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Immunostimulation Versus Immunosuppression after Multiple Vaccinations: the Woes of Therapeutic Vaccine Development

□□ *Commentary on Faries et al., p. 7029, Slingluff et al., p. 7036 and La Celle et al., p. 6881*

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Three articles in this issue of *Clinical Cancer Research* show how multiple vaccinations can lead to immunosuppression. Moreover, two studies in patients show that granulocyte macrophage colony-stimulating factor (GM-CSF) as an adjuvant immunostimulant to different kind of vaccines can lead to adverse outcome in terms of relapse-free and overall survival. Modulation of regulatory T-cell activity may be required to overcome this outcome and may be crucial for the successful development of therapeutic vaccines. (Clin Cancer Res 2009;15(22):6745–7)

Three studies in this issue of *Clinical Cancer Research* show once more that the development of therapeutic cancer vaccines is very complex. Stimulation and suppression are the two sides to the coin of manipulation of the immune system: the latter may get the upper hand after multiple vaccinations and thus lead to a detrimental outcome. Two studies in patients show that even relatively straightforward questions about the utility of a well-known immunostimulant can still bring unexpected results (1, 2). A study in mice by La Celle and colleagues, shows that multiple vaccinations induce as much immunosuppression by regulatory T cells (Tregs) as immunostimulation. The study indicates that Treg-mediated immune suppression must be tackled to restore and maintain immunostimulatory activity of vaccines (3). These three articles also remind us of the notorious failures and detrimental outcomes of vaccines in the adjuvant setting in a number of large clinical trials, that need to be understood and avoided in the future.

Most disquieting are the results of the studies by Faries and colleagues and by Slingluff and coworkers on the use of granulocyte macrophage colony-stimulating factor (GM-CSF) as immuno-adjuvant in an allogeneic cell-based vaccine and a peptide-based vaccine, respectively. In the study by Faries and colleagues 97 patients with resected stage IIB-IV melanoma were randomized to receive either a cell-based vaccine with or without GM-CSF (1). It was shown that delayed-type hypersensitivity (DTH) response, immunoglobulin M (IgM) response, and other immune parameters were all increased after vaccination, including GM-CSF; but this result did not correlate with a better outcome, rather, with a significantly worse outcome. This finding sheds doubts on the value of the surrogate immune-monitoring endpoints and indicates that current clinical endpoints must remain an integral part of studies at all phases of vaccine development (4).

Similarly, the studies by Slingluff and colleagues are highly interesting. In this trial, 121 resected melanoma stage IIB-IV patients were randomized to receive 12 different melanoma peptides in combination with a tetanus helper peptide in complete Freunds adjuvant with or without GM-CSF. In this study, significantly better CD8+ and CD4+ responses were obtained in the absence of GM-CSF. Also 3-year survival rates were higher without GM-CSF than with GM-CSF (76% versus 52%), but there were too few events to classify this difference as statistically significant. Both studies clearly challenge the value of GM-CSF as a vaccine adjuvant in humans and suggest that GM-CSF may even be detrimental.

A matched control study on the potential value of adjuvant single agent therapy with GM-CSF in stage III-IV melanoma has suggested a beneficial role for GM-CSF (5). This finding has lead to a randomized phase III trial comparing GM-CSF with a multipeptide vaccine + GM-CSF (ECOG4697). Of note is the absence of an observation arm in this study. In other words a potential negative effect of GM-CSF will remain undetected in this trial. Based on the results of the studies reported in this issue of *Clinical Cancer Research*, such a detrimental impact of GM-CSF is quite possible. It would remain undetected until a comparative study of GM-CSF versus observation only would be performed. This situation is reminiscent of the observations with the ganglioside GM2 vaccine (GMK). The results of the large phase III EORTC 18961 trial comparing adjuvant vaccination with the ganglioside vaccine GMK versus observation in 1,314 patients with stage II melanoma were reported in 2008 (6). This trial was stopped early by the Independent Data Monitoring Committee (IDMC) because of the futility of the primary endpoint (RFS) and inferior survival in the vaccine arm. This difference in survival at the second interim analysis was quite similar to that observed at the second interim analysis of the ECOG1694 trial, in which 880 stage IIB-III patients were randomized between high-dose interferon (IFN) therapy and the

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GMK-vaccine (7). That trial did not have an observation comparator arm and thus the results were interpreted as having a significant impact on survival mediated by high dose IFN therapy. Now it is clear that the outcome of this trial is difficult to interpret because of the detrimental impact of the GMK-vaccine in the EORTC18071 trial comparing the vaccine to observation. It is clear that the results of these large adjuvant trials are a significant setback to the development of a vaccination strategy in melanoma (8).

Another important point is the notorious unreliability of matched control or matched pair analysis of recently treated patients with historic control patients matched for important prognostic factors. As it is impossible to match for the impact of improved imaging procedures in the staging of patients, such studies must be viewed with great caution and can only be considered as hypothesis building. The Canvaxin story provides an important example. The allogeneic cancer vaccine, Canvaxin, developed from three cell lines, was evaluated in large randomized trials in 1,166 patients with stage III melanoma and in 496 patients with resected stage IV melanoma who were randomized to Canvaxin plus BCG or placebo plus BCG after surgery. Matched pair analyses on patients who had received this vaccine after melanoma metastasis resection suggested efficacy and important survival benefit (9). The randomized trials would tell a very different story; in 2007 D. L. Morton reported on these trials that were closed prematurely by the IDMC (10). There was a survival disadvantage in patients receiving Canvaxin treatment in both studies. The median survival in the stage III study had not been reached, but the 5-year survival was 59% for those receiving Canvaxin and 68% for the untreated patients. In the stage IV study, the median survival was 32 months for the patients treated with Canvaxin and 39 months for patients receiving placebo, with respective 5-year survival rates of 40% and 45%. The outcome of these four largest adjuvant trials conducted with vaccines in stages II, III, and IV patients are problematic to say the least, especially because vaccines are believed to have their best chance of demonstrating efficacy in the adjuvant setting.

Demonstration of efficacy of vaccines in stage IV melanoma patients is a chronic problem, and very little guidance has come

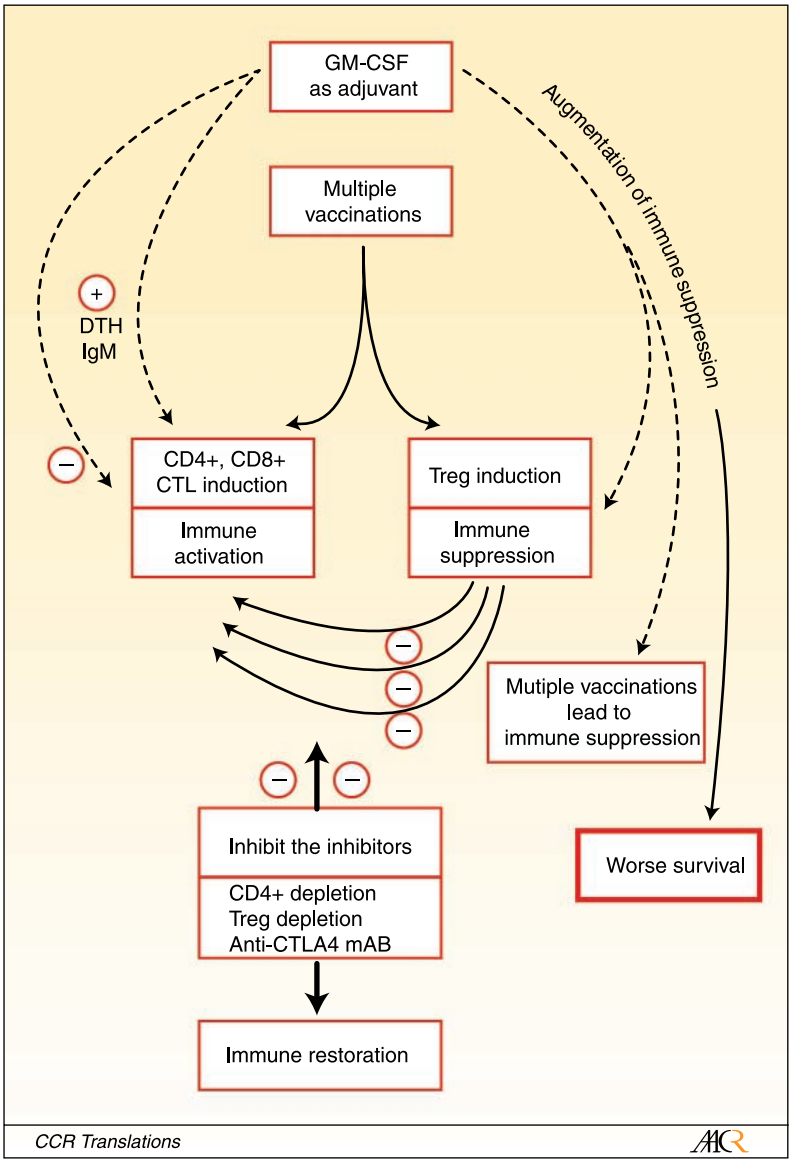

Fig. 1. Diagram shows the immune activation and immunosuppressive actions of multiple vaccinations and of GM-CSF as an adjuvant. It shows that an intervention by inhibition of the inhibitors by agents such as anti-CTLA4 monoclonal antibodies can lead to immune restoration.

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of these trials for testing in the adjuvant setting. No effective therapy for most patients with advanced metastatic melanoma is currently available. Interleukin-2 is effective in only very few patients, and the 10% response rate-chemotherapeutic drug DTIC is still the comparator drug in most pivotal phase III trials (11). Most vaccine trials have failed to show an important response rate or an impact on survival (12). Of note is the single exception, reported by Schwartzentruber and coworkers in 2009. The combination of high dose IL-2 with a gp100 peptide vaccination schedule had a significantly better progression-free survival than treatment with IL-2 alone (13). The overall situation, however, is problematic, and it has become clear that the induction of cytotoxic T-cell activity goes hand in hand with the induction of Treg activity. In other words, immune activation is always followed by immunosuppression.

Therefore, the observations reported in *Clinical Cancer Research* by La Celle and coworkers are important and instructive (3). In a murine tumor model with a GM-CSF-secreting B16BL6-D5 melanoma cell line they showed that multiple vaccinations, rather than boosting the immune response, significantly reduced the therapeutic efficacy of adoptive immunotherapy. They showed that multiple vaccinations induced an increase of Treg activity and that this could be reversed by partial depletion of CD4+ cells before the second and third vaccination. This result led to the restoration of immunity, as T-effector cells generated from these mice were highly significantly more therapeutic than T cells from multiply vaccinated mice.

In other words, multiple vaccinations lead to an immunosuppressive status and can be harmful, and reducing Treg activity, which could also be achieved by anti-CTLA4 antibodies in patients, can restore and or maintain the immune response.

Thus, this article by Lacelle and coworkers (3) suggests that monoclonal antibodies to CTLA4, such as ipilimumab and tremelimumab, may be crucial to the successful development of vaccination-based therapy. These antibodies can break self tolerance, and thus mediate antitumor effects, but at the same time result in autoimmunity in some tissues, also called immune-related adverse events (14). In stage IV patients, slowly developing, long-lasting complete remissions have been observed, both in first and second line with interesting 2-year survival rates of about 50% and 30%, respectively, suggesting that these agents may also slow down the progression of the disease in nonresponders (15). Other candidates are the PD-1 antibody with a somewhat similar mode of action (16), the anti-OX44 and anti-1-4BB, which have an agonistic action on T-cell activation, and the anti-CD25 antibody, which targets T-regulatory cells that constitutionally overexpress CD25. These antibodies allowing new approaches in immune modulation may be crucial to the successful development of vaccines in the future (17).

In summary the three articles published in this issue of *Clinical Cancer Research* and discussed here show that multiple vaccinations in solid tumors can be harmful. Therefore, this topic and this observation are in need of further study and underscore the need of safeguards in the design of vaccine trials (18). The putative mechanisms involved are depicted in Fig. 1.

Disclosure of Potential Conflicts of Interest

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