Combining tumor-reactive mAbs with cytokines to induce ADCC in patients

iSBTc mAb Workshop
October 1, 2010
Washington DC

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IL2 Facilitated ADCC of LAN5 Neuroblastoma (NBL)

Hank et al., Cancer Res. 50:5234, 1990
CCG-0901

IL-2 + 14.G2a FOR REFRACTORY NEUROBLASTOMA AND MELANOMA

MoAb 14.G2a

IL-2

1

8

15

22

TREATMENT DAY

Frost et al, Cancer 80:317, 1997
Published 14.18 phase I studies: PK, Tox., MTD, Biologic effects, but little **measurable** antitumor effect

- **Melanoma - UWCCC**
  - M. Albertini Chair
  - 14.G2a + IL2
  - Ch14.18 + IL2
  - Influence of IL2 on HACA
  - ch14.18 + R24 + IL2

- **Neuroblastoma - COG**
  - 14.G2a + IL2
  - Ch14.18 + GM-CSF after ASCT
  - Ch14.18 + GM-CSF + IL2 after ASCT
2 Major Types of Activating FcR for IgG

- **FcγRIIA (CD32)**
  - Expressed on:
    - Macrophages
    - PMNs
  - Functions:
    - Phagocytosis
    - ADCC
  - Activate with
    - GM-CSF

- **FcγRIIIA (CD16)**
  - Expressed on:
    - NK Cells
  - Functions:
    - ADCC
  - Activate with
    - IL2
CCG-
Pilot Phase-I study of ch14.18 + IL2 + GM-CSF following ABMT for NBL

- Day 0 ABMT
- Day 35 Ch14.18  + GM-CSF
- Day 56 Ch14.18  + IL2
- Day 77 Ch14.18 + GM-CSF
- Day 98 Ch14.18 + IL2
- Day 119 Ch 14.18 + GM-CSF


Overall survival ~75% at 2 years
Schema: C.O.G. NBL Study ANBL0032

(2003) - A. Yu Chair

High Risk Newly Diagnosed NBL

Induction

Ablation + Stem Cell Rescue

Randomize

Accrual of 386 randomized patients needed

Observe

ch14.18 + GM-CSF + IL2

Cis-Retinoic Acid
Event free survival for 226 children randomized to ImmRx vs CRA

Implications of this result for neuroblastoma clinicians:

Simon et al (J.C.O 22:3549, 2004) 334 pts treated after consolidation, 166 got ch14.18 (no cytokines). Multivariate analyses showed no benefit in OS or EFS.

"Because of these results, the MAB ch14.18 treatment is not continued in the current German NBL trial”.

Why did the COG trial show the ch14.18 + cytokine regimen provides clear benefit for OS and EFS?

Might it be the addition of the IL2 + GM-CSF?
Implications of this result for other cancers:

1. Rituxan, Herceptin, Erbitux mediate ADCC

2. Trials combining these mAbs with IL2 or GM-CSF to augment ADCC have been for patients with bulky (measurable) relapsed disease

3. Based on this COG result of ch14.18 + GM-CSF + IL2, it may be appropriate to consider combining these other mAbs with IL2 + GM-CSF in a randomized trial for patients in remission but at high risk of relapse.
Hu14.18-IL2, a genetically engineered fusion protein linking IL2 to hu14.18 mAb

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

IMMUNOCYTOKINE (IC)
Efficacy of ch14.18-IL2 Immunocytokine against Murine Neuroblastoma Liver Metastases


![Graph showing the number of liver mets for PBS Control, ch14.18 + IL-2, and ch14.18-IL-2 treatments. The values are 123 ± 69, 34 ± 21, and 0 ± 0, respectively.](image)
hu14.18-IL2 (10ug/d) for 5 days starting on day 5, 7, 9, or 11 following $5 \times 10^5$ NXS2 cells injected on day 0, and harvested on day 28.
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?
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.

Arens, Buhtoiarov et al: submitted 2010
FITC-IC Distribution

Tumor Cell

NK Cell

Synapse Formation

NK Cell

FITC-IC  IL2R  GD2
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.
- IL2R-facilitated ADCC.
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

• ** Shusterman et al-JCO In Press, 2010
<table>
<thead>
<tr>
<th>Pt. #</th>
<th>Response</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>CR</td>
<td>BM disease only at study entry (10/05). BM clear and ICC negative following course 2. Completed 6 courses antibody at full dose with NED (4/06). CRA post treatment. Recurred 12/06 with BM and abdominal disease (10 mo CR)</td>
</tr>
<tr>
<td>10</td>
<td>CR</td>
<td>BM disease only at study entry (6/06) although ICC negative. BM clear following course 2. Completed 4 courses with NED (10/06). No further rx given due to hypotension at 50% dose. Recurrence by marrow and bone scan 4/07 (8 mo CR).</td>
</tr>
<tr>
<td>22</td>
<td>CR</td>
<td>R tibia MIBG avid at study entry (10/06). MIBG clear after course 2. Competed 6 courses of treatment with NED 3/07. F/u MIBG 1/08 with NED. Recurrence 6/08 at tibial site (18 mo CR).</td>
</tr>
<tr>
<td>27</td>
<td>CR</td>
<td>BM disease only at study entry 11/06. BM clear following course 2. Completed 6 courses of treatment with NED 6/07. Recurrence 4/09 in scalp (28 mo CR)</td>
</tr>
<tr>
<td>29</td>
<td>CR</td>
<td>BM disease and MIBG at 4 sites at study entry. After course 2, BM morphology negative and MIBG cleared, but ICC slightly positive. All clear after courses 4 and 6. NED. F/U 12/08 NED. (35+ mo CR)</td>
</tr>
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*Shusterman et al, JCO, In Press, 2010*
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)

• 5 of 24 responses (stratum 2) > 0 of 13 (stratum 1) (p= 0.07)

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

Shusterman et al, JCO, In Press, 2010
Potential role of genotypes related to NK and ADCC functioning in anti-NBL Phase-II effects of hu14.18-IL2?

- **KIR (killer inhibitory receptors) and their ligands**
- **FcR polymorphisms for Fc\(\gamma\)R2A and Fc\(\gamma\)R3A**
“Missing Self Hypothesis” & KIR Mismatch

KIR Match = NK cell Inhibition → Tumor cell survival

KIR Mismatch (Missing self) → = Tumor cell death

French and Yokoyama Arthritis Res Ther 2004 6:8-14
KIR ligand mismatch helps ABMT

155 neuroblastoma pts: those with KIR mismatch w/ 45% lower risk of death.
Hypothesis: Autologous KIR/Ligand mismatch will influence response to hu14.18-IL2 in completed COG Phase II study

Mismatch vs. Response/Improvement (Stratum 1 & 2)

<table>
<thead>
<tr>
<th></th>
<th>KIR-Mismatch</th>
<th>KIR-Match</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response/improvement</td>
<td>7 (29%)</td>
<td>0 (0%)</td>
<td>7</td>
</tr>
<tr>
<td>No Response/No</td>
<td>17 (71%)</td>
<td>14 (100%)</td>
<td>31</td>
</tr>
<tr>
<td>improvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24 (63%)</td>
<td>14 (37%)</td>
<td>38</td>
</tr>
</tbody>
</table>

P = 0.03

Demonstrates an association between “mismatch” and clinical response

Consistent with in vivo role for NK cells in the anti-tumor response to hu14.18-IL2

Summary: Potential role for IV ICs in standard therapy

• Include a IC containing regimen (possibly combined with other therapy) in the standard care for patients with high-risk cancers in remission (i.e. likely to relapse)

• **Goal** – to prevent recurrence
  - **Who is most likely to benefit?**
  - **When is the best time to treat?**
Collaborators in our Anti-GD2 Research-2010

• UWCCC
  – J Hank
  – M Albertini
  – E Ranheim
  – A Rakhmilevich
  – J Gan
  – I Buhtoiarov
  – B Soto
  – J Kostlevy
  – J Haldeman
  – KM Kim
  – J Eickhoff
  – S Seo
  – J Kimball
  – Z Neal
  – J Arens
  – M Patankar
  – D Delgado
  – K DeSantes
  – R Yang
  – L Scardino
  – K Alderson

• C.O.G and N.A.N.T.
  – S Shusterman
  – A Yu
  – J Maris
  – W London
  – R Seeger
  – Many Pediatric Oncologists

• Provenance
  – S Gillies and colleagues

• EMD-Merck
  – S McMillan
  – Jean Henslee-Downey

• Scripps
  – R Reisfeld

• NCI-
  – Toby Hecht
  – Malcolm Smith

• Several others involved
The following slides are available to address questions that may arise
Hypothesis: FcR polymorphisms for FcγR2A and FcγR3A will influence response to hu14.18-IL2

- Result: For the FcγR2A (on PMNs and macrophages) there is a weak association (p=0.06) between high affinity genotype (HH) and response/improvement.
- This suggests (but clearly doesn’t prove) that even with monotherapy by hu14.18-IL2, some endogenous GM-CSF might be induced and pmns and macrophages may be making ADCC with the IC *(and doing so more effectively with the right FcR genotype)*

*(Delgado et al-Cancer Research In Press, 2010)*
Hypothesis: FcR polymorphisms for FcγR2A and FcγR3A will influence response to hu14.18-IL2

- Result: For the FcγR3A (on NK cells) there is no hint of any association ($p=0.40$) between high affinity genotype (VV) and response or improvement.

- This would be consistent with the hypothesis that the hu14.18-IL2 IC molecule potentially mediates effective ADCC even with the “lower affinity” FcγR3A genotypes (VF and FF), by interacting with IL2 receptors on NK cells and mediating ADCC

EpCAM-Bearing Tumor cell (breast, colon, prostate, ovarian, etc.)

KS-IL2 a genetically engineered fusion protein linking IL2 to KS mAb (recognizes EpCAM)

S. Gillies et al
Fig. 7. KS-IL2 mediates NKL-OVCAR-3 binding via IL-2 receptor. Calcein AM–labeled CD16<sup>neg</sup> NKL cells were added to confluent cultures of OVCAR-3 in the presence or absence of the designated reagents. After 25 min incubation, cultures were washed three times and fluorescence in individual wells was determined on a fluorescence plate reader. Data shown is mean of 6 repeats.

NKL cells use their IL2Rs to bind to tumor via KS-IL2

Gubbels et al, submitted 2010
ADCC via IL2Rs requires IC for FcR-/IL2R+ NK Cells (NKL and RL12)

M21 (GD2+)

K562 (GD2-)

Buhtoiarov et al;
Submitted 2010
Hu14.18-IL2 binds to tumor cells, and its cell-bound IL2 can activate AND polarize NK cells via their IL2Rs.
Mechanistic Hypotheses* for greater killing by IC than by mAb + IL2:

• 1. IC enables ADCC via conventional FcR interactions, while simultaneously further activating effectors via IL2Rs*

• 2. IC enables “novel ADCC” mediated via FcRs (enables cells without FcRs to mediate ADCC)

• 3. Both mechanisms (ie: 1 + 2) can occur simultaneously, to generate greater tumor killing (and greater localized cytokine release at tumor sites in vivo)

• * These need to be tested further in our lab
Next clinical steps for COG

• Obtain additional data with hu14.18-IL2 treatment in stratum 2 NB patients to confirm efficacy of single agent in MRD setting

• Compare hu14.18-IL2 with GM-CSF and CRA as an experimental arm vs ch14.18 + IL2 + GM-CSF + CRA immunotherapy in subsequent Phase III trial.

• Both studies approved by COG-NBL committee
2 Major Types of Activating FcR for IgG

- **FcγRIIA (CD32)**
  - Expresses on: Macrophages, PMNs
  - Functions: Phagocytosis, ADCC

- **FcγRIIIA (CD16)**
  - Expresses on: NK Cells
  - Functions: ADCC
Importance of FcγRIIIA on NK cells in Rituxan Therapy

Fig. 2

Kaplan-Meier estimates of progression-free survival by immunoglobulin G fragment C receptor IIIa (Fc RIIIa) 158 valine (V)/phenylalanine (F) polymorphism.

Importance of FcgRIIA on Mφs and PMNs cells in Rituxan Therapy

Fig. 3

Kaplan-Meier estimates of progression-free survival (PFS) by immunoglobulin G fragment C receptor IIa (Fc RIIa) 131 histidine (H)/arginine (R) polymorphism.

Fig 2. Progression-free survival (PFS) by immunoglobulin G (IgG) fragment C receptor IIIa (FcγRIIIa) 158 valine (V)/phenylalanine (F) and FcγRIIa 131 histidine (H)/arginine (R) polymorphisms.