Serial Immune Monitoring: Essential to Resolve Immune Dynamics for Improving Clinical Effectiveness of Immunotherapy

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Royal Adelaide Hospital, Adelaide, South Australia &

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University of Melbourne, Melbourne, Australia
Main Aim

Monitoring & Targeted Therapy to Induce Complete Responses and Long-Term Survival in Advanced Cancer
Advanced Cancer Response Rates

1 Year

PD       ~30%
CR        5-10%
PR        ~30%
SD       ~30%

5 Years

CR ~4.5%
PR ~3%
SD ~3%

90% PD
## Meta Analysis Data Set

160 trials / studies = 9,964 patients

### IL2

<table>
<thead>
<tr>
<th>Agent</th>
<th>% CR Rate</th>
<th>Study No.</th>
<th>Authors</th>
<th>No. of Patients / Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL2 High Dose Combo M/A</td>
<td>4.40%</td>
<td>14</td>
<td>Coventry, Ashdown 2013</td>
<td>1066 in 14 trials</td>
</tr>
<tr>
<td>IL2 High Dose Mono M/A</td>
<td>6.60%</td>
<td>13</td>
<td>Coventry, Ashdown 2013</td>
<td>2741 in 19 trials</td>
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<tr>
<td>IL2 Intermediate Dose Comb M/A</td>
<td>2.60%</td>
<td>16</td>
<td>Coventry, Ashdown 2013</td>
<td>400 in 5 trials</td>
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<tr>
<td>IL2 Intermediate Dose Mono M/A</td>
<td>1.60%</td>
<td>15</td>
<td>Coventry, Ashdown 2013</td>
<td>184 in 2 trials</td>
</tr>
<tr>
<td>IL2 Low Dose Combo M/A</td>
<td>5.20%</td>
<td>18</td>
<td>Coventry, Ashdown 2013</td>
<td>362 in 11 trials</td>
</tr>
<tr>
<td>IL2 Low Dose Mono M/A</td>
<td>4.50%</td>
<td>17</td>
<td>Coventry, Ashdown 2013</td>
<td>286 in 13 trials</td>
</tr>
<tr>
<td>IL2 Meta Analysis (M/A) 62 Trials</td>
<td>5.60%</td>
<td>12</td>
<td>Coventry, Ashdown 2013</td>
<td>5312 in 62 trials, 1988-2012</td>
</tr>
</tbody>
</table>

### CTLA4

<table>
<thead>
<tr>
<th>Agent</th>
<th>% CR Rate</th>
<th>Study No.</th>
<th>Authors</th>
<th>No. of Patients / Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipilimumab + Nivolumab BMS</td>
<td>6%</td>
<td>29</td>
<td>Wolchok 2013</td>
<td>86</td>
</tr>
<tr>
<td>Ipilimumab</td>
<td>10%</td>
<td>2</td>
<td>Farolfi 2012</td>
<td>36</td>
</tr>
<tr>
<td>Ipilimumab + gp120 esc</td>
<td>6%</td>
<td>5</td>
<td>Prieto 2012</td>
<td>85</td>
</tr>
<tr>
<td>Ipilimumab + surgery</td>
<td>7.50%</td>
<td>6</td>
<td>Ku 2010</td>
<td>53</td>
</tr>
<tr>
<td>Ipilimumab +gp120</td>
<td>7%</td>
<td>3</td>
<td>Prieto 2012</td>
<td>56</td>
</tr>
<tr>
<td>Ipilimumab +IL2</td>
<td>17%</td>
<td>4</td>
<td>Prieto 2012</td>
<td>36</td>
</tr>
<tr>
<td>Tremelimumab</td>
<td>15.70%</td>
<td>24</td>
<td>Huang 2011</td>
<td>19</td>
</tr>
<tr>
<td>Tremelimumab</td>
<td>0%</td>
<td>25</td>
<td>Kirkwood 2010</td>
<td>241</td>
</tr>
<tr>
<td>Tremelimumab</td>
<td>5.10%</td>
<td>27</td>
<td>Ribas 2008</td>
<td>39</td>
</tr>
<tr>
<td>Tremelimumab</td>
<td>2.20%</td>
<td>28</td>
<td>Comacho 2009</td>
<td>89</td>
</tr>
<tr>
<td>Tremelimumab + INFα2b</td>
<td>11.40%</td>
<td>23</td>
<td>Tahini AA 2012</td>
<td>35</td>
</tr>
<tr>
<td>Tremelimumab MART-1 DC</td>
<td>12.50%</td>
<td>26</td>
<td>Ribas 2009</td>
<td>16</td>
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</tbody>
</table>

### ChemoRx

<table>
<thead>
<tr>
<th>Agent</th>
<th>% CR Rate</th>
<th>Study No.</th>
<th>Authors</th>
<th>No. of Patients / Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meta Analysis, cytotoxic agents</td>
<td>7%</td>
<td>21</td>
<td>Coventry, Ashdown 2013</td>
<td>2756 in 68 trials, 2000-2008</td>
</tr>
<tr>
<td>Tomozolomide Meta Analysis</td>
<td>7.12%</td>
<td>22</td>
<td>Yatomi Clarke 2013</td>
<td>541 in 9 trials 2010-2013</td>
</tr>
</tbody>
</table>

### Braf

<table>
<thead>
<tr>
<th>Agent</th>
<th>% CR Rate</th>
<th>Study No.</th>
<th>Authors</th>
<th>No. of Patients / Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabrafenib (Break II)</td>
<td>7%</td>
<td>11</td>
<td>Ascierto 2013</td>
<td>75</td>
</tr>
<tr>
<td>Dabrafenib /Mek150/1</td>
<td>6%</td>
<td>8</td>
<td>Flaherty 2012</td>
<td>54</td>
</tr>
<tr>
<td>Dabrafenib Mono</td>
<td>4%</td>
<td>9</td>
<td>Flaherty 2012</td>
<td>54</td>
</tr>
<tr>
<td>Dabrafenib/Mek 150/2</td>
<td>9%</td>
<td>7</td>
<td>Flaherty 2012</td>
<td>54</td>
</tr>
<tr>
<td>Vemurafenib</td>
<td>6.25%</td>
<td>1</td>
<td>Ravan 2012</td>
<td>32</td>
</tr>
<tr>
<td>Vemurafenib</td>
<td>6%</td>
<td>10</td>
<td>Sosman 2012</td>
<td>132</td>
</tr>
</tbody>
</table>

### PD-1/L

<table>
<thead>
<tr>
<th>Agent</th>
<th>% CR Rate</th>
<th>Study No.</th>
<th>Authors</th>
<th>No. of Patients / Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-1 Mab (mono) BMS</td>
<td>3.30%</td>
<td>30</td>
<td>Wolchok, 2013</td>
<td>30</td>
</tr>
<tr>
<td>PD-1 Mab Merck</td>
<td>9.70%</td>
<td>20</td>
<td>Hamid O, 2013</td>
<td>103</td>
</tr>
<tr>
<td>PD-1L Mab Genentech</td>
<td>7%</td>
<td>19</td>
<td>Gordon, AACR 2013</td>
<td>30</td>
</tr>
</tbody>
</table>

Av 6.6% CR rate
"You won't know how to vaccinate until you know how to immunize.

And you won't know how to immunize until you know how to monitor."

- Lloyd J. Old, M.D. Director, Cancer Vaccine Collaborative, CRI, NYC....2003
Examples of repeating Bio-rhythms

The ~ 28 day Menstrual Cycle

* Well documented and understood due to close serial daily data

The 24Hr Cortisol Cycle
CANCER PATIENT MONITORING WITH THERAPIES

Animal expts - inadequate monitoring

Human expts - inadequate monitoring

Successful Essential Close Serial Monitoring
• Fertility
• Diabetes
• Cortisol
• Antibiotics
• Cardiac
Robert J North - T Cell Mediated Murine Tumor Regression
Trudeau Institute, NY.

- Others.

Control mice all dead by 45 days

Tumor Size (mm)

Single Rx dose Vinblastine day ~15

T cell Nos.

<table>
<thead>
<tr>
<th>Tumor Size (mm)</th>
<th>T cell Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

nm

mice cured

Single Rx dose Vinblastine day ~15

Others.
Critical Timing Effect

Klatzman JCI et al., 2009
“Monotherapy cps (Tg) treatment of B16F10 also stands out from many other immunotherapy approaches by relying on manipulation of the endogenous immune response in vivo.”
So what’s happening here from day 10-21...!!!!...???
IL-2 RECEPTOR EXPRESSION ON T-CELLS

Acute Antigen Stimulation → IL2R

~ 48 hrs

Feedback induced; Homeostatic response

T-EFF >80%

>5%

IL-2 RECEPTOR EXPRESSION ON T-CELLS

Chronic Persistent Antigen Stimulation

- **T-EFF**
  - >80%
  - ~ 48 hrs

Feedback induced; Homeostatic response

Chronic Persistent Antigen Stimulation

- **T-REG**
  - >5%
  - >80%
C- Reactive Protein (CRP) – a surrogate biomarker of immune kinetics

**CRP**
- Pentraxin
- Opsonin
- Functional analogue of Ig
- Binds cellular debri
- Binds Fcγ IIIR on DCs
- Initiates the adaptive IR
- Rises/ Falls with IR initiation/ termination
- Elevated in cancer patients

---

**CRP kinetics in the acute response**

**CRP kinetics in the late stage cancer patient**

**Serial CRP, Advanced Melanoma Patient**
Serial hs-CRP over 4 weeks, Stage IV Melanoma

Days
14/03/2004
16/03/2004
18/03/2004
20/03/2004
22/03/2004
24/03/2004
26/03/2004
28/03/2004
30/03/2004
1/04/2004
3/04/2004
5/04/2004
7/04/2004
9/04/2004
11/04/2004
13/04/2004
15/04/2004

hs-CRP mg/L
0
10
20
30
40
50
60
70
80
90
100
110
120
Serial hs-CRP over 4 weeks, Stage IV Melanoma
Vaccine Therapy for Malignant Melanoma Metastases

Vaccinia Melanoma Cell Lysate (VMCL) – Peter Hersey, NU

- No toxicity, tested in over 400 patients previously
- Simple protocol, I/D Vax fortnightly
VMCL VACCINE STUDY

- DATA ANALYSIS TO END DEC 2010, All Evaluable Patients

RESPONSE RATES  (N = 54 patients)

• CR 9 patients (16.7%)

• SD 25 patients (46.3%)

• PR 8 patients (14.8%)

• Progressive Disease 12 patients (22.2%)

Does timing WRT immunological cycles/oscillations affect efficacy ....??
Continued Repetitive Vaccination Events
– Persistent Immune Stimulation
Continued Repetitive Vaccination Events
– Persistent Immune Modulation
Major Dilemma in Control of the Immune System

How are either **Tolerance** and **Responsiveness** controlled and determined *in-vivo*?

How is the CRITICAL BALANCE orchestrated?

How is ‘Immunological Homeostasis’ achieved?

Implications for Cancer Immuno-Chemotherapy?
Multiple Vaccinations: Friend or Foe

Sarah E. Church, Shawn M. Jensen, Chris Twitty, Keith Bahjat, Hong-Ming Hu, Walter J. Urba, and Bernard A. Fox

Robert W. Franz Cancer Research Center, Earle A. Chiles Research Institute, Providence Cancer Center, Providence Portland Medical Center, Portland, Oregon
Departments of Molecular Microbiology and Immunology, Oregon Health and Science University, Portland, Oregon
Cancer Research and Biotherapy Center, The Nanjing Second Hospital, Nanjing, Jiangsu. China

Homeostatic Regulated Immune Kinetics in Cancer
- How to Break Tolerance.

Drivers
Ag*
IL-2*
IL-4
IL-10*
IL-12*
IL17*
IL-21*
TGFb1
CTLA4*
GITR*
VLA4*
CD134*
PD-1*
INFγ*

Tolerance

Drivers
Ag*
IL-2*
IL-10*
IL-12*
IL17*
IL-21*
CTLA4*
GITR*
VLA4*
CD134*
PD-1*
INFγ*

Responsiveness

T-regs
CRITICAL BALANCE
T-effs

"Nature exists in a delicate balance, the immune system being no exception."

Many Drivers for Responsiveness same as for Tolerance
The Functional Unit of the Adaptive Immune Response

Stimulus – chronic antigen

~ Time (Hours / Days)

Lymphocyte Nos. & Immune Dynamics

Chronic Antigen Persistence

Repeats / oscillates

Responsiveness

Tolerance

Responsiveness
Patient CG Vax treatment date & relative position on CRP cycle 2007-2009

~7 Days

CRP

30/06/09

VAX
Patient CG Vax treatment date & relative position on CRP cycle 2007-2009

- 27/02/07
- 13/03/07
- 27/03/07
- 24/04/07
- 19/06/07
- 3/07/07
- 31/07/07
- 28/08/07
- 25/09/07
- 26/08/08
- 20/10/09
- 20/11/07
- 6/05/07
- 1/07/07
- 18/12/07
- 4/12/07
- 13/03/07
- 30/06/09
- 25/09/07
- 18/11/08
- 3/06/08
- 19/06/07
- 3/06/08
- 27/03/07
- 12/02/08
- 24/04/07
- 18/11/08
- 31/07/07

Patient CG Vax treatment date & relative position on CRP cycle 2007-2009

~7 Days

CRP
Vaccinia Melanoma Cell Lysate (VMCL) vaccine therapy for advanced melanoma

Timing Vaccine administration WRT CRP Cycle

Serial monitoring hs-CRP the week before and the day of vaccination. Note how the cycle kinetics can be approximately resolved with a week of serial data.

Number of vaccinations and their approximately position on the CRP cycle, either near a peak or a trough, etc. Patient #JM
Patient #EG Chemotherapy treatment date & relative position on CRP cycle

CRP (Relative position on the stylised CRP curve at the time of each dose, plotted on the standard CRP curve).
C- Reactive Protein (CRP) – a potential surrogate biomarker of immune kinetics

![Graph showing CRP levels and immune cell numbers over time with annotations for Teff stimulation and Treg ablation.](image)

- Teff stimulation with vaccine (green)
- Treg ablation with chemotherapy (red)
C- Reactive Protein (CRP) – a potential surrogate biomarker of immune kinetics

Teff stimulation with vaccine (green)

Treg ablation with chemotherapy (red)

12 Hour Therapeutic Window : 7-Day Cycle; 1 in 14 or 7%
Patient: S S (Colorado USA) Melanoma, hs-CRP

Predicted extrapolated immune cycle

Days

hs-CRP mg/L

~7 days
Advanced Prostate Ca

LC hs-CRP – Prostate Ca

Predicted extrapolated immune cycle

Rx 15/8

Days

hs-CRP mg/L

0 1 2 3 4 5 6 7 8 9 10

DN CRP CYCLE SERIES - THERAPY

- **VAX**
- **CHEMO**

### CRP Level
- 5 days: 11/03/10
- 9 days: 05/04/10
- 8 days: 07/06/10
- 4 days: 15/06/10
- 7 days: 17/07/10
- 24/07/10: Complete Response

### Cycle Days
- A
- B
- C


The dynamic human immune response to cancer: it might just be rocket science. Holtan etal. Immunotherapy (2011) 3(9), 1021–1024


- Successful Cancer Therapy remains an international unsolved problem.

New Trial Commencing

‘TIMED’ vs ‘UNTIMED’ Vaccine +/- Oral Chemotherapy
Candidate Molecules for Serial Monitoring

- CRP
- SAA
- IL-2R soluble
- IL-2 cytokine
- γIFN
The Complete Response Probability Equation

\[
0.07_{(P_{CR})} \approx \frac{W_{Rx}}{\lambda_{IC}}
\]

\(\lambda_{IC} = \) Immune Cycle Periodicity (~7 days)

\(W_{Rx} = \) Width of Therapeutic Window (~12hrs or 0.5 day)

\(P_{(CR)} = \) Probability of a CR (0.5/7; 1:14 or 0.07, or 7%)

Conclusion

- MONITORING
- IMMUNE SYNCHRONISATION OF THERAPY
- 2 POSTERS
CONCLUSIONS

• Antigen Recognition does not appear to be the problem

• Repeated Persistent Vaccinations / Cell Damage
  – Re-Directs the In-vivo Immune Response

• Immune Oscillation from Chronic Stimulation
  – offers repeated therapeutic opportunity

• Missing the ‘window’ for immune re-direction
  – can be corrected by repeated dosing

• Cure is likely to reside in the TIMING of dosing
Close SERIAL MONITORING IS ESSENTIAL
to
determine when to vaccinate or treat
with immuno-modulatory agents
Serial CRP waveforms......experienced so far.

Wavelength ~7 days
"...why hasn't Coley's approach been forged into a widely available therapy with a **predictable** benefit for cancer patients.......

The best reason,...

...science had to catch up with the Coley phenomenon and that the cellular and molecular language of inflammation and immunity had to **be understood** before the forces that Coley unleashed could be predictably translated into tumor cell destruction.”

The Value of Health and Longevity

*Murphy & Topel, Uni Chicago. Journal of Political Economy Vol 114, No.5: 2006*

“... a permanent 1 percent reduction in mortality from cancer has a present value to current and future generations of Americans of nearly $500 billion, whereas a cure (if one is feasible) would be worth about $50 trillion.”
Patient FO. Serial acute phase marker, cytokine & cancer marker fluctuations in a late stage asymptomatic ovarian cancer patient (as marked) over a 4 week period indicating a periodicity of ~7 days (Quinn MA & Ashdown ML).
## Complete Response Rates – various modalities

<table>
<thead>
<tr>
<th>Agent</th>
<th>Indication</th>
<th>Reported CR Rate (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coley Toxins (c 1903)</td>
<td>Sarcomas / Carcinomas</td>
<td>~ 10%</td>
</tr>
<tr>
<td>Std Cytotoxic agents</td>
<td>Various Solid Cancers</td>
<td>~ 7%</td>
</tr>
<tr>
<td>IL2 (1988 – 2010)</td>
<td>Advanced Melanoma/ RC (OvCa)</td>
<td>~7% (10%)</td>
</tr>
<tr>
<td>Ontak (2003- 2010)</td>
<td>Advanced Melanoma/CTCL</td>
<td>~ 5-10%</td>
</tr>
<tr>
<td>DC Vaccine (QIMR)</td>
<td>Advanced Melanoma</td>
<td>~ 10%</td>
</tr>
<tr>
<td>Provenge (Dendreon)</td>
<td>Prostate</td>
<td>~ 0.3%</td>
</tr>
<tr>
<td>CSL/ Ludwig (NYESO-1)</td>
<td>Melanoma</td>
<td>~ 0%</td>
</tr>
<tr>
<td>Median CR rate</td>
<td></td>
<td>7%</td>
</tr>
</tbody>
</table>
When considered by cancer type and by drug type there was no evidence that any particular cancer or drug was higher or lower than 7% CR \( [n = 68 \text{ Chemotherapy Trials 2000-2007}] \).
Our meta-analysis: 53 Studies; 5312 patients; CR rate = 5.60% (unpublished)
<table>
<thead>
<tr>
<th>Agent</th>
<th>Indication</th>
<th>Reported CR Rate (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-Raf Inhibitor Roche</td>
<td>Melanoma</td>
<td>6%</td>
</tr>
<tr>
<td>B-Raf Inhibitor GSK</td>
<td>Melanoma</td>
<td>4%</td>
</tr>
<tr>
<td>B-Raf Inhibitor GSK/ Mek</td>
<td>Melanoma</td>
<td>6%/ 9%</td>
</tr>
<tr>
<td>CTLA-4 Mabs</td>
<td>Advanced Melanoma</td>
<td>~ 0.2%/ 1.5%</td>
</tr>
<tr>
<td>PD-1/ PD-L1</td>
<td>Melanoma</td>
<td>1%/ 6%</td>
</tr>
<tr>
<td>CTLA4/ PD-1</td>
<td>Melanoma</td>
<td>9.6%</td>
</tr>
<tr>
<td><strong>Median CR rate</strong></td>
<td></td>
<td><strong>6 %</strong></td>
</tr>
</tbody>
</table>
## Complete Response Rates – various modalities

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<thead>
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<th>Reported CR Rate (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCML Vaccine</td>
<td>Advanced Melanoma</td>
<td>~18% (n=37); 18(n=54)</td>
</tr>
<tr>
<td>CTLA-4 / IL-2</td>
<td>Advanced Melanoma</td>
<td>17%</td>
</tr>
<tr>
<td>Median CR rate</td>
<td></td>
<td>17.5 %</td>
</tr>
</tbody>
</table>
The Price We Pay for Progress: A Meta-Analysis of Harms of Newly Approved Anticancer Drugs

Saroj Niraula, Bostjan Seruga, Alberto Ocana, Tiffany Shao, Robyn Goldstein, Ian F. Tannock, and Eitan Amir

38 RCT Studies of 38 targeted agents 2000-2010 for therapy of advanced solid malignancies

Agent Toxicity-related death increased - OR 1.40
Treatment discontinuation greater - OR 1.33
Grade III/IV Toxicity increased - OR 1.52

Conclusion: ‘Off target’ effects of ‘targeted’ agents overall appear more severe and extensive than many ‘non-targeted’ agents.
Acknowledgements

Derek Abbott, Engineering, University of Adelaide

Tony Michele, Peter Hersey, Medical Oncology

Carrie Cooper, Research Nurse, Royal Adelaide Hospital

Richard Bright, Research Assistant

Svetomir Markovic, Medical Oncologist, Mayo Clinic

Cancer Patients