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CD40 therapy and surgery: a potential immunological partnership

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Significant strides have been made in the last two years in the field of cancer immunotherapy, including the observation of potent and durable cancer regressions following administration of monoclonal antibodies (mAbs) against negative checkpoint molecules such as CTLA-4¹, PD-1², and PD-L1³. In this issue of the *Journal of Immunotherapy*, Khong *et al.* highlight the potential of using immunomodulatory mAb against CD40 as another approach for immune based therapy of cancer. This work builds on a large body of preclinical experimentation and clinical investigation that demonstrate the immune stimulatory power of agonistic (not blocking) CD40 antibodies^{4,5}. Khong *et al.* provide interesting data showing that when administered to mice in a post-surgical setting, CD40 mAb protects against both local and distant tumor rechallenge. As agonistic CD40 mAb continue to be advanced in the clinic^{6–8}, these data suggest new approaches in combining surgery with novel immunotherapies.

The cell-surface molecule CD40, a member of the tumor necrosis family receptor superfamily, is upon ligation a vigorous activator of both the innate and adaptive arms of the immune system^{9,10}. CD40 is expressed by antigen presenting cells (APC), including dendritic cells, monocytes, and B cells, and binds to CD40 ligand, expressed by activated CD4 T helper cells and platelets^{9,10}. Ligation of CD40 results in the upregulation of costimulatory molecules and MHC molecules on the APCs, promoting effective T cell priming and activation of macrophages, B cells, and NK cells^{9,10}. Thus, CD40 ligation promotes broad activation of the innate immune response, in addition to priming of humoral and cellular adaptive immune responses.

Agonistic CD40 mAb can substitute for endogenous CD40 ligand *in vivo*^{11–13}. Although agonistic CD40 mAb treatment can have direct tumor killing effects—for example, in B cell lymphoma, for which CD40 ligation inhibits proliferation of CD40⁺ tumor cells¹⁴—CD40 expression by tumor cells is not required for efficacy of mAb treatment. Instead, the potency of agonistic CD40 mAb lies in the indirect killing of tumor cells via the myriad of activated immune cells. Anti-tumor T cell responses in particular are improved when agonistic CD40 mAb is combined with a tumor antigen vaccine^{15–19}. But the nature of this “vaccine” appears quite flexible, even including standard therapies that kill tumors and mobilize release of tumor antigen. This is quite clearly the case for chemotherapy^{20–22} or radiation therapy^{23,24} when used in combination with CD40 mAb. The manuscript by Khong *et al.*

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makes the suggestion that CD40 therapy in combination with surgery may also be immunologically synergistic.

Surgical excision is often the fastest and most straightforward way to eliminate solid tumors and remains the chief modality for cure in patients with such malignancies. Beyond removing the cancerous cells, surgical excision of the tumors may alleviate tumor-induced immunosuppression²⁵. Moreover, there may also be a “vaccine effect” of mechanically disrupting the tumor microenvironment in the process of its removal. Khong *et al.* completely resected subcutaneous mesotheliomas in tumor-implanted mice and then reinjected tumor cells at the surgical site to model local recurrence. Upon detection of secondary tumors, mice were treated with either CD40 mAb or isotype control via local or systemic administration. Decreased regrowth of local tumors, as well as improved survival, was observed in mice treated with CD40 mAb. Some mice were cured with this therapy and fully rejected a third tumor challenge, likely indicating the generation of T cell immunity after CD40 mAb therapy. Similar tumor protection was seen if mice were reinjected with tumors on the opposite flank in the post-surgical setting, modeling distant metastases. The authors do not formally demonstrate an immunological synergy between surgery and CD40 mAb therapy, nor is the “value added” from surgery measured relative to the contribution of CD40 mAb, but the study does highlight the potential use of agonistic CD40 as an adjuvant therapy.

Interestingly, regional lymph node dissection performed at the same time as surgical resection did not ablate the therapeutic effects of CD40 mAb, a surprising finding considering that T cell activation in the tumor draining lymph node could reasonably be expected to play a major role in the observed immunological effect. If tumor primed T cells had already trafficked out of the tumor and its draining lymph nodes, one might predict that the cells would be tolerized against tumor antigens. Even if this occurred, however, the administration of agonistic CD40 mAb appears to have reversed this effect. It would be interesting to determine if any T cell response generated in these studies represents newly recruited effector cells or reactivated cells (suboptimally) primed during the growth of the first tumor.

Alternatively, the authors speculate that CD40 mAb therapy might activate innate components of the immune system, outside the draining lymph node, to explain the mechanism of action. The authors, however, did not perform T cell depletion, macrophage depletion, or genetic studies to discern these possibilities. Nevertheless, the interesting hypothesis that agonistic CD40 mAb does not rely on T cells is supported by experimental evidence from several previous studies in other models^{20,26–29}. For example, we have shown that in the genetically engineered KPC model of pancreatic ductal adenocarcinoma (PDA), CD40 mAb treatment triggers activated macrophages to deplete tumor stroma and leads to cancer cell death and tumor regression in a T cell-independent fashion²⁰.

Overall, these studies highlight the potential for agonistic CD40 mAb as an adjuvant therapy with surgery in the clinical setting, with potential mechanisms explained by activation of the innate or adaptive arms of the immune response—or both. For patients with PDA, for whom early dissemination of tumor cells to metastatic niches results in most patients relapsing after resection of the primary tumor, these findings suggest immunotherapy in conjunction with surgery may be highly beneficial, providing long-lived protection that prevents recurrent or metastatic lesions from arising.

Surgery is not the only potential partner for agonistic CD40 mAb in the clinic; other methods of reducing or removing tumors have also been shown to synergize with the immunostimulatory mAb, as shown in Figure 1. Significant work has been done combining

agonistic CD40 mAb and various chemotherapies, such as gemcitabine²² and cyclophosphamide²¹, where both innate and adaptive immune cells mediate anti-tumor responses. Clinically, in patients with metastatic PDA, CD40 mAb used in combination with gemcitabine led to a 24% rate of objective tumor responses, associated with macrophage infiltration—not T cell infiltration—in the microenvironment of regressing tumors²⁰. Radiation has long been known to trigger an abscopal effect, and adding CD40 mAb to total body irradiation improves adaptive immune responses against tumors^{23,24}. Finally, combining immunotherapies is an attractive approach given the success of anti-CTLA-4 and PD-1/L-1 antibodies in the clinic^{1–3}. Indeed, combining the immunostimulatory effects of agonistic CD40 mAb with negative checkpoint regulators (e.g. anti-CTLA-4) promotes adaptive immune responses against tumors in preclinical studies¹⁷, and is currently the focus of an ongoing clinical trial at the University of Pennsylvania ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01103635), #NCT01103635). Furthermore, partnering agonistic CD40 mAb with immunostimulatory treatments drives significant tumor regression in preclinical studies^{16,19,29–32}. Thus, partnering agonistic CD40 mAb with a variety of therapies has the potential for elevating cancer patient responses and outcomes.

The ability of agonistic CD40 mAb to activate both the innate and adaptive arms of the immune system promotes rapid responses that have the potential to develop into long-lived T cell memory. The advantages of removing or reducing the tumor—in conjunction with liberation of tumor antigen via surgery, chemotherapy, radiation, or immunotherapy—provide a unique opportunity for synergy with agonistic CD40 mAb treatment. Thus, the partnership between current therapies and agonistic CD40 mAb represents an effective and realistic approach for treating cancer with long-term benefits for patients.

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Figure 1. Partnerships for agonistic CD40 immunotherapy against tumors

Agonistic CD40 mAb treatment can partner with a number of therapies (listed in figure) to drive enhanced CD8 T cell priming by dendritic cells (DC), resulting in adaptive immune responses that directly target tumor cells. CD40 mAb treatment can also promote direct tumor targeting by natural killer (NK cells) after priming by DCs. Additionally, combination treatments with CD40 mAb therapy can promote indirect tumor cell death, via reeducated macrophages (Mac) that target tumor stroma, or ligation of CD40 on endothelial cells, which promotes long-term responses when combined with adoptively transferred, tumor-specific CD8 T cells.