Boosting anticancer vaccines
Too much of a good thing?

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Therapeutic anticancer vaccines have eventually reached the bedside, but their clinical effects are limited. This might (at least in part) reflect our limited knowledge on the behavior of tumor-specific T lymphocytes in cancer patients. Indeed, it can be argued that T cells are stimulated by the vaccine on one hand, while—on the other hand—sensing the endogenous antigen either directly on tumor cells or via antigen-presenting cells. Such a continuous antigen stimulation in a microenvironment that is often immunosuppressive may favor T-cell exhaustion. Thus, whether, how and how frequently a cancer patient should be boosted upon vaccination remains an open conundrum.

The therapeutic potential of anticancer vaccines stems from their ability to stimulate a strong and long-lasting memory T-cell response against tumor-associated antigens (TAAs). Memory T cells can be distinguished in central memory (T_CM) and effector memory (T_EM) cells, which have different functional and phenotypic characteristics.¹ In particular, a greater antitumor function has been attributed to T_CM cells compared with T_EM cells.²

On the basis of these clues, we have recently investigated the impact of dendritic cell (DC)-based vaccines and different vaccination schedules on the persistence and antitumor activity of T_CM cells, in both prophylactic and therapeutic settings. Assuming that fully activated T_EM cells immediately respond to an antigenic challenge whereas quiescent T_CM cells must get activated first,³ we have set up a long (24 h) ex vivo intracellular interferon γ (IFNγ)-specific assay to better detect the latter population.⁴ Adopting this strategy, we have been able to demonstrate that, in healthy mice, a single DC-based vaccination elicits an antigen-specific immune response that lasts for at least 5 mo in the absence of subsequent antigen stimulation, confirming what has been reported for healthy humans⁵ and extending this concept to T_CM cells.⁴

We have also found that boosting has a considerable impact on the pool of IFNγ-producing cytotoxic CD8+ T_CM cells, which exceeds by more than 2-fold the pool detected in non-boosted mice.⁴ This holds true for both exogenous and endogenous antigens, which are recognized by T cells bearing high- and low-affinity TCR, respectively.⁴

However, the timing of boosting is critical. Indeed, a lag of at least 4 weeks was required to obtain the most potent T_CM response, correlative with the ability of vaccinated mice to reject a challenge with B16F1 melanoma cells.⁴ When mice received booster injections at earlier time points (i.e., after a 2-week interval; tight boosting), a reduced amount of T_CM cells was found in the spleen and the survival curve of these mice resembled that of mice that received only the priming injection.⁴ Unexpectedly, also boosting with complete and incomplete Freund’s adjuvants (CFA and IFA, respectively), even when performed at 4-week intervals, was detrimental for the pool of T_CM cells.⁴ These findings are in line with a recent report showing that IFA leads to the trapping of tumor-specific CD8+ T cells at the vaccination site, where they become dysfunctional and undergo apoptosis.⁶

The effect of boosting was totally unexpected in the context of minimal residual disease, which most likely benefits of vaccination. Indeed, when mice were challenged with B16F1 cells and the first dose of vaccine was given one day later, when a well-defined mass of viable melanoma cells is clearly visible at the inoculation site, no difference was found in the overall survival of mice primed and either boosted (at 2- or 4-week intervals) or not.⁷ Strikingly, a very tight (i.e., weekly) boosting schedule reduced the survival of vaccinated melanoma-bearing mice (Fig. 1). Even more surprisingly, while priming was indispensible, a 4-week boosting schedule was detrimental for the treatment of transgenic adenocarcinoma of the mouse prostate (TRAMP) mice bearing advanced autochthonous prostate cancers.⁸ Along similar lines, a tight boosting regimen has been shown

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are not aware of any study in humans that has directly compared different vaccination schedules. However, the results of two subsequent studies on the efficacy of a bacillus Calmette-Guérin (BCG)-adjuvanted vaccine in advanced melanoma patients upon surgical resection of the primary lesion suggest that an increase in the frequency of booster vaccinations is associated with a reduced median survival (36 vs. 32 mo), reinforcing the results obtained by us and others. Thus, the activation of a TAA-specific immune response with prime vaccine injections appears to be essential to promote tumor eradication. Conversely, boosting strategies in subjects with residual disease or undergoing tumor recurrence should be carefully revisited.

Why is boosting so detrimental in therapeutic settings? We speculate that the release of antigens from neoplastic lesions naturally boosts vaccine-induced immune responses, while exogenous boosts may expose T cells to excessive antigen stimulation. Indeed, the chronic exposure of T cells to TAAs may drive exhaustion.

Altogether, our results should warn against including excessively tight boosting schedules in the design of preventive vaccines and should also prompt clinical trials that specifically address the impact of boosting on tumor-specific TCM cells in cancer patients.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Figure 1. A very tight boosting schedule has a negative impact on the therapeutic efficacy of a dendritic cell-based anticancer vaccine. (A) Mice were challenged s.c. with 5 x 10^4 B16F1 melanoma cells and, one day later, either given PBS (n = 5) or primed with dendritic cells (DCs) pulsed with the cytotoxic T lymphocyte epitope TRP2180-188 (DC-pep, n = 15). A fraction of vaccinated mice (n = 10) was thereafter boosted every week with the same vaccine (Very Tight Boost). Animals were followed for disease progression, and they were euthanatized when tumors reached a diameter of 10 mm or became ulcerated. (B) Kaplan–Meier survival curves representative of 2 independent experiments are reported. Long-Rank test: No Boost vs. PBS, p = 0.01; Really Tight Boost vs. PBS, p = 0.0003; Really Tight Boost vs. No Boost, p = 0.01.

to negatively influence the therapeutic potential of adoptively transferred cytotoxic T lymphocytes when compared with a single inoculation. Thus boosting is either dispensable or detrimental in these preclinical scenarios.

We are not aware of any study in humans that has directly compared different vaccination schedules. However, the results of two subsequent studies on the efficacy of a bacillus Calmette-Guérin (BCG)-adjuvanted vaccine in advanced melanoma patients upon surgical resection of the primary lesion suggest that an increase in the frequency of booster vaccinations is associated with a reduced median survival (36 vs. 32 mo), reinforcing the results obtained by us and others. Thus, the activation of a TAA-specific immune response with prime vaccine injections appears to be essential to promote tumor eradication. Conversely, boosting strategies in subjects with residual disease or undergoing tumor recurrence should be carefully revisited.

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