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.


Durable complete responses in heavily pretreated patients with metastatic melanoma

Survival of patients with metastatic melanoma treated with autologous TILs and IL-2

(median follow-up 62 mo)

REP for ex vivo expansion of TILs

Dudley ME, et al, JCO 2005
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)
REP for ex vivo expansion of TILs

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.

- Up to 1000-fold TIL expansion in melanoma.
- Maintenance of anti-tumor function by expanded T cells.
- Heterogeneous antigen specificity and subset (CD4/CD8)
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\textsuperscript{nd} stimulation.
  - Preferential CD4 expansion; substantial number of CD8\textsuperscript{+} 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)

P<0.001

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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**REP:**
OKT-3 anti-CD3 Ab (30ng/mL), allogeneic feeders (200-fold excess) from three independent donors and rhIL-2.
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/10⁶ cells at 4°C 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
Optimization for aAPC-based ex vivo expansion of IL-2 cultured TILs

2:1 aAPC: TIL Ratio

Dependent on anti-CD3 loading and IL-2
Optimization for aAPC-based ex vivo expansion of IL-2 cultured TILs

2:1 aAPC: TIL Ratio

Dependent of anti-CD3 loading and IL-2

OvTILs are CD28\textsuperscript{low}

...and effector memory phenotype
CD28 crosslinking impacts PBL, not TIL, expansion & IL-2 production
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CD28 crosslinking impacts PBL, not TIL, expansion & IL-2 production
Comparison to other conventional expansion methods

Cell Division

<table>
<thead>
<tr>
<th>Method</th>
<th>TILs</th>
<th>autoPBLs</th>
</tr>
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<tbody>
<tr>
<td>IL-2</td>
<td>[Graph]</td>
<td>[Graph]</td>
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<tr>
<td>CD3/28 beads</td>
<td>[Graph]</td>
<td>[Graph]</td>
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<tr>
<td>aAPC (0.5)</td>
<td>[Graph]</td>
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<tr>
<td>aAPC (2)</td>
<td>[Graph]</td>
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<tr>
<td>aAPC (5)</td>
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<tr>
<td>REM</td>
<td>[Graph]</td>
<td>[Graph]</td>
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</tbody>
</table>

CFSE (day 6)
Comparison to other conventional expansion methods

**Cell Division**

*Method*
- IL-2
- CD3/28 beads
- aAPC (0.5)
- aAPC (2)
- aAPC (5)
- REM

**Expansion**

*Comparison to other conventional expansion methods*
- Single round; Day 9-11 counts (n=6)
Comparison to other conventional expansion methods

Cell Division

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<td>IL-2</td>
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Expansion

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

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<tr>
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<th>CD3/28</th>
<th>IL-2</th>
<th>None</th>
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<td>Fold expansion</td>
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<td></td>
<td>≈200</td>
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200:1 **2:1**
Comparison to other conventional expansion methods

Cell Division

Expansion

Secondary Expansion

Comparison to other conventional expansion methods

Cell Division

Expansion

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
Favorable TIL subsets following expansion with aAPC

**CD4:CD8 Ratio**

- REM
- CD3/28 Beads
- IL-2
- aAPC

**CD4:CD8 ratio is effected by aAPC: T ratio**

**Favorable Treg (FOXP3+CD4+) cell frequency**
Favorable TIL subsets following expansion with aAPC

CD4:CD8 Ratio

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

![Graph showing maintenance of tumor antigen-specific TILs after expansion with aAPCs](image)
Maintenance of tumor antigen-specific TILs after expansion with aAPCs

(a) Graph showing cell number over time (days) with ~220-fold increase.

(b) Flow cytometry images of TIL-A and TIL-B showing CD8 expression and MART/IA2, HER2/A2 tetramers.
Maintenance of tumor antigen-specific TILs after expansion with aAPCs
Maintenance of tumor antigen-specific TILs after expansion with aAPCs

(a) Graph showing the total cell number (x10⁶) over time (days) for TIL-A and TIL-B, with a ~220-fold increase after 12 days.

(b) Flow cytometry images showing the percentage of CD8+ T cells positive for MART1/A2 Tetramer and HER2/A2 Tetramer in TIL-A and TIL-B before (PRE) and after (POST) expansion.

(c) Graph showing IFN-γ levels (pg/ml) after tumor stimulation with different cell lines (No 624 mel, 935 mel, OVCAR3, SKOV3) with Pretreatment and aAPC+CD3/28 mAbs conditions.
Inhibition of tumor outgrowth using aAPC-expanded TILs
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

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

Tran, K et al J Immunother. 2008.
Tumor regression after administration of endogenous tumor-reactive T cells

Tran, K et al J Immunother. 2008.

Test for high levels of production of IFNγ after stimulation in an IFNγ ELISPOT ELISA

Rapid clonal expansion with IL-2 and CD3-specific antibody

Multiple cultures of TILs in the presence of IL-2

Adoptive transfer of antitumor lymphocytes

Tumor excision

Non-myeloablative lymphodepleting chemotherapy before ACT

A

CD25+ (percent of CD8+ T cells)

B

CD38+ (percent of CD8+ T cells)

C

CD71+CD57+ (percent of CD8+ T cells)

Age (days)

Age (days)

Mean telomere length (kb)

Age (days)

p < 0.0001

p = 0.003

p < 0.0001

p < 0.001

Tran, K et al J Immunother. 2008.
Can “young” TILs be expanded directly from enzyme digested solid tumor?

Harvest → IL-2 culture → Expansion

- 1mm³ fragments
- + IL-2 (6000IU/ml)
- 3-4wks (1e6)
- (CD3/28 + IL-2 + aAPCs)

~40 Days

~9 Days
Can “young” TILs be expanded directly from enzyme digested solid tumor?

Harvest

IL-2 culture

1 mm³ fragments

+ IL-2
(6000 IU/ml)

3-4wks
(1e6)

Expansion

a

~40 Days

Harvest

O/n enzyme digestion

Expansion

a

~9-11 Days

aAPC

(CD3/28 + IL-2 + aAPCs)
Can “young” TILs be expanded directly from enzyme digested solid tumor?

**Harvest**
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**IL-2 culture**
- 3-4wks
- ~9 Days

**Expansion**
- aAPC
- ~9-11 Days

**Harvest**
- o/n enzyme digestion

**Expansion**
- aAPC
- ~9 Days

**EpCAM**
- PRE-EXP
- POST-EXP

**CD45**
- PRE-EXP
- POST-EXP

**CD14**
- PRE-EXP
- POST-EXP

**CD3**
- PRE-EXP
- POST-EXP
Can “young” TILs be expanded directly from enzyme digested solid tumor?

Harvest

IL-2 culture

Expansion

1mm³ fragments

+ IL-2 (6000IU/ml)

3-4wks (1e6)

aAPC

(Y)

YaAPC (CD3/28 + IL-2 + aAPCs)

~40 Days

~9 Days

Harvest

o/n enzyme digestion

Expansion

aAPC

~9-11 Days

PRE-EXP

POST-EXP

EpCAM

CD45

CD14

CD3

9.32

61.98

23.24

33.87

0.18

99.26

1.55

88.02
“Young” TILs can be expanded *directly* from enzyme digested solid tumor

**Total Cell Number**

- **Fold T cell Expansion**

- **EpCAM**
  - 24.0
  - 7.1

- **CD3**
  - 0.18
  - 99.26
“Young” TILs have favorable phenotype

CD4:8 ratio  %CD27+  %CD28+  %FP3+CD4+

![Graphs showing changes in CD4:8 ratio, %CD27+, %CD28+, and %FP3+CD4+ before and after treatment with IL-2 and aAPC.](image-url)
“Young” TILs can be expanded directly from enzyme digested solid tumor

![Graph showing IFN-γ levels with Auto Tu and none conditions for IL-2 and aAPC](chart.png)
Adoptive T Cell Therapy for Ovarian Cancer Using TIL

1) Dissociate tumors

2) Identify tumor-reactive TIL cultures

3) Expand TIL w/ aAPCs to 4e10

Freeze

Thaw

Phase I → Pilot Phase II

Surgery

Standard Chemotherapy

CT

Thaw

IA

CT

Apheresis

T Cell Infusion

Tumor pulsed DCs

CVPF

Monitoring

IA

IA

IA

IA
ECCE expand human CD8+ TIL in vitro

(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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Caitlin Stashwick

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Jim Riley
Megan Suhoski
George Coukos
Lana Kandalaft
Mark Dudley, NCI
Steven Rosenberg, NCI

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YOUR BODY'S OWN CELLS CAN FIGHT YOUR CANCER

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