Combination opportunities in immunotherapy with immunostimulatory mAb.
SYNERGY

syn-ergos, συνεργός, meaning 'working together'.
SYNERGY
TWO AGENTS ACTING TOGETHER
SUCH THAT THE WHOLE IS GREATER THAN THE SUM OF THE PARTS
1. Different mechanism of action.
2. Cooperative effect
What is an immunostimulatory monoclonal antibody?

- Receptor agonist: CD137, CD40, OX40, GITR, CD27

- Receptor antagonist: CTLA-4, PD1, B7-H1, BTLA, TGF-β, IL-10
Effective Tumor Immunotherapy: Start the Engine, Release the Brakes, Step on the Gas Pedal, ... and Get Ready to Face Autoimmunity

INIGO TIRAPU, GUILLERMO MAZZOLINI, MERCEDES RODRIGUEZ-CALVILLO, AINHOA ARINA, BELEN PALENCIA, IZASKUN GABARI and IGNACIO MELERO*
REASONS TO PLAY WITH INDUCIBLE TARGETS

1. Less toxicity (remember about the supra-agonist anti-CD28 mAb)

2. More specificity and avidity

Surface expression

TCR avidity for Tumor antigen

Selective advantage for the TCR-fittest CTL clones

Anti-CD137 or Anti-CTLA-4
Radiotherapy

Chemotherapy

Vaccination

T-reg Depletion/inactivation

Adoptive T-cell immunotherapy

α-CD137

α-CTLA-4

α-CD40

α-OX40

α-PD1

**Figure 1** Multiple points of intervention to engender successful cancer immunotherapy. Successful strategies will involve the integration of multiple elements that activate dendritic cell presentation of antigens to most effectively initiate immune responses, block immunologic checkpoints to amplify these responses and, finally, enhance the traffic and activity of T cells against metastatic tumors. The specific examples of molecular pathways shown for each step do not represent a comprehensive list. DC, dendritic cell; TLR, toll-like receptor.
COMBINATION WITH STANDARD TREATMENTS (chemotherapy)

• Will happen anyway
• Maybe not the most efficacious combinations

• Interesting points of synergy:
  - Immnogenic cell death
  - Lymphopenia
  - Nice surprises
Priority Report

Combination Therapy with Cisplatin and Anti-4-1BB: Synergistic Anticancer Effects and Amelioration of Cisplatin-Induced Nephrotoxicity

Young H. Kim, Beom K. Choi, Kwang H. Kim, Sang W. Kang, and Byoung S. Kwon
CTLA-4 blockade synergizes with tumor-derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma

Arthur A. Hurwitz*, Tina F.-Y. Yu, Dana R. Leach, and James P. Allison

Provision of antigen and CD137 signaling breaks immunological ignorance, promoting regression of poorly immunogenic tumors

Ryan A. Wilcox, 1 Dallas B. Flies, 1 Gefeng Zhu, 1 Aaron J. Johnson, 1 Koji Tamada, 1 Andrei I. Chapoval, 1 Scott E. Strome, 2 Larry R. Pease, 1 and Lieping Chen 1, 3

Table 1
Treatment of mice bearing established C3 tumors

<table>
<thead>
<tr>
<th>Ab</th>
<th>Peptide</th>
<th>Tumor-free/total (%)</th>
<th>Mean tumor diameter (mm)</th>
<th>P valueC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A</td>
<td>E7 (aa 49-57)</td>
<td>16/38 (42%)</td>
<td>7.6 ± 2.4</td>
<td>-</td>
</tr>
<tr>
<td>Rat IgG</td>
<td>E7 (aa 49-57)</td>
<td>0/11 (0%)</td>
<td>10.5 ± 3.0</td>
<td>0.017</td>
</tr>
<tr>
<td>2A</td>
<td>Vp2 (aa 121-130)</td>
<td>2/23 (9%)</td>
<td>11.4 ± 4.0</td>
<td>0.004</td>
</tr>
<tr>
<td>Rat IgG</td>
<td>Vp2 (aa 121-130)</td>
<td>0/8 (0%)</td>
<td>11.5 ± 3.5</td>
<td>0.005</td>
</tr>
</tbody>
</table>

aMice were injected with 1 × 10⁶ C3 cells. Two weeks later, mice were immunized with the indicated peptide, as previously described. On the day of immunization and again 3 days later, mice were given 100 μg of either mAb 2A or a control rat IgG intraperitoneally. Tumor size was assessed weekly. Data shown were pooled from several experiments. bTwenty-one days after treatment, the mean tumor diameter was calculated for those tumors that had failed to completely regress. cThe unpaired Student t test was used to calculate P values, comparing the mean tumor diameter of the treatment group that received both the E7 peptide and mAb 2A with that of the control group.
Improved Survival with Ipilimumab in Patients with Metastatic Melanoma

F. Stephen Hodi, M.D., Steven J. O'Day, M.D., David F. McDermott, M.D., Robert W. Weber, M.D., Jeffrey A. Sosman, M.D., John B. Haanen, M.D., Rene Gonzalez, M.D., Caroline Robert, M.D., Ph.D., Dirk Schadendorf, M.D., Jessica C. Hassel, M.D., Wallace Akerley, M.D., Alfons J. van den Eertwegh, M.D., Ph.D., Jose Lutzky, M.D., Paul Lorigan, M.D., Julia M. Vaubel, M.D., Gerald P. Linette, M.D., Ph.D., David Hogg, M.D., Christian H. Ottensmeier, M.D., Ph.D., Celeste Lebbe, M.D., Christian Peschel, M.D., Ian Quirt, M.D., Joseph I. Clark, M.D., Jedd D. Wolchok, M.D., Ph.D., Jeffrey S. Weber, M.D., Ph.D., Jason Tian, Ph.D., Michael J. Yellin, M.D., Geoffrey M. Nichol, M.B., Ch.B., Axel Hoos, M.D., Ph.D., and Walter J. Urba, M.D., Ph.D.
Combinations with vaccines

- Dependent on the type of vaccines.
- Optimal timing for immunization and immunostimulation.
- Commercially difficult to find a partner.
- Especially important in the minimal residual disease setting.

CCR Translations

Palettes of Vaccines and Immunostimulatory Monoclonal Antibodies for Combination

Commentary on Li et al., p. 1623

Ignacio Melero,1,2 Ivan Martinez-Forero,1 Juan Dubrot,1 Natalia Suarez,2 Asis Palazón,1 and Lieping Chen3

- Especially important in the minimal residual disease setting.
....What about turning one/some of the existing tumor lesions into a vaccine?

1. Chemotherapy-induced immunogenic cell death.

2. Intratumoral injections of TLR agonists or cytokines While causing some tissue destruction:  
   CpG, Poly I:C, Imiquimod, IL-12, IFNα, GM-CSF…

Follow research by Lawrence Zitvogel, Ron Levy…
COMBINATIONS AMONG IMMUNOSTIMULATORY MAB
Eradication of established tumors in mice by a combination antibody-based therapy

Tomoyasu Uno¹,², Kazuyoshi Takeda¹,³, Yuko Kojima⁴, Hirohisa Yoshizawa⁵, Hisaya Akiba¹, Robert S Mittler⁶, Fumitake Gejyo², Ko Okumura¹, Hideo Yagita¹ & Mark J Smyth³

Trail-like induced tumor cell apoptosis + DC maturation + Tcell costimulation
Furthermore...
Combination Therapy with Anti–CTL Antigen-4 and Anti-4-1BB Antibodies Enhances Cancer Immunity and Reduces Autoimmunity

Ergun Kocak,1,2 Kenneth Lute,1 Xing Chang,1 Kenneth F. May, Jr.,1 Katie R. Exten,1 Huiming Zhang,1 Shahab F. Abdessalam,2 Amy M. Lehman,3 David Jarjoura, Pan Zheng,1 and Yang Liu1
Blockade of B7-H1 and PD-1 by Monoclonal Antibodies Potentiates Cancer Therapeutic Immunity

Fumiya Hirano,¹ Katsumi Kaneko,¹ Hideto Tamura,¹ Haidong Dong,¹ Shengdian Wang,¹,² Masao Ichikawa,¹,² Cecilia Rietz,¹,² Dallas B. Flies,¹,² Julie S. Lau,¹ Gefeng Zhu,¹,² Koji Tamada,¹,² and Lieping Chen¹,²
Optimising anti-tumour CD8 T-cell responses using combinations of immunomodulatory antibodies

Juliet C. Gray¹, Ruth R. French¹, Sonya James¹, Aymen Al-Shamkhani¹, Peter W. Johnson² and Martin J. Glennie¹

Figure 5. Endogenous SIINFEKL-specific T-cell responses to immunisation with OVA and mAb. Mice received OT-I cells, mAb and OVA on day 0 as in Fig. 1. Splenocytes were harvested on day 7 and assessed for IFN-γ secretion by ELISPOT. For each mouse, triplicate wells, each containing $4 \times 10^5$ splenocytes, were incubated for 24h with and without 0.1 μM SIINFEKL peptide. The histogram shows the mean and range of the number of IFN-γ spots per $4 \times 10^5$ splenocytes. Data are from one of two experiments, each with 2 mice per group.
COMBINATIONS AMONG IMMUNOSTIMULATORY MABS

- Non-overlapping mechanism of action.
- Non-overlapping mechanism of toxicity.
- Driven by immune correlates and biomarkers.

PRE-POST TUMOR BIOPSIES.

- Business reasons weigh a lot but should follow good scientific reasons
- Dose optimization
- (and let us be very empirical)
Synergism of Cytotoxic T Lymphocyte–associated Antigen 4 Blockade and Depletion of CD25+ Regulatory T Cells in Antitumor Therapy Reveals Alternative Pathways for Suppression of Autoreactive Cytotoxic T Lymphocyte Responses

Roger P.M. Sutmuller,¹ Leonie M. van Duivenvoorde,¹ Andrea van Elsas,¹ Ton N.M. Schumacher,² Manon E. Wildenberg,¹ James P. Allison,³ Rene E.M. Toes,¹ Rienk Offringa,¹ and Cornelis J.M. Melief¹
Anti-CTLA-4 is not a good anti-Treg treatment.

• CTLA-4 is brightly expressed on Treg cells
• Selective genetic deficiency of CTLA-4 in FOXP-3 cells leads to autoimmunity
• Paradoxically, selective CTLA-4 blockade on Treg with mAb has a modest effect.
• Patients under ipilimumab or tremelimumab treatment have largely normal Treg numbers and function.
CD4$^+$ cells (gated out FoxP3)

- Red: DC+PBL
- Green: DC+[PBL-Treg]

CD8$^+$ cells

- Red: DC+PBL
- Green: DC+[PBL-Treg]
We have neglected lymphocyte traffic to tumors

Enhanced traffic and activity of tumor specific T cells at sites of metastases

Enhanced antigen presentation by dendritic cells

Antigen-specific engineered vaccines

Incorporation of dendritic cells, differentiators or activators into vaccines

Incorporation of B7 family costimulatory molecules

Antigen coupled to DC targeting molecules

Mobilization of dendritic cells (Flt3L, CD40L, TLR agonists)

CTLA-4 blockade

PD-1 blockade

Stat3 inhibition

Blockade of immunologic checkpoints

Inhibition of regulatory T cells

Enforced costimulation CD40, CD137, OX40, GITR

IL-15

B7-H1 blockade

B7-H4 blockade

Target proinflammatory signals to neovascular endothelium

Immunotherapy + blockade of anti-apoptosis pathways in tumors
Clinical development of combination strategies in immunotherapy: are we ready for more than one investigational product in an early clinical trial?

Jose L Perez-Gracia†, Pedro Berraondo†, Ivan Martinez-Forero†, Carlos Alfaro†, Natalia Suarez†, Alfonso Gurpide†, Bruno Sangro†, Sandra Hervas-Stubb†, Carmen Ochoa†, Jose A Melero† & Ignacio Melero††

Immunotherapy (2009)

‘Shortcut’ combination clinical trials in immunotherapy: early assessment of safety, mechanism of action & clinical response for combination immunotherapies

Figure 1. Flow chart for clinical development of trials that combine various immunotherapeutics agents testing for synergy. IND: Investigational new drug.
Dr. Melero can you predict the future a little bit?

Disclaimer:
1) Almost all I have to predict the future with are mice.
2) I am glad to inform you that mice are similar, but not identical to humans.

Standard treatment
And
Local intervention to enhance immunogenicity in some tumor lesions
Or
A vaccine in minimal residual disease status
Do something to Treg

Check point blockade:
-CTLA-4
-PD1
-B7-H1

Enforce costimulation:
-CD137
-OX40
-GITR
-CD40

The sequence of these treatments?:
-concomitant
-Sequential

……to be empirically learnt
My lord, some used to say so, but it must be done. Let me talk to friends in Pharma industry, Regulatory agencies, and funding agencies at ISB TC and let us see what happens...

Sancho, my friend: is it a Quixotean task to COMBINE THESE IMMUNOTHERAPIES?