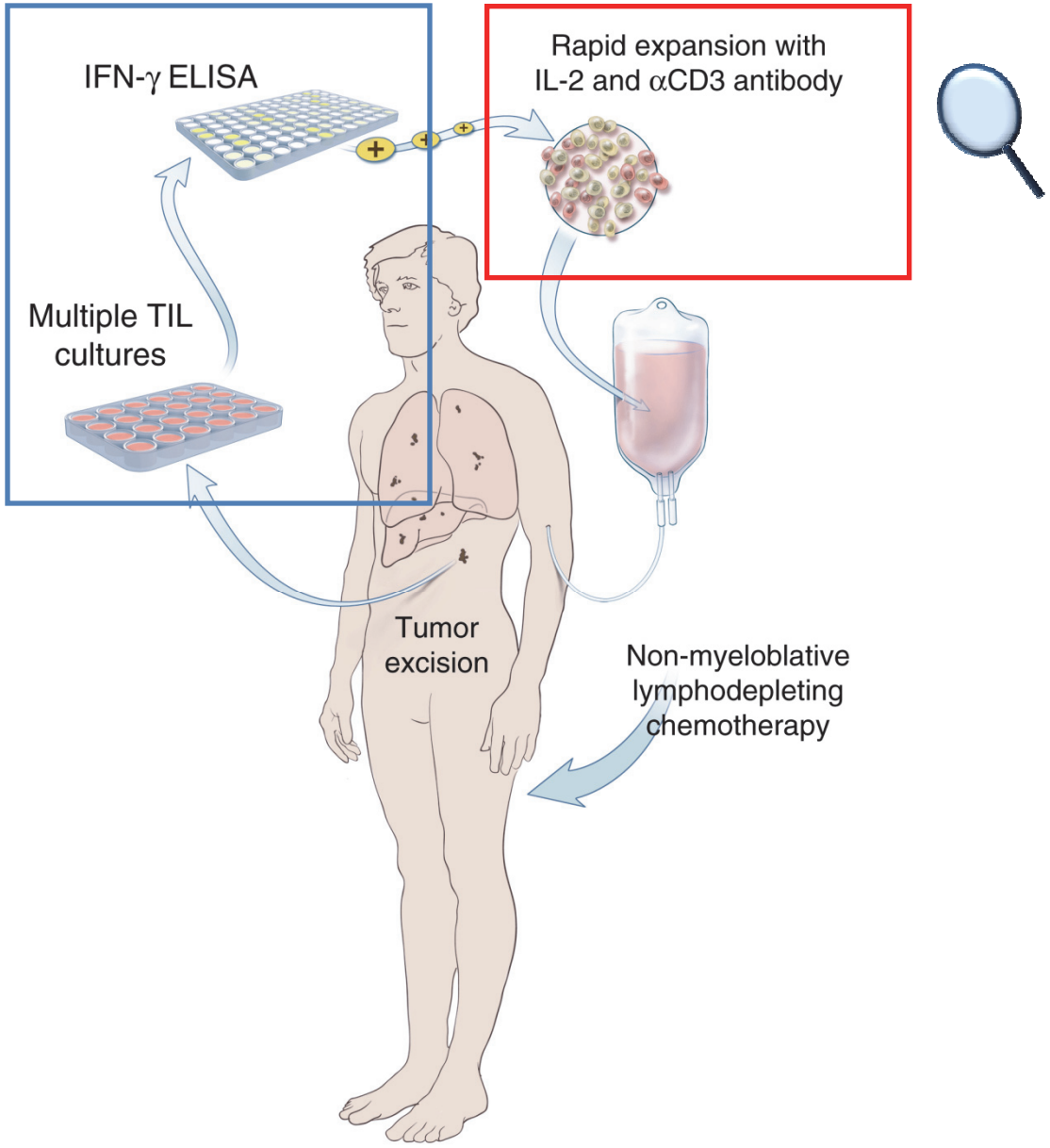
(*Left*) Gattinoni L, Powell DJ Jr, Rosenberg SA, Restifo NP. Nat Rev Immunol. 2006 May;6(5):383-93.

Durable complete responses in heavily pretreated patients with metastatic melanoma



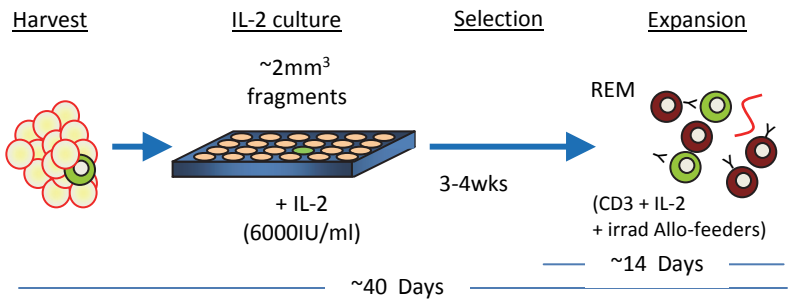
Rosenberg SA et al. Clin Cancer Res 2011;17:4550-7.

REP for ex vivo expansion of TILs



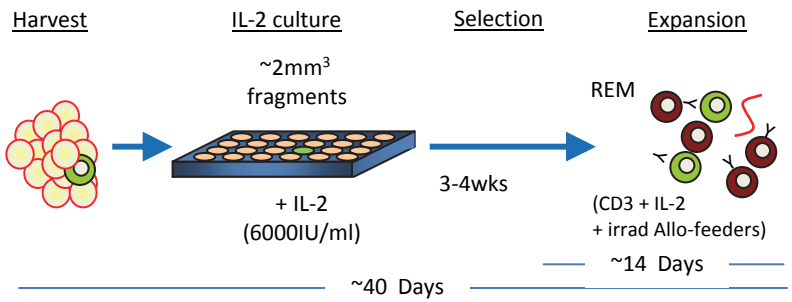
Dudley ME, *et al*, Science 2002
Dudley ME, *et al*, JCO 2005
Gattinoni, Powell *et al.* Nature Rev Immuno 2006

REP for ex vivo expansion of TILs



- Adapted from REM of Riddell and colleagues.

REP for ex vivo expansion of TILs



- Up to 1000-fold TIL expansion in melanoma.
- Maintenance of anti-tumor function by expanded T cells.
- Heterogeneous antigen specificity and subset (CD4/CD8)

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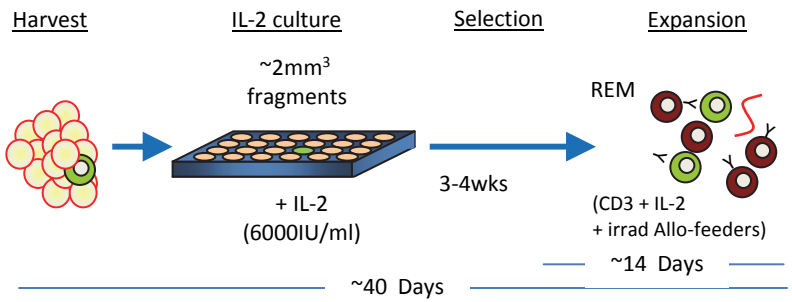

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Inherent technical, regulatory, and logistic challenges of REP that could limit its widespread application.

1. ***Large numbers*** of allogeneic feeders (100 to 200-fold excess), often from ***multiple donors***.
2. Allogeneic feeder cells harvested by large-volume leukapheresis from healthy donors exhibit ***donor to donor variability*** in their viability after cryopreservation and capacity to support TIL expansion, thus test expansions are often required.
3. Process necessitates extensive and ***costly*** laboratory testing of each individual donor cell product to confirm sterility and lack of opportunistic pathogens.

Alternative (Common) Clinical Strategies for Polyclonal T cell expansion

❑ **rhIL-2 (T cell Growth Factor) Cytokine**

- Promotes T cell proliferation
- Requires prolonged culture duration – progressive T cell differentiation

❑ **CD3/CD28 Beads**

- Paramagnetic Beads coated with Anti-CD3 and Anti-CD28 antibodies.
- Potent stimulation of peripheral T cells results in 50-1000 fold expansion; up to 10,000-fold with 2^{nd} stimulation.
- Preferential CD4 expansion; substantial number of $CD8^{+}$ do not divide.
- Largely has been restricted to PBL studies.

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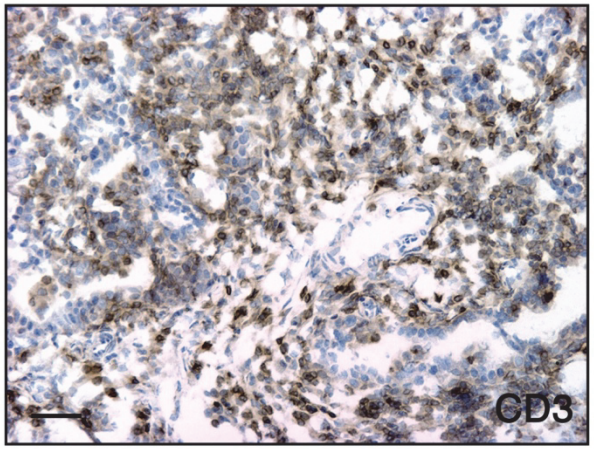
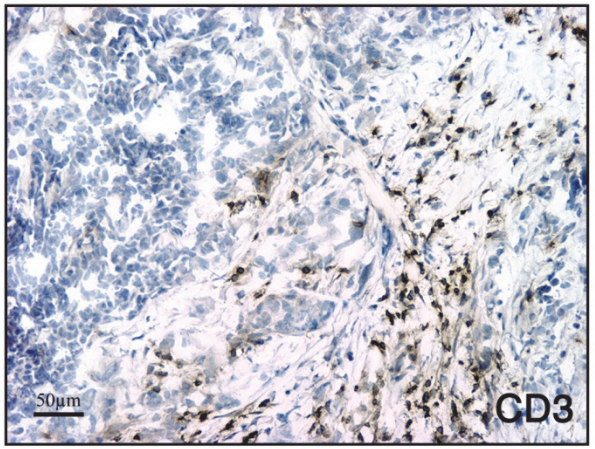
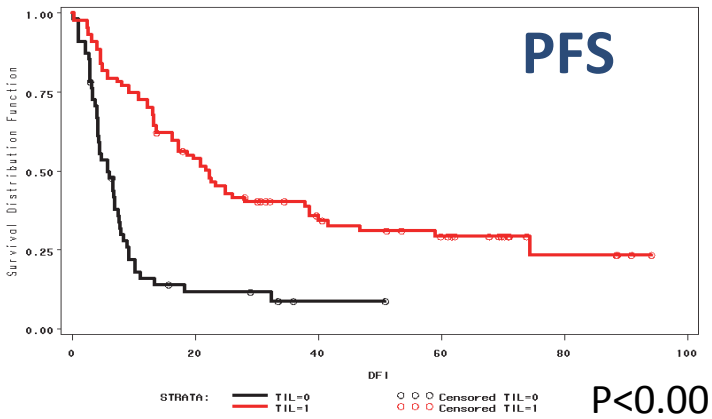
Currently there is no standardized expansion platform for TIL.

Impact of TILs on Outcome in Ovarian Cancer
Stage III/IV – All patients (n=174)







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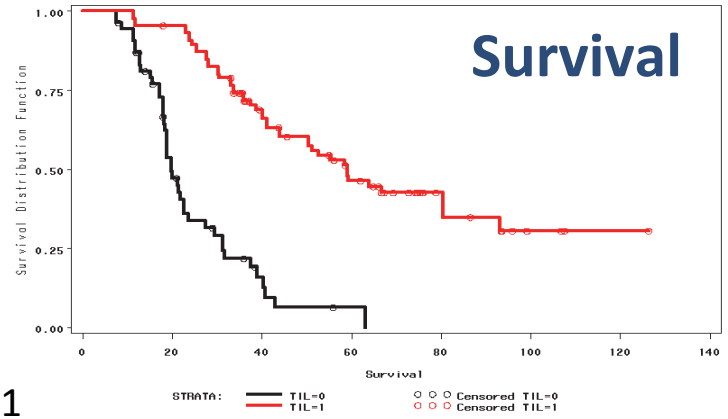
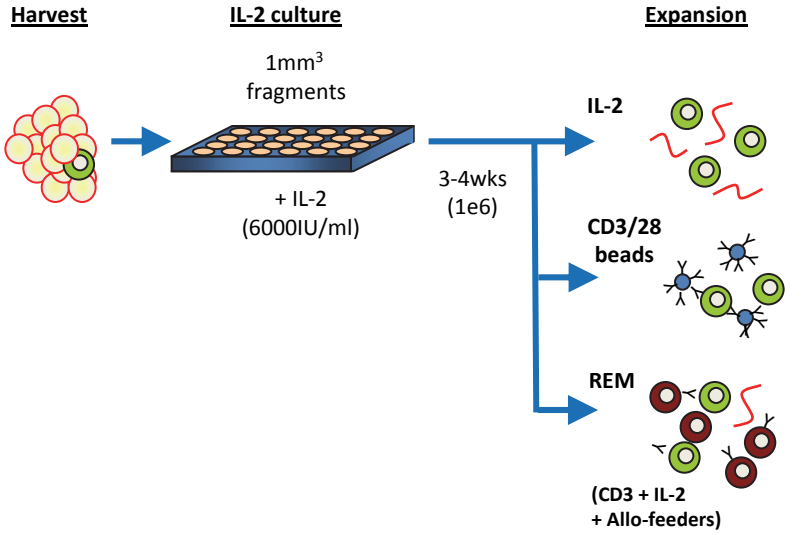


+ TILs
- TILs

n=174

Zhang et al, NEJM, 2003; 348: 203

Comparison of common expansion methods for TILs



IL-2:

6000 IU/mL or 600 IU/mL rhIL-2

CD3/28 beads:

3:1 bead to TIL ratio; plus rhIL-2

REP:

OKT-3 anti-CD3 Ab (30ng/mL), allogeneic feeders (200-fold excess) from three independent donors and rhIL-2.

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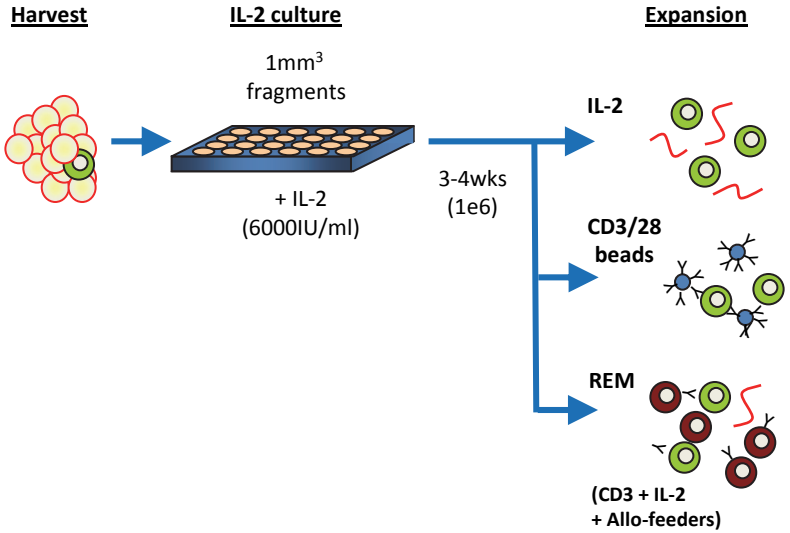

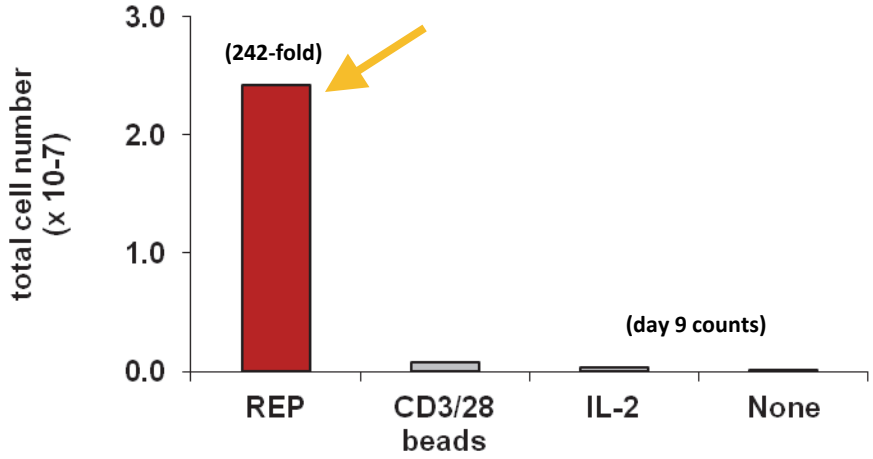

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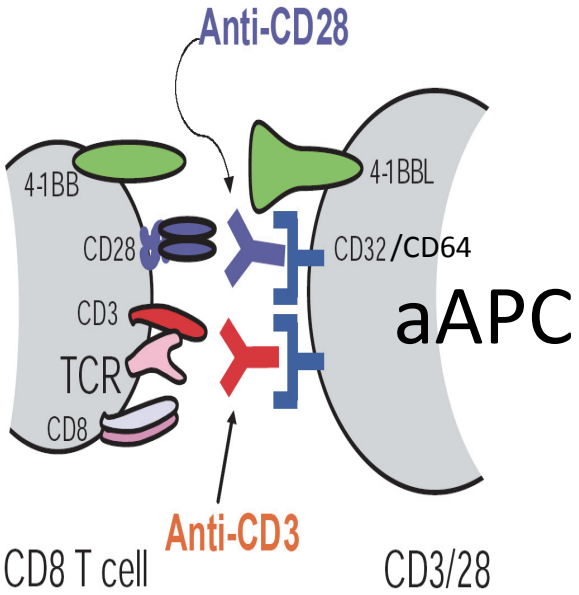
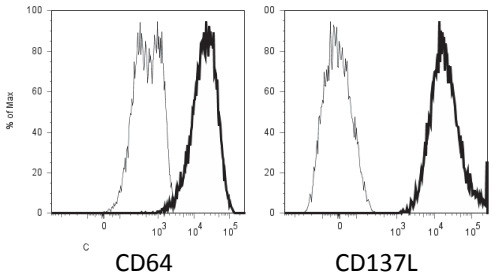
Ideal features of a Standardized TIL Expansion Methodology

- Single source “off-the-shelf” clinical grade-reagent.
- Renewable/reproducible.
- Rapid expansion.
- Expanded TILs should have favorable:
  - Level of expansion
  - Phenotype differentiation status (CD27, CD28, telomere, CD62L, etc.)
  - Maintenance of anti-tumor function

Evaluation of aAPC platform for ex vivo expansion of TILs





1. For PBLs, K562 (human erythroleukemia line) cell-based aAPCs bearing the costimulatory ligand CD137L are more efficient at activating and expanding antigen-experienced CD28- CD8+ T cells, than the magnetic bead-based aAPC.

2. Expand antigen-specific T cells from peripheral blood.

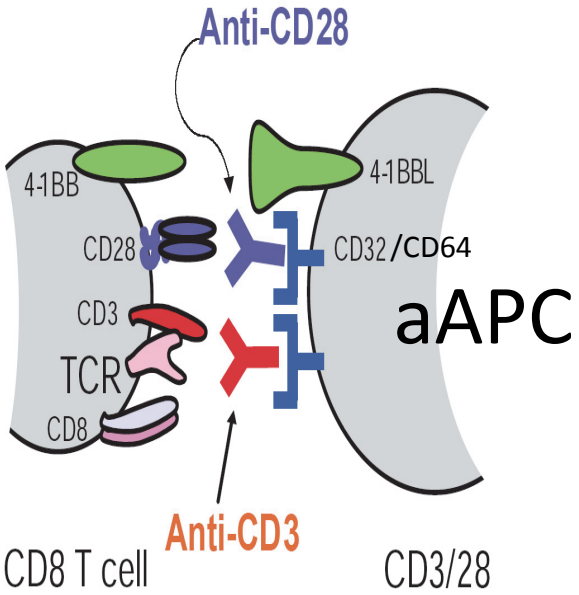
3. Ex vivo costimulatory signals mediated by CD137L reinforce maintained expression of CD28 on antigen-experienced circulating T cells in vitro and support their in vivo persistence and antitumor activity adoptive transfer of tumor-specific T cells in mice.

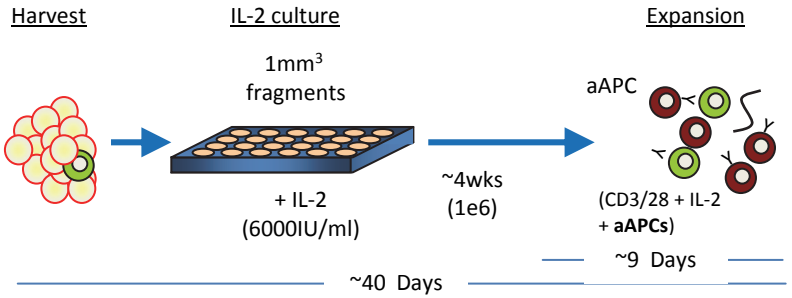
(Maus MV, Nat Bio, 2002; Suhoski M, Mol Ther, 2007)

Objective

- **Cell-based aAPCs represent a standardized platform for successful expansion of antigen (Ag) specific Tumor-infiltrating lymphocytes (TIL) and Tumor associated lymphocytes (TAL) of suitable phenotype and function for use in adoptive immunotherapy.**

Evaluation of aAPC platform for ex vivo expansion of TILs





1. Fc-binding receptors on KT64/BBL aAPCs were pre-cleared of serum immunoglobulins by culture in serum free AIM-V medium (SFM) overnight and then irradiated at 10,000 rad.

2. Anti-CD3 (OKT-3) with or without anti-CD28 (clone 9.3) mAbs were loaded on aAPCs at 0.5 ug/10^6 cells at 4^oC for 30 minutes.

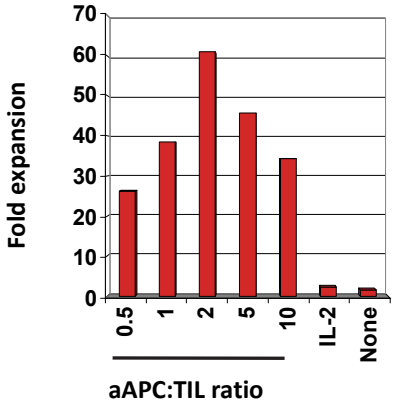
3. Before use, aAPCs were washed twice with SFM.

4. Loaded aAPC were used fresh (but are effective when cryopreserved in Batch quantities).

(Maus MV, Nat Bio, 2002; Suhoski M, Mol Ther, 2007)

Optimization for aAPC-based ex vivo expansion of IL-2 cultured TILs

2:1 aAPC: TIL Ratio



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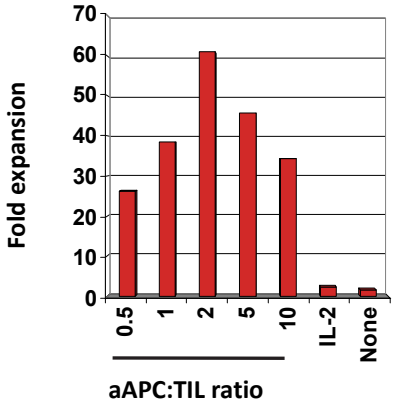
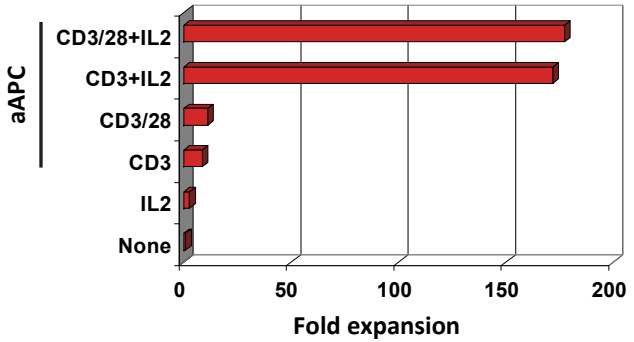


Dependent on anti-CD3 loading and IL-2



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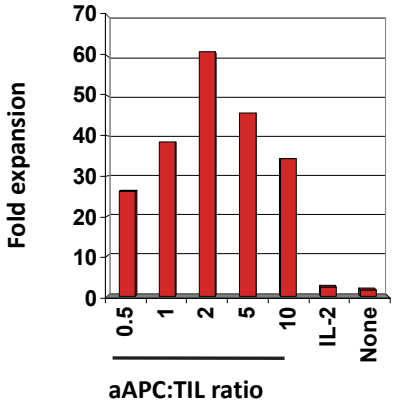

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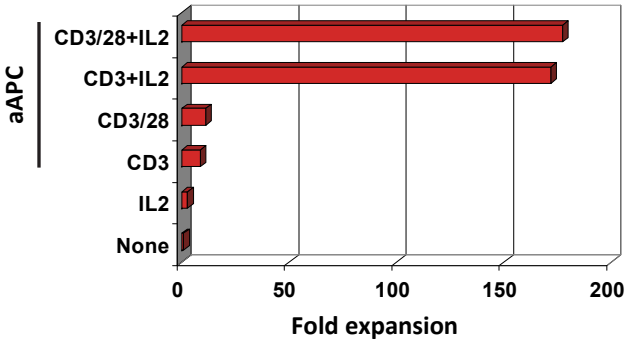
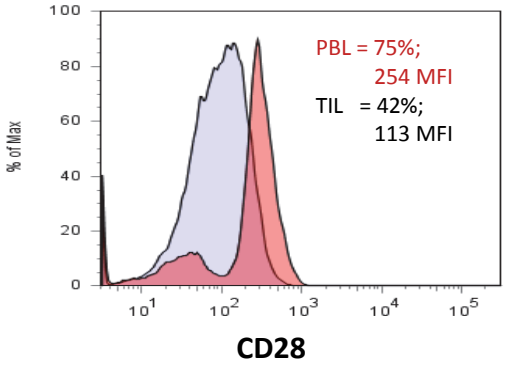
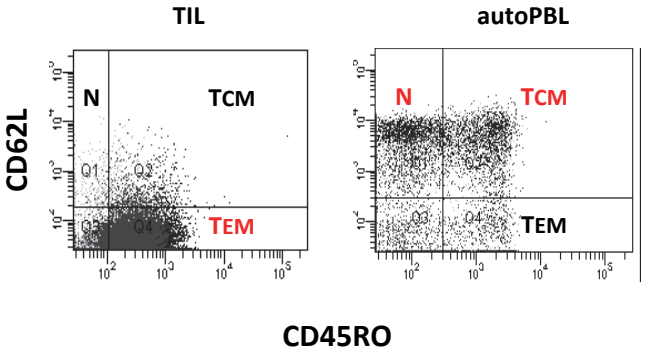


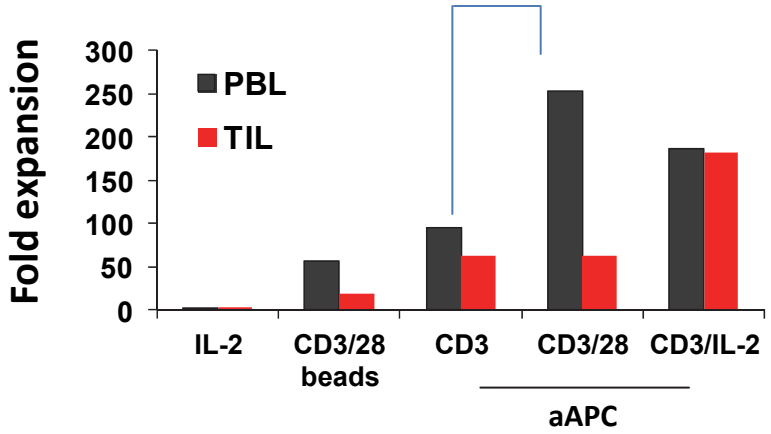
OvTILs are CD28^{low}



…and effector memory phenotype



CD28 crosslinking impacts PBL, not TIL, expansion & IL-2 production



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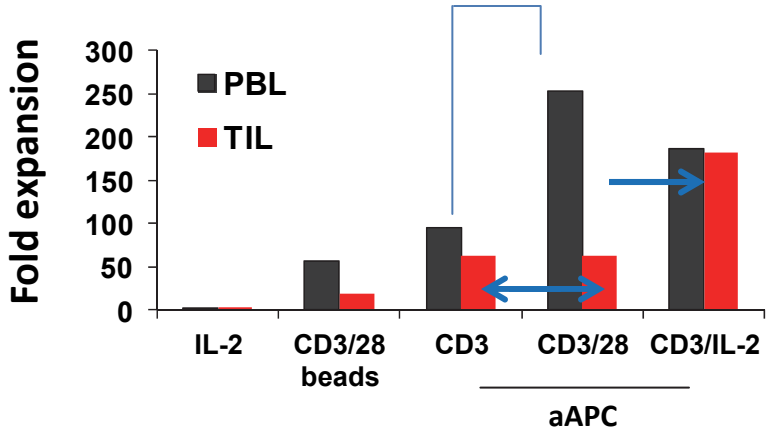

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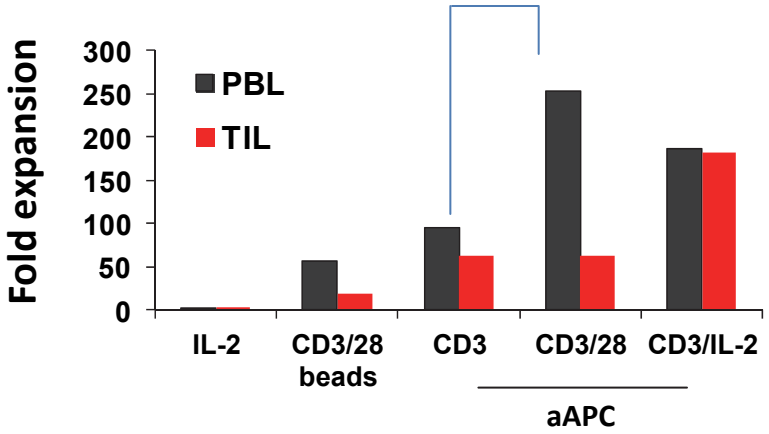
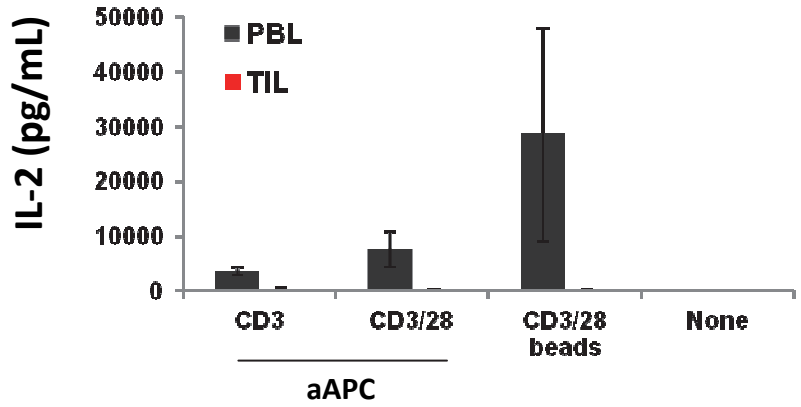
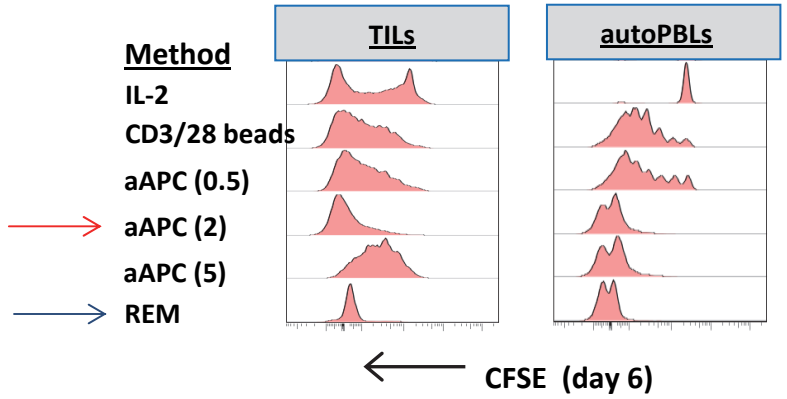




Comparison to other conventional expansion methods

Cell Division



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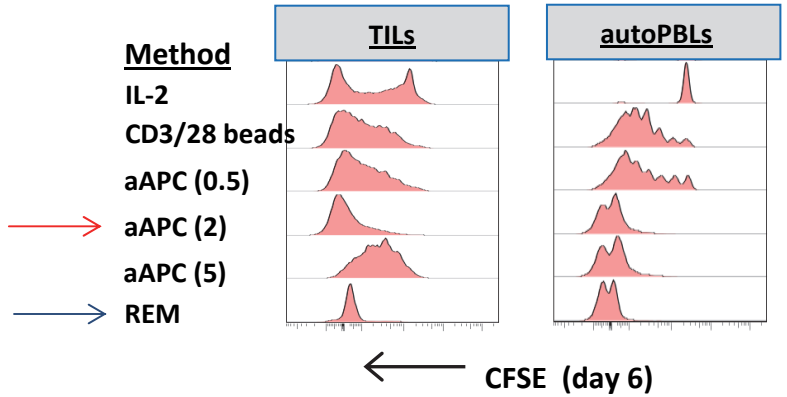
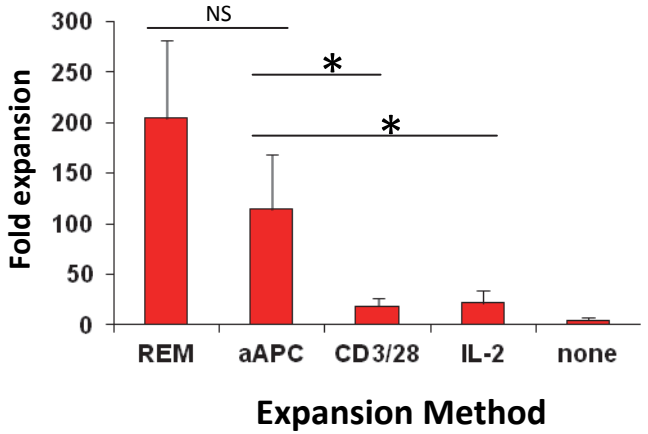


Expansion

Single round; Day 9-11 counts (n=6)



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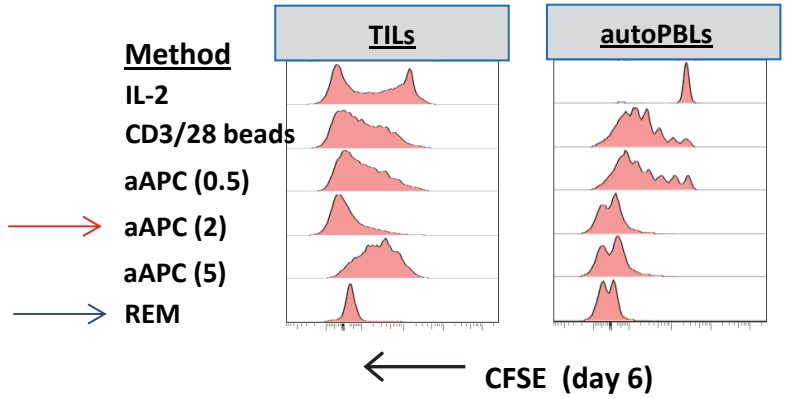

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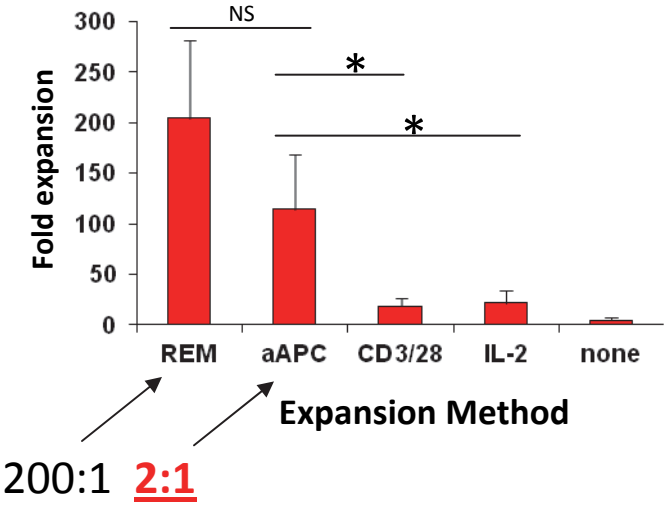

200:1 **2:1**

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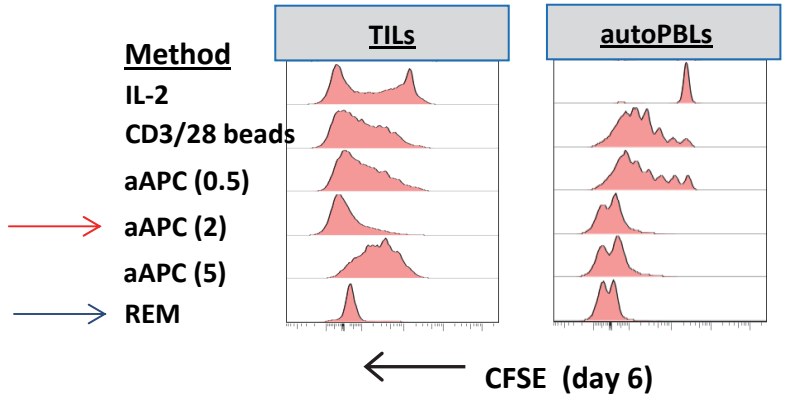

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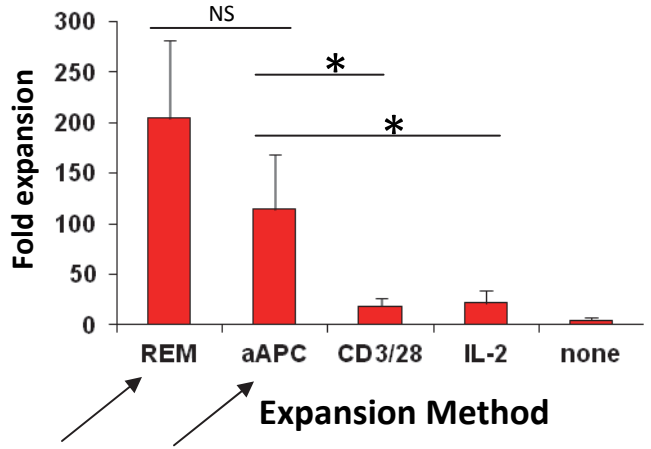
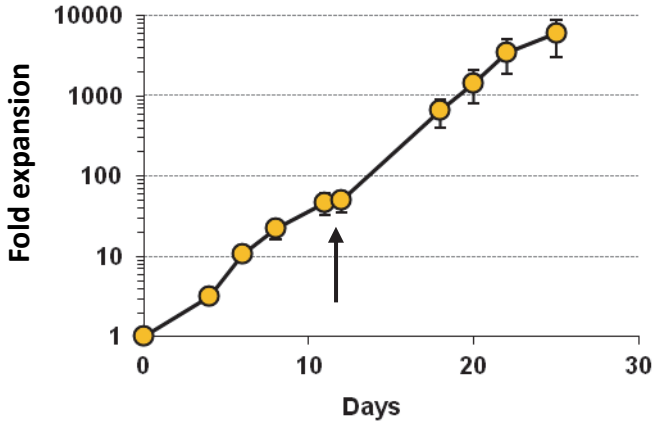


200:1 **2:1**

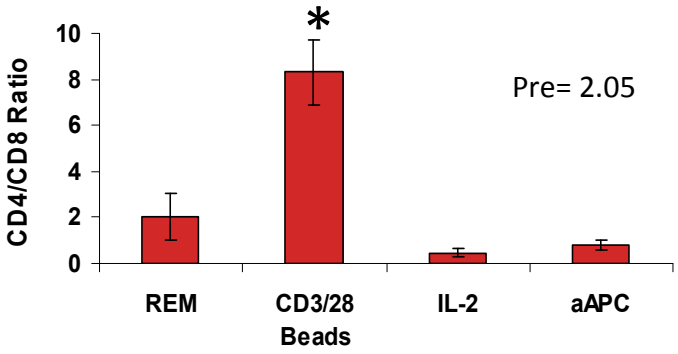
Secondary Expansion



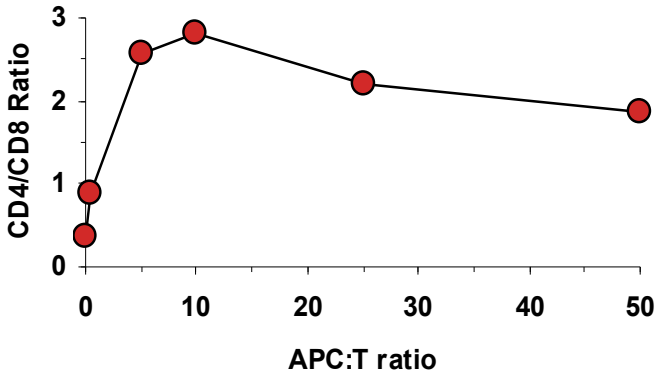
OVC TIL and Normal Peripheral T Cells Expanded with IL2, aAPC and REP (day6)

Favorable TIL subsets following expansion with aAPC

CD4:CD8 Ratio



CD4:CD8 ratio is effected by aAPC: T ratio



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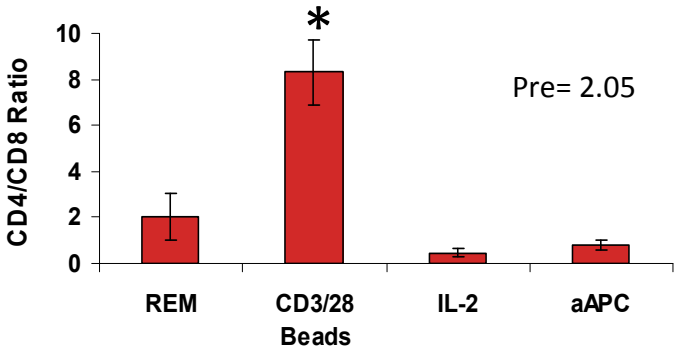

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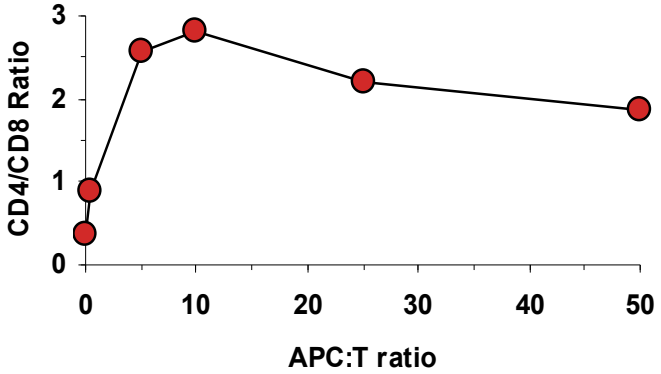
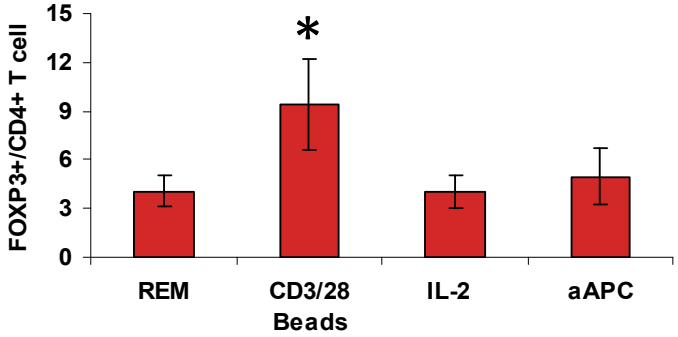


Favorable Treg (FOXP3+CD4+) cell frequency



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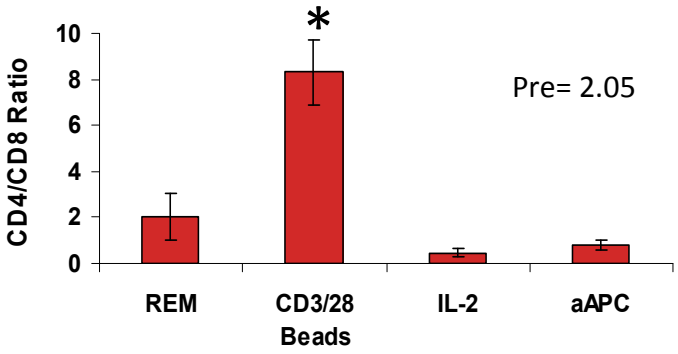
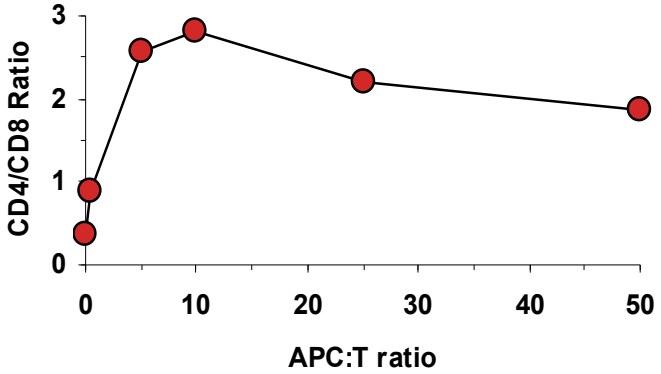
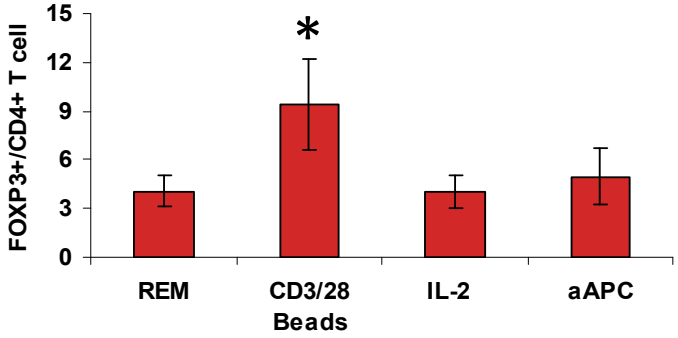
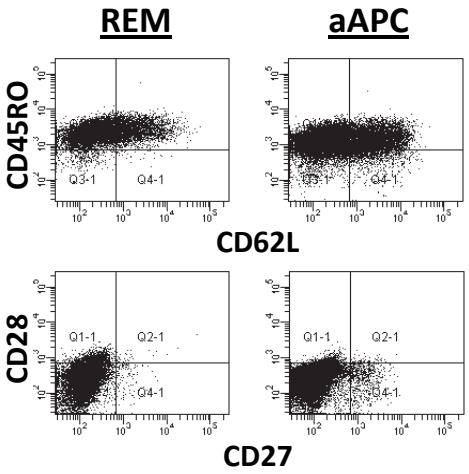


CD4:CD8 ratio is effected by aAPC: T ratio



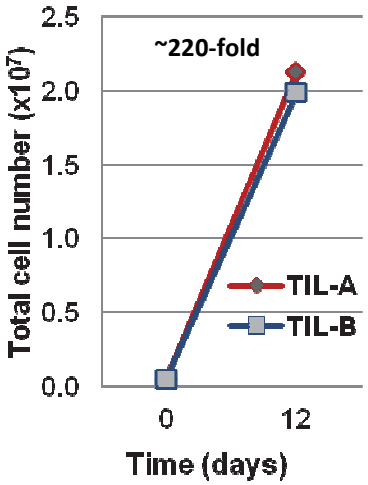
Favorable Treg (FOXP3+CD4+) cell frequency





Maintenance of tumor antigen-specific TILs after expansion with aAPCs

a



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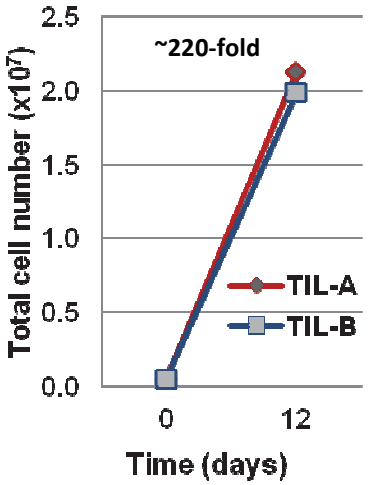

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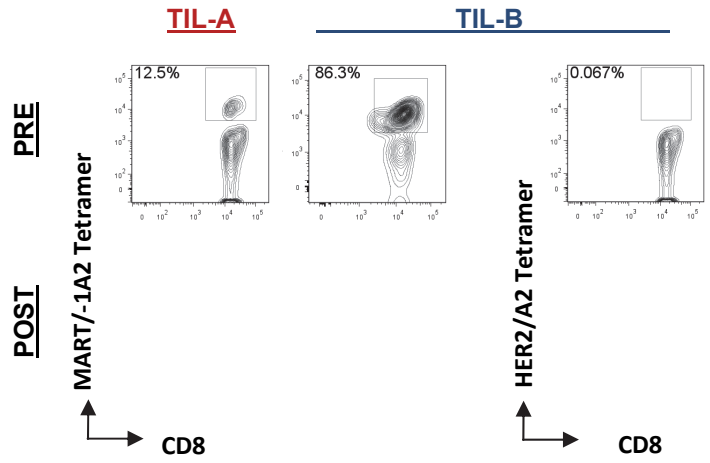
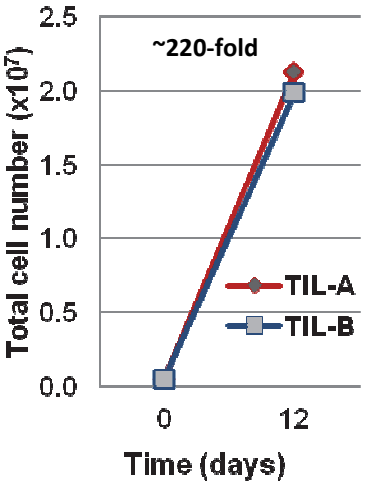


Maintenance of tumor antigen-specific TILs after expansion with aAPCs

a



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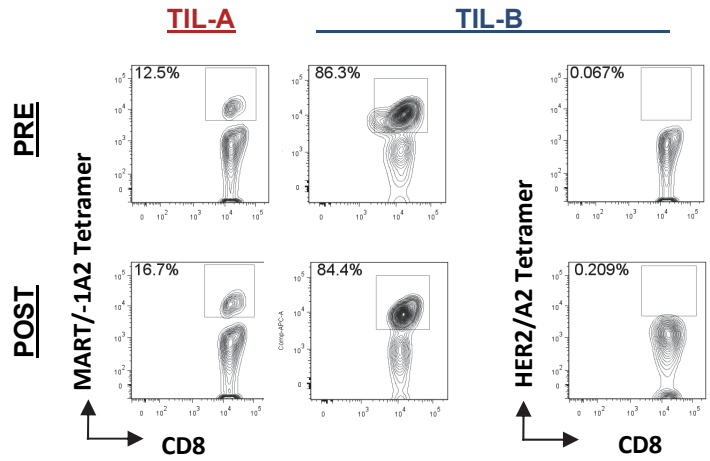
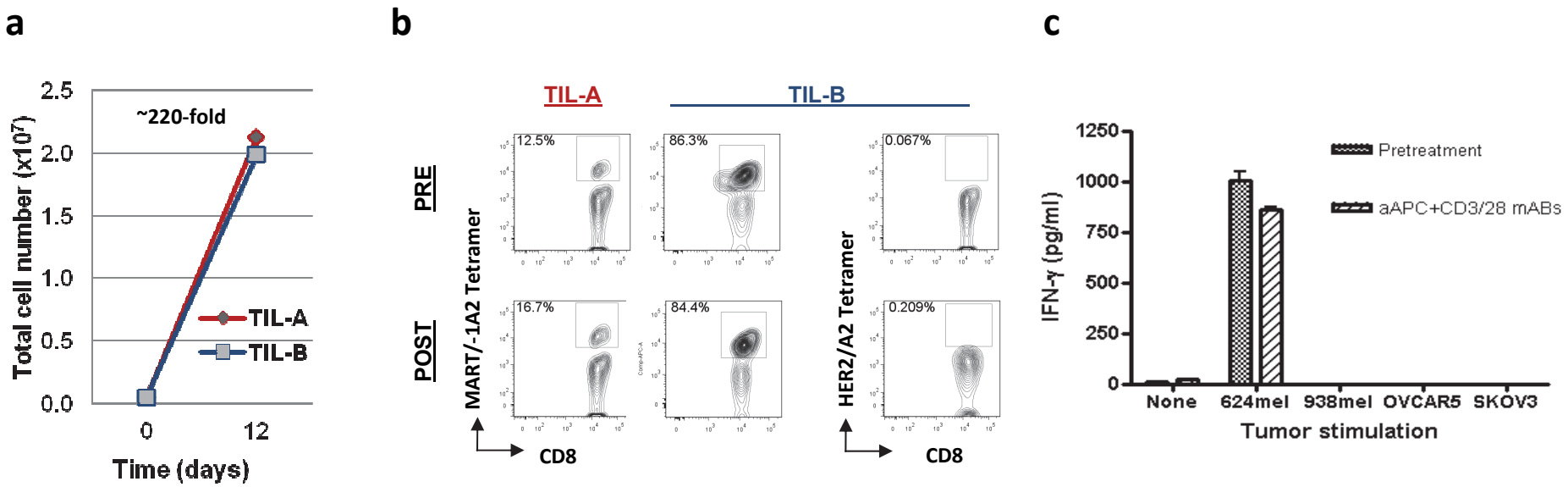
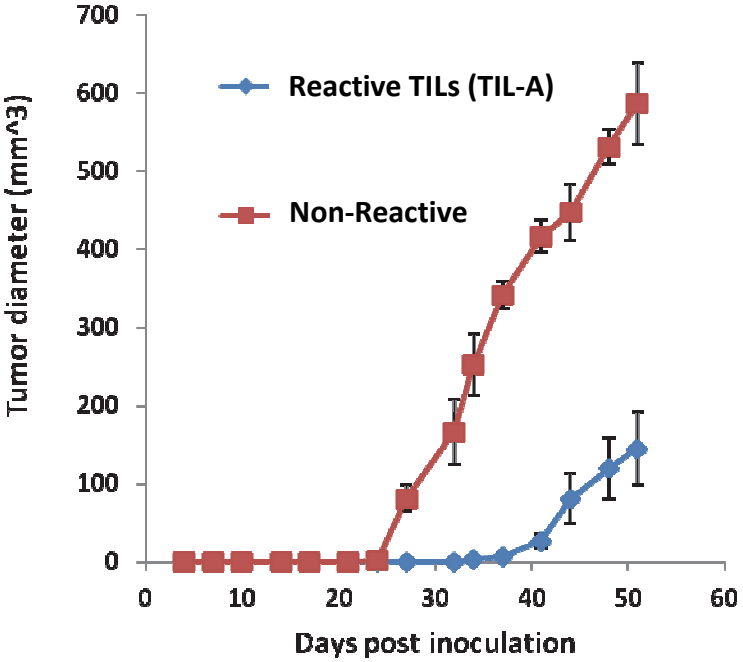
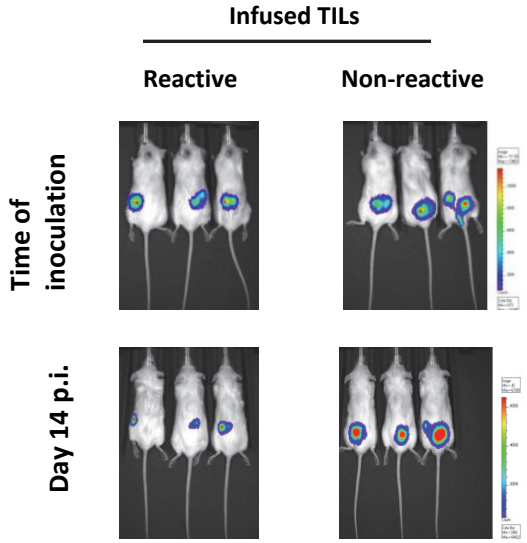


Maintenance of tumor antigen-specific TILs after expansion with aAPCs



Inhibition of tumor outgrowth using aAPC-expanded TILs





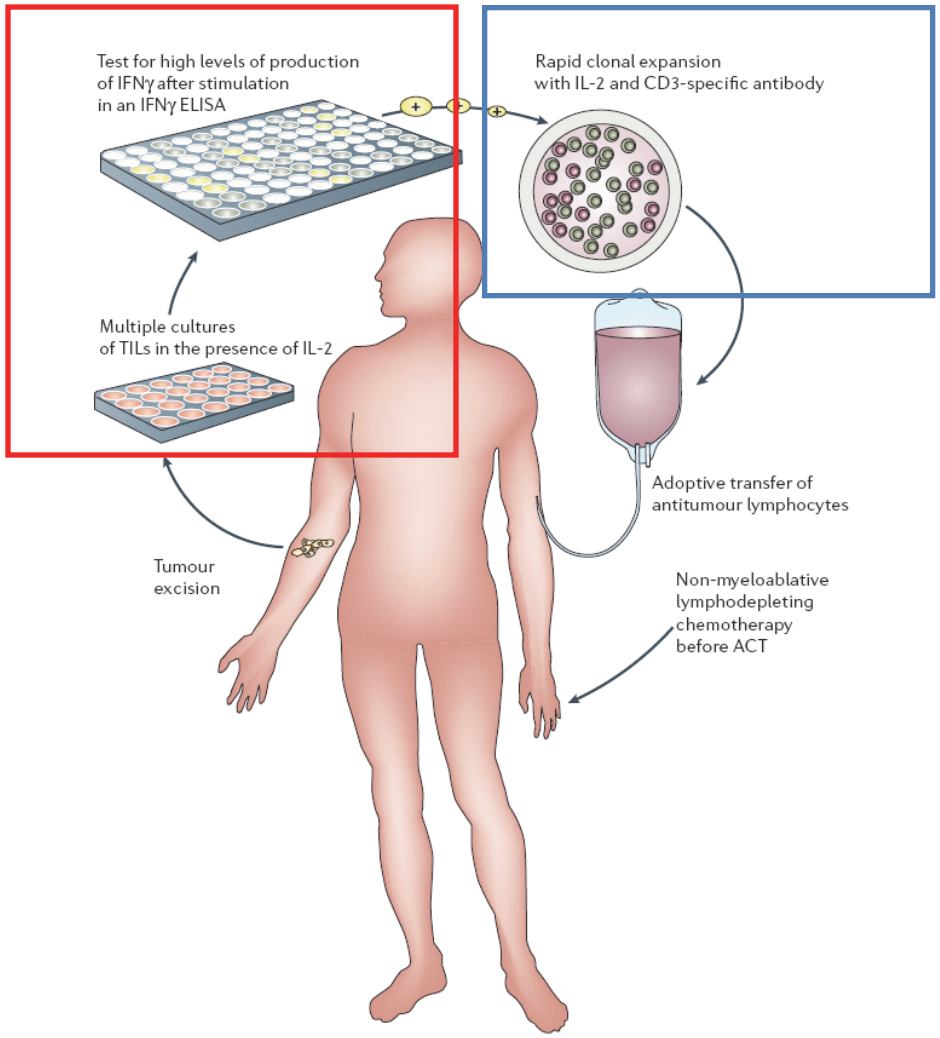
Initial SUMMARY

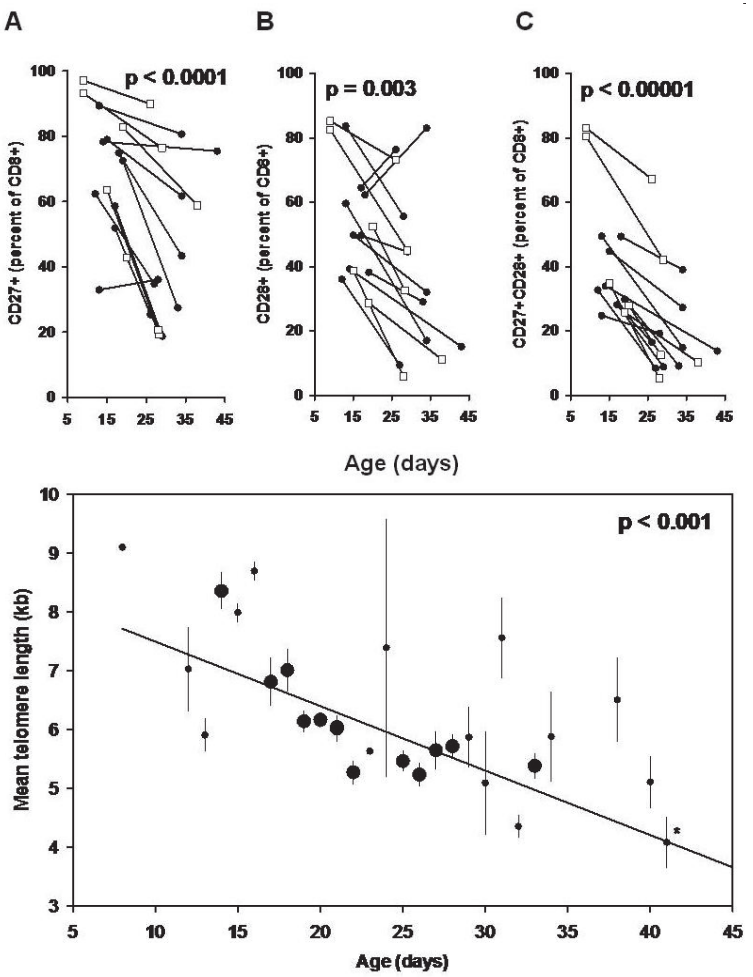
K652 cell-based aAPCs allow for the efficient expansion of IL-2 cultured TILs from solid cancer (and ascites); similar to REP.

- aAPC-expanded TILs have a favorable CD4/CD8 ratio, and low FOXP3+ CD4+ Treg cell number and frequency.

- Antigen-specific TILs with anti-tumor function are maintained following aAPC expansion.

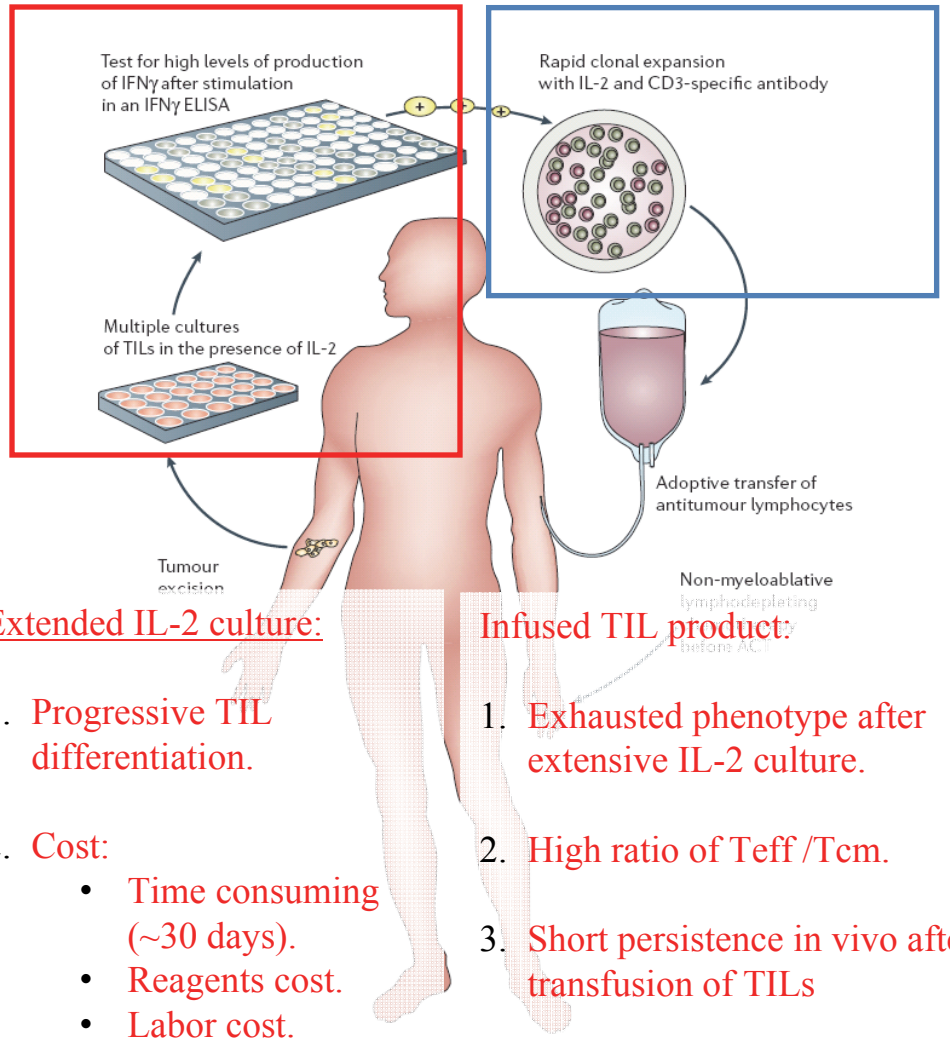
Tumor regression after administration of endogenous tumor-reactive T cells

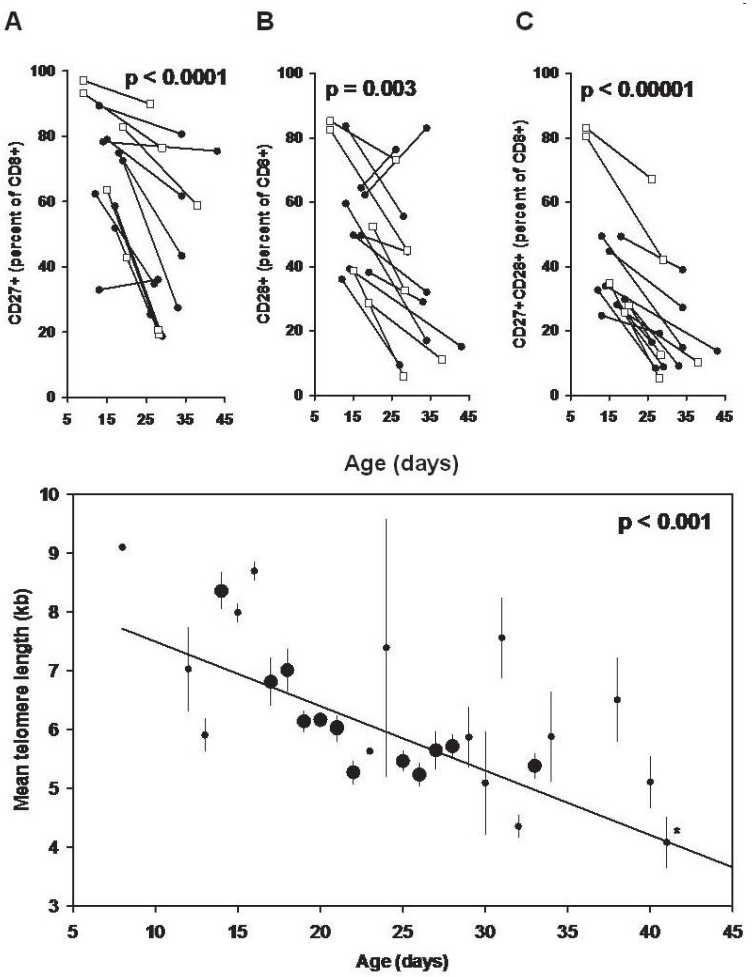




Tran, K et al J Immunother. 2008.

Tumor regression after administration of endogenous tumor-reactive T cells





Tran, K et al J Immunother. 2008.

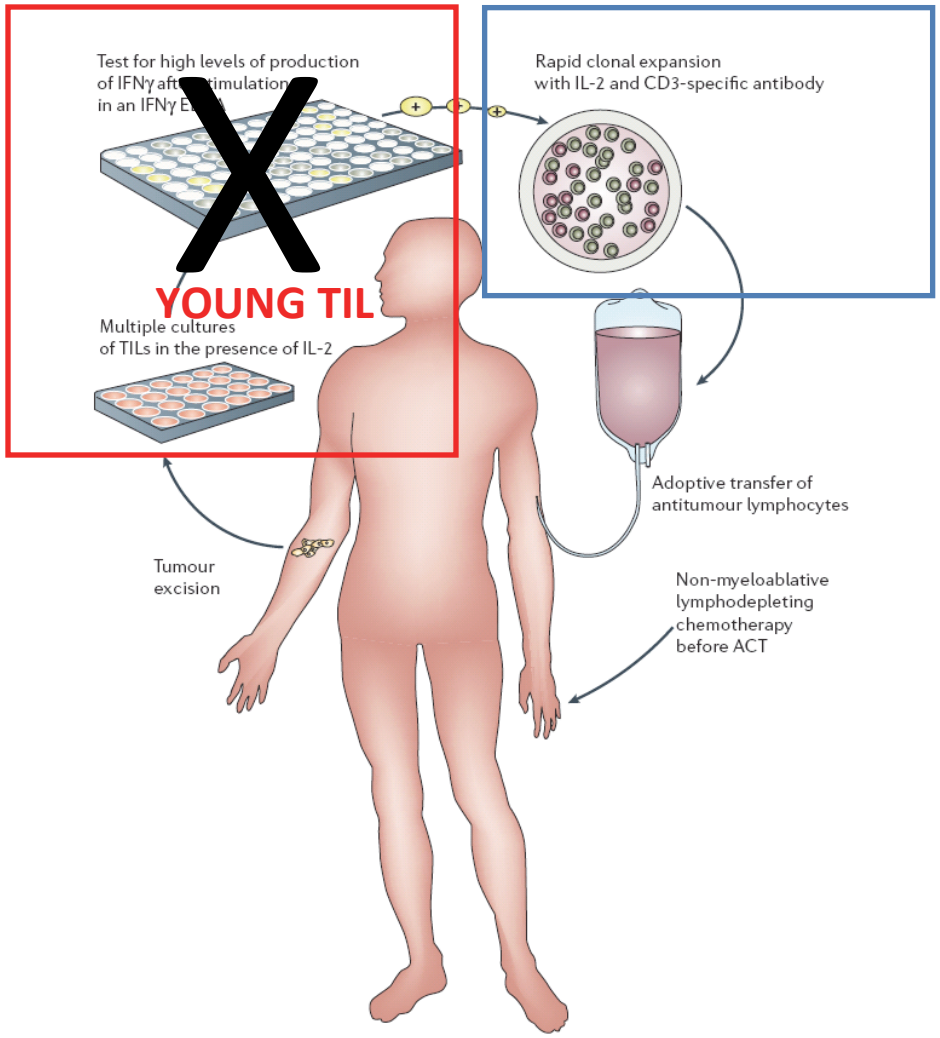
Extended IL-2 culture:

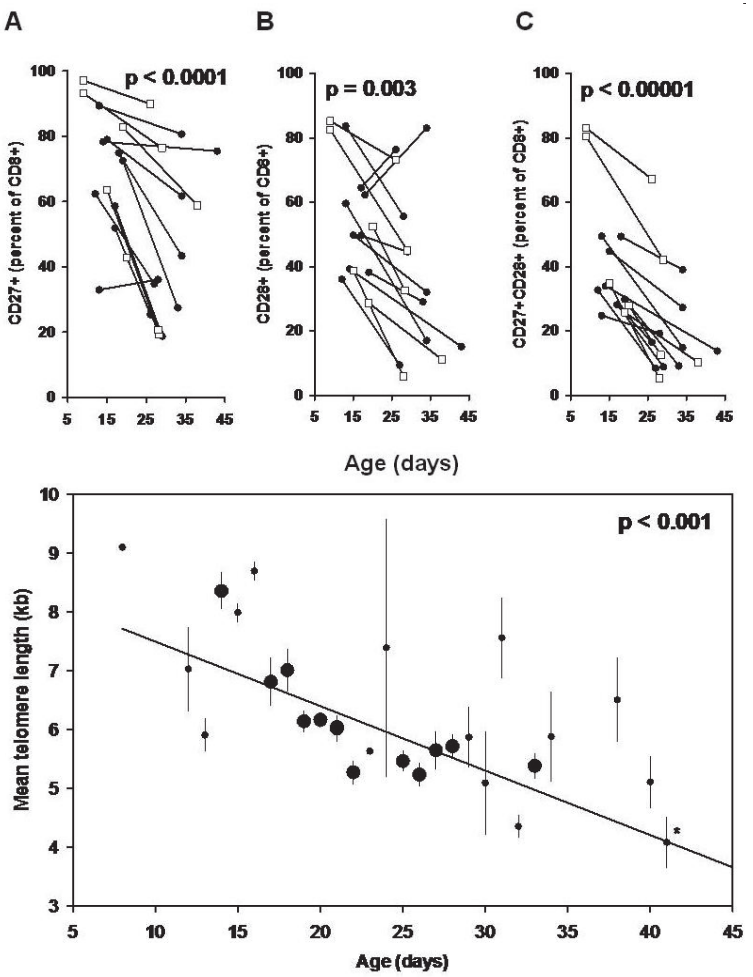
1. Progressive TIL differentiation.
2. Cost:
   - Time consuming (~30 days).
   - Reagents cost.
   - Labor cost.

Infused TIL product:

1. Exhausted phenotype after extensive IL-2 culture.
2. High ratio of Teff /Tcm.
3. Short persistence in vivo after transfusion of TILs

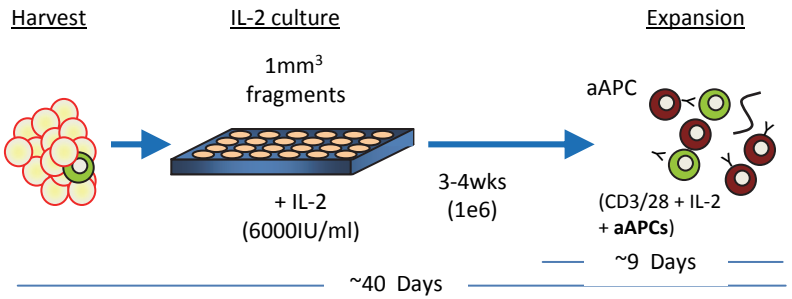
Tumor regression after administration of endogenous tumor-reactive T cells





Tran, K et al J Immunother. 2008.

Can “young” TILs be expanded directly from enzyme digested solid tumor?



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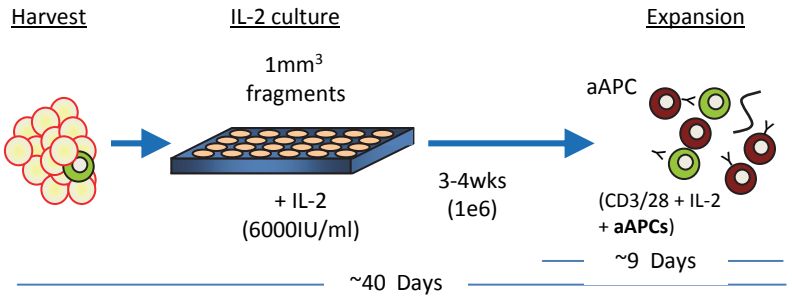

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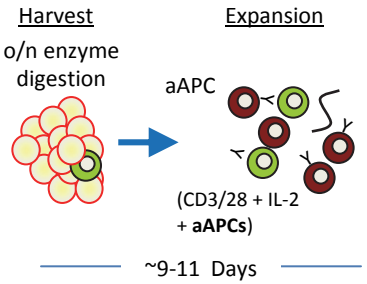

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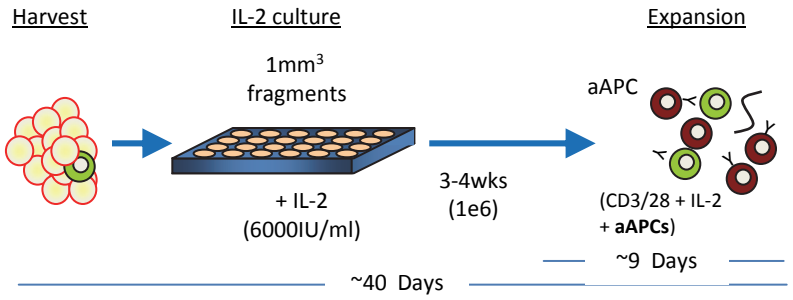

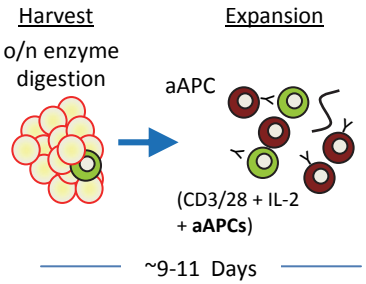

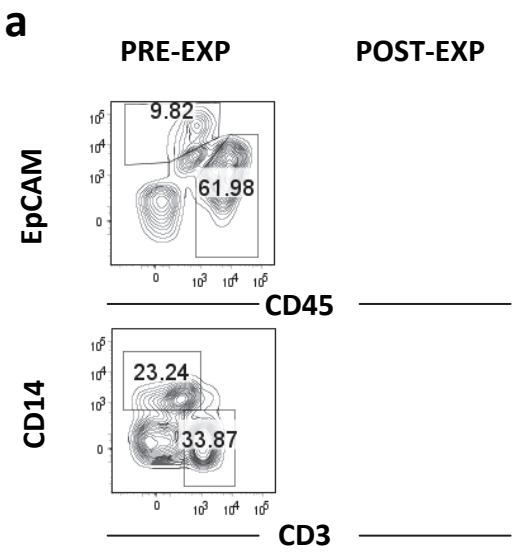

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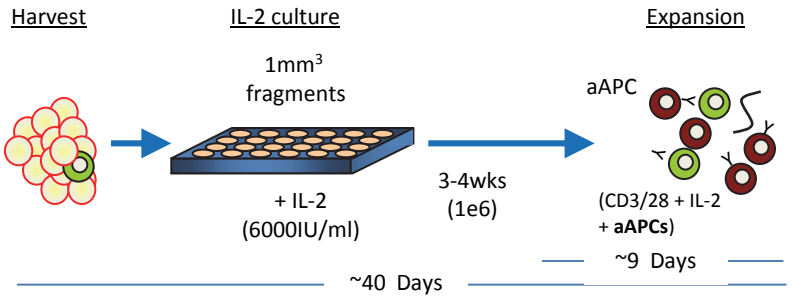
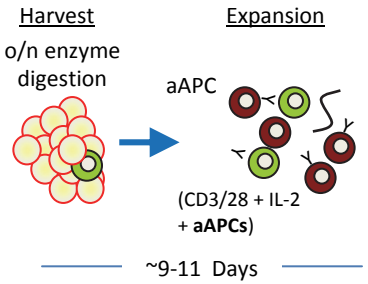
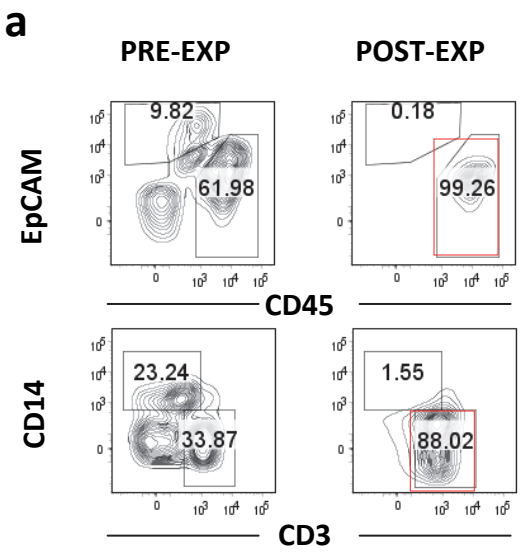
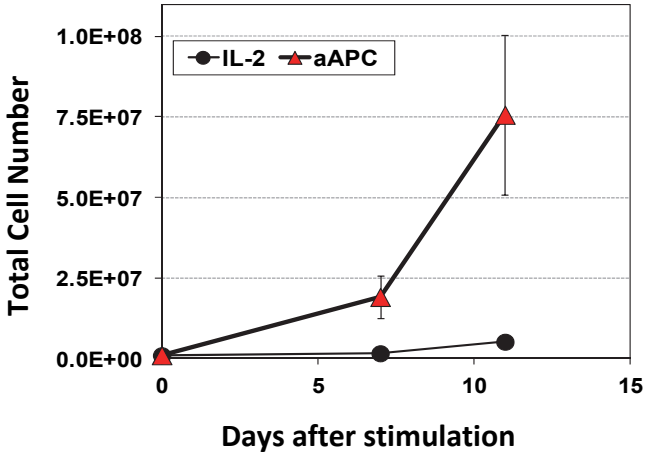




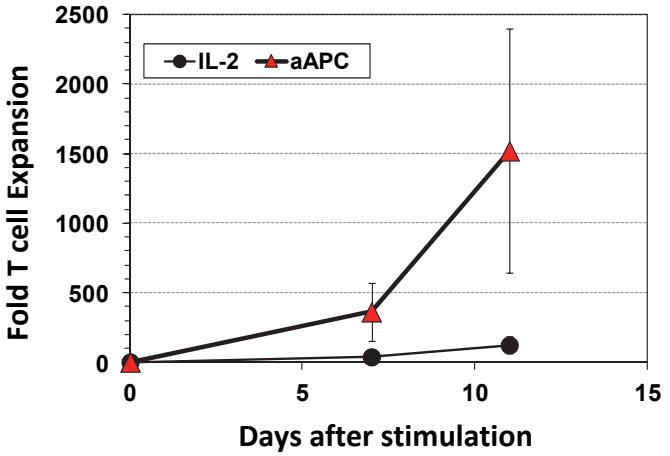


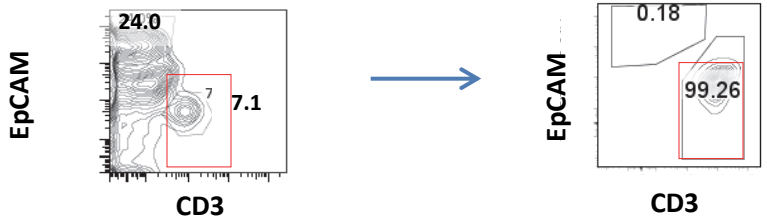
"Young" TILs can be expanded directly from enzyme digested solid tumor

Total Cell Number



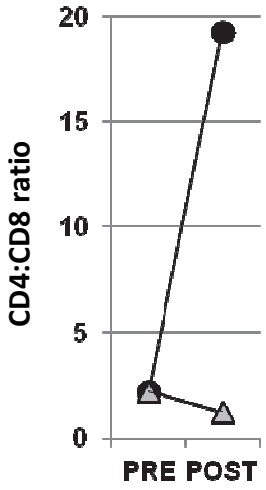
Fold T cell Expansion



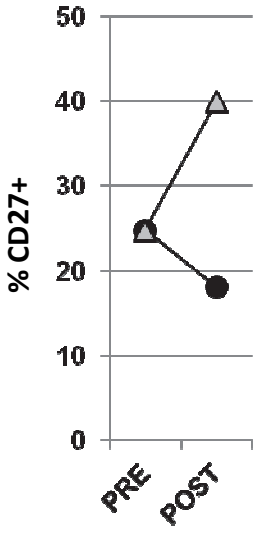


"Young" TILs have favorable phenotype

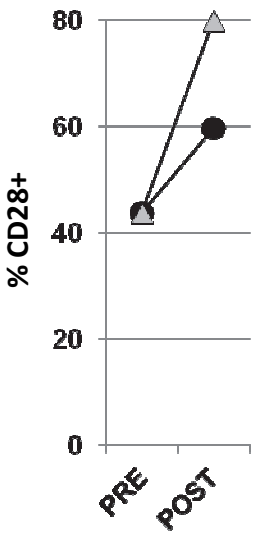
CD4:8 ratio



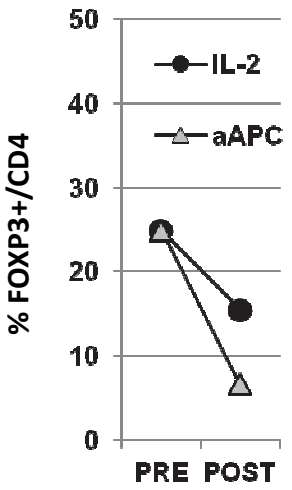
%CD27+



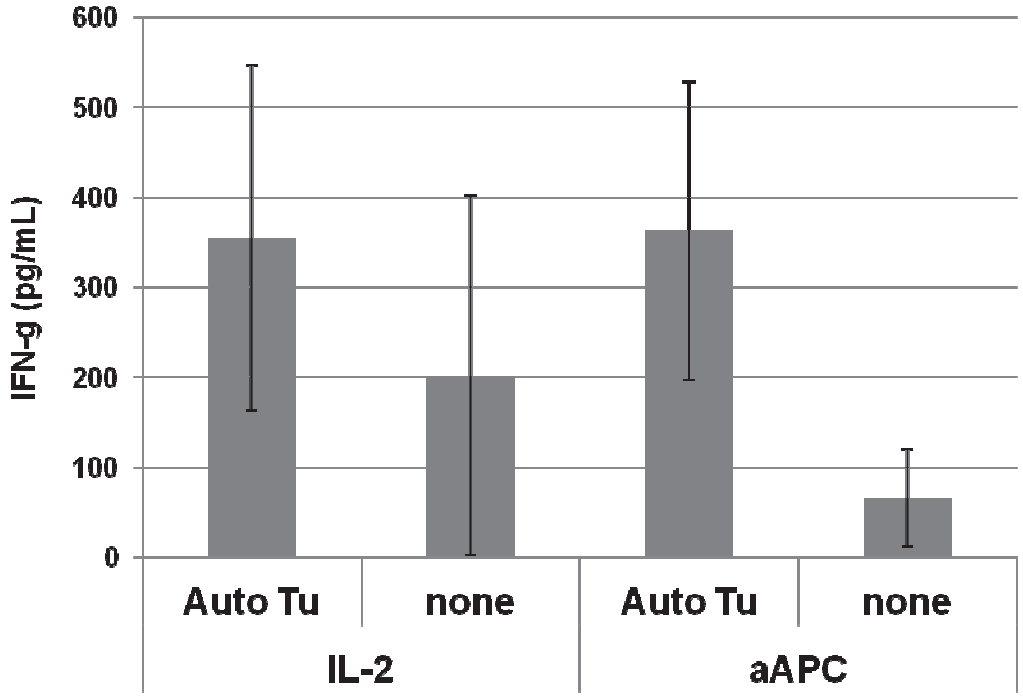
%CD28+



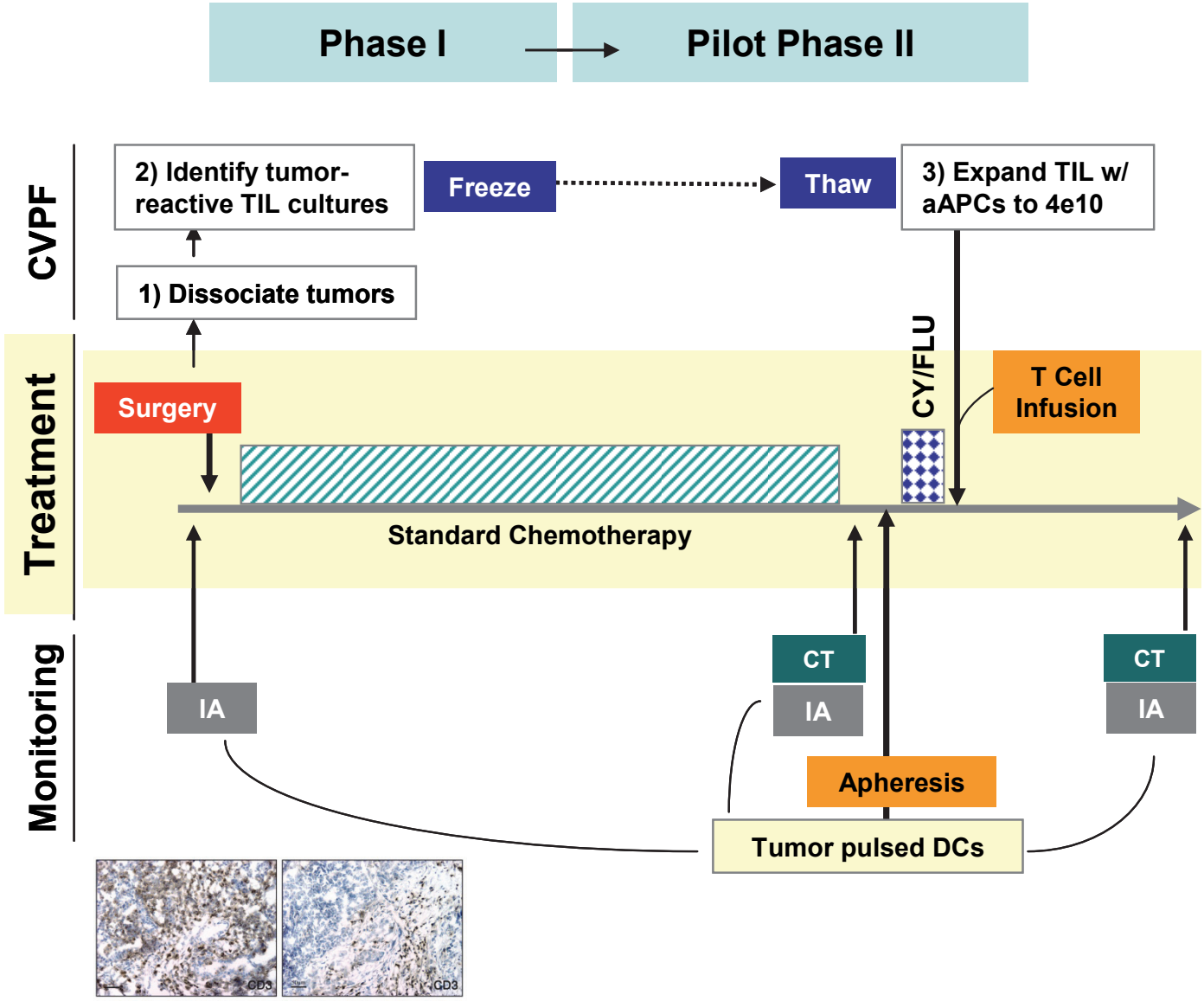
%FP3+CD4+



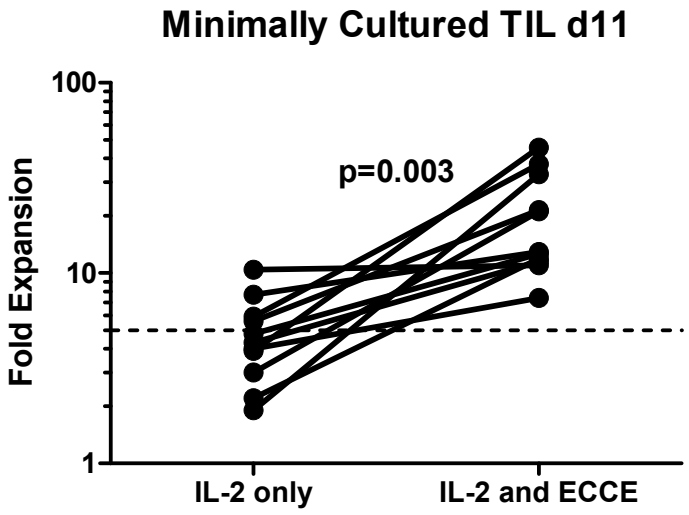
“Young” TILs can be expanded directly from enzyme digested solid tumor

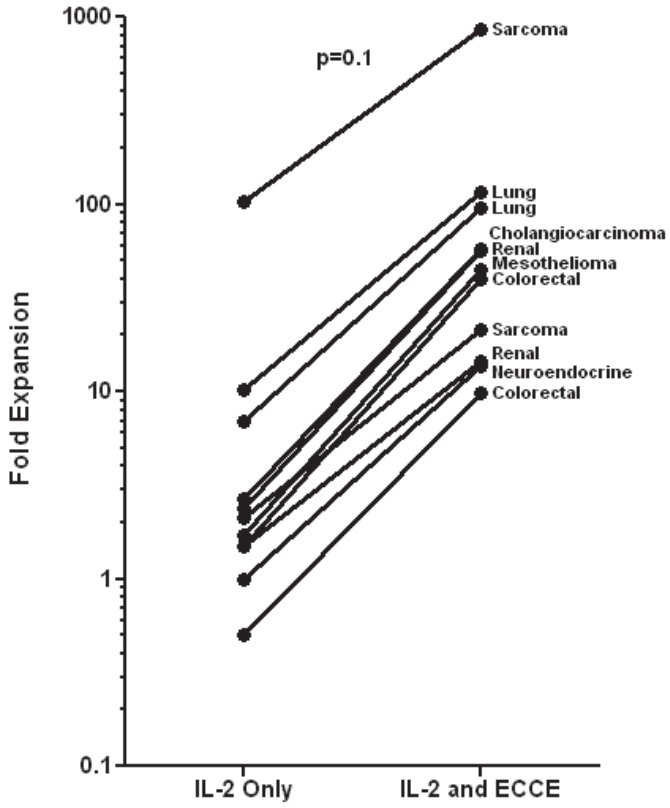


Adoptive T Cell Therapy for Ovarian Cancer Using TIL



ECCE expand human CD8+ TIL in vitro





Friedman KM, et al 2011, J Immunother 34:651-61
(Courtesy of Mark Dudley, NCI)

Summary

The engineered KT64/BBL aAPC line represents an attractive "off-the-shelf" platform for ex vivo TIL expansion.

- aAPC can efficiently expand tumor-reactive TILs and TALs that were established in long-term IL-2 culture, similar to REP, but at lower APC:TIL ratio. These TILs have low CD4/CD8 ratios and Treg numbers.

- "Young" TILs can be rapidly expanded directly from heterogenous cell suspensions established from solid tumor by enzymatic digestion using aAPC : ~1500 fold.

- TILs and TALs expanded by aAPC are comprised of favorable CD4:CD8+ and Foxp3 CD4+ ratios and express higher CD27, CD28 phenotypes than IL-2 cultured cells; favorable for use in adoptive immunotherapy for cancer.

Summary

- Cell-based aAPCs:
  - (i) can be grown to large number and cryopreserved for the establishment of master and working cell banks, thus meeting the needs of even the largest cell cultures,
  - (ii) reduce sample variability, preparative time requirements and regulatory issues that surround the use of donor PBMCs as a feeder cell source,
  - (iii) are amenable to further genetic engineering or antibody loading to broaden or fine-tune the spectrum of costimulatory or adhesion molecules expressed (to become single agent),
  - (iv) lack endogenous MHC expression thus eliminating issues of HLA-compatibility,
  - and (v) alleviate possible infectious agent concerns related to the use of donor PBMC as feeder cells.

Impact

aAPCs represent an efficient cellular platform for the rapid expansion of TILs or TALs:

- Reduced variability, increased flexibility.
- Reduced technical, regulatory, logistic challenges.
- Capacity to generate “young” TILs –improved cell survival.
- Potential cost savings.
- Exportable – more widespread application.

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