Published in final edited form as:

Clin Cancer Res. 2013 November 15; 19(22): . doi:10.1158/1078-0432.CCR-13-2424.

Harnessing immune responses in the tumor microenvironment: All signals needed

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Summary

An agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine is well tolerated in patients with advanced pancreatic adenocarcinoma. The combination results in induction of cytokines, B cell activation, and clinical responses. These findings support testing immunotherapies in combination with other established and targeted therapies.

In this issue of *Clinical Cancer Research*, Beatty and colleagues report their findings from a phase I study of an agonist CD40 monoclonal antibody (CP-870,893) given in combination with gemcitabine to 22 patients with previously untreated advanced pancreatic ductal adenocarcinoma (PDA)(1). Most patients experienced transient cytokine release syndrome and showed an induction of systemically released inflammatory cytokines (interleukin (IL)-6, IL-8, IL-10), an increase in co-stimulatory molecules on B cells (HLA-DR, CD86)), and a transient depletion of B cells. The radiographic partial response rate (19%) and median overall survival (8.4 months) were higher than expected for single agent gemcitabine (9.4% and 6.8 months, respectively)(2) providing encouraging preliminary evidence of clinical activity.

PDA remains one of the deadliest cancers with the incidence nearly approximating the death rate and novel therapies are desperately needed. While immunotherapy is promising for a number of solid tumors, this promise has yet to be realized for PDA. Single agent therapy using blocking antibodies against negative regulatory pathways, including Yervoy (anti-cytotoxic T lymphocyte antigen-4, CTLA-4)(3) and BMS-936559 (anti-programmed death ligand-1, PD-L1)(4) have yielded little activity in PDA patients. Preclinical studies suggest that combination strategies with traditional therapies including chemotherapy and radiation when given to modify the tumor microenvironment and release tumor antigens, or with other immunotherapies including vaccines that activate T cells and other immune checkpoint antagonists that block more than one inhibitory signal within the tumor microenvironment (TME) at the same time, will likely be required to effectively treat PDA(5).

CD40, a member of the tumor necrosis factor (TNF) receptor superfamily, is present on antigen presenting cells (APCs) including dendritic cells, B cells, macrophages, and non-hematopoietic (endothelial cells and some tumor cells). CD40 stimulation enhances T cell dependent anti-tumor immunity by enhancing the capacity of APCs to prime and activate tumor specific T cells, and T cell independent immunity by activating tumoricidal tumor associated macrophages(6) (Figure 1). The design of this study was driven by the same group's preclinical data establishing the collaborative interactions of gemcitabine chemotherapy with CD40 agonist therapy. While it may seem counterintuitive to use

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No conflicts to report or funding sources applicable to this manuscript.

potentially immunosuppressive chemotherapy with immunotherapeutic agents, the enhanced clinical activity of the combination may be the result of the agonist antibody's activation of the non-T cell dependent and/or T cell dependent immune responses which are further enhanced by the chemotherapy's role as a tumor lytic agent simulating an *situ* vaccine by assisting tumor antigen release in the context of CD40 antibody mediated costimulation of the tumor residing APCs. Clinically, full dose carboplatin and taxol has also been combined with CP-870,893 for the treatment of advanced solid tumors(7). In addition, Yervoy has been given with dacarbazine for the treatment of melanoma(8), and with carboplatin and taxol for the treatment of non-small cell lung cancer(9), all without causing detrimental effects to the activity of the immunotherapy. Interestingly, in the lung cancer study, the therapy was most efficacious when the immunotherapy was started during the third cycle of chemotherapy as opposed to being started concurrently, suggesting that sequencing plays an important role mechanistically.

Prior publications by this same group emphasized the important role of tumor associated macrophages in the anti-tumor effects of CD40-based therapy(6). Resected specimens from 2 patients on this clinical study showed a predominance of macrophages and not lymphocytes. Although interesting, these specimens were obtained several months after start of therapy and represent a single time point within the TME. Although always a challenge in clinical trials, repetitive biopsies of regressing tumors would provide the best opportunity to accurately elucidate the mechanism of action of CD40-based therapy.

In this case like in other diseases, macrophages may be "scavengers" after the response to therapy rather than the mediators of anti-CD40-based therapy. The authors did address this further in an earlier publication using spontaneous tumors in the KPC model, a genetically engineered PDA mouse model, and found that tumor regressions required macrophages and not T cells or gemcitabine(6). This latter finding does bring into question the role of gemcitabine in this combination. F4/80+ tumor associated macrophages express CD40. The CD40 antibody engages CD40 on peripheral blood macrophages that then infiltrate the tumor microenvironment. MHC class II and the costimulatory molecule CD86 are upregulated on these macrophages. Macrophage depletion studies suggested that CD40 therapy required macrophages for stromal depletion and anti-tumor activity. The emphasis on the role of macrophages also underscores the importance of the effects of CD40-based treatment on the stroma. CP-870,893 is thought to bind to peripheral blood monocytes which then infiltrate the tumor and promote degradation of the stroma. Interestingly, [18F]-fluorodeoxyglucose-positron emission tomography/computed tomography (FDG-PET) responses were more consistently found in the primary tumor (6 of 8 patients) with more heterogeneous responses in the metastatic lesion. Improved overall survival correlated with a decrease in FDG uptake in the hepatic lesions. Primary pancreatic tumors more often have a dense desmoplastic reaction in comparison to metastatic lesions(10) (personal communication, Chris Iacobuzio-Donahue). This difference in the stromal contribution to the tumor mass in the primary versus metastases might explain the more consistent FDG responses observed in the primary tumors in this study.

On the contrary, prior work in a transplantable KPC tumor model showed that T cells and gemcitabine were required to achieve tumor regressions(11). Perhaps the spontaneous tumor model is more biologically relevant and the role of T cells should be minimized. Alternatively, the need for gemcitabine and T cells in the transplantable model might suggest that a "vaccine" consisting of gemcitabine and tumor cells, is most effective when administered peripherally and not as *situ* release in the inhibitory TME. T cells are necessary for immune memory and induction of durable responses.

CD40-based therapy is unique in that the antibody is agonistic and provides costimulatory effects as opposed to the antagonistic approach of anti-CTLA-4 and anti-PD-1/anti-PD-L1 therapies which block negative signals. Unlike these other agents, CD40 therapy is showing preliminary efficacy without the immune-related autoimmune-like toxicities. Additional studies should focus on both understanding mechanism and the contribution of the individual components to the therapeutic effect. Gemcitabine does have single agent activity in PDA patients and preclinical data suggest that CP-870,893 has single agent activity. As the authors point out, this is a small study with large confidence intervals. Future studies would also need to address how the agent would be developed within the rapidly changing treatment landscape of PDA. Combination chemotherapy using FOLFIRINOX (oxaliplatin, irinotecan, leucovorin, fluorouracil) in good performance status patients resulted in a median overall survival of 11.1 months(2). Gemcitabine and nab-paclitaxel has recently received FDA approval for metastatic PDA given a median overall survival of 8.5 months(12). If results from this current study were reproduced in a larger study in which the combination were randomized against one of these standard therapies, this less toxic therapy would be comparable and possibly preferable to the current standards. Combinations with other immunotherapies may be another possibility. The authors suggest the possibility of combining CP-870,893 with these newer chemotherapy regimens. While immunosuppression remains a concern, these treatments have a higher response rate which would lead to better antigen release and stromal breakdown. Given signs of clinical activity and the myriad of downstream stimulatory effects, targeting CD40 is an attractive target for further development for the treatment of PDA.

References

1. Beatty GL, Torigian DA, Chiorean EG, Saboury B, Brothers A, Alavi A, et al. A phase I study of an agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma. *Clin Cancer Res*. Epub 2013/08/29.
2. Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med*. 2011; 364(19):1817–25. [PubMed: 21561347]
3. Royal RE, Levy C, Turner K, Mathur A, Hughes M, Kammula US, et al. Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. *J Immunother*. 2010; 33(8):828–33. [PubMed: 20842054]
4. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012; 366(26):2455–65. [PubMed: 22658128]
5. Le DT, Lutz E, Uram JN, Sugar EA, Onners B, Solt S, et al. Evaluation of Ipilimumab in Combination With Allogeneic Pancreatic Tumor Cells Transfected With a GM-CSF Gene in Previously Treated Pancreatic Cancer. *J Immunother*. 2013; 36(7):382–9. [PubMed: 23924790]
6. Beatty GL, Chiorean EG, Fishman MP, Saboury B, Teitelbaum UR, Sun W, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science*. 331(6024):1612–6. [PubMed: 21436454]
7. Vonderheide RH, Burg JM, Mick R, Trosko JA, Li D, Shaik MN, et al. Phase I study of the CD40 agonist antibody CP-870,893 combined with carboplatin and paclitaxel in patients with advanced solid tumors. *Oncoimmunology*. 2(1):e23033. [PubMed: 23483678]
8. Robert C, Thomas L, Bondarenko I, O'Day S, MDJ, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med*. 2011; 364(26):2517–26. [PubMed: 21639810]
9. Lynch TJ, Bondarenko I, Luft A, Serwatowski P, Barlesi F, Chacko R, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 30(17):2046–54. [PubMed: 22547592]

10. McDonald OG, Maitra A, Hruban RH. Human correlates of provocative questions in pancreatic pathology. *Adv Anat Pathol.* 19(6):351–62. [PubMed: 23060061]
11. Vonderheide RH, Bajor DL, Winograd R, Evans RA, Bayne LJ, Beatty GL. CD40 immunotherapy for pancreatic cancer. *Cancer Immunol Immunother.* 62(5):949–54. [PubMed: 23589109]
12. Von Hoff DD, Ervin TJ, Arena FP, Chiorean G, Infante JR, Moore MJ, et al. Randomized phase III study of weekly nab-paclitaxel plus gemcitabine versus gemcitabine alone in patients with metastatic adenocarcinoma of the pancreas (MPACT). *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2013; 30(suppl 34):abstr LBA148.

Figure 1.

CD40 agonist therapy in combination with chemotherapy results in simultaneous antigen release and CD40-mediated activation of antigen presenting cells including dendritic cells, B cells, and macrophages. Licensing of antigen presenting cells results in downstream activation of T cells. CD40 agonist therapy results in systemic release of cytokines such IL-6, 9, 10 and tumor stromal breakdown thereby enhancing chemotherapy delivery.