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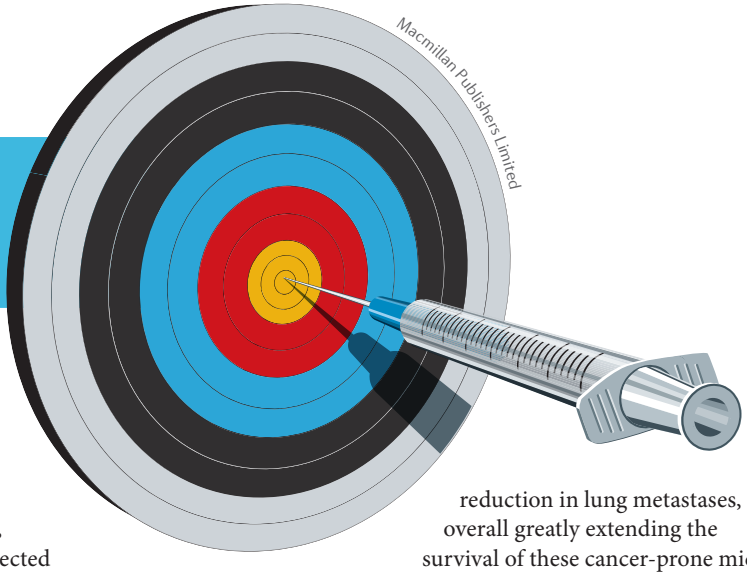
IMMUNOTHERAPY

Direct shot



Harnessing the immune system to fight tumours is providing real hope in treating patients with cancer. Yet several immunotherapy approaches are limited by the need for prior knowledge of tumour antigens or large doses of expensive antibodies. In a recent study published in *Science Translational Medicine*, researchers use a non-customized approach that involves intratumoural injection with low doses of immuno-enhancing agents that induce T cell responses that are able to eliminate both the injected tumour and tumours at distant sites. Injection with a combination of Toll-like receptor 9 (TLR9) ligand and agonistic anti-OX40 antibody proved highly effective in eradicating spontaneous malignancy.

To identify agents with potential anticancer effects, the authors injected various immunostimulatory agents directly into established tumours in mice and analysed the effects on T cells. After intratumoural injection with the TLR9 ligand CpG oligodeoxynucleotides (ODNs), there was an upregulation of expression of the activating receptor OX40 on $CD4^+$ effector T cells and regulatory T (T_{reg}) cells infiltrating the injected tumour. A similar upregulation of T cell OX40 expression was seen in humans after intratumoural injection with CpG ODNs. *In vitro* culture analysis indicated that OX40 upregulation by T cells was dependent on the presence of myeloid cells secreting cytokines such as IL-12, interferon-γ and tumour necrosis factor.

Next, the authors tested whether ligation of OX40 with an agonistic anti-OX40 antibody might further enhance CpG ODN-induced antitumour immune responses. Mice were implanted with A20 B cell lymphoma tumours at two different sites, one of which was injected with CpG ODNs with or without the anti-OX40 antibody. Only the combination of both immune stimulants induced regression of both the injected tumour and, several days later, the distant (noninjected) tumour. Tumour regressions were long-lasting and led to cure of most of the mice. Moreover, therapeutic effects were achieved by extremely low doses of both CpG ODNs (typically 50 μg) and anti-OX40 antibody (typically 8 μg).

The systemic antitumour response required the presence of both $CD4^+$ and $CD8^+$ T cells. The proportions of effector memory T cells increased in the treated site immediately after CpG ODNs and anti-OX40 co-injection, in the spleen after 24 hours and in the distant tumour after 5 days, which is consistent with the kinetics of a T cell response. Notably, anti-OX40 antibody was more effective than immune checkpoint antibody against programmed cell death 1, which delayed tumour growth in the nontreated site but was not curative.

The combination of CpG ODNs and anti-OX40 antibody was then tested in a model of highly invasive mammary ductal carcinomas that give rise to spontaneous lung metastases — namely, PyVt/PyMT mice. Treatment at a single tumour site protected against the occurrence of new independent mammary tumours and led to a significant reduction in lung metastases, overall greatly extending the survival of these cancer-prone mice.

> “therapeutic effects were achieved by extremely low doses of both CpG ODNs … and anti-OX40 antibody”

Importantly, the vaccination-induced immune response seemed to be antigen specific: treated mice were immune to a secondary challenge with the same tumour but not to an antigenically distinct tumour. T cell responses against multiple tumour antigens could be achieved by injecting a tumour comprising a mixture of tumour cell types at one site.

In terms of a mechanism, the authors show that the immuno-enhancing activity of the anti-OX40 antibody was mediated by impaired T_{reg} cell function (but not T_{reg} cell depletion) as well as by stimulation of effector T cells. An Fc-competent version of the anti-OX40 antibody was required for its stimulatory effect, through a mechanism dependent on Fc receptors expressed by natural killer cells.

Together, these data identify a practical strategy for immunotherapy of cancer that takes advantage of a pre-existing T cell immune repertoire in the tumour environment that can be boosted by *in situ* vaccination with a TLR agonist and anti-OX40 antibody.

Lucy Bird

ORIGINAL ARTICLE Sagiv-Barfi, I. *et al.* Eradication of spontaneous malignancy by local immunotherapy. *Sci. Transl Med.* **10**, eaan4488 (2018)
FURTHER READING Weiden, J., Tel, J. & Figdor, C. G. Synthetic immune niches for cancer immunotherapy. *Nat. Rev. Immunol.* https://doi.org/10.1038/nri.2017.89 (2017)