Immunotherapy: A New Era in Cancer Treatment

This is to acknowledge that Dr. Deepak Nijhawan M.D. PhD has disclosed that he does not have any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Nijhawan will not be discussing off-label uses in his presentation.

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Dr. Nijhawan supervises a laboratory interested in discovering the mode of action for chemicals that are toxic to cancer cells. These discoveries have the potential to not only unveil new biology, but also lead to potential therapies.

Purpose: A review of the discovery and development of immunotherapeutic drugs for cancer.

Overview: Drugs that manipulate the immune system have recently been shown to have durable clinical responses in patients with melanoma. In this protocol and presentation, we discuss the scientific and clinical milestones that led to that achievement. Each of these milestones was surrounded by controversy and there are lessons to be learned in how the challenges were overcome.

Educational objectives:

- **Learn the experimental rationale for cancer immunosurveillance**
- **Learn how CTLA-4 was discovered and the rationale for targeting CTLA-4 in cancer**
- **Review clinical trials for immunotherapeutic agents**

Cancer continues to be a common cause for death, and there is an urgent need for new treatments. Most of our attempts to treat cancer have yielded modest improvement in outcomes measured in weeks to months of prolonged survival. These modest gains are further compromised by severe, sometimes fatal adverse events.

For more than a century, it has been proposed that the immune system could be harnessed to fight cancer. In 1957, Burnett proposed that tumors might be recognized as foreign by the immune system and that one strategy to eradicate cancer would be to activate that immune response. In the 50 years since his proposal, immunotherapeutic agents have been developed and are beginning to show durable responses lasting more than three years in some cancer patients. (Figure 1) In this protocol, I will focus on the controversies surrounding three major milestones in the development of these agents. These milestones are the following: 1) establishing a biological rationale in mouse models that the immune system has a role in cancer surveillance, 2) the discovery of cytotoxic t cell lymphocyte 4 (CTLA-4) as a protein involved in immune checkpoint, and 3) the success of ipilimumab in improving the overall survival of patients with metastatic melanoma in a randomized controlled trial. By achieving these

three milestones, immunotherapy is poised to become a cornerstone in the treatment of cancer.

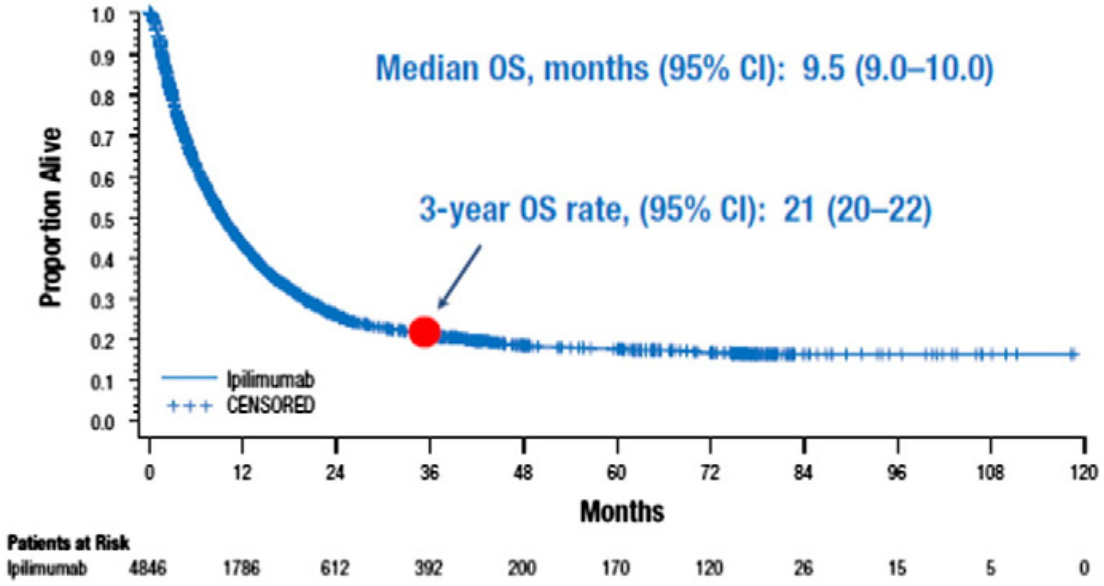

Figure 1. Long term clinical outcomes in over 4,800 metastatic melanoma patients that have received ipilimumab. Nearly 20% of patients are alive at least three years after treatment. [1]

A rationale for cancer immunosurveillance.

The discovery of the athymic nude mouse in 1962 provided a critical reagent to determine the role of the immune system in cancer. The athymic nude mouse is a spontaneous mutant that lacks a thymus and as a result lacks mature T lymphocytes. The mouse also lacks hair, which is why it is called nude. These mice are immune deficient evidenced by their inability to reject skin

grafts from unrelated mouse strains. Stutman in 1974 administered methylcholanthrene (MCA), a chemical carcinogen that leads to sarcomas in mice, to a nude mouse (homozygous), nude mouse (heterozygous), or a wild type mouse that readily produces sarcomas after intramuscular injections into mice. [2] The homozygous nude mouse lacked the ability to reject skin graft which confirmed that they were immune-deficient. Nonetheless, all three experimental groups had the same frequency of tumor formation. These observations suggested that mature T lymphocytes and a functional thymus had no impact on tumor formation. The strength of this data silenced much of the enthusiasm for harnessing the immune system to attack cancer.

In the 1990's there was a resurgence of interest in cancer immunosurveillance new results using genetically engineered mouse models of immune deficiency. By using gene-targeting technology in mice, several groups were able to generate mice lacking genes essential for immune function. These included genes essential for lymphocyte survival such as recombination activating gene (RAG) that results in complete loss of T, B, and natural killer (NK) lymphocytes. In addition, mice were generated that lacked mediators of immune activation such as the interferon gamma receptor or its downstream effector STAT. By eliminating

either all lymphocytes or required mediators, these mouse models were more immune-deficient than the athymic nude mouse. Hence, they provided an opportunity to retest Stutman's original hypothesis. Surprisingly, Schreiber and colleagues, as well as others found that genetically engineered immune deficient mice lacking RAG, STAT, or IFN gamma receptor all were more likely to form a tumor after an injection of MCA. (Figure 2-3) [3,4] These studies overruled Stutman's original claim and resurrected the field of tumor immunology.

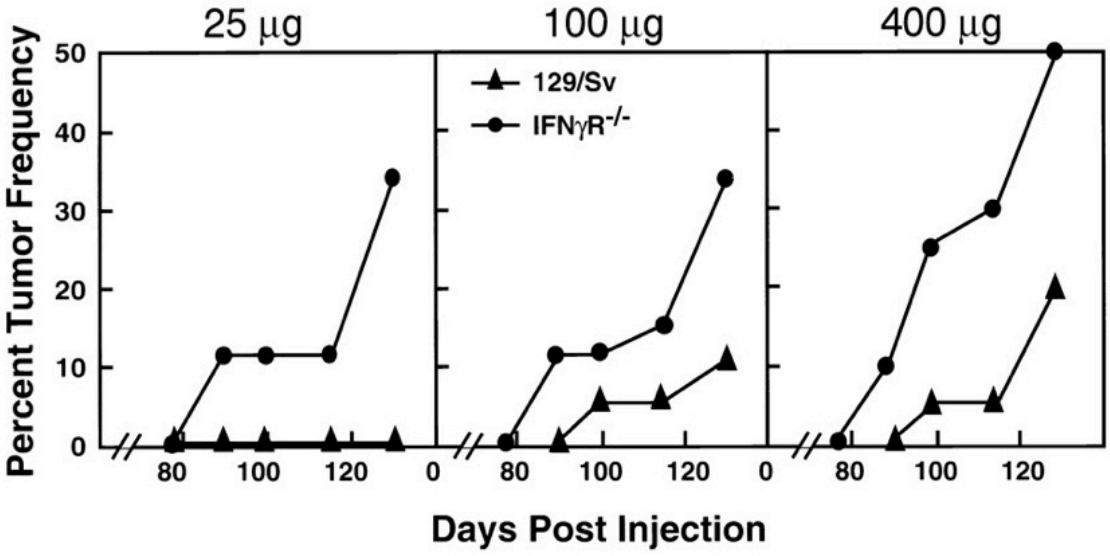

Figure 2. Wild type mice (129/Sv) and IFN gamma receptor null mice were injected with increasing concentrations of MCA, a chemical carcinogen. The percent of mice with tumors was higher in the mice lacking IFN gamma receptor.

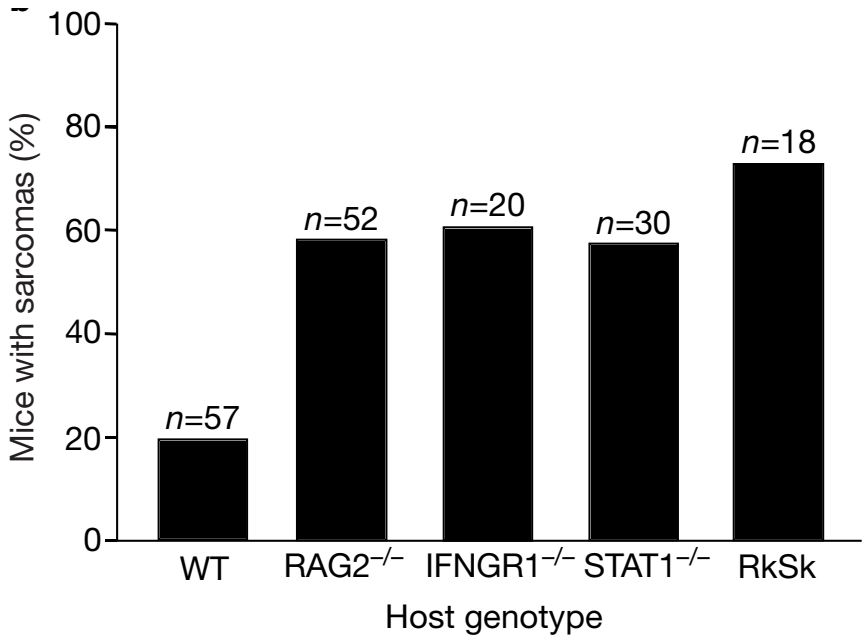

Figure 3. Mice immune deficient as a result of loss of RAG2 (recombination activating gene), IFN gamma receptor, STAT, or both of the latter two gene products have higher tumor frequencies in response to chemical carcinogens.

Coincident with the mouse studies, there was mounting evidence of cancer immunosurveillance in humans. Patients whose immune system is compromised have an increased incidence of cancer. Most of these cancers have a viral etiology and include squamous cell cancer related to human papilloma virus (HPV) and epstein bar virus (EBV). However, the risk of other solid tumors not know to have a viral etiology are also higher. For instance in a epidemiologic analysis of cancer outcomes in patients who

are received a cadaveric renal transplant and are medically immunosuppressed, the risk of melanoma is 4-5 fold higher.[5]

A more direct link between the immune system and cancers comes from studies analyzing and quantifying the number of lymphocytes in a tumor, so called tumor infiltrating lymphocytes (TIL). (Figure 4) The number of lymphocytes invading the tumor correlated with improved survival outcomes in both melanoma and colorectal carcinoma. [6] [7]

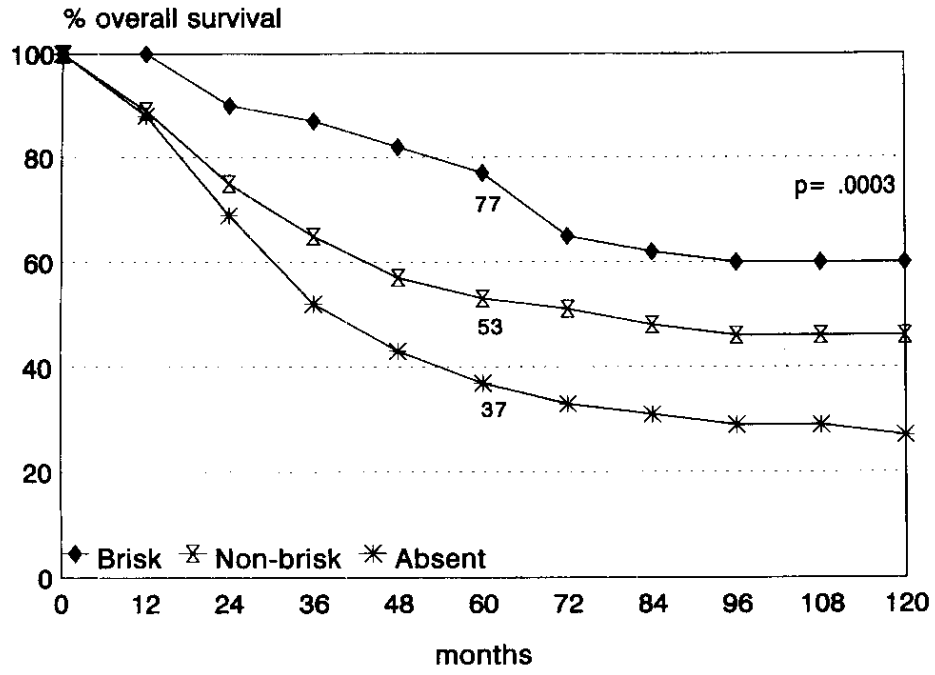

Figure 4. The number of lymphocytes infiltrating a melanoma lesion was quantified and designated “brisk”, “non-brisk”, or absent. Patients with a “brisk” response had better survival outcomes.]

The discovery of CTLA-4 as an immune checkpoint regulator.

In order to effectively manipulate the immune system in order to target cancer required a detailed understanding of the proteins involved in the immune response. The activation of a specific T cell clone as an initial response to a foreign agent depends on the binding of the major histocompatibility complex protein in complex with a peptide to the T cell receptor (TCR). Activation of the immune system requires a co-stimulatory signal in addition to the MHC-TCR interaction. The MHC-TCR interaction is, however, not sufficient to activate T cells. T cell activation requires a second signal through the binding of B7-1 or B7-2 (hereafter referred to as B7) to CD28, which is constitutively expressed on T lymphocytes. (Figure 5) [8] This second signal acts as a necessary co-stimulator to activate a particular T cell clone.

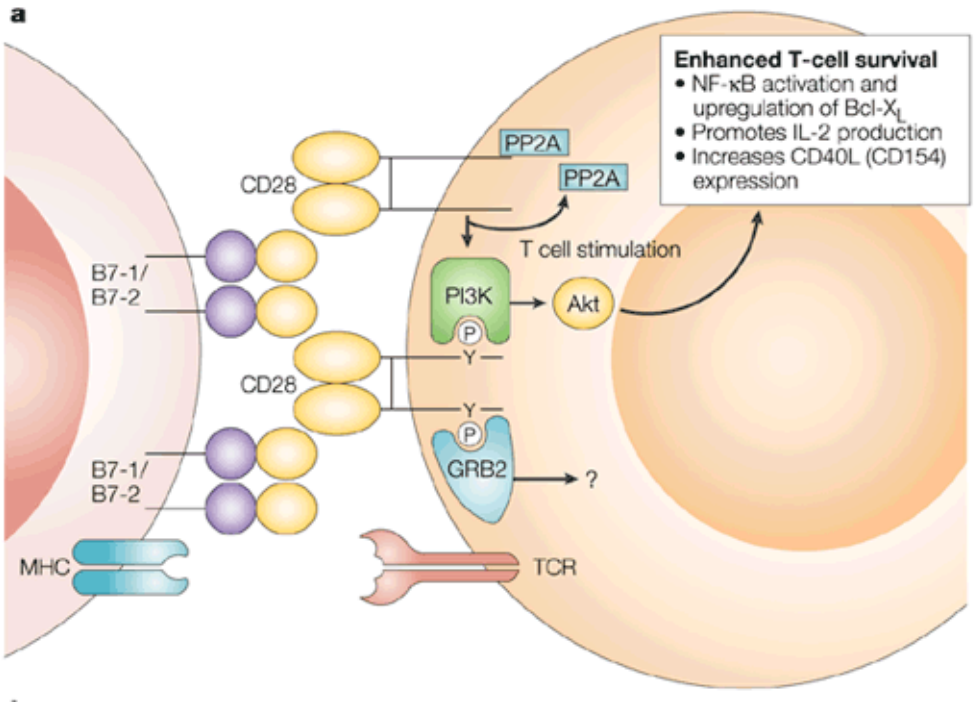

Figure 5. The two signal model of T cell activation. CD28 binding to B7-1/2 is a necessary co-stimulatory signal to activate T cells.

CTLA-4 was discovered as a mRNA that was upregulated in activated T cell lymphocytes [9], and the first clue to its function came from its similarities to CD28. CTLA-4 is 75% identical at the protein sequence to CD28, and like CD28, binds to B7 proteins with high affinity. [10] Allison and his colleagues developed an in vitro system of T cell activation in which they triggered interleukin 2 release by crosslinking antibodies to CD28 and TCR. Antibodies that activate CTLA-4 inhibit IL-2 release by T cells. [11] These observations suggested that CTLA-4 binds to B7 as an

antagonist to CD28 leading to a dampening of T cell activation. (Figure 6)

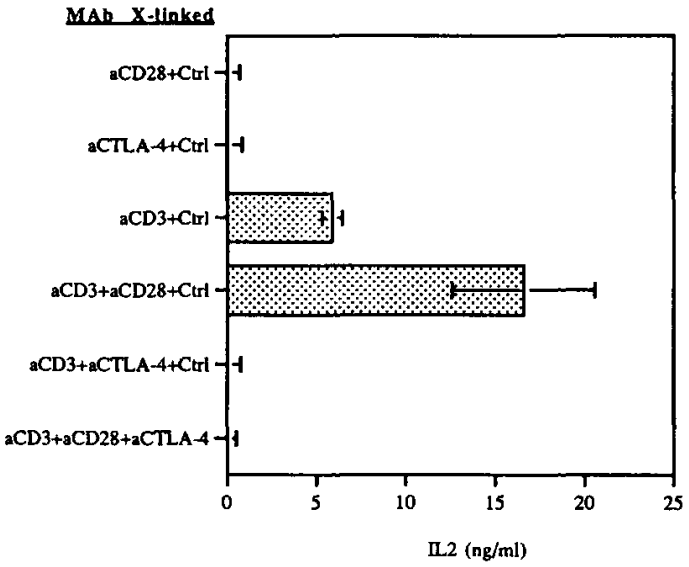

Figure 6. Activating antibodies of CD3 (TCR complex) and CD28 lead to release of IL2, a marker of T cell activation. This is inhibited by activation of CTLA-4.

The upregulation of CTLA-4, therefore, is considered a "checkpoint" on the degree of the immune response. Consistent with this hypothesis, Mak and colleagues generated mice lacking CTLA-4 and showed that they develop a massive expansion of their lymphocytes and manifest symptoms of auto-immune disease. [12]

Allison and colleagues proposed that antagonism of CTLA-4 might stimulate the immune system to attack an existing cancer. In a landmark experiment, they discovered that tumors derived from mouse cancer cell lines regressed

after treatment with neutralizing antibodies to CTLA-4. Moreover, when these mice were rechallenged with the same cancer cells, they were refractory to tumor formation. (Figure 7) [13]

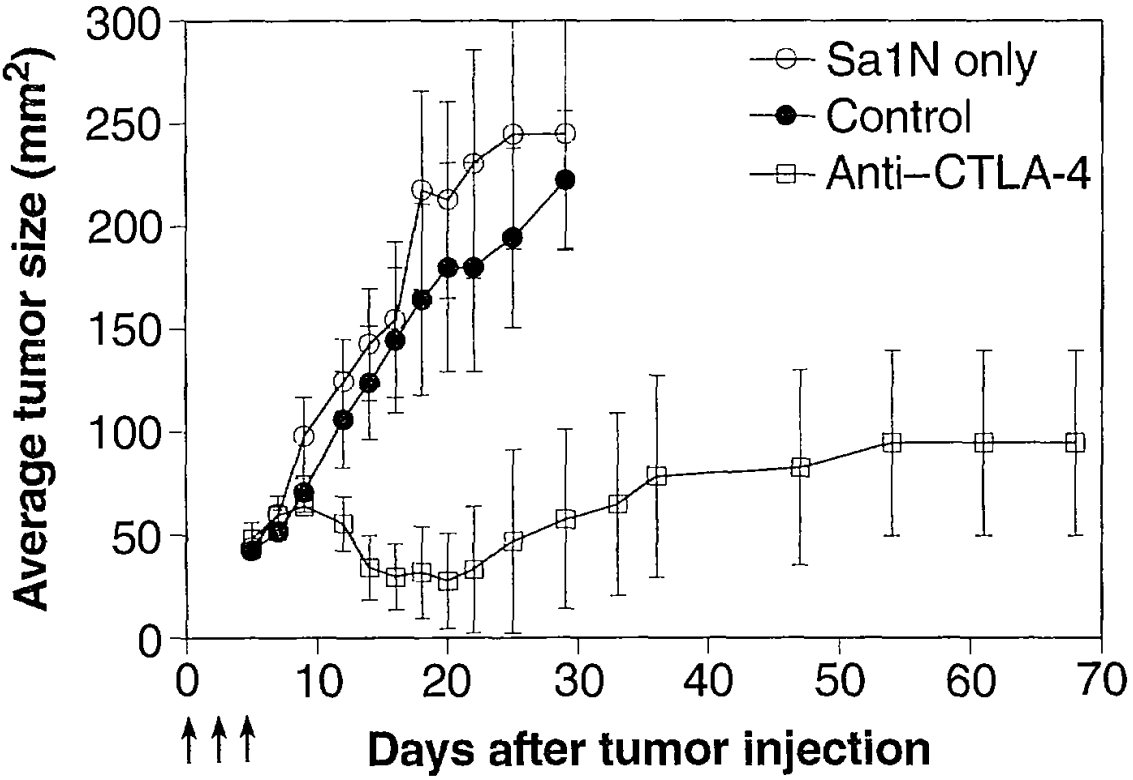

Figure 7. Cancer cell lines derived from a mouse were injected into syngeneic animals who were either untreated, treated with a control antibody, or treated with hamster antibodies to CTLA-4. Arrows indicated treatment time points. Mice treated with CTLA-4 showed evidence of tumor regression.

These findings were consistent with an immune attack on the tumor stimulated by CTLA-4 inactivation. These

studies inspired the clinical development of analogous humanized antibodies – Tremelimumab (Pfizer) and Ipilimumab (Bristol Myers Squib – BMS) for the treatment of patients with malignant melanoma.

The clinical development of Ipilimumab and the failure of Tremelimumab.

CTLA-4 knockout mouse as mentioned earlier have a profound expansion of lymphocytes leading to early lethality, which led to apprehension about the development of CTLA-4 antagonists for the treatment of patients. Nonetheless, two different companies, Pfizer and Bristol Myers Squibs, advanced tremelimumab and ipilumumab, respectively, to the clinic.

Tremelimumab is a IgG2 humanized antibody, which is less likely to activate complement in an antibody dependent cytotoxicity. In early clinical development, investigators used plasma IL-2 levels following injection of tremelimumab as an in vivo pharmacodynamics marker for efficacy. [14] Using this marker, they established that the pharmacokinetics efficacy of tremelimumab could last 90 days after a single dose at 15 mg/kg. Phase I/II testing of tremelimumab compared 10 mg/kg dosed monthly versus 15 mg/kg dosed every

90 days. Both doses had a 10% objective response rate, however, the 15 mg/kg dose had an improved toxicity profile and was chosen as the dosage in phase III testing. Of note in phase II testing, the median time to a response was 21 weeks and there were numerous durable responses lasting over 2 years. Based on these results, tremelimumab was tested in a randomized control Phase III trial against standard of care chemotherapy at a dose of 15 mg/kg with the primary endpoint of overall survival. Although there were ~10% objective and durable response rates in the tremelimumab arm, the change in overall survival was not significant (p=0.12). [15]

In contrast, a similar phase III trial using ipilimumab, a IgG1 antibody targeting CTLA-4, did result in improved overall survival and received fast track approval by the FDA. Given that these agents were both antibodies to the same target, CTLA-4, it is worth considering why one trial succeeded and the other failed.

In early phase I/II testing with ipilimumab, investigators noted that patients were exhibiting delayed responses, sometimes even after treatment started. (Figure 8) [16] For example after 12 weeks of treatment, 63/186 patients had stable disease and of these 45 patients showed

a steady decline in their overall tumor

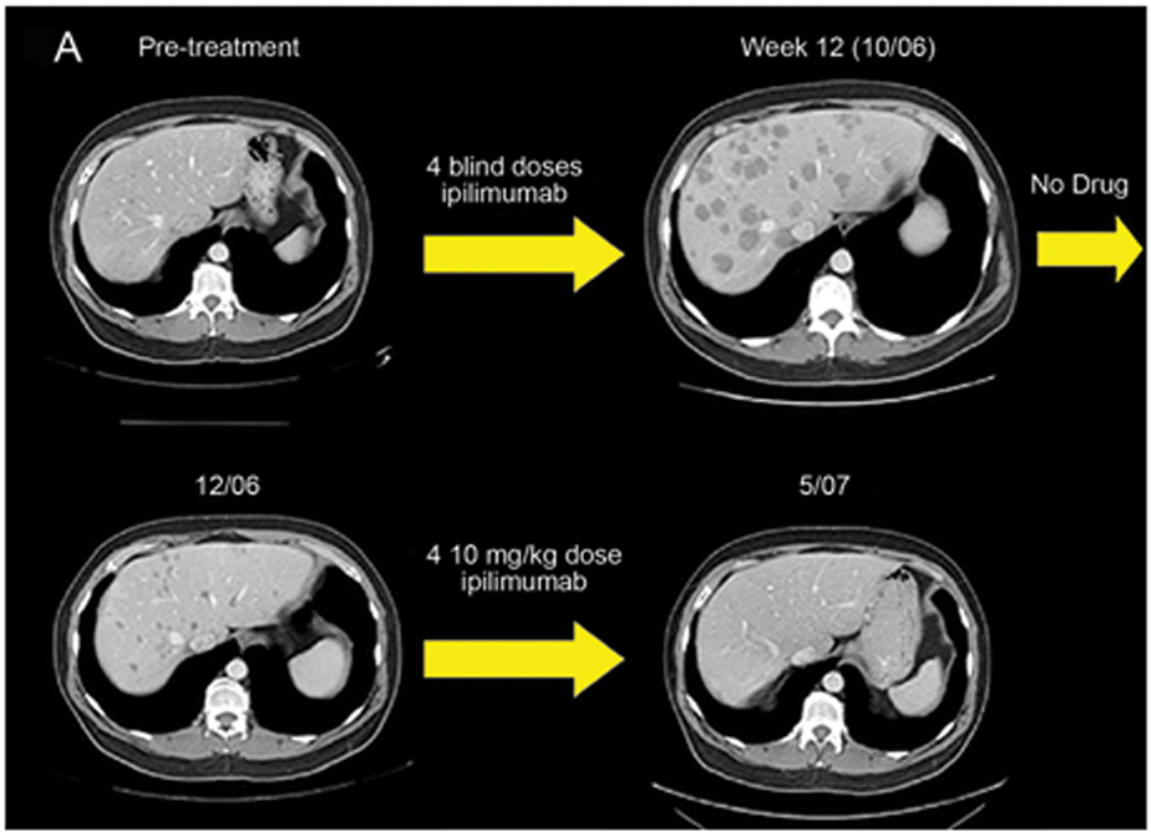

Figure 8. The case of a 50 yo man with melanoma metastatic to the liver who received ipilimumab monotherapy. After treatment, he exhibited radiographic progression (top right) even though other markers of disease (LDH) were reduced. These "lesions" responded over the course of several months. One year later, he had no evidence of disease.

burden.

Even more surprising, 10 of 57 patients with progressive disease at 12 weeks eventually showed a partial response. Based on these observations, these

investigators established new criteria for measuring the response following immune therapy that is based on total tumor burden. In this case, evidence of a new lesion but stable or partial responses in existing lesions would be considered stable disease based on a change in total tumor burden. These experiences influenced the protocol for the phase III ipilimumab trials. For example, patients were given four cycles of therapy every 3 weeks unless they had a decline in performance status, high grade toxicity, or clear progressive disease. Unlike in the tremelimumab, immune related toxicities were aggressively managed with high dose steroids and/or anti-TNF agents, and if the toxicity resolved, therapy was restarted. Furthermore, patients who showed delayed responses were offered a reinduction regimen in which they received four additional cycles of therapy. Ultimately, at least in part because of these elements in the protocol, ~60% of patients completed Ipilimumab treatment were as ~13% completed tremelimumab treatment. [17] It is my opinion that this is the primary reason why trememlimumab failed. Other possibilities noted by the authors are that no crossover was allowed from the chemotherapy to tremelimumab arm and that several patients in the chemotherapy arm received ipilimumab leading to improvements in survival in that arm.

Immunotherapeutic agents have begun to cure patients with malignant melanoma. The development of new immunomodulatory agents that antagonize either PD-1 or its ligand PD-L1 have shown even better clinical outcomes either alone or in combination with ipilimumab. Furthermore, the types of cancers that are responding to these agents have expanded beyond melanoma to also include lung, bladder, and colorectal cancers. Now, there is little doubt that immunotherapy will become a mainstay in cancer treatment and there is optimism that some patients will achieve durable remissions. In my opinion, there were three key milestones in this achievement which we have discussed here: the basis for cancer immunosurveillance in mice, the discovery of CTLA-4 and the proof of principle experiments of anti-CTLA-4 anti-tumor efficacy in mice, and finally the clinical trial of ipilimumab in melanoma.

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