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REVIEW ARTICLE

Paradox-driven adventures in the development of cancer immunology and immunotherapy

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Abstract After more than one hundred years of documented trials, immunotherapy has become a standard of care in the treatment of human cancer. Much of the knowledge that led to recent breakthroughs seems quite logical from today's point of view. However, what we now cite as facts were originally considered *paradoxes*, meaning something contrary to expectations or perceived opinion at the time. In order to make gains in the field of immunotherapy, one had to be willing to confront ideas and concepts that seemed to contradict one another, and reconcile how each could be true. This is what led to new knowledge and advances. Here, we highlight some of these paradoxes and the milestone discoveries that followed, each one critical for our understanding of immune checkpoint pathways. By outlining some of the steps that we took and the challenges that we overcame, we hope to inspire and encourage future generations of researchers to confront the paradoxes that still permeate the field.

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Introduction

Cancer, in its simplest description, is the uncontrolled division of abnormal cells. It is a disease that has been afflicting mankind for millennia, first described medically as early as 1600 BC (Edwin Smith Papyrus). Through the centuries, we have gained a better understanding of the molecular underpinnings of malignant transformation, but the treatments available for patients have largely stayed the same. Surgical resection, radiation, and/or cytotoxic therapy have remained the mainstays, with drugs becoming somewhat more targeted in recent decades with the introduction of kinase inhibitors. Until roughly 15 years ago, drug developers were focused on the tumor itself: what pathways were intrinsic to its survival, how to target these pathways with small molecules and chemotherapy, and how to overcome the inevitable resistance to these treatments. Still, a relative minority of researchers believed that the immune system, and not the tumor, held the key to defeating cancer and continued to pursue this idea in the face of sometimes harsh opposition. Then in 2011, and later in 2014, as the first immune checkpoint therapies were approved, these researchers were rewarded with stories of exceptional responses to these new drugs targeting the pathways they had helped discover. The world began to take notice, and now the entire landscape of cancer therapy has shifted. As evidence of this shift, more than 1500 different clinical studies using checkpoint inhibitors (anti-PD-1/PD-L1) were ongoing as of 2017, and they have been approved for more than 10 different cancer indications.[1]

But how, exactly, did we get here? Can the recent breakthroughs in immunotherapy be traced to a single "Aha!" moment in which everything became clear? The answer is a resounding "No." The journey to where we now stand was much more of an adventure, filled with many stops and starts, new discoveries, and frustrations along the way. Every few miles, a paradox would confront the field — like a roadblock. These roadblocks were the puzzles that had to be solved before the group could move forward and make progress. The word "paradox" is derived from the Greek word *paradoxon*, which describes something that is contrary to expectations, existing belief, or perceived opinion (*New World Encyclopedia*). In order to make gains in the field of immunotherapy, one had to be willing to confront ideas and concepts that seemed to contradict one another, and reconcile how each could be true. This is what led to new knowledge and advances. In this short discussion, we will attempt to highlight some of these paradoxes and the milestone discoveries that followed, each one critical for our understanding of immune checkpoint pathways. We will particularly focus on PD-1/PD-L1 signaling and our own perspective of how this field has evolved over the years, although much of this history is broadly applicable. We should not fail to mention that work in the field of tumor biology, such as defining the hallmarks of cancer and elucidating the pathways that are altered within cancerous cells, also greatly contributed to the development of immunotherapies, though these areas of study are not mentioned in this more focused review.

By outlining some of the steps that we took and the challenges that we overcame to get to where we are today, we hope to inspire and encourage future generations of researchers to confront the paradoxes that still permeate the field. Anti-PD-1/PD-L1 therapeutics have reached the clinic, and while revolutionary, their use has also left us with many new questions that need to be addressed with the very same sense of curiosity, heretical thinking, and persistence that was required to make it to this point.

First paradox: giving someone a disease (infection) can cure another (cancer)

In 1909, Nobel Prize-winning German physician Paul Ehrlich (1854—1915) proposed the idea that our bodies are fighting constant battles with cancer. Currently, this concept is well defined in cancer immunoediting where in the first phase the body is constantly eliminating transformed cells.[2] This can progress to the equilibrium phase where the rate of clearing transformed cells is similar to the rate at which new malignant cells are being produced. If this equilibrium is disturbed, then the transformed cells continue to grow and mutate to form a tumor. Alternatively, the body can get rid of these cells via the activity of cytotoxic lymphocytes. Based on this idea, though the mechanism was not known then, Paul Ehrlich attempted to generate immunity to cancer by injecting weakened cancer cells, analogous to vaccination. Thus, some of cancer immunotherapy's earliest roots stem from Ehrlich's description of the body's built-in defense system. Around the same time, Dr. William B. Coley (1862—1936), a bone sarcoma surgeon, was intrigued by the seemingly contradictory idea that by creating an infection, you could cure cancer.[3] Finding anecdotal evidence for this in the literature, he decided to inject streptococcal organisms into a patient with an inoperable bone tumor. The outcome was successful for the patient, and marked the beginning of Dr. Coley's career studying perhaps the first true cancer immunotherapy. Over the next 40 years, he went on to treat hundreds of inoperable cases with his "toxins," various forms of heat-killed and live bacteria, and reported the results in primary literature. It is interesting to note that when records are revisited today, it appears that Dr. Coley's toxin may have had an approximately 10%—20% response rate.[4] These numbers are strikingly close to the response rates we see with current immunotherapy for solid tumors, but at the time, his results were highly criticized as ineffective. In addition, there was no known cellular mechanism to explain the cases in which the toxin was successful. With the advent of radiation and chemotherapy, Coley's toxin gradually fell out of use.

In 1960, the field of immunology took a step forward when a piece of the cellular mechanism that Coley's therapies lacked was uncovered. Cytotoxic lymphocytes were first described in human peripheral blood.[5] Andre Govaerts was searching for "circulating antibodies" in dogs that would cause them to reject kidney transplants. His experiments using lymphocytes from the recipient animal showed that these cells could kill cultures from the kidney of the donor. To that point, humoral immunity had been one of the main focuses of the field, but this new knowledge of what would later be described as $CD8^+$ cytotoxic T lym-

phocytes was critical for explaining Ehrlich's and Coley's ideas about the body's defense system and the ability to enhance or activate cellular immunity to kill tumors.

Second (Hellstrom) paradox: the coexistence of growing tumors and tumor-specific effector T cells

Once cytotoxic T lymphocytes were discovered, it was only a few years later, in 1968, that Dr.'s Karl and Ingegerd Hellstrom provided the first evidence of the presence of tumor-reactive immune cells in the peripheral blood of cancer patients.[6] In laboratory experiments, they observed decreased plating efficiency of tumor cells after they were incubated with lymphocytes derived specifically from tumor-immune animals. No inhibition was observed with antigen non-specific lymphocytes. They also carried out experiments using plasma from adenocarcinomas of the colon and showed a complement dependent inhibition of colony formation compared to plasma from other tumors that were not antigen-specific. Given that the source of lymphocytes and plasma used in some of their experiments were from patients who had evidence of either persistent or progressive neoplastic disease, the Hellstroms found it paradoxical that there would be cytotoxic lymphocytes present in cancer patients, able to kill tumors *in vitro*, but obviously unable to effectively kill the tumors *in vivo*. It would have made much more sense if the cytotoxic lymphocytes were simply not present in the blood of patients harboring a tumor. Nevertheless, they very astutely hypothesized the following concerning this paradox:

> *"One explanation of this finding seems to be that tumours can grow in vivo in spite of an immunological reaction against their antigens, which may destroy many of their cells but not be potent enough for tumour eradication. It may also be speculated that certain factors, such as enhancing antibodies, are present in tumour patients and counteract the destructive effect of immune lymphocytes".*[6]

It would be nearly 30 years before the "certain factors" they speculated might be present in tumors to counteract the immune response would be identified (Fig. 1). First, the

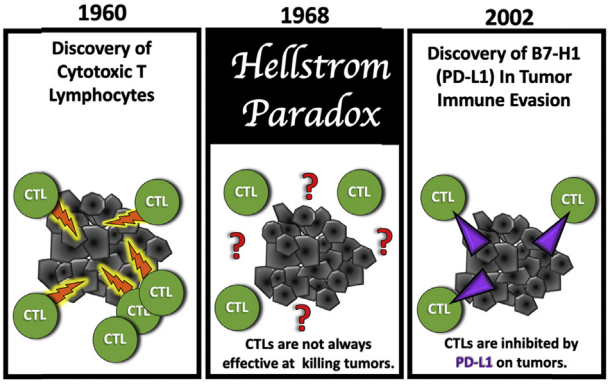

Figure 1 The Hellstrom paradox and its resolution: cytotoxic T Lymphocytes (CTLs) play a key role in tumor immunity.

field needed to characterize and better define functions of T lymphocytes, and other signals, both intrinsic and extrinsic, that controlled these important cells during an adaptive immune response.

Third paradox: binding to B7 ligands can cause T cell stimulation *and* inhibition

By the early 1990's, knowledge of T lymphocytes had progressed, and a "two-signal" model had been described for their activation. Co-stimulatory molecules B7-1 (CD80) and B7-2 (CD86), were identified on APC's and B cells, and were known to bind to CD28 and CTLA-4 on T-cells.[7,8] It was at this point that Chen et al hypothesized that one reason why immunogenic tumors were able to escape host immunity might be that tumor-reactive T cells receive inadequate co-stimulation. In their 1992 manuscript, they showed that anti-tumor immunity could be boosted by forcing tumor cells to express B7 co-stimulatory molecules.[9] By 1996, a series of papers had shown that co-stimulation was more complex than originally thought and that CTLA-4 was actually an inhibitory co-receptor that bound B7-1 and B7-2 with higher affinity than CD28, resulting in decreased T cell proliferation and IL-2 production.[10,11] The fact that binding to B7 molecules could result in either activation or inhibition, depending on the receptor/ligand pair was a paradox that took time and intense research to understand. However, once this was known, Dr. James Allison's group took the first steps in applying this new knowledge to cancer therapeutics. He blocked CTLA-4 and B7 interactions via a monoclonal antibody which resulted in tumor rejection in pre-clinical mouse models.[12] This was a huge breakthrough in terms of establishing the potential for immune checkpoint blockade to be clinically useful as an anti-tumor treatment. Still, there were limitations to this strategy that would become apparent over the next few years. It was clear that enhancing co-stimulation by blocking CTLA-4/B7 inhibitory interaction was highly effective at stimulating cytotoxic T lymphocytes in lymphoid organs (the main site of co-stimulatory activity), but there still seemed to be factors present in the local tumor environment that were detrimental to these same T cells, limiting their ability to infiltrate and eliminate neoplastic cells. Those factors still remained unknown.

Around this time, Dr. Lieping Chen and his research team at Mayo Clinic were identifying additional co-stimulation molecules relevant to T cell activation. By searching for proteins that shared homology with the immunoglobulin V and C domains of B7-1 and B7-2, one of their key discoveries was a previously unknown gene called *B7—H1* (B7 homolog 1), which they went on to clone and characterize in 1998.[13] Importantly, they found a large amount of B7—H1 mRNA in several normal organs such as lung and placenta that are usually protected from unwanted inflammatory or immune reactions, and determined that co-stimulation of T cells mediated by B7—H1 led to secretion of IL-10. These were the first hints that B7—H1 could play a role in inhibiting immune responses. Shortly after, Freeman et al published their manuscript in which they identified B7—H1 (they termed it PD-L1) as the ligand for PD-1, an immunoinhibitory receptor they had previously discovered.[14] In T cell

assays, they showed that engagement of PD-1 by PD-L1 led to the inhibition of TCR-mediated lymphocyte proliferation and cytokine secretion. They speculated that PD-1/PD-L1 signaling was likely to be an important checkpoint *later* in the immune response than CTLA-4, and that if a T cell were to reencounter antigen in the periphery in the presence of PD-L1 but not B7-1 or B7-2, this might limit its expansion and ability to clear tumors.

This raised an important question: was PD-L1 the "certain factor" mentioned in the Hellstrom paper, able to protect tumor cells, despite the presence of T lymphocytes specific to tumor antigens? Two years later, Dr. Lieping Chen's group confirmed that indeed, this was the case. In their follow-up publication, they showed that B7—H1 (PD-L1) protein was present to some degree in most human cancers that they examined.[15] *In vitro*, they saw that PD-L1-expressing tumor cells co-cultured with cytotoxic T cells were resistant to T cell killing and actually resulted in greater T cell apoptosis when compared to B7—H1 negative tumor cells. This was confirmed *in vivo*, as PD-L1-transfected tumor cells growing in the peritoneum of mice resulted in the deletion of activated T cells when the lymphocytes were adoptively transferred. This suggested a mechanism by which tumors could evade immune destruction and explained why sufficiently activated T cells were still vulnerable to inhibition and apoptosis once they made it to the tumor site. Shortly after, Iwai et al reported that PD-L1 binding antibody could restore antitumor immunity *in vivo* in preclinical mouse models.[16] Collectively, these findings indicated that in order to mount an effective anti-tumor immune response, killer T cells actually needed to be "protected," yet another paradox the field had to accept (Fig. 2). This protection would ensure their survival both in the periphery and within the tumor microenvironment. Thus, a new cancer immunotherapy concept, i.e. PD-L1 and PD-1 blockade, emerged from these original observations and was confirmed in follow-up studies.

Fourth (PD-1) paradox: biochemical responses do not always lead to clinical responses

Realizing that cytotoxic T cells themselves actually needed to be protected was a major change in the way we thought about the anti-tumor immune response. Once this was proven, we had two important pieces of information: (1) there were T cells already within patients that could kill their tumors, but (2) these T cells were being overwhelmed by inhibitory signaling pathways at the tumor site. With the CTLA-4 inhibitor ipilimumab already FDA-approved, pharmaceutical companies began developing immune checkpoint inhibitors that would bind to PD-1 and PD-L1. There was enough pre-clinical work to show that blocking these ligand/receptor interactions might be able to stimulate T cell responses against a tumor, but even as drug candidates moved into phase I trials, basic science was still trying to further define the mechanism by which these interactions resulted in T cell exhaustion and death. Previous work had shown that PD-1 signaling through PI3k/Akt and Ras/MEK/Erk pathways regulated cell cycle pathways in T cells.[17,18] However, this alone did not adequately address the questions present. In 2012, we discovered that Bim, a pro-apoptotic Bcl-2 family member, was the downstream mediator of PD-1-induced T cell apoptosis.[19] At the peak of the expansion phase following antigen stimulation, $CD8^+$ T cells expressed lower levels of Bim in PD-L1-deficient mice than in wildtype mice. In addition, *in vitro* assays revealed that stimulation by plate-bound PD-L1 led to Bim upregulation in activated $CD8^+$ T cells, and antibodies which inhibited the PD-L1/PD-1 or PD-L1/CD80 interaction blocked this upregulation. This filled in some of the blanks in terms of how PD-L1 binding to PD-1 was resulting in T cell death, and provided further evidence that PD-1/PD-L1 blocking antibodies could be clinically useful.

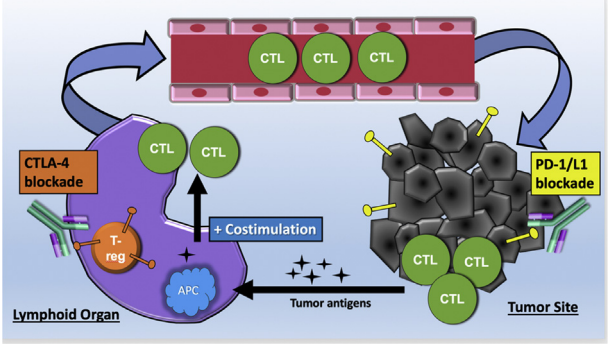

Figure 2 T cells need protection at tumor sites.

Over the next few years, as PD-1 and PD-L1 checkpoint inhibitors were FDA-approved and more widely used in the clinic, it became apparent that only a subset of patients would respond to these drugs. Unfortunately, there was no *a priori* test that could determine which patients would respond and which patients would fail (and even to the present day, no such test exists). Worse still, there were no molecular or biochemical markers to monitor drug activity while on treatment. Only tumor shrinkage vs. growth could be tracked, a metric that was extremely complicated to interpret given that some tumors exhibited "pseudoprogression," meaning they actually got larger before ultimately regressing, and some non-responders could go on to become responsive as late as 36 weeks after treatment began. This made it extremely difficult for oncologists to manage these new therapies, and still remains a challenge today. It was around this time, in 2016, while considering what we knew about the downstream pathways of PD-1/PD-L1 signaling, we realized that levels of Bim within activated $CD8^+$ T cells might be a potential marker for $PD\text{-}1^+$ T cells "at risk" of dying, if they encountered PD-L1 in tumor tissue, resulting in an even higher level of Bim and pro-apoptotic signals. Unsurprisingly, this turned out to be the case. We found that high levels of Bim in circulating tumor-reactive ($PD\text{-}1^+CD11a^{hi}CD8^+$) T cells were prognostic of poor survival in patients with metastatic melanoma who did not receive anti—PD-1 therapy and were also predictive of clinical benefit in patients with metastatic melanoma who were treated with anti—PD-1 therapy.[20] Responders showed a significant decrease in the level of Bim in their activated $CD8^+$ T cells 12 weeks following anti-PD-1 therapy, whereas overall, the non-responders showed little change from baseline in the level of Bim. It is plausible that in responders, the $CD8^+$ T cells' high level of Bim expression at baseline is an indication that these cells have been active

and effective against the tumor cells, and when PD-1 inhibition is removed, these cells are able to function again, resulting in response. In the case of non-responders with low levels of Bim at baseline, this could be an indication that these effector cells have never truly entered into an "effector mode" and removal of inhibition does not change their function.

Though Bim levels in circulating $CD8^+$ T cells are not yet being monitored as a standard clinical test, our findings echoed the first observation made by the Hellstroms, who showed that the lymphocytes in peripheral blood can reflect antitumor immunity within the human body.[6] Recently a burst of circulating $Ki67^+$ $CD8^+$ T cells has been observed in cancer patients shortly after PD-1 blockade therapy,[21,22] suggesting that PD-1 signals may suppress early fate decision of T cells as well as recall of memory T cell responses.[23–25] Thus, Ki67 and Bim expressed by $CD8^+$ T cells in the peripheral blood may provide both early (Ki67) and late (Bim) pharmacodynamic or biochemical biomarkers for monitoring patient responses to PD-1 blockade therapy.

Working on this project also presented us with another paradox, one that we, and others, are still trying to resolve: when looking at the distribution of Bim levels before and after treatment, there is a "tail" of outliers in the non-responder group that actually do have a decrease in Bim after treatment. This suggests that certain individuals categorized as "non-responders" may in fact have a measurable biochemical response, but do not go on to have a clinical response in the form of tumor shrinkage (Fig. 3). This subset of patients is intriguing, and hard to explain. It may be that they present a unique opportunity for further therapy: a group of patients who are poised to reject their tumors, but who need an additional stimulus to actually succeed. Whether this additional push might be a tumor vaccine, radiation, or chemotherapy remains to be seen, but certainly the field has begun to turn towards combination therapies, hoping that this may be the key to converting non-responders into responders.

To this end, our recent manuscript described the phenomenon of chemo-withstanding immune cells as a rationale for adding chemo to anti-PD-1 therapy in certain patients who do not initially respond to anti-PD-1

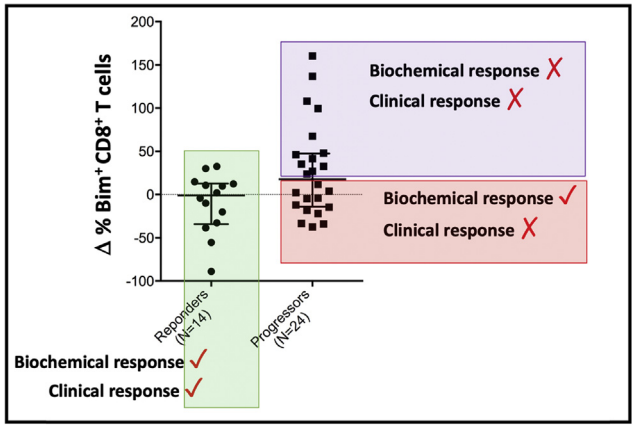

Figure 3 The PD-1 blockade paradox: Biochemical responses do not always lead to clinical responses.

alone.[26] At first, it seemed "paradoxical" to utilize chemo *after* immune therapy, given that chemotherapy typically depletes highly-proliferative immune cells. However, we were able to identify a population of T effector cells that could withstand chemotherapy, expand afterwards, and then provide more effective anti-tumor immune responses following paclitaxel and carboplatin treatment in patients with metastatic melanoma. We believe that these chemo-resistant $CX3CR1^+CD8^+$ T cells, which demonstrate an effector memory phenotype, may eventually execute tumor rejection due to their abilities of drug efflux (ABCB1 transporter), cytolytic activity (granzyme B and perforin), and migration to and retention (CX3CR1 and CD11a) at tumor sites. In this regard, chemotherapy's cytotoxic effects may function at two layers for promotion of immunotherapy: one is to cause tumor cell death for releasing more tumor-rejecting antigens or chemokines; the other is to deplete immune regulatory cells (T-regulatory cells, for example) and create more space (lymphopenia) for effector T cells ($CX3CR1^+$) to expand and migrate to tumor sites.

Beyond just chemotherapy, there are other avenues of combination immunotherapy being pursued, one of which has only recently been explored. As we have outlined above, monoclonal antibodies targeting immune checkpoint molecules like PD-1 and PD-L1 were developed to enhance T cell responses that *already exist* within patients through protecting their anti-tumor T cells from death and helping them to remain activated and effective. However, it is important to realize that over a similar timeframe in the 1990's and early 2000's, others in the immunotherapy field decided to take a different approach. Working from the same foundational knowledge that T cells could kill cancer but were failing to accomplish this task, they sought to solve the problem through cellular engineering and adoptive cell therapy. Pioneers in this area, including Dr.'s Rosenberg, Eshhar, Sadelain, and June, had the idea that T cells could be modified outside the body to target specific cancer antigens and then be delivered back to patients as "living" therapeutics.[27–30] Over time, they developed an effective construct: a single-chain variable fragment (scFv) derived from the variable domain of an antibody that recognizes a tumor marker (independent of MHC) was conjugated to the signaling domains of the CD3ζ chains to activate downstream TCR signaling along with additional co-stimulatory receptors (CD28 or 4-1BB) to sustain activation once the target antigen was bound. Cultured T cells from patients were made to express these chimeric receptors, effectively turning them into weapons against target antigen-expressing cancer cells once returned to the circulation. These engineered T cells were termed "CAR-T cells" (Chimeric Antigen Receptor - T cells).

In 2017, the first two CAR-T cell therapies were approved by the FDA, both of which employ CD19 as the target antigen. One is used to treat refractory, relapsed B-ALL in children and young adults and the other is used to treat refractory and relapsed DLBCL. Results have been impressive, especially given that these patients were no longer responsive to any other treatment regimen: overall remission rate within 3 months was 81% in a phase I/II B-ALL trial and a phase I DLBCL trial had a complete response rate of

54%.[31,32] These studies show that CAR-T cells can be effective in a subset of B cell malignancies, but there are still hurdles to overcome. Many of the B-ALL and DLBCL patients did not have a durable response to therapy and became resistant over months to years following CAR-T infusion. In addition, CAR-T cells have not been as successful in other cancer types, especially with regard to solid tumors. One contributor to this ineffectiveness is the fact that engineered CAR-T cells suffer from the same problem that endogenous T cells face: they can be inhibited and even killed within the tumor microenvironment via immune checkpoint signaling[33–35] (Fig. 1). Recognizing this connection, two recent clinical trials have reported positive results when combining anti-PD-1 inhibitors with CAR-T therapy. Six patients with B-ALL were treated with a PD-1 inhibitor following CAR-T cell infusion (after resolution of cytokine-release syndrome) with the hopes that checkpoint blockade would improve CAR-T cell persistence.[36] The combination was found to be relatively safe and initial results are encouraging. A separate study enrolled twelve patients with progressive or relapsed B-cell Non-Hodgkin's Lymphoma who had previously received CD19-targeted CAR T-cell therapy.[37] They were treated with pembrolizumab (anti-PD-1) with the hopes of "jump-starting" the previously infused CAR-T's. Accordingly, they noted re-expansion of CAR T-cell populations in 8 of 11 patients after receiving pembrolizumab. Additional trials are ongoing both in the US and internationally (reviewed by Yoon et al[38]), including both co-administration of CAR-T cells with anti-PD-1 or anti-PD-L1 therapy as well as engineered constructs that force expression of anti-PD-1/L1 antibodies by the CAR-T's themselves. Immune checkpoint blockade and CAR-T cells represent the two major arms of cancer immunotherapy in clinical use today, and this recent data indicates that a partnership between these two could be a powerful strategy for forward progress.

Fifth (hyperprogression) paradox: removing inhibitory immune signals can result in tumor acceleration rather than regression

September of 2019 will mark five years since the anti-PD-1 antibody pembrolizumab was first approved by the FDA for treatment of advanced melanoma. The passing of this amount of time has allowed a significant number of patients to be treated, not only with pembrolizumab but also with other PD-1/PD-L1 blocking antibodies such as nivolumab, atezolizumab, and avelumab. We can now begin to analyze larger and larger groups of patient outcomes following treatment with these new therapies. Most trials find that approximately 20–40% of patients have a response to these immune modulators, and the remaining 60–80% of patients are eventually designated as non-responders for whom the drug simply did not work. However, in 2016 Champiat et al published a report describing a third possible outcome which they termed "hyperprogression".[39] They showed that a subset of patients actually had a significant *increase* in growth kinetics of solid tumors when treated with anti-PD-1/PD-L1 therapies, voicing what many oncologists had suspected for some time. Several other confirmatory reports have followed. Estimates differ from study to study, and variability may be due to tumor type, cohort size, and method of assessment. However, hyperprogression occurrence can range from 4 to 29% of patients treated.[40] Evidence for hyperprogression is still being brought forward, but many have pointed to the fact that in several Phase III clinical trials of immune checkpoint inhibitors (CheckMate 057, CheckMate141, Keynote 045, and IMvigor211), the survival curves cross at about 3 months of treatment. This means that in the first few months of therapy patients were more likely to die in the immunotherapy arm than in the chemotherapy cohort, while in the long-term immunotherapy had better outcomes. This may reflect a group of patients who respond poorly to PD-1/PD-L1 inhibitors very early on for a yet unknown reason. It may also reflect the aggressive nature of some tumors which would have had accelerated growth regardless of treatment modality. This illustrates one of the challenges with studying hyperprogression: defining the kinetics of tumor growth prior to treatment and then reliably measuring a change in growth once on therapy. This process was recently reviewed by Champiat et al here.[41] In their review, the authors also outline some of the immunological hypotheses for what may be causing this phenomenon. To date, no mechanism has been defined. Possible explanations include PD-1/PD-L1 blockade inducing regulatory T cell expansion, up-regulation of other checkpoint inhibitor pathways on cytotoxic effector T cells, modulation of innate immune cell subsets such as dendritic cells and macrophages, a shift to TH_{17} type immunity along with influx of neutrophils, or oncogenic pathway activation in tumors expressing PD-1. All of these theories bear consideration, and it is likely that more than one of them may prove to be involved in hyperprogression, depending on the unique immune environment of each patient.

We would further highlight macrophages as potential key players in hyperprogressive disease. Macrophages are known to express both PD-1 and PD-L1 within the tumor microenvironment, and therefore will likely be bound by anti-PD-1/PD-L1 antibody drug treatments. What the ultimate downstream effects of this binding might be has been largely unaddressed to this point, although we do know that host PD-1 and PD-L1 expression on macrophages is critical for an anti-tumor response in pre-clinical models.[42] Macrophages are known for their plasticity and can significantly shift an immune environment to either pro- or anti-tumor through the release of inflammatory or suppressive cytokines and chemokines as well as play a role in vascularization through the expression of VEGF. In addition, one recent report found that M2-like $CD163^{+}CD33^{+}PD\text{-}L1^{+}$ macrophages were clustered within the tumors of patients which met criteria for hyperprogression and suggests that the Fc portion of anti-PD-1/PD-L1 antibodies may be modulating macrophage function within tumors.[43]

In order to better inform our studies, clinical-pathological features that correlate with hyperprogresion are being diligently sought. Some have been noted such as advanced patient age, specific tumor mutation profiles, or higher number of metastatic foci prior to treatment (>2), though none have been consistent across studies.[40] Using hints that are gained from clinical data, the next critical step will be to establish pre-clinical models of

hyperprogression where theories can be tested and signaling can be dissected more easily.

Conclusion

The field of immunotherapy has come a long way in the past century, and it is impossible to ignore the great success stories that have resulted from the hard work of many, victories both for those in research who persevered and discovered new science, and more importantly for cancer patients who now have new options for treatment. Because of the attention it has garnered, and because immune checkpoint drugs have gained approval and are regularly given in cancer centers across the world, it might be easy to think that the adventure is over and all of the details have been worked out, but this is far from the truth. In reality, we have only just begun to understand how to manipulate the immune system to counteract anti-tumor defenses. Puzzles like defining biochemical responses vs. clinical responses, converting non-responders to responders, designing rational combination therapy, and explaining the phenomenon of hyperprogression urgently need to be considered and solved for the field to continue forward. This will require a great deal of courage, persistence, and careful study, but the rewards for patients are certainly worth the effort, and there is no doubt that an exciting journey lies ahead.

Acknowledgements and conflicts of interest

The authors declare no conflicts of interest.

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