Strategies to Augment Antitumor Efficacy by Inducing Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

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Disclosure:
Neither I nor any member of my family has a financial relationship or interest with any proprietary entity producing health care goods or services related to the content of this presentation.
Our Goal: Use “off-the-shelf” broadly applicable agent(s) that can effectively engage all these mechanisms.
IL2 activates NK cells to kill neuroblastoma cells coated with an anti-GD2 mAb (ADCC)

1. NK cells from healthy donors kill best with IL2 AND anti-GD2 mAb
2. NK cells from cancer patients receiving IL2, kill best with anti-tumor antibody AND IL2

These in vitro studies showed that adding IL2 + mAb augments NK-mediated ADCC

How to move this into effective clinical treatment? (Phase I/II studies at UWCCC and COG)
COG’s approach to Innate Immunity and ADCC for NBL (ANBL0032)

1. Activate Multiple Pathways of ADCC (ie: stimulate and engage several different populations of ADCC effector Cells)
2. Administer Immunotherapy in Minimal Residual Disease [ie: patients in remission, at risk of relapse, *to circumvent poor penetration, Tregs, myeloid derived suppressor cells (MDSCs)*]
Event Free Survival for 226 Children Treated Immunotherapy vs No Immunotherapy

- New Standard” for Neuroblastoma
- Dinutuximab (ch14.18 mAb) approved by FDA March 2015
- Event-free survival (for patients that enter remission) still only 60%; more improvements needed (for patients in remission, and those that don’t achieve remission)!
Tumor Cell

Melanoma or Neuroblastoma

Hu14.18-IL2 a genetically engineered fusion protein linking IL2 to hu14.18 mAb

S. Gillies and R. Reisfeld
PNAS 89:1428, 1992

GD2 Antigen

IL-2 Receptor

hu14.18-IL2

T Cell or NK Cell
Improving outcome in the setting of MRD (remission)

Efficacy of ch14.18-IL2 Immunocytokine against Murine Neuroblastoma Liver Metastases

Effective anti-GD2 Immunotherapy: Dependence on Minimal Tumor Status
Neal ZC, et al Clinical Cancer Research, 10:4839-4847, 2004

hu14.18-IL2 (10ug/d) for 5 days starting on day 5, 7, 9, or 11 following 5 X 10^5 NXS2 cells injected on day 0, and harvested on day 28.
IC is a tri-functional targeting agent, binding via GD2, Fc and IL2.

NKL cells have IL2Rs but no FcRs.
All IL2Rs on NKLs localize to immune synapse induced by hu14.18-IL2

Form conjugates with NKL + M21 + HU14.18-IL2, Then stain IL2Rs with anti-CD25 mAb.

Proves that all IL2Rs on NKL cells go to synapse

Suggests that hu14.18-IL2 mediates:
Conventional ADCC, and
IL2R-facilitated ADCC

Gubbels, Buhtojarov et al: CII, 2011
COG Phase II NBL Trial**- includes minimal residual disease (MRD)

Stratum*

- **Stratum 1**: residual/refractory NBL measurable by standard radiographic criteria

- *Stratum 2*: residual/refractory NBL not measurable by standard radiographic criteria, but evaluable by MIBG scanning or by bone marrow histology

Hu14.18-IL2 as a MRD agent

- **Stratum 1**: 0 of 13 patients respond
- **Stratum 2**: 5 of 24 patients with CR, (+ 2 with clear improvement)

7 (improved) of 24 (stratum 2) > 0 of 13 (stratum 1)  
\((p= 0.03)\) as hypothesized by preclinical data

**IMPLICATION**: Clinical studies confirm biology from preclinical studies IF the clinical study simulates the setting of the preclinical trial.


Shusterman S. et al, ASCO 2015; follow up phase-II clinical study with similar activity.
Preclinical and clinical conclusions from IV ADCC therapy of solid tumors

1. Systemic delivery is most effective against microscopic disease (MRD)
Increase efficacy of IC against macroscopic solid tumors:

1. Local delivery (intratumoral injection),
2. Combine IC with immunomodulators

3. Goal: *Make the Tumor an In Situ Vaccine*
IT IC is More Effective than IV IC in the Treatment of Murine Melanoma

Conclusion: better response with IT than IV IC (5 daily doses). (Johnson et al, Canc. Imm. Immunother. 57:1891, 2008)
Intratumoral hu14.18-IL2 is distinguished by Many Increased Tumor (NXS2-NBL) Infiltrating Lymphocytes (TILs)

Representative immunohistochemical (IHC) stains of subcutaneous NXS2 tumor frozen sections were digitally photographed. Sections were stained and quantification of IHC pictures used the manual counting method. IT-IC treated tumors are characterized by higher tumor leukocyte infiltration percentages (CD45+, CD3+, CD8a+, NKG2A+, F4/80+) than IT-PBS and untreated tumors. (Results confirmed also by flow cytometry studies- not shown).

Yang RK et al. JI. 189:2656, 2012
T cell and NK cell Depletion Disrupts Full IT-IC Induced Anti-NXS2 NBL Effects

T cell or NK cell depleted, but IT-IC treated mice bearing NXS2 tumor are characterized by increased tumor growth and worse survival outcomes compared to non-depleted IT-IC treated mice bearing NXS2 tumor. (C) Kaplan-Meier survival curves of IT-IC treated subcutaneous NXS2, with and without NK and T cell depletion.

Yang RK et al. JI. 189:2656, 2012
IT-IC Shows 100-fold Augmented IC Localization and Increased IC Retention Compared to IV-IC

Tumor-bearing mice given hu14.18-IL2 IT or IV were sacrificed at varying times and their tumors disaggregated. (A) Flow cytometric measurements of levels of human IgG FcG antibody fragment on NXS2 tumor cells ex-vivo at various times post treatment. (B) Flow cytometric measurements of levels of human IL-2 on NXS2 tumor cells ex-vivo at various times post treatment. All values of MFI (mean fluorescent intensity) are normalized to an intratumoral non-specific control immunocytokine (IT-KS-IL-2).

Yang RK et al. Jl. 189:2656, 2012
Can augmented activity to macroscopic disease be obtained by combination with local therapy (ie: local radiation therapy)?

Zach Morris MD PhD
Experimental design

**Tumor cells**

**B78 melanoma** – poorly immunogenic line derived from B16 melanoma by stable transfection with β-1,4-N-acetylgalactosaminyltransferase inducing a constitutive plasma membrane expression of GD2

**5 weeks**

Tumor cells injected in syngeneic mice

**DAYS 1.** Macroscopic tumors radiated to 12 Gy

**DAYS 6-10.** Daily intra-tumor injections of IC

Tumor growth and animal survival monitored
Cooperative interaction of radiation and intratumoral injection of tumor-specific antibody or immunocytokine

**REQUIRES:**
1. GD2 antigen
2. FCY-receptor
3. FC mAb
4. NK cells

**REQUIRES:**
1. GD2 antigen
2. FCY-receptor
3. T cells

**No effect on:**
1. *In vitro* clonogenic survival
2. GD2 expression
3. Animal weight

**Complete tumor regression:**
73% with 12 Gy + hu14.18-IL2
14% in 12 Gy + hu14.18
0% in other groups
12 Gy + hu14.18-IL2 improves animal survival

* p < 0.001

B78 Melanoma
Radiation: 12 Gy x 1 – Day 1
mAb and IC: 50 µg /mouse IT - daily Days 6-10
Radiation and IT hu14.18-IL2 results in cure of most 5-week (200mm³) B78 tumors

**73% (11/15) of mice had durable complete tumor regression vs. none of the control mice**

* p < 0.05

Day 30 mean tumor volume (mm³) +/- SE

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Tumor Volume (mm³) +/- SE</th>
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<tbody>
<tr>
<td>12 Gy + hu14.18-IL2</td>
<td>1161 +/- 233</td>
</tr>
<tr>
<td>12 Gy + IgG</td>
<td>619 +/- 106</td>
</tr>
<tr>
<td>hu14.18-IL2</td>
<td>462 +/- 29</td>
</tr>
<tr>
<td>IgG</td>
<td>61 +/- 26**</td>
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</tbody>
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Log-rank p < 0.001

n = 15

n = 9

n = 9
XRT + hu14.18-IL2 increases tumor infiltration by CD8+ T cells

Day 12 post radiation
B78 melanoma tumors

*p < 0.05
Mice rendered disease-free by treatment with radiation and hu14.18-IL2 exhibit a tumor-specific memory T cell response.

B16 is parent to B78, but is GD2- (9 of 12 mice that resist B78 now resist B16)
Panc02 is syngeneic to but a distinct tumor from B16/B78
Cancer is seldom an isolated primary tumor. What about metastases? The abscopal response to radiation is thought to be immune-mediated

“The abscopal effect”

- Mole, Br J Radiology 1953
A second un-radiated tumor suppresses the primary tumor response to radiation and hu14.18-IL2

Primary (treated) tumor responses

Radiation: 12 Gy x 1 - Day 1
hu14.18-IL2: 50 µg/mouse daily - Days 6-10

* p < 0.001
Tumor-specific suppression of primary tumor response to radiation and intratumoral hu14.18-IL2 by a second non-treated site of disease

Primary (treated) tumor responses

- B78 Melanoma
- Panc02

* p < 0.001
Inhibition of primary tumor response to combined radiation and intratumoral hu14.18-IL2 may be overcome by secondary tumor radiation

Radiation: 12 Gy x 1 – Day 1
hu14.18-IL2: 50 µg /mouse IT - daily Days 6-10

* p < 0.001
Regulatory T cells from a distant site of disease may suppress the response to combined radiation and intratumoral hu14.18-IL2:

**Circumvention by Treg depletion**

*C57BL/6-Tg(Foxp3-DTR/EGFP)23.2Spar/Mmjax* “DEREG mice”

B78 Melanoma

Radiation: 12 Gy x 1 – Day 1

hu14.18-IL2: 50 µg /mouse daily – Days 6-10

Diphtheria toxin: 1 µg IP – Day 1
Inhibition of primary tumor response to combined radiation and intratumoral hu14.18-IL2 may be overcome by Treg depleting (IgG2a) anti-CTLA-4
Combined radiation, intratumoral hu14.18-IL2 and checkpoint blockade with anti-CTLA4 antibody can augment local and systemic response.

**Primary tumors**

<table>
<thead>
<tr>
<th>Radiation: 12 Gy x 1 – Day 1</th>
<th>Alive at 60 days: 12 Gy + hu14.18-IL2 + CTLA4 – 75% (12/16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hu14.18-IL2: 50 µg /mouse IT - daily Days 6-10</td>
<td>12 Gy + CTLA4 – 30% (3/10)</td>
</tr>
<tr>
<td>anti-CTLA4 IgG2A: 200 µg /mouse IP – Days 3, 6, 9</td>
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</table>
Overall Conclusions: ADCC/Cytokine-Based Immunotherapies for Solid Tumors

• Clinically effective in microscopic setting (COG study)
• Preclinically, synergize with checkpoint blockade (anti-CTLA4), and other immunomodulators (Radiotherapy)
• Combined treatments can:
  – Activate innate and adaptive immunity
  – Overcome tumor induced immune suppression
  – Use existing tumors as in situ vaccines
• Clinical translation is being planned
• While most of our work involves anti-GD2 mAb in Melanoma and/or Neuroblastoma these results should translate to other tumor-specific mAbs
Collaborators in our Immunotherapy Research: 2016

- **UWCCC (partial list)**
  - J Hank
  - M Albertini
  - E Ranheim
  - A Rakhmilevich
  - J Gan
  - KM Kim
  - Z Morris
  - J Kimball
  - M Patankar
  - K DeSantes
  - R Yang
  - A Erbe
  - K McDowell
  - C Capitini
  - M Otto
  - W Wang
  - Z Perez-Horta
  - A Hofges
  - P Harari
  - Several Energetic Undergrads

- **C.O.G. (Many Pediatric Oncologists)**
  - S Shusterman
  - A Yu
  - J Maris
  - J Park
  - W London
  - R Seeger

- **St. Jude**
  - F Navid
  - V Santana
  - W Furman

- **Provenance**
  - S Gillies

- **BMS**
  - Alan Korman

- **Apeiron**
  - H Loibner
  - O Mutschlechner

- **Scripps**
  - R Reisfeld

**Current Research Support**
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- Alex’s Lemonade Stand
- MACC Fund
- INBRACED/SKC Foundation
- University of Wisconsin ICTR Grant

INBRACED Consortium
- J. Gray
- M. Gaze
- H. Lode
Front Row (L to R): J. Hank, P. Sondel, A. Rakhmilevich, J. Gan
University of Wisconsin’s Childhood Cancer Reunion

**KIDS WITH COURAGE V**

September 29, 2013
Kalahari Resort and Convention Center
Wisconsin Dells, WI

**PROOF THAT CANCER RESEARCH MAKES A DIFFERENCE!**

Our Goal: Use Improved Therapy (like Immunotherapy) to help cure cancer for many more children (and adults)!
Preclinical Conclusions for hu14.18-IL2

1. NK cells and T cells can be involved in the response
2. Antibody Dependent Cellular Cytotoxicity (ADCC) is involved
3. Efficacy in MRD setting

4. 14.18-IL2 is more effective than 14.18 + IL2

WHY?
Flow cytometric detection of IC- facilitated conjugates between NKL cells (FcR-negative / IL2R-pos) and M21 (GD2-pos) requires IC and IL2Rs.

Buhtoiarov IN, Neal ZC, Jan J, Buhtoiarova TN, Hank JA, Yamane B, Rakhmilevich AL, Patankar MS, Gubbels JAA, Reisfeld RA, Gillies SD, Sondel PM. J. Leukocyte Bio. 2011
Hu14.18-IL2 (FITC) localizes at immune synapse of NKL-M21 conjugates

Form conjugates with Hu14.18-IL2-FITC + NKL + M21, and stain with actin.

IC gives “ring staining” on M21 (via GD2), but localizes to synapse on NKL (CD25-pos., CD16-neg.)

Cell-bound IL2 induces IL2Rs to cause activating synapses.

Gubbels et al: CII, 2011
Treatment of Established Murine GD2+ Tumors with IV vs. intratumoral (IT) hu14.18-IL2 (Johnson et al, Canc. Imm. Immunother. 57:1891, 2008)

<table>
<thead>
<tr>
<th></th>
<th>Tumor Free Mice</th>
<th>% Tumor free</th>
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<tbody>
<tr>
<td></td>
<td>Expt 1</td>
<td>Expt 2</td>
</tr>
<tr>
<td>14.18 IL2 Dose</td>
<td>15 μg</td>
<td>15 μg</td>
</tr>
<tr>
<td>Saline</td>
<td>0 of 3</td>
<td>0 of 6</td>
</tr>
<tr>
<td>IV</td>
<td>0 of 5</td>
<td>3 of 6</td>
</tr>
<tr>
<td>IT</td>
<td>5 of 5</td>
<td>5 of 6</td>
</tr>
</tbody>
</table>

**IT-IC is superior to IV-IC in measurable tumors**

(Johnson et al, Canc. Imm. Immunother. 57:1891, 2008)
**CTLA-4 vs. PD-1: Distinct immune checkpoints**

![Diagram showing signal 1 from APC to Naïve/resting T cell and APC to Experienced T cell, involving B7.1/2, CD28, CTLA-4, PD-1, PD-L1, and costimulation ligands and receptors.]

*Naïve/resting T cell*

*Costim. ligand* → *Costim. receptor*

*APC* → *Signal 1*

*CTLA-4 to surface*

*APC* → *Signal 1*

*CTLA-4*

*T cell priming*

*APC* → *Signal 1*

*Tissue* → *Traffic to periphery*

*APC* → *Signal 1*

*PD-L1* → *PD-1*

*Inflammatory cytokines*

*Topalian et al., Curr Opin Immunol 2012*
Regulatory T cells from a distant site of disease may suppress the response to combined radiation and intratumoral hu14.18-IL2

B78 melanoma tumors harvested from C57BL/6 mice on day 6 after radiation