

Combinations of immunostimulatory antibodies with synergistic effects against spontaneous cancer

Aizea Morales-Kastresana¹, Sara Labiano¹, Ines Gütgemann², and Ignacio Melero^{1,3,*}

¹Departamento de Inmunología; Centro de Investigación Médica Aplicada (CIMA); Universidad de Navarra; Pamplona, Spain;

²Department of Pathology; University of Bonn; Bonn, Germany; ³Departamento de Oncología; Clínica Universidad de Navarra; Pamplona, Spain

Keywords: B7-H1 (PD-L1), CD137 (4-1BB), immunotherapy, OX40 (CD134), T lymphocytes

Abbreviations: HCC, hepatocellular carcinoma; IFN γ , interferon, γ ; IL-2, interleukin-2; mAb, monoclonal antibody; NK, natural killer; OVA, ovalbumin; TCR, T-cell receptor; TAA, tumor-associated antigen; TNFRSF, tumor necrosis factor receptor superfamily; PD-1, programmed cell death 1; CTLA-4, cytotoxic T lymphocyte-associated protein 4

Immunostimulatory monoclonal antibodies can be given in combinations, hence modulating the activity of 2 or more receptors of the immune system. Some of these combinations have been shown to synergize at the elicitation of therapeutically relevant immune responses in transgenic mice developing spontaneous, oncogene-driven tumors, including multifocal hepatocellular carcinomas expressing ovalbumin as a surrogate tumor-associated antigen.

Making the most of the immune system to tackle cancer needs the simultaneous or sequential modulation of multiple immunological mechanisms. The concept of combinatorial immunotherapy originates from the fact that it is virtually impossible to develop a single molecule that would impact all such mechanisms. Hence, efforts should be (and are being) refocused on the use of a palette of interventions¹ to identify the most suitable synergistic approaches. Immunotherapeutic approaches can be combined not only in pairs, but also in triplets and quadruplets.^{2,3} In this setting, improved efficacy can certainly be attained, but the possibility that the combinatorial approach may result in synergistic adverse effects should be carefully monitored.

Transplanted tumors are no longer the workbench to test the efficacy of combinatorial immunotherapy, mainly because in this setting treatments are often curative and the predictability of using each agent in suboptimal conditions is not

likely to render solid preclinical evidence. Rather, investigation should be based on transgenic mice that accurately mimic human oncogenesis, tumor progression and response to therapy. Many such models are available, though often they involve multifocal carcinogenesis with weak evidence of immunoediting,⁴ mainly because of the limited accumulation of mutations that may give rise to tumor-associated antigens (TAAs) and short disease latency.

We have used a very aggressive model of multifocal hepatocellular carcinoma (HCC) based on the inducible, liver-specific expression of human *MYC*.⁵ If deprived of doxycycline at birth, animals develop multifocal HCCs in 3–4 wk, and usually die before 8–10 wk of age. Importantly, in this model, a bidirectional transgene ensures that cancer cells express *MYC* together with chicken ovalbumin (OVA) as a surrogate TAA. Previous work had unveiled the strong tolerogenic properties of OVA as a result of its expression on the surface of cancer cells.⁵

In fact, upon adoptive transfer to these mice, OT-1 T lymphocytes (which express an OVA-targeting TCR) become anergic along with TCR downregulation.

In this dire scenario, we tested a triple combination of immunostimulatory monoclonal antibodies,⁶ encompassing molecules targeting CD274 (best known as PD-L1 or B7-H1), tumor necrosis factor receptor superfamily member 9 (TNFRSF9, best known as CD137 or 4-1BB), and TNFRSF4 (best known as OX40 or CD134).⁷ PD-L1 and its receptor, programmed cell death 1 (PDCD1, best known as PD-1), were expressed within neoplastic lesions and conceivably were repressing antitumor immune responses.⁸ CD137 and OX40 were also available for antibody ligation and these interactions are known to act as agonists for T-cell and NK-cell co-stimulation.⁹

To our surprise, our combinatorial immunotherapeutic regimen prolonged the survival of *MYC*- and OVA-expressing mice. Such an effect was totally dependent on CD8⁺ T cells as well as on

*Correspondence to: Ignacio Melero; Email: imelero@unav.es

Submitted: 01/07/2014; Accepted: 01/11/2014; Published Online: 01/16/2014

Citation: Boisgerault N, Vile RG. Trick and treat: Ambushing recurrent tumors before they get out of the door. Oncoimmunology 2014; 3:e27811; <http://dx.doi.org/10.4161/onci.27811>

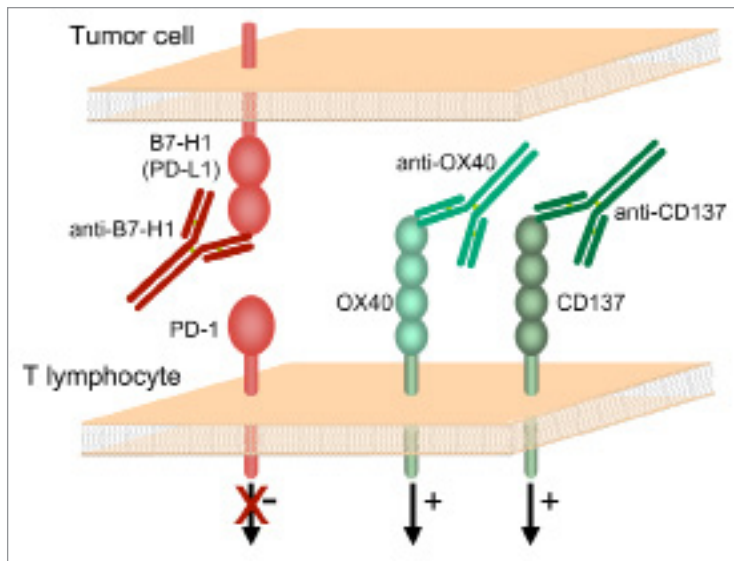


Figure 1. Molecular mechanisms underlying the therapeutic efficacy of anti-PD-L1, anti-CD137, and anti-OX40 monoclonal antibodies. Interactions between a T lymphocyte and a cancer cell. In this setting, monoclonal antibodies (mAbs) targeting PD-L1 (B7-H1) are de-repressing T-cell activation while anti-CD137 (4-1BB) and anti-OX40 (CD134) mAbs provide robust co-stimulatory signals.

NK1.1⁺ lymphocytes. Conversely, CD4⁺ T cells were dispensable, despite expressing the targets for all antibodies. The combinatorial administration of anti-PD-L1, anti-CD137 and anti-OX40 mAbs mediated long-term antineoplastic effects in 20% of the animals. In this setting, the adoptive transfer of activated OT-1 and OT-2 cells (which also express an OVA-specific TCR) is totally ineffective even when supported by intraperitoneal interleukin-2 (IL-2). However, adoptive T-cell therapy with activated OT-1 and OT-2 T lymphocytes combined with anti-PD-L1, anti-CD137 and anti-OX40 mAbs achieved an impressive efficacy, resulting in the survival in more than 70% of mice at day 250. Of note, a mAb specific for cytotoxic T lymphocyte-associated protein 4 (CTLA4) did not improve further the efficacy of our immunotherapeutic intervention in this model.

References

1. Melero I, Grimaldi AM, Perez-Gracia JL, Ascierto PA. Clinical development of immunostimulatory monoclonal antibodies and opportunities for combination. *Clin Cancer Res* 2013; 19:997-1008; PMID:23460531; <http://dx.doi.org/10.1158/1078-0432.CCR-12-2214>

Experiments addressing the mechanisms underlying our observations revealed a dramatic infiltrate of tumor lesions by CD4⁺ and CD8⁺ T cells. Both such lymphocyte subsets expressed the targets of our immunostimulatory mAbs, namely, PD-L1, CD137, and OX40. Interestingly, the co-administration of anti-PD-L1, anti-CD137 and anti-OX40 mAbs promoted the expression of their targets on T cells.

The cellular response orchestrated around interferon γ (IFN γ), perforin, and granzyme B is the ultimate effector mechanism of anticancer immunity and is significantly inhibited by the profound tolerogenic nature of the most tumors. The spontaneous immune reactivity against OVA is completely suppressed in our model, implying that the effector immune response elicited by the combinatorial administration of

2. Takeda K, Kojima Y, Uno T, Hayakawa Y, Teng MW, Yoshizawa H, Yagita H, Gejyo F, Okumura K, Smyth MJ. Combination therapy of established tumors by antibodies targeting immune activating and suppressing molecules. *J Immunol* 2010; 184:5493-501; PMID:20400706; <http://dx.doi.org/10.4049/jimmunol.0903033>

anti-PD-L1, anti-CD137, and anti-OX40 mAbs must be directed to other TAAs. We also ruled out the possibility that such response may be directed against the bacterial protein that regulates the tetracycline-repressible expression cassette employed or human MYC. Hence, an immune response targeting other, weak antigens must be involved. The nature of such antigens, however, remains elusive.

As a whole, our work demonstrates the efficacy of a triple immunostimulatory mAb-based approach acting on interrelated target molecules (Fig. 1). The efficacy of such an approach mainly relies on the release of PD-1-dependent immunosuppression coupled to dual, CD137- and OX40-driven co-stimulation.

The field of HCC therapy is potentially very fertile for immunotherapy. For instance, an anti-CTLA4 mAb has recently been associated with signs of clinical activity in HCC patients,¹⁰ and an anti-PD-1 mAb is currently being tested in a multicenter clinical trial (NCT01658878). Our proof of concept study based on murine models supports the therapeutic potential of triple immunostimulatory mAb combinations.⁷ The addition of adoptive T-cell therapy further enhanced the efficacy of our approach, suggesting the value of testing immunotherapeutic regimens to the limit. Combinations are nowadays perceived as the most suitable strategy when seeking superior efficacy.

Disclosure of Potential Conflicts of Interest

I.M. has a commercial research grant and honoraria from speakers' bureau from Bristol Myers Squibb and is a consultant/advisory board member of Merck, Bristol Myers Squibb, and Medimmune. No potential conflicts of interest were disclosed by the other authors.

3. Uno T, Takeda K, Kojima Y, Yoshizawa H, Akiba H, Mittler RS, Gejyo F, Okumura K, Yagita H, Smyth MJ. Eradication of established tumors in mice by a combination antibody-based therapy. *Nat Med* 2006; 12:693-8; PMID:16680149; <http://dx.doi.org/10.1038/nm1405>

4. DuPage M, Mazumdar C, Schmidt LM, Cheung AF, Jacks T. Expression of tumour-specific antigens underlies cancer immunoediting. *Nature* 2012; 482:405-9; PMID:22318517; <http://dx.doi.org/10.1038/nature10803>
5. Ney JT, Schmidt T, Kurts C, Zhou Q, Eckert D, Felsher DW, Schorle H, Knolle P, Tüting T, Barchet W, et al. Autochthonous liver tumors induce systemic T cell tolerance associated with T cell receptor down-modulation. *Hepatology* 2009; 49:471-81; PMID:19105207; <http://dx.doi.org/10.1002/hep.22652>
6. Melero I, Hervas-Stubbs S, Glennie M, Pardoll DM, Chen L. Immunostimulatory monoclonal antibodies for cancer therapy. *Nat Rev Cancer* 2007; 7:95-106; PMID:17251916; <http://dx.doi.org/10.1038/nrc2051>
7. Morales-Kastresana A, Sanmamed MF, Rodriguez I, Palazon A, Martinez-Forero I, Labiano S, Hervas-Stubbs S, Sangro B, Ochoa C, Rouzaut A, et al. Combined immunostimulatory monoclonal antibodies extend survival in an aggressive transgenic hepatocellular carcinoma mouse model. *Clin Cancer Res* 2013; 19:6151-62; PMID:24030703; <http://dx.doi.org/10.1158/1078-0432.CCR-13-1189>
8. Sznol M, Chen L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer--response. *Clin Cancer Res* 2013; 19:5542; PMID:24048329; <http://dx.doi.org/10.1158/1078-0432.CCR-13-2234>
9. Melero I, Hirschhorn-Cymerman D, Morales-Kastresana A, Sanmamed MF, Wolchok JD. Agonist antibodies to TNFR molecules that costimulate T and NK cells. *Clin Cancer Res* 2013; 19:1044-53; PMID:23460535; <http://dx.doi.org/10.1158/1078-0432.CCR-12-2065>
10. Sangro B, Gomez-Martin C, de la Mata M, Iñarrairaegui M, Garralda E, Barrera P, Riezu-Boj JI, Larrea E, Alfaro C, Sarobe P, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J Hepatol* 2013; 59:81-8; PMID:23466307; <http://dx.doi.org/10.1016/j.jhep.2013.02.022>