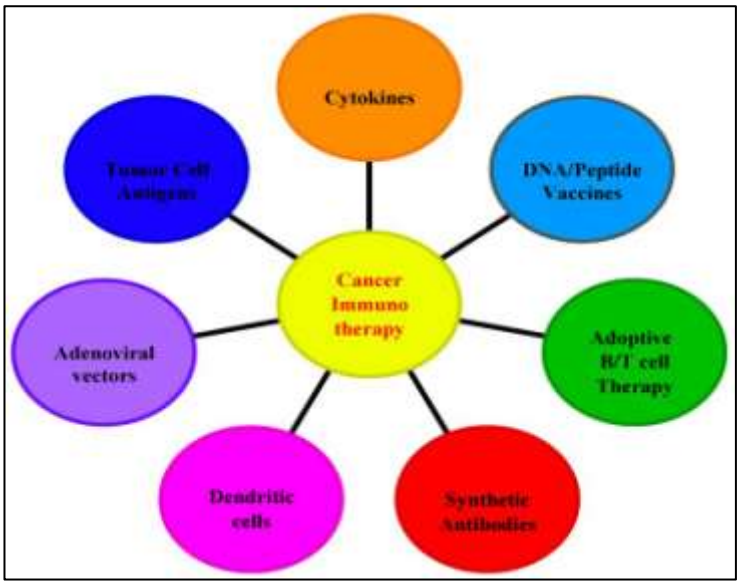
CANCER IMMUNOTHERAPY

Immunotherapy is a new class of cancer treatment that works to harness the innate powers of the immune system to fight cancer. Because of the immune system's unique properties, these therapies may hold greater potential than current treatment approaches to fight cancer more powerfully, to offer longer-term protection against the disease, to come with fewer side effects, and to benefit more patients with more cancer types.

Passive transfer of anticancer monoclonal antibodies and donor T cells in the context of allogeneic bone marrow transplantation are effective treatments for a variety of haematological and solid malignancies. Although not always thought of as ‘immunotherapy’, the success of these biotherapeutics probably reflects the ability of the donor cells or antibodies to induce an immediate immune reaction against the cancer, bypassing the requirement to activate endogenous immunity. Nine monoclonal antibodies targeting six cancer-associated proteins (Her2/neu, EGFR, VEGF, CD20, CD52 and CD33) are approved for the treatment of solid and haematological malignancies. In addition to antagonizing oncogenic pathways, these biotherapeutics may act by opsonizing tumour cells and triggering their death or removal by antibody-dependent cellular cytotoxity or phagocytosis. Ongoing investigations in murine models and patients raise the possibility that they may also stimulate adaptive immune responses in some settings. Recently, the successful conjugation of toxins to antibodies has been achieved, and these have induced a clinical response in patients who are refractory to the naked antibody. The concurrent administration of immunostimulatory cytokines such as IL-2 and GM-CSF may also enhance the efficacy of antibody therapy. Other immune treatments that have received the FDA approval include recombinant cytokines, such as IL-2 (Proleukin), which is used for melanoma and renal cell cancer. Response rates are low (15%) and the significant risk of serious systemic inflammation requires administration as an in-patient. Interferon-α is another agent that gained approval for melanoma or renal cell cancer. Although also associated with low response rates and high-dose toxicity, a small subset of melanoma patients, who are also predisposed to autoimmunity, has been shown to exhibit an impressive survival response. These immune treatments have been well established in oncology for several decades, and continued

advances in antibody and T-cell engineering should further enhance their clinical impact in the years to come (Nature, vol 1480, 22, Dec 2011).



In contrast to these passive immunotherapy strategies, the active stimulation of specific and durable antitumour immunity has proved elusive. In 1891, William Coley began intratumoral injections of live or inactivated *Streptococcus pyogenes* and *Serratia marcescens* in an effort to reproduce the spontaneous remissions of sarcomas observed in rare-cancer patients who had developed erysipelas. Given Elie Metchnikoff's contemporaneous work demonstrating the immune system's ability to cause inflammation and destroy invading bacteria, 'Coley's toxins' made sense by acting to stimulate antibacterial phagocytes that might kill bystander tumour cells. Some significant responses were recorded over the ensuing 40 years, but successes were sporadic, difficult to reproduce and not obtained in a scientifically rigorous fashion. The approach was never embraced by oncologists who continued to rely on surgery and, increasingly, on effective new methods, such as radiation therapy and ultimately chemotherapy.

Before continuing, however, it is useful to summarize what must happen to elicit a protective immune response to cancer, and why overcoming these barriers has been so difficult. There are three distinct steps that must be achieved to mount effective antitumour immune response:

- **Dendritic cells must sample antigens derived from the tumour**, which can be ingested in situ or delivered exogenously as part of a therapeutic vaccine. These antigens might reflect one or more of the many mutated proteins that are typical of cancer, the products of nonmutated genes that are preferentially expressed by cancer cells (for example, cancer-testis antigens), or differentiation antigens associated with the cancer's tissue of origin, but against which thymic or peripheral tolerance has not been completely established (for example, melanosome associated proteins in melanoma). On antigen encounter, the dendritic cells would also have to receive a

suitable activation (‘maturation’) signal, allowing them to differentiate extensively and present tumour-antigen-derived peptides. Activation signals could be therapeutically supplied exogenously (for example, Toll-like receptor (TLR) ligands or agonist antibodies against activating receptors such as CD40 or endogenously: dying or necrotic tumour cells release factors (for example, high mobility group proteins or ATP) that are thought to result in the immunogenic maturation of dendritic cells.

- In lymphoid organs, tumour-antigen-loaded **dendritic cells must generate protective T-cell responses**. The precise type of T-cell response needed is unknown, but they certainly include the production of CD81 effector T cells with cytotoxic potential. Dendritic cells may also trigger antibody and natural killer (NK) or natural killer T (NKT)- cell responses, which may contribute to tumour immunity. The lymph node is a second potential site for therapeutic intervention, providing agents that may help guide the T-cell response. However, the dendritic cells are again key players because they must have been matured by a stimulatory adjuvant to have a chance at eliciting the desired T cells. Presentation of antigens by dendritic cells at the steady state (that is, dendritic cells that have not received an immunogenic maturation signal) promotes tolerance by regulatory T cell (Treg) production, which would oppose an antitumour response.
- Cancer-specific T cells must enter the tumour bed to perform their function at which point immune suppression becomes a challenge. Tumours may (presumably by skewing dendritic cell maturation) prevent immunization, trigger the ‘wrong’ immune response or enable the local accumulation or expansion of Treg cells that would oppose the activity of effector T cells. Indeed, infiltration of Treg cells correlates with poor prognosis in a variety of epithelial tumour types.

Thus, the success of immunotherapy depends on overcoming several significant barriers:

- Tumour-associated antigens are typically closely related or identical to self antigens. Thus antitumor activity should safely encompass the tolerance of self antigens.
- To separate therapeutic responses from pathological autoimmune responses.
- Both central and peripheral tolerance would have conspired to deplete or inactivate the relevant T-cell repertoire.
- The tumour microenvironment is inherently immunosuppressive.

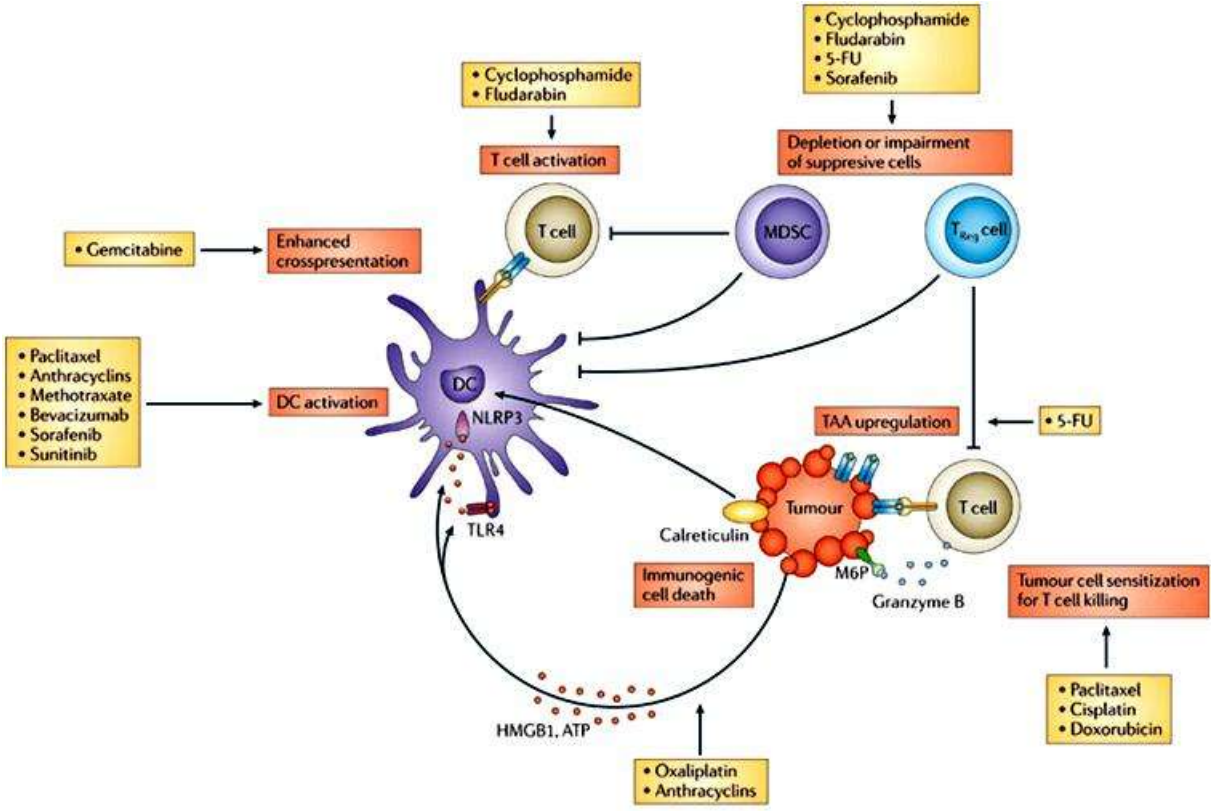


Fig: Multiple chemotherapeutic drugs targeting the different components of immune system to initiate Tumor cell killing (W. Joost Lesterhuis et. al. *Nature Reviews Drug Discovery* August 2011 **10**, 591-600).

CANCER VACCINES

The idea of a therapeutic cancer vaccine originated with the discovery that patients can harbour CD81 and CD41 T cells specific for antigens expressed in their tumours. Vaccination might reasonably be expected to amplify the frequency and strength of these pre-existing responses or perhaps induce some *de novo* reactions. Additionally, clinicopathological studies have demonstrated a strong association between prolonged patient survival and the presence of intratumoral CD31 or CD81 cytotoxic T cells and an IFN-γ gene signature. Thus, if vaccination could trigger these types of T-cell responses, then a clinical benefit might be expected.

Many initial attempts were compromised by a poor understanding of the mechanism of immunization, specifically the role of dendritic cells. However, there now seems to be a path to clinical success due to rational design of immunotherapy based on better understanding of dendritic cells. One potentially promising approach involves the use of peptides (20-mer) derived from viral oncoproteins in the presence of a suitable dendritic-cell

activating adjuvant, these peptides are thought to be more efficient at generating effector T cells. A recent study of peptides derived from the HPV-16 E6 and E7 viral oncoproteins administered in incomplete Freund's adjuvant showed clinical responses in 15 of 19 women with vulvar intraepithelial neoplasia. Tumour regressions were associated with the generation of HPV-specific, IFN-γ-producing CD41 and CD81 T cells. These favourable results might reflect in part the selection of viral gene products for immunization, because these proteins might be more readily recognized as foreign by the host. Full-length proteins are also being explored as targets for cancer vaccinations, as they contain a broader profile of epitopes that might be presented by dendritic cells. Indeed, a small trial of long peptides derived from TP53 (previously known as p53), a tumour suppressor often mutated in cancer, delivered in Montanide (an emulsion-adjuvant) induced a weaker IFN-γ production.

Viral vectors encoding tumour antigens are another vaccine platform undergoing evaluation. These strategies exploit the strong immune response directed against viral components to enhance reactivity against the cancer antigen. Similarly, configured fowlpox vector was administered subsequently in a prime-boost strategy, and GM-CSF was administered with the vectors for further immune stimulation (the entire vaccine was termed as PROSTVAC). Another strategy for vaccine therapy involves the use of cell-based approaches. One of the ideas underlying this strategy is that an actual cancer cell would already harbour a wide range of tumour-associated antigens (including mutant proteins), so that if used as a vaccine the problem of antigen selection would be reduced.

There has also been considerable interest in developing dendritic-cell-based vaccines. In this approach, dendritic cells are isolated from a cancer patient, loaded with antigens (peptides or even tumour cell lysates) ex vivo, activated and then reinfused back into the patient. In general, there are many barriers to success, or at least to quantifying the success, of cancer vaccines administered as 'single agents'.

- The criteria for defining optimal tumour antigens remain to be fully defined. Mere expression in the target tumour population may be inadequate for predicting the ability to generate protective T-cell response.
- The optimal adjuvant for producing antitumour CD81 T-cell responses that can be used safely and effectively in humans is not yet clear.
- The effectiveness of a tumour-specific T-cell population may be limited by the multiple mechanisms of immunosuppression used by tumours to guard against T-cell killing.

IPILIMUMAB'S EMERGENCE AS AN EFFECTIVE THERAPY

The most significant development for cancer immunotherapy was the recent readout of the ipilimumab phase III trials in late-stage metastatic melanoma. Not only it quoted a clear survival advantage observed for a patient group with no other therapeutic options, but it was achieved with an agent whose mechanism of action is virtually certain to involve the modulation of endogenous T-cell responses. Based on these results, FDA in March 2011 granted broad approval for use in patients with metastatic melanoma, either as initial therapy or after relapse.

Ipilimumab is a monoclonal antibody to CTLA4, whose role in regulating T-cell function has been studied for many years. CTLA4 is a key negative regulator that is recruited to the plasma membrane on T-cell activation where it binds to members of the B7 family of accessory molecules expressed by dendritic cells and other antigen-presenting cells. CTLA4 ligation effectively inhibits further activation and expansion, thereby controlling the progress of an immune response and attenuating the chances for chronic autoimmune inflammation. The negative regulation is overcome by use of a blocking antibody. Pre-clinical studies using mouse models were promising, which led two companies (Pfizer and BMS/Medarex) to put two different anti-CTLA4 antibodies into the clinic. Phase II trials failed to reach their endpoints of tumour regression, but BMS/Medarex felt there was sufficient potential for long-term benefit, and so a lengthy randomized phase III trial was initiated in relapsed-refractory metastatic melanoma patients to determine overall survival. A twofold survival benefit was detected at 12–15 months which was still durable after 2.5 years and included a complete response in some patients.

The use of ipilimumab does present some clinical and scientific challenges. First is the significant rate of on-target toxicities. Up to 23% of the ipilimumab-treated patients developed serious colitis and hypophysitis due to induced inflammation. A second clinical challenge with ipilimumab relates to the kinetics of the antitumour response. In contrast to conventional cytotoxic therapies that may trigger rapid tumour shrinkage due to direct killing of cancer cells, the stimulation of T-cell response with ipilimumab may take several months to occur. Tumours may increase in size during this period, and some component of this growth may be a result of the evolving inflammatory reaction. Despite these limitations, ipilimumab provides realistic hope for melanoma patients and a clear clinical validation for cancer immunotherapy in general.

THE NEXT GENERATION T-CELL IMMUNOMODULATORS

The success of anti-CTLA4 in melanoma should create interest in evaluating other antibodies that can be used to activate T-cell responses. There are a number of known receptors that could serve as targets for agonist antibodies, including 4-1BB, OX40, GITR, CD27 and CD28. The latter, however, introduces a cautionary note owing to an early clinical trial of an agonist anti-CD28 (TGN1412) in which severe toxicities and even death resulted from unexpected cytokine release. These serious events emphasize the power of the immune system and the need for extreme care and a conservative trial design when using any immune activator. The use of agents that clear more rapidly from the circulation than intact IgGs may help mitigate the potential for such toxicities, or at least enable the more rapid removal of the inducing drug. The same consideration may apply to anti-CTLA4 therapy, where alternative dosing strategies may serve to increase its therapeutic index. LAG-3 is another T-cell receptor that, likeCTLA4, is largely suppressive. Not as well studied asCTLA4, LAG-3 appears similar in that it acts to limit the activity of CD41 and CD81 T cells, and augment the activity of Treg cells.

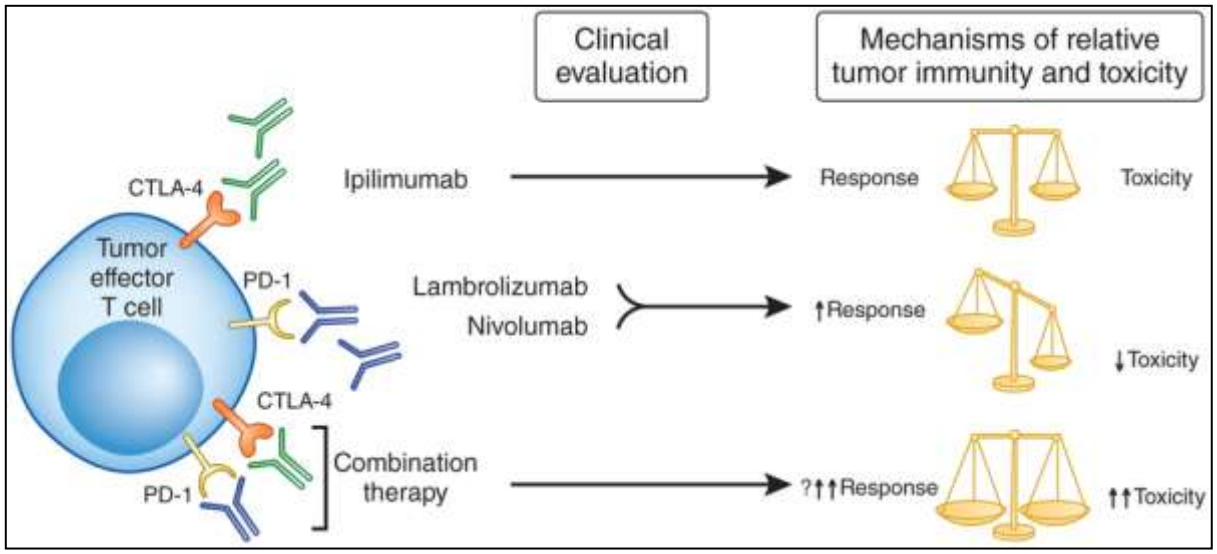


Fig: Different targets of T cell immunomodulators. (*Nature Medicine* **19**, 1100–1101, 2013)

PERSPECTIVE

In addition to specific antigen recognition through the TCR, T-cell activation is regulated through a balance of positive and negative signals provided by co-stimulatory receptors. These surface proteins are typically members of either the TNF receptor or B7 superfamilies. Agonistic antibodies directed against activating co-stimulatory molecules and

blocking antibodies against negative co-stimulatory molecules may enhance T-cell stimulation to promote tumour destruction.

Cancer immunotherapy has come to lime light at the right time. The advent of a cohort of inhibitors that target oncogenic pathways with ever greater specificity is starting to reveal significant and sometimes spectacular responses in several indications. Yet, even in diagnostically defined populations, these responses can be transient or require continued dosing. If such drug regimens can be matched to appropriate immunotherapies, activating a patient's immune system during a time of tumour reduction and diminution may be the best way to ensure that responses are converted to a long-term and durable benefit. The mechanism of action in immunotherapy is so distinct, both mechanistically and temporally, compared with conventional cytotoxic drugs, that it cannot be expected to perform according to standards developed a generation ago, even though the result may ultimately be curative. Thus a dire need exists to create a multi-disciplinary platform including drug designers, oncologist and pharmacologist to work together with a single prime target of developing a curative immunotherapy.