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Further Clinical Evidence for the Depletion Model of Immune Checkpoint Inhibitor Therapy for Cancer

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Abstract

Despite that immune checkpoint inhibitor (ICI) therapy has achieved some of the most dramatic and durable antitumor responses in multiple clinical trials in the past decade since its birth, the true working mechanism behind these efficacies remains unconfirmed in clinical setting. The currently adapted working model for ICI therapy is seriously flawed in that it more and more clinical observations challenge instead of support it. On the other hand, accumulated evidence has indicated that ICI therapy used in the real-world clinical setting has caused many accelerated disease progression and death. This phenomenon, although well known among clinicians who administer ICI therapy often, is rarely mentioned in public and its true mechanism remains unexplained. Another two clinical observations associated with ICI therapy also remain unexplained: One is the "trigger effect" of ICI therapy observed in many patients who dropped out of continued antibody administration due to various reasons. Despite the discontinuation of antibodies to PD1/PDL1, the single administration of the drug brought sustained response that sometimes last months or even years. The other observation is the severe autoimmune attack incited by the therapy in some cases. There is no satisfactory explanation for such event by the accepted model of ICI therapy. We have previously proposed a "depletion" model for the ICI therapy that can explain all clinical perplexing observations. Here we continue to present case evidence supporting our depletion model. Our observations show that the most durable and dramatic antitumor immunity activated by anti-PD1 antibody is carried by activated PD1-nagetive T cells. This observation is ironic based on the currently established "blocking" model in that antibody to PD1 activates PD1-nagative antitumor T cells, how can this take place by the blocking model? On the contrary, this observation is expected based on our depletion model. Furthermore, our findings from true clinical cases raise fundamental questions regarding the current clinical use of ICI therapy and points to a future direction for the search of ways to activate PD1-nagative T cells for durable antitumor response.

Keywords: Cancer, Immune Checkpoint Inhibitor, Pd1 Blocking Therapy, Hyper-Progression

Introduction

Thanks to ICI therapy in the past decade, there is no doubt today that the magnitude of immune responses against cancer is real and powerful that if activated in a right way, it can eradicate almost any commonly seen tumor burdens existing in a clinical setting. Yet, despite the high hopes and hypes in the past few years, ICI therapy so far is mostly effective in various clinical trial settings, but not in real world use [1, 2]. Why there is such a huge difference between these two settings is not clear. Our own experience pointed to a confusion on the mechanism and, as a result, the wrong application in about 40% cases, causing harm instead of benefit [3].

According to the established mechanism of ICI therapy, T cells attacking tumor releases IFN-g, causing tumor cells to express PDL1, this in turn down regulates immune response, thus preventing immune destruction of tumor [4]. ICI antibodies block the interaction between T cells expressing PD1, and tumor cells expressing PDL1, thus saving T cells from being inhibited by PDL1. Not only us, others had also experienced ICI-induced hyper-progressive disease [5-8]. This serious adverse effect of ICI therapy was not explained by the established "blocking" model, because that the reason behind hyper-progression is loss of anti-tumor immunity that was previously present, although not highly effective in terms of total control of tumor progression. With this week immunity, a case before ICI therapy-induced hyper-progression at least maintained control over newly established metastases. During ICI therapy-induced hyper-progression, this ability is removed and metastases establish feely. Why a therapy intended to activate antitumor immunity turns into depletion of antitumor immunity in some cases? How is this depletion related to the "blocking" function of the antibody? Despite the widespread observation of such harmful effect estimated to account for about 40% of randomly selected users in the real-world clinic setting (our own experiences and observations), no explanation has come forward to answer these questions.

In addition, two other perplexing clinical observations could not be explained by the "blocking" model either. One is the "trigger effect" observed in some patients who for various reasons only

got the chance to use the therapy once. Their tumors responded to this single treatment persistently, some time over a year. Most durable responders also demonstrated continued tumor regression long after stop of therapy for two years [9-12].

Why is a single administration of ICI antibodies caused persistent responses? How can this effect be explained by the "blocking" model? Without periodic antigen release and stimulation, how can this persistent response be explained by immunological principles? If a single antibody dosing could trigger such effect, why frequently (once every three-weeks) repeated administration of ICI antibody is recommended by companies that developed ICI therapy drugs? Don't they notice this trigger effect? Or they are ignoring it because they have not investigated this phenomenon and do not want to? Again, not only there are no answers to these questions, there are even no mentioning of these questions in the literature despite many thousands of publications on ICI therapy use and mechanisms. The other perplexing observation is the autoimmunity associated with ICI therapy, a treatment associated adverse effect that is well-known and cannot be ignored among treating clinicians [13].

It cannot be explained by the "blocking" model because unlike tumor cells, there is no expression of immune checkpoint ligands (such as PDL1) on the target cells for autoimmunity. Take the example of autoimmunity in the lung, the most common immune adverse effect associated with ICI therapy, according to the blocking model, immune T cells attacking the lung should be inhibited by immune checkpoint pathway during normal time and activation of autoimmunity is similar to activation of antitumor immunity through blocking the interaction between PD1 on T cells and PDL1 on normal lung cells. But there is no PDL1 expression on normal lunge tissue regardless with or without autoimmune response. No PDL1 expression is found by other autoimmunity-affected tissue and organs also. The fact that antibody to PDL1 may also cause autoimmunity, albeit at a lower chance, is even more confusing. Then, how can ICI antibodies incite autoimmunity? Again, there is no answer to this question. Because that there is no understanding of the true mechanism behind ICI therapy-induced autoimmunity, there is no criteria to select patients who may suffer by the therapy-induced autoimmune disease that could be lethal.

Recently, through our own investigation, we have come up with an alternative working model for ICI therapy. Based on this new model which we call depletion model the location of tumor-infiltrating T cells is critical in that PD1-positive T cells located in the stromal and interstitial space are bond by the antibody and are depleted by various mechanism including ADCP while T cells deeply infiltrating tumor mass are spared due to lack of antibody penetration and/or lack of PD1 expression[3,14]. This depletion causes quick drop of T cells and is followed by homeostasis-driven expansion of residual T cells. This is the reason behind non-antigen specific activation of antitumor T cells. This initial activation results in the expansion of those T cells that could deeply infiltrate tumor mass to result the most effective responses. This model, although could explain the three most perplexing observations the blocking model could not explain, still leaves some perplexing questions to be answered.

The most challenging one is about the continued and durable responses in the presence of continued antibody doing in a clinical setting. If T cell location could provide the initial hide-out place for some T cells infiltrating deeply in a tumor mass, the subsequent expansion will require these T cells to migrate out of tumor mass and into draining lymph nodes for most effective expansion. This change of location will expose these T cells to antibody-mediated depletion unless these T cells do not express PD1. But clinical observations suggest that these T cells may as well express PD1 and susceptible to antibody-mediated depletion. In quite a few cases, we have seen the initial robust responses following the initial ICI antibody dosing turned into hyper-progression following subsequent dosing (described in detail below). On the other hand, there are those durable responders that maintain long-term responses in the presence of continued antibody dosing while the same time in the absence of continued antigen release. How to explain the differences in these two situations is a challenge, too. In the following sections of this report, we cite four real world cases to illustrate few points that together form a bigger picture. The combined observations and reasonable deduction provide solid support to the depletion model proposed by us. Further, they point to a direction in which we may find out a way to activate the most effective antitumor response.

Case Evidence

Case 1: Repeated anti-PD1 Dosing Results in Depletion of Antitumor T Cells and Hyper-Progression

A 59-year-old male with a large swollen mass in the neck (Fig. 1-1) was diagnosed with melanoma upon biopsy. PET-CT examination showed additional bone metastases (Fig. 1-2). The patient had an elevated black mole in the forehead, but resected biopsy did not show malignant cells, thus the primary location of this melanoma was unknown. Patient went to us for help with treatment plan. We first examined the biopsy sample to evaluate the mode of tumor replication and status of antitumor immunity. Analysis showed that the tumor structure is typical of melanoma with packed tumor cells and lack of interstitial space between tumor cells (Fig. 1-3, HE). Tumor replication was active in that 40-70% of tumor showed strong Ki-67 staining depending on area (Fig. 1-3, Ki67). There was a large number of dispersed T cells in the tumor mass (Fig. 1-3, CD3). These T cells are of the CD8 subtype and some show activated status.

Figure 1-1: The large (8x6cm) Neck Swollen Nodule at the Time of Diagnosis.

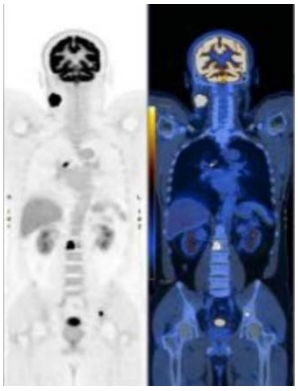

Figure 1-2: PET-CT at the Time of Diagnosis Showing the Neck Nodule and a L4 Bone Metastasis.

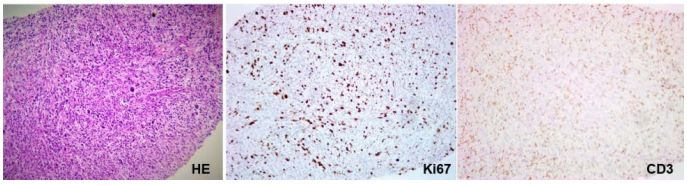

Figure 1-3: Biopsy tissue at diagnosis stained for Ki-67 and CD3, showing a compact structure of melanoma cells without interstitial space (left). Tumor replication is active with 40-70% tumor cells expressing Ki-67 (middle). There are large number of T cells mixed with tumor cells (right). Most T cells are of the CD8 subtype, some show signs of activation (concentrated CD3 membrane location).

These T cells seemed to have antitumor activity in that tumor replication was most active in the area where there were fewer T cells while in the area where there were more T cells, tumor replication was much less active. Based on these observations we believed that this was a case of highly active tumor replication with a concomitant antitumor immunity. The levels of the antitumor immunity in this case are relatively strong compared to most tumors at the time of diagnoses, especially some of the CD8 T cells inside the tumor showed activated state and there was a clear antagonism between T cells and tumor replication. Furthermore, the pattern of T cell infiltration in this case is a "mixed" type, indicating that it is likely to benefit from ICI therapy with antibody to PD1 based on the depletion model of ICI therapy [3]. On the other hand, our observation of T cell-mediated suppression of tumor replication indicated that there was no tumor expression of PDL1 due to immune attack, which usually enhances Ki-67 staining [3]. This was confirmed by a commercial third-party assay on PDL1 expression that concluded no tumor expression of PDL1 (not shown). The reason why tumor cells under such strong immune attack did not express PDL1 is not clear. Inasmuch as PDL1 expression is stimulated by IFN-g [15, 16], it could not be the lack of IFN-g release because we saw clear suppression of tumor replication, which is the hallmark of T cell-released IFN-g. There must be other factors that prevented tumor cells from expressing PDL1.

Regardless of tumor PDL1 expression, our depletion model for selecting patients for ICI therapy predicted that this would be a beneficial case [3]. We therefore recommended anti-PD1 treatment. Unlike the mainstream use of PD1 antibody, our use based on the depletion model depends on the trigger effect of the antibody, and does not require repeated dosing unless necessary. Because PD1-positive T cells would be depleted, and this depletion is likely variable among patients who may have expressed different alleles of their FC receptor gene that affect IgG1 binding by macrophage and T cell removal, we monitored the blood cell counts from the patient before and after administration of anti-PD1 (Keytruda, 200mg). Blood cell counts indicated that there was a 23% drop of lymphocytes one day following antibody dosing (no drop of other white blood cells seen at the same time). This is not a large drop among the patients monitored for ICI therapy, which is often more than 30% drop immediately following the antibody dosing (our unpublished results), indicating that T cell depletion may not be severe. Since T cell activation depends on homeostasis-driven recovery by residual T cells, small depletion would drive a small recovery and probably less T cell activation.

Two weeks following the treatment, we could witness a response on the neck tumor nodule. By 5 weeks, this nodule had shrunk significantly to <20% of previous volume (Fig. 1-4). This response began to wean down by the 6th week and neck tumor relapsed slowly. Other physicians the patient and his family members had consulted all blamed this lack of continued response on lack of scheduled antibody dosing (once every three weeks) and lack of combined chemotherapy. Because that the first PD1 antibody treatment did not show any sign of temporary tumor progression, a phenomenon associated with temporary depletion of PD1-positive antitumor T cells according to our depletion model, we thought a subsequent repeat of the treatment two months later should be safe, but we were against repeated dosing every three weeks due to the possibility of over-depletion of antitumor T cells and loss of control on tumor progression entirely leading to hyper-progression. Despite our warning and explanation, patient went on with his family and took the advice of the other physicians. By 6 weeks following the resumed PD1 antibody, accelerated tumor progression become obvious in a daily basis in that the neck tumor quickly became hard and larger, previous single nodule had split into four protruding nodules occupying large area of the neck (Fig. 1-5). The patient also experienced back and leg pains. Based on our previous warning, we realized that the patient had experienced depletion of antitumor immunity and a hyper-progression as a result. Yet, the treating physician insisted that this is caused by the development of drug-resistant clones of tumor variation, not a loss of antitumor immunity. They insisted on continued antibody dosing.

To resolve this dispute on the cause of tumor relapse, we asked for another biopsy on the neck tumor and another PET-CT to see whether tumor progression was limited to the neck tumor or new metastases had established. As Fig. 1-6 shows, this PET-CT showed a massive presence of new metastases in many locations of the body. The biopsy of the progressing tumor showed active proliferation tumor cells without T cells inside the tumor mass (Fig. 1-7). Together, these observations support the conclusion of a total loss of antitumor immunity in the entire body, a result only explainable by antibody-mediated T cell depletion, supporting our depletion model for ICI therapy.

Figure 1-4: The Neck nodule (a mirror imaging shown here) Had Shrunk to Almost Flat 5 Weeks following First anti-PD1 Therapy.

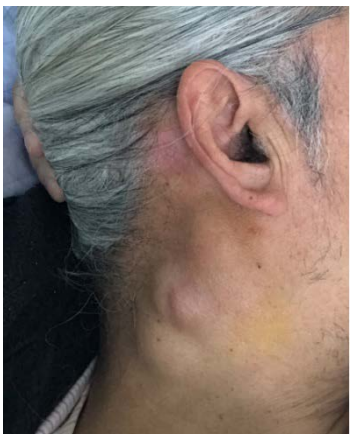

Figure 1-5: The Neck Nodule After Hyper-Progression Following Repeated PD1 Antibody Treatment.

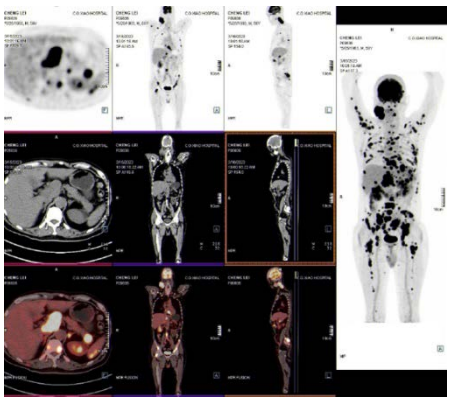

Figure 1-6: Second PET-CT showing massive newly established metastases in the liver, lung, many bones and muscle locations.

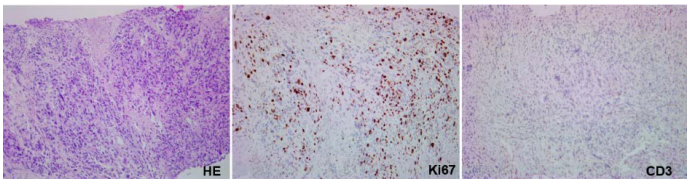

Figure 1-7: Biopsy Tissue of the Neck Nodule Following Hyper-Progression. the Lack of T Cell Forms Clear Contrast to Previous Biopsy Tissue at the Time of Diagnosis (Fig.1-3).

Upon these findings, all previously involved physicians gave up on this patient. We explained to the patient and family members that the depletion of antitumor T cells was temporary as long as no more antibody was given. Immunity could recover eventually with time (2-3 months). In order to prevent more metastases from establishing, we suggested intermittent chemotherapy to suppress freshly established metastases. Yet our advice of chemotherapy was not carried out due to lack of cooperation by area hospitals. During this waiting time, around 9 weeks following the last antibody dosing, the patient started to experience regular 39°C fever that lasted few hours every day for more than two weeks. Despite the high fever, patient felt mostly normal. This was clearly different from the commonly seen "cancer fever" that is associated with terminal stage cancer patients. With this fever, we noticed the partial softening of the neck tumor, indicating the return of antitumor immunity. In order to confirm this, we recommended another biopsy of the neck tumor. The biopsy indeed confirmed the return of T cells inside the tumor (Fig. 1-8).

Based on this observation, we suggested two options: 1) return to PD1 antibody treatment one more time, but only once at a time; 2) Use chemotherapy to activate antitumor immunity. Patient and his physicians did not accept the idea of using ICI therapy again due to the previous bad experience, so they opted to try one course of chemotherapy. The response from that chemotherapy was so dramatic, that not only the neck tumor shrunk quickly, a large degree of depigmentation appeared around the neck tumor following its regression also. At the same time, CT and MRI exams showed regression of many previously established metastases (not shown). With this massive tumor regression, the patient entered a state of rapid body weight loss accompanied by severe malaise resembling cancer cachexia. We believed that this was caused by a heightened immune response against the large tumor burden, and it should be suppressed partially to save the patient's life. Despite our advice on using immune suppressive measurements (for example, corticosteroids), the patient and his physicians did not intervene accordingly. The patient died soon after.

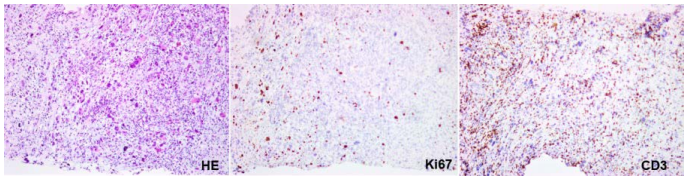

Figure 1-8: Biopsy tissue of the neck nodule 10 weeks following last PD1 antibody dosing. Compared to the last biopsy taking at the peak of hyper-progression (Fig. 7), large number of T cells returned to tumor mass. Tumor replication was clearly suppressed by these T cells, too.

What was the reason behind the roller-coaster swing in responses following ICI therapy from one extreme to the other? Our analyses based on the depletion model point to the initial activation of antitumor T cells following one single administration of anti-PD1 antibody. Continued dosing of the same antibody caused the near complete depletion of the activated T cells and hyper-progression. Subsequent return of antitumor T cells after stopping giving more antibody resulted in spontaneous tumor control. But we did not expect the dramatic sustained antitumor response following a single course of chemotherapy with paclitaxel that eventually caused the death of the patient. Depig-

mentation of melanocytes following melanoma immunotherapy has been described before. It is usually associated with self-sustained antitumor responses that often resulted in cancer eradication [17].

Apparently, this type of sustained response is not usually associated with chemotherapy, less to say a single course of chemotherapy with a common drug. The true reason for this sustained response seen in this case comes not from the selection of chemotherapy drug, but the activation of returned antitumor T cells. Since we have seen the best responses following ICI to be mediated by PD1-negative T cells (see later section on case 3), we went back to check the PD1 expression status of T cell in the first and the third biopsy samples (since the second biopsy did not contain T cells). As Figure 1-9 illustrated, in the sample of the first biopsy taken at the time of diagnosis, nearly all T cells inside the tumor mass expressed PD1 marker. In clear contrast, in the third biopsy taken at time of spontaneous tumor control 9 weeks following cessation of repeated anti-PD1 antibody, there was large number of T cells in the tumor, but less than half of these T cells expressed PD1.

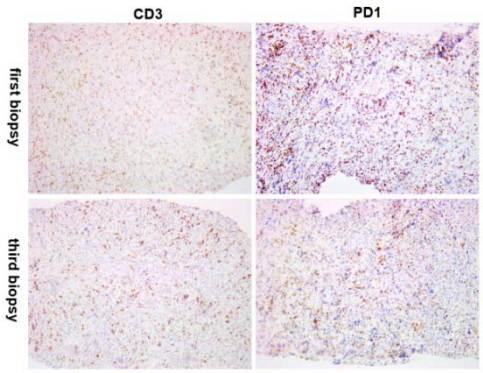

Figure 1-9: PD1 expression ratio in T cells infiltrating tumors in the first and third biopsy samples. As shown, nearly all T cells in the first biopsy taken at the time of diagnosis expressed PD1, whereas in the third biopsy taken at a time when spontaneous tumor control retuned following anti-PD 1 antibody induced hyper-progression, less than half of T cells inside the tumor expressed PD1.

We could not conclude that these PD1-negative T cells would remain PD1-nagative after chemotherapy during sustained antitumor response, but based on our observation from other cases (for example Case 3), we believe so. It is not even clear whether the sustained antitumor response following chemotherapy was activated by the single course of chemotherapy, it could as well be the continuation and expansion of the spontaneous T cell recovery process already observed before chemotherapy. In Case 3 and 4, we would be witnessing a selective process of PD1 antibody for PD1-negative T cells to expand only. Had the treatment with anti-PD1 antibody not stopped upon observing hyper-progression in this case, we may actually see the subsequent tumor regression after the PD1-nagative T cells caught up eventually. In as much as some of the most durable responses following ICI therapy are carried out under continued antibody administration, this would be a reasonable explanation.

Case 2: PDL1 Expression by Tumor Is Not a Safe Indication to Avoid Hyper-Progression Following ICI Therapy

In the above case, the status of PDL1 expression was negative both by our evaluation and by a third-party immunohistochemistry analysis. This is not the reason anti-PD1 should not be used in that case, because that ICI treatment was given and was highly effective following the first dosing. On the other hand, mainstream guideline for selection of ICI therapy candidates often uses the status of tumor expression of PDL1. A correlation between the expression levels of PDL1 by tumor cells and responses to ICI therapy has been established by clinical data [18]. Although many studies have since demonstrated that patients with PDL1-negative status may benefit from ICI therapy as well, but high expression of PDL1 by tumor is generally a better indicator of better responses [19].

In light of the finding that tumor expression is stimulated by IFN-g released by T cells this high expression of PDL1 by tumor cells at least indicates the nearby location of antitumor T cells and the ability to release IFN-g, a hallmark for the preferred Th1 antitumor response [15, 16]. Even by our depletion model, this nearby location of T cells to tumor often points to a mixed T cell infiltration within the tumor mass, an indicator of potential benefit following ICI therapy. But the status of tumor expression of PDL1 is not a guaranty that depletion of antitumor T cells by ICI antibodies would not take place. The protective factors for depletion are 1) T cell location inside tumor mass; and 2) lack of PD1 expression on T cells, but not that whether T cells stimulated tumor cells to express PDL1. The following case is an illustration for this point.

A 52-year-old man was diagnosed with lung cancer following symptoms of persistent coughing and chest pain. A PET-CT exam showed a 4CM primary tumor in the left lung and multiple metastases all over the body (Fig. 2-1), securing a stage IV designation. Analysis on driver gene mutation and any potential use of targeted therapy did not yield any hope. The patient who was a physician by training and who had familiarized himself with current treatment guidelines on stage IV lung cancer went to us for assessment of prognosis and treatment plan suggestions. We asked to evaluate the status of his concomitant antitumor immunity by looking into the biopsy sample for the mode of tumor replication and the presence of antitumor immunity. The analysis with his biopsy samples showed (Fig. 2-2) a low-differentiated adeno carcinoma (Fig. 2-2, HE) with few autonomously replicating tumor cells (Fig. 2-2, Ki67) that with enlarged nucleus and stained heavily with Ki-67 expression, a sign of extremely active in recruiting local inflammation (the reason for heightened symptoms).

There were large number of T cells present in the biopsy sample (Fig. 2-2, CD3). The distribution of T cells was mainly in the interstitial space surrounding small patch of tumor mass, but some clearly infiltrated inside the tumor mass to form a mixed pattern of infiltration with tumor cells. Most of these T cells are of the CD8 subtype and did not show activated status. Together, these observations put this case into a category of relatively strong concomitant antitumor immunity with a widely metastasized tu-

mor distribution. By the TNM staging, this is a Stage IVb, very late-stage cancer with the worst prognosis, whereas by our compiled staging system incorporating the status of antitumor immunity, this case is not desperate as it seems and if antitumor immunity can be activated to eradicate most metastases, the case could be salvageable with a good long-term prognosis. Based on this assessment, we suggested to activate antitumor immunity with ICI therapy using one single treatment of anti-PD1 antibody.

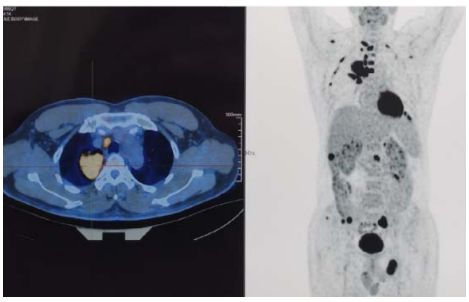

Figure 2-1: PET-CT Showing the Primary Tumor in The Lung (Left) And the Multiple Metastases (Right) All Over the Body Including Lung and Nearby Lymph Nodes, Peritoneal Metastases, Liver, Bone and Muscles.

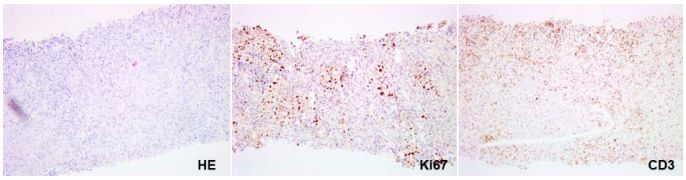

Figure 2-2: Biopsy Tissue at Diagnosis Stained for Ki-67 And Cd3, Showing A Low Differentiated Adeno Carcinoma of the Lung (left). Tumor Replication Was Active as Indicated by Enlarged Ki-67-Stained Tumor Cell (middle). There are Large Number of T Cells Mixed with Tumor Cells (right). Most T Cells are of the CD8 Subtype.

It should be pointed out that the selection of ICI therapy was also supported by a third-party analysis on tumor PDL1 expression that showed >90% tumor cells expressed PDL1 (Fig. 2-3). However, based on our observation of his biopsy samples, we made it clear to the patient that anti-PD1 therapy could only be given once at a time.

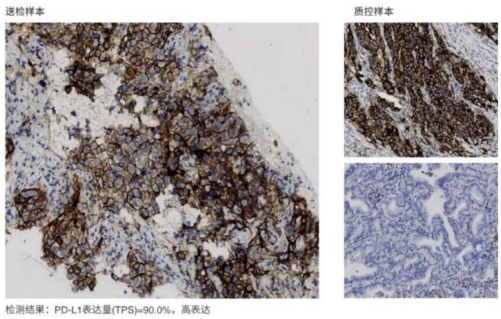

Figure 2-3: Tumor Expression of PDL1 as Tested by a Third-Party Commercial Laboratory. The Positive (top) and Negative (bottom) Controls Are Shown in The Right Panels Whereas the Tested Sample with High PDL1 expression on >90% of Tumor Cells is Shown on the Left.

Fig. 2-4 is the change of sensitive tumor markers before and at various times after the first dosing of anti-PD1 antibody. All three sensitive markers showed a temporary rebound 2 weeks after the administration of antibody, a phenomenon often seen with ICI therapy. This is explained by the depletion model as the short-term effect when those interstitial infiltrating T cells were removed by the antibody. Since these T cells were responsible for controlling tumor progression, their removal would result in tumor rebound. Subsequently all tumor markers dropped quickly and continuously for the next 12 weeks at which time a rebound of only marker CEA was seen. The sustained response following a single anti-PD1 antibody treatment was expected based on the depletion model we have described before but the rebound of CEA without the other two markers rebounding was unexpected and pointed to an escape event rather than general decaying of antitumor immunity activated by ICI therapy [3]. In order to confirm this, we asked for a second PET-CT exam. Figure 2-5 shows the comparison between the two PET-CT results. There were dramatic differences in tumor burdens between these two tests, illustrating a dramatic antitumor response activated by a single dose of anti-PD1 antibody. This dramatic and durable response supports the trigger-effect as explained by the depletion model [3].

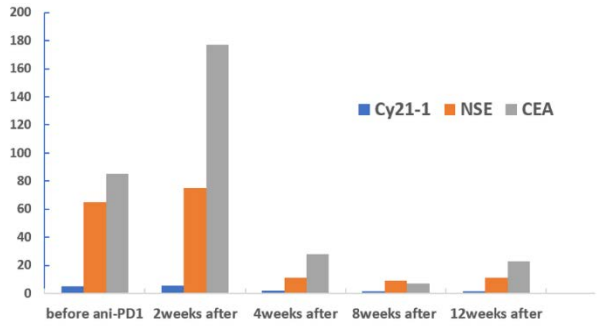

Figure 2-4: Change of Sensitive Tumor Markers (Cyfra21-1, NSE and CEA) Before and after Initial Anti-PD1 Treatment.

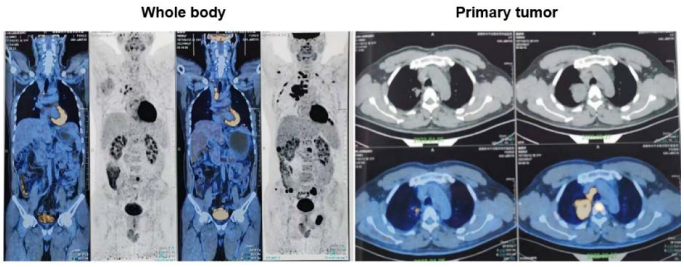

Figure 2-5: Comparison between PET-CT images before and 13 weeks after ICI therapy with a single dose of anti-PD1 antibody. The left side shows the whole-body image comparison. The initial PET images are on two the right-hand side panels, while the after-treatment PET images are next to the left. The regression of primary tumor is presented on the right side as labeled. Again, the before treatment images are on the two right-side panels and the after images are on the left.

Further, we also found the reason for CEA rebound as there was one newly established bone metastasis (Fig. 2-6) among all previously identified nodule regressing. This is a clear demon-

stration that the ongoing antitumor immunity, regardless the strength, could not recognize this nodule. Since the other two tumor markers (Cyfra21-1 and NSE) did not rebound, replication of this nodule was not represented by these two markers, thus was likely a new variant in replication and an immune escape as well. In light of the overall tumor regression with one escape metastasis, we suggested a radiation treatment of this bone metastasis while leaving the rest tumors to continue regressing. But other physicians the patient and his family consulted insisted on giving more antit-PD1 antibody. While we explained the reason why ICI therapy has trigger effect and that the three-month response pattern from the initial anti-PD1 antibody supported this view, and that T cell infiltration pattern in this tumor may not withstand repeated dosing of ICI antibody, the patient chose to do radiation treatment on the newly established bone metastasis while the same time taking repeated dosing of anti-PD1 antibody. Two months later after radiation therapy and two consecutive anti-PD1 antibody treatment, tumor markers showed rapid rebound, indicating a loss of tumor control. Patient went back to us for explanation and suggestion. We asked for a third PET-CT to see the changes of tumor burden. As Figure 2-7 shows, there was clear relapse of some of the previously regressing tumors including the primary tumor by the time of the third PET-CT exam.

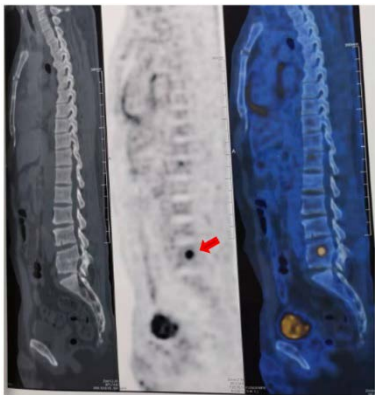

Figure 2-6: The New Bone Metastasis as Indicated by the Arrow Found by the 2nd PET-CT Despite Massive Tumor Regression Following ICI Therapy as Shown in Figure 2-5.

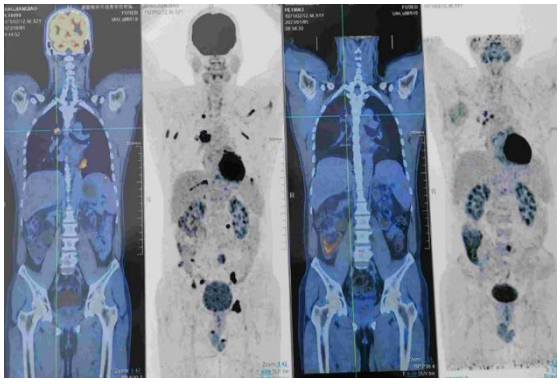

Figure 2-7: Comparison Between the Third (left-hand side two panels) to the Second (right-hand side two panels) PET-CT Images, Showing Rebound of Previously Regressing Tumors and Appearance of New Metastasis.

In addition, there were also numbers of newly established metastasis. The single bone metastasis identified by the second PET-CT (Fig. 2-6), which was treated by radiation showed reduced metabolism. Together with rapidly rebounding tumor markers, these observations indicate that T cells that were responsible for suppressing tumor was removed by repeated anti-PD1 antibody, thus we saw the rapid regrew of the primary tumor and the appearance of new metastases. In contrast, since the single bone metastasis identified by the second PET-CT was an immune escape, T cell depletion would not affect its growth. Indeed, this metastasis was suppressed by radiation treatment and showed reduced metabolic activity.

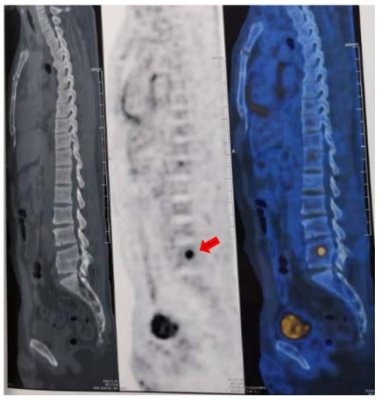

Figure 2-8: The New Bone Metastasis as Indicated by the Arrow Found by the 2nd PET-CT Despite Massive Tumor Regression Following ICI Therapy as Shown in Figure 2-5.

This case was designated as potential high-responder to ICI therapy by extremely high tumor expression of PDL1 (Fig. 2-3). On the other hand, it was also recognized by the depletion model as potential beneficiary of ICI therapy by the structure of lowly differentiated tumor and presence of mixed T cell infiltration of tumor mass (Fig. 2-2). The actual response form ICI therapy was a dramatic antitumor effect as witnessed by the two PET-CT tests before and after the initial ICI therapy (Fig. 2-5). The subsequent dosing of anti-PD1 antibody was carried out three months later at a time when tumor control was still apparent except for one variant escape. It is difficult to blame a second dosing of ICI antibody for the subsequent reverse from dramatic response to hyper-progression, not even by the depletion model. What caused the dramatic reverse should be the third antibody dosing spaced three weeks away from the second.

According to the depletion model, T cells not hiding inside solid tumor mass and present in the interstitial and stromal space are subjected to antibody binding and removal unless they do not express PD1. Following 2nd antibody dosing, T cells hiding deeply in the tumor migrated out of the tumor mass for expansion, this was the time when they were most accessible by anti-PD1 antibody for removal. Thus, a repeated antibody dosing given at this time would result in massive removal of T cells responsible for tumor control, causing total loss of tumor control. The actual hyper-progression supported this speculation. This event, therefore, predicted that all of the antitumor T cells following initial anti-PD1 antibody still retained PD1 expression, therefor was susceptible for removal by anti-PD1 antibody. To test this pre-

diction, we went back to look for PD1 expression in the biopsy sample shown in Figure 2-2. Figure 2-8 shows that at the time of diagnosis, all T cells infiltrating the tumor expressed PD1. One may assume from this observation that upon removal of interstitial T cells, T cells that came out of tumor mass for homeostatic expansion may retain their PD1 expression status. These T cells therefore were susceptible for antibody-mediated depletion. Had this case not taken subsequent anti-PD1 antibody, whether T cells activated by the initial ICI therapy treatment could sustain the antitumor response till complete tumor regression is an interesting question. Together, observations from this case again support the trigger effect of ICI therapy and the depletion of antitumor T cells by continued administration of ICI antibodies.

Case 3. T Cells Surviving Persistent Anti-PD1 Antibody are PD1-Negative

Although the above two cases demonstrated T cell depletion and hyper-progression as result of repeated ICI antibody dosing, clinical trials have demonstrated some cases that received many doses of antibody without loss of responses. What are the reasons behind these cases? Is the repeated antibody actually necessary or responsible for the durable response? One prediction based on the depletion model is that surviving T cells during the durable response under repeated ICI antibody must be PD1-negative or else they would have been depleted way early. Based on what we see in the above two cases and in some other cases where hyper-progression takes places, if T cell depletion is set to happen by ICI antibodies, it shall take place no more than two repeated doses of antibody. If a case was treated with two consecutive doses and no T cell depletion took place, it will not take place with more antibody treatments. The following case is a good illustration of this point.

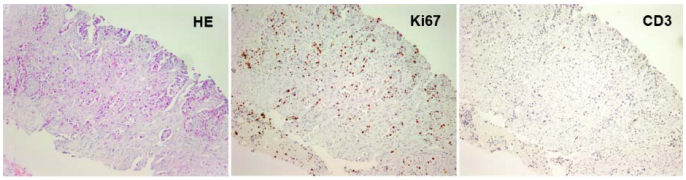

Figure 3-1: Biopsy Tissue at Diagnosis of Case 3 Stained with HE, Ki-67 and CD3. The Tumor is Squamous Carcinoma by Morphology (HE) With Large Area of Interstitial Space and Stroma. Tumor Replication was Medium active with 30-40% of Tumor Cells Expressed Ki-67. There is Almost no sign of T Cells in the Tumor (CD3).

A 60-year-old man was found to have a large lump in the left lung during a normal physical check-up. A biopsy showed that it was a lowly differentiated adenocarcinoma. There were few possible local lymph node metastases and no distant metastasis was identified. Since the primary tumor was large (>7CM in diameter), the treating surgeon proposed to carry out a few rounds of neoadjuvant chemotherapy combined with anti-PD1 antibody to see whether they could shrink the tumor to make surgery easier. A family member wen to us for advice. We asked to see the biopsy tissue for the mode of tumor replication and the status of antitumor immunity. As Figure 3-1 shows, this is a lowly differentiated adenocarcinoma (HE) with relatively active replication (Ki-67). The important observation is that there is almost no presence of T cells in the biopsy sample (CD3).

This finding was consistent with the lack of any symptoms despite the rather large size of the primary tumor, but somewhat inconsistent with the lack of distant metastasis, two features heavily influenced by presence or absence of antitumor immunity. Based on these observations, we worried that the lack of concomitant antitumor immunity may be true. We have since proposed to remove the primary tumor with surgery and use the resected tumor tissue for vaccine preparation. Protection against recurrence would rely on vaccine-induced antitumor immunity.

The family and the patient chose to accept arrangement by the hospital and went for chemotherapy and anti-PD1 antibody. Few months later, the family member went back to report that the patient had accomplished three rounds of the chemotherapy and anti-PD1 antibody treatments, tumor size showed >30% reduction and the treating surgeon was ready to perform surgery. We advised that tumor should be saved in case that antitumor immunity was not activated by the neoadjuvant therapy. Following surgery, we examined the sample of primary tumor for signs of antitumor immunity. As Figure 3-2 shows, there were still some tumors remaining with similar lowly differentiated structure (HE). The replication of the tumor remained active, similar to the biopsy sample (Ki-67). To our surprise, in clear contrast to the lack of T cell presence in the biopsy sample, there was a large accumulation of T cells in the tumor, mostly located in the interstitial space between tumor structure (CD3). This was a surprise because based on our 9-year practice and experiences, neoadjuvant chemotherapy could only elevate antitumor immunity in adenocarcinoma cases where presence of antitumor immunity is confirmed, not in cases where there is no antitumor immunity like in this case. The second surprise was that T cell activation leads to initial PD1 expression and in the presence of continued anti-PD1 antibody, PD1-positive T cells face depletion. In addition, the large number of T cells in the surgical sample survived three repeated dosing of anti-PD1, they should be mostly, if not totally, PD1 negative if the depletion model for ICI therapy is correct. To check this prediction, we have stained the surgical sample for PD1 expression.

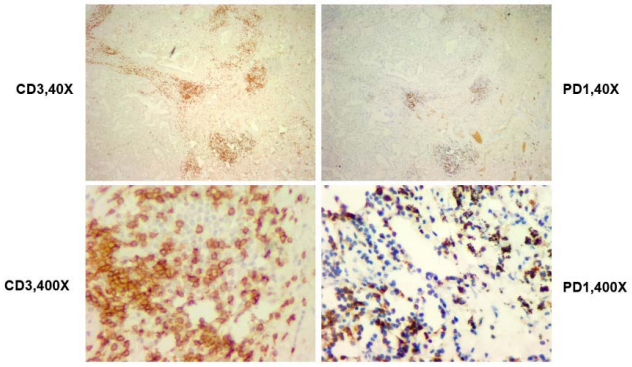

Figure 3-3: Most Of the T Cells in the Surgical Sample Did Not Express PD1 (compare the same area 40x micrographs of CD3 and PD1). Most CD3 Cells Show Activated Staining Pattern (round and circular staining, CD3,400x), While the Few PD1-posiotive T cells all showed inactivated State with Irregular Shape and whole Cell Evenly Staining Pattern (PD1,400x).

As Figure 3-3 shows, comparison between slides stained with CD3 and PD1 indicated that most CD3 positive T cells in the tumor did not express PD1. These T cells were of both CD4 and CD8 subtype, mostly in activated state (round cell shape with circular staining pattern). Interestingly, there were a few T cells among the cluster of T cells that showed PD1 positive staining. But most of these T cells were not activated in that they showed elongated and irregular shape with whole cell even staining pattern. Together, these observations support our depletion model for the ICI therapy in that activated T cells surviving repeated antibody dosing must be PD1-negative. The few PD1-positive T cells may survive repeated antibody dosing due to their location inside the tumor. It is likely that these T cells were PD1-negative before they enter tumor and turned PD1-posittive inside the tumor. There have been some reports to show that PD1 expression is a feature of T cell exhaustion inside tumor [20,21]. It should be pointed out that the use of ICI therapy in this case was not justified by pre-screening criteria, not by PDL1 expression based on mainstream accepted method, neither by our depletion model-depicted method. The reason we did not recommend ICI therapy was that this case lacked antitumor immunity all together. ICI therapy cannot activate something that is not in existence to begin with. Secondly, the tumor structure, although lowly differentiated by structure, did contain large area of interstitial space and stroma that could be easily penetrated by antibody to PD1 leading to T cell depletion. Only if we saw mixed infiltration of T cells in the tumor that we might recommend the use of ICI therapy. In this case, we did not see the proper condition for ICI therapy, thus we did not recommend its use. In this sense, this case was lucky compared to the above two cases in that the family went ahead against our recommendation and ended with a good ending.

Based on the strong antitumor response we observed in the surgical sample, we told the family that this case is likely cured after surgery and no additional adjuvant therapy was necessary. The reason why a large number of PD1-negativeT cells was activated remains unclear. They could be expanded from a few PD1-nagetive T cell inside the tumor that we did not see in the biopsy sample. Or they could be activated from a few PD1-positive T cells inside the tumor and turned PD1-negative, expanded under continued antibody dosing. We could not tell because we could not see T cells in the biopsy sample.

Case 4. The Source of PD1-Nagative T Cells

In the above case, large number of PD1-nagetive T cells was present in the final surgical tumor sample. Since there was no T cells present on the biopsy sample before ICI therapy, we could not tell where were those PD1-nagative T cells derived. By our depletion model, T cells do not have to be negative in order not to be depleted by anti-PD1 antibody as long as their location are inside the tumor, not in the interstitial or stroma space. But if they are PD1-negative to begin with, their activation and expansion would not be affected by antibody. Case 1 and 2 also demonstrated that T cell activation by ICI therapy does not necessarily turn T cells into PD1-negative or subsequent antibody dosing would not deplete an activated antitumor response. Then what factor(s) decides the fate of PD1 expression on ICI therapy-activated T cells? Is de novo PD1-nagetive population required for subsequent expansion or the PD1-nagetive T cells can be converter from PD1-positive T population upon ICI therapy-induced T cell activation? In the following case, we looked for clue.

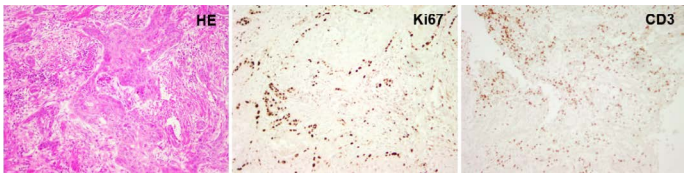

Figure 4-1: The primary tumor surgical sample form Case 4 stained with HE, Ki-67 and CD3. The tumor is a typical squamous carcinoma by structure with patches of tumor buried in large interstitial space (HE). Tumor replication is active at the border between tumor and interstitial space (Ki-67). There are large number of T cells present in the tumor, mostly in the interstitial space. No clear suppression of tumor replication or destruction of tumor structure by the presence of T cells was evident.

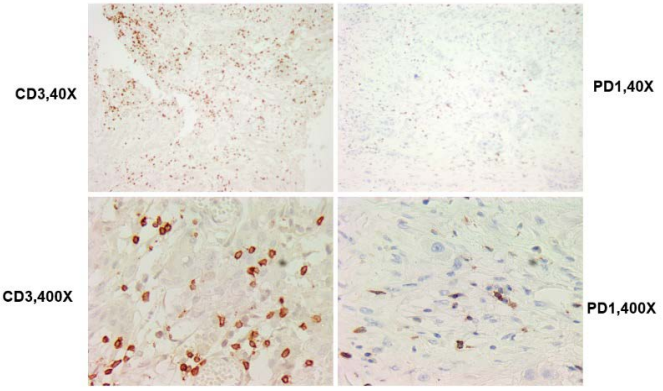

Figure 4-2: Most T cells in the surgical sample did not express PD1 (compare CD3 and PD1 staining, 40x). Most T cells show activated status with round and circular staining pattern (CD3, 400x). The few PD1 positive T cells did not show activated tatus (PD1, 400x).

A 50+ years-old man was diagnosed with esophageal cancer and went through neoadjuvant therapy composed of chemotherapy combined with ICI therapy. After two rounds of treatments, tumor response was not obvious and his treating surgeon went ahead with surgery to remove the primary tumor. Following surgery, we were consulted for the risk of recurrence. We therefore looked into the surgical sample for clue. Because we knew that the patient was treated by two consecutive rounds of chemotherapy combined with anti-PD1 antibody, we expected to see that most or all surviving T cells from such sample should be PD1-nagative. This was confirmed as Figure 4-1 and Figure 4-2 shows. The remaining tumor showed typical morphology of a squamous cell carcinoma with patches of irregular shaped tumor conjugate buried in large space of interstitial tissue (HE). Tumor replication was active in that >70% of tumor cells along the edge of tumor conjugates were heavily stained with Ki-67. There were moderate number of T cells clustered in the interstitial space surrounding tumor conjugates (CD3). These T cells are mostly CD8, mostly activated, and most did not express PD1. The few that stained with anti-PD1 were not in activated

states (Figure 4-2). Based on these observations, we believed that this case would have a good post-surgery prognosis, most likely to achieve clinical cure. At the same time, we were curious about the source of these PD1-negative T cells surviving anti-PD1 antibody-mediated depletion. They could be expanded from a population of PD1-negative or converted from PD1-posotive T cells during homogenesis-mediated activation. In order to answer this question, we went back to examine the biopsy sample before neoadjuvant therapy.

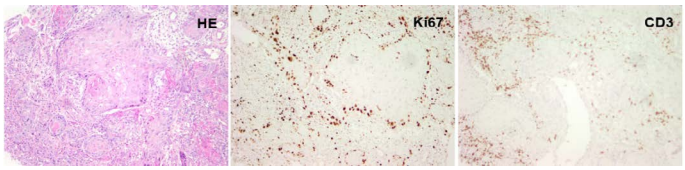

Figure 4-3: Biopsy Tissue at Diagnosis of Case 4 Stained with HE, Ki-67 and CD3. Compared with the Surgical Sample, the Tumor Structure is More Packed with Less Interstitial Space. Tumor Replication Pattern Was Similar to The Surgical Sample in That Only Tumor Cells Alone the Boarder with Interstitial Space Showed Active Replication (Ki-67). There Were Some T Cells in The Sample, Clearly Less Than the Number of T cells in the Surgical Sample. Most T Cells are of the CD8 Subtype, Some Showed Activated Status. No clear Suppression of Tumor Replication or Destruction of Tumor Structure by The Presence of T cells was evident.

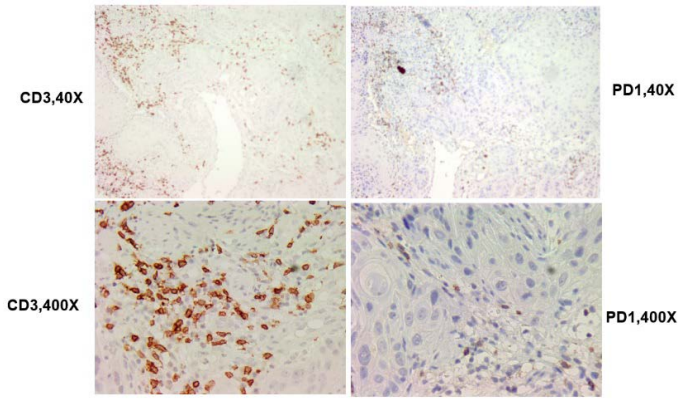

Figure 4-4: The T Cell Activation and PD1 Expression Status of the Biopsy Sample. Comparing the Signals from CD3 and PD1 Staining, Clearly Some T Cells (about 30-40%) expressed PD1. Some T Cells Showed Activated or Simi-Activated Status (CD3, 400x), and none of the PD1-positive T Cells Showed Activated status.

As Figure 4-3 and 4-4 show, the tumor has a structure of typical squamous cell carcinoma (HE) with active tumor replication (Ki-67). There were some T cells in the tumor area, but mostly located in the interstitial space (CD3), and most of these T cells were PD1-negative. This observation thus explained why these T cells survived the repeated anti-PD1 antibody treatments without being depleted, albeit that they were located mostly in the interstitial space. In addition, comparing the T cell number and activation status between biopsy and surgery samples showed that there was an expansion in number and increase in activation status (more round shaped T cells in the surgery sample). This increased T cell response could be the result of ICI therapy. If so, large number of PD1-nagetive T cell following ICI therapy could be expanded from previously PD1-nagetive population.

Discussion

ICI therapy with blocking antibodies to PD1/PDL1 has been in clinical use for more than a decade. Many exciting applications have been reported based on clinical study results. Yet the real-world use of the therapy did not show similar efficacies as reported from clinical trials [1, 2]. Many off-label use of ICI antibodies, especially in China where several domestically produced antibodies are available for relatively low costs, has been adapted in cancer clinic as if it is the "last hope" for desperate patients (our observations). Despite such massive and abusive use of ICI therapy, more and more physicians began to realize the limit of this therapy and its harmful effects (our personal communication). The harmful aspects of ICI therapy have not been well discussed because that these harmful effects cannot be explained by the adapted mechanism for ICI therapy. It is confusing to see that a therapy designed for activating antitumor immunity may actually harm antitumor immunity leading to accelerated tumor progression, thus most physicians chose to ignore it, especially after they found that hyper-progression seemed prevented by combining chemotherapy. But the reality is that the harmful effects of ICI therapy have been with the therapy since day one and chemotherapy only delay tumor progression (including establishment of new metastases) to a few months later, thus appearing not related to ICI antibody administration, but from "normal" ineffectiveness. If followed closely by monitoring sensitive tumor markers, one can still see rebound of tumor replication due to depletion of T cells with or without chemotherapy (our unpublished observation). If the current adapted working model for ICI therapy (the blocking model) cannot explain the harmful effects of the therapy, it must be wrong or incomplete. As such, we have previously proposed another working mechanism for ICI therapy dubbed "depletion model" to replace the mainstream-adapted working model which we call "blocking model [3].

The new model, although could explain all of the perplexing clinical observations that cannot be explained by the blocking model, left some critical predictions to be confirmed. Among these predictions, one stands out unavoidably ironic: the antitumor responses that activated by repeated anti-PD1 antibody dosing and last durably must be mediated by PD1-negative T cells. The reason for this paradoxical prediction is based on the crux of the depletion model: all T cell expressing PD1 shall be bond and removed by macrophage-dependent mechanism such as ADCP [22].

While we believed that this must be the case, we refused to prove it though repeated use of anti-PD1 antibody in our own cases because we knew that it is not necessary, but could also be harmful as Case 1 and 2 in this report showed. Nevertheless, repeated use of ICI antibodies is a common practice in clinical setting, and we have some opportunities to observe the effects when some patients came to use for advice. Like the patients in Case 3 and 4, they were treated by repeated anti-PD1 antibody

before surgery to remove primary tumors. If they were our patients, we would not recommend repeated use of ICI therapy, or not even ICI therapy like in Case 4 because the location of T cells was mostly in the interstitial space (Figure 4-3). Nevertheless, these patients had been treated without consulting our suggestions, thus we could observe the effects of repeated ICI therapy in cases where no hyper-progression took place after ICI therapy. The results indicate that under such outcome, the surviving T cells are indeed mostly PD1-negative. This observation alone confirms our prediction based on the depletion model. It needs to be pointed out that what we are relying on to prove our prediction is by looking for any exception. Even a single case where large expanding number of surviving T cells immediately after repeated ICI therapy are PD1-positive, our depletion model is flawed. On the contrary, if more than seven consecutive cases show similar result as what we see in Case 3 and 4, it reaches statistical significance ($p<0.01$) and the depletion model is proven. We are collecting cases to test this prediction and will report the conclusion when it is available.

The observation that PD1-nagative T cells are mainly responsible for the durable responses following ICI therapy is an interesting one. PD1 is a molecule expressed on the surface of activated T cells [23]. But many studies also show that PD1 expression is a hallmark for exhausted T cells in tumor environment [20] [21]. The overall analyses on PD1 expression tend to show that it is a negative regulator of T cell function. On the other hand, tumor cells express PDL1 by the stimulation of IFN-g [15, 16].
Therefore, we have a situation where tumor-infiltrating T cells inside tumor mass are met with tumor cells expressing PDL1. The net effect is the survival of tumor with presence of antitumor T cells, a situation we call concomitant immunity. The antitumor immunity inside a growing tumor may be "exhausted" as many tend to believe, but also "functional" as very few realize. The functions of these tumor-infiltrating T cells include: 1) to restrict the growing (proliferating/replicating) rate of the tumor; and 2) to restrict the establishment of new metastasis. These functions are easily seen in animal models when T cells are removed, but hardly recognized in human cancer patients until recently when ICI therapy bring many hyper-progression cases. The essence of ICI therapy-induced hyper-progression is the depletion of antitumor T cells, those many thought to be exhausted cells that co-existing with growing tumor. By this measurement, we should not consider these co-existing T cells "exhausted" and "functionless", but recognize their important role as functional antitumor T cells.

These tumor-infiltrating T cells can be activated to overthrow the balance between tumor and immunity in that for a short or longer period, antitumor immunity becomes dominant over tumor progression to cause tumor regression. When such control continues, tumor regression may lead to complete tumor eradication. In many times, this activation of antitumor immunity is achieved by chemotherapy or radiation therapy occasionally. This activation seems to depend on synchronized antigen release during the killing process of tumor reductive therapies [24,25]. This is consistent with general rule of immunology that states that adaptive response is driven by specific antigen. ICI therapy, by the current blocking model, is not an immunity activation therapy, because it is working by blocking the interaction between immune checkpoint molecules, thus to release the inhibited T cells to resume attacking tumor antigen. By the general rule of antigen-driven response, the T cells released by ICI therapy should have been activated though other process of antigen release, but are not able to destroy tumor cells due to down regulation by tumor expression of immune checkpoint ligands (PDL1, for example). But in reality, ICI therapy may activate massive and persistent T cell response in the absence of any antigen-releasing interventions such as chemotherapy. Then, how does ICI therapy achieve this T cell activation? The blocking model cannot explain this effect. The depicted "negative regulation" and the release of such regulation by ICI antibody have not been demonstrated in patients using ICI therapy. Instead, our observation has repeatedly shown that tumor expression of PDL1 does not inactivate T cells, but just makes tumor cells themselves insensitive to inhibition of T cell-released factors such as IFN-g [3].

The blocking model cannot explain why blocking PD1 on T cells will make the tumor cells sensitive again to T cell-mediated attack. On the other hand, the depletion model proposed by us could provide explanation. PD1-positive T cells bound by anti-PD1 antibody is removed by macrophage-dependent process (ADCP, for example). The depletion causes a state of temporary homeostatic disbalance of T cells and a subsequent expansion of any surviving T cells. When all antitumor T cells are PD1-positive and are depleted, there will be a short-term loss of tumor control, and a possible tumor outgrowth. This is the reason for hyper-progression even under a single ICI antibody administration in many cases. But when some T cells, although PD1-positive, hide inside a tumor mass not accessible to antibody binding, they may be activated through homeostasis recovery. This activation results in T cell number expansion, and changes the activation status of the T cells. These T cells in turn infiltrate and attack tumor, resulting in antitumor response.

In Case 1 and 2, this seemed to be the case following initial anti-PD1 antibody. But from the subsequent hyper-progression in these two cases, homeostatic activation perse does not seem to lead to permanent PD1-nagative T cells. The question how PD1-negative T cells arise remains a mystery. But there is always this possibility that there are some naturally occurring PD1-nagative T cells in a concomitant antitumor immunity, which will expand over PD1-positive T cells under repeated anti-PD1 antibody-mediated depletion. Case 4 has illustrated this possibility, even though the T cells location exclusively in the interstitial space was a factor favoring complete T cell depletion by anti-PD1 antibody. Thus, the depletion model explains how T cells are activated without antigen release and the reason for over depletion and hyper-progression.

Under the new evidence, especially presence of PD1-nagative T cell-led responses, the depletion model is further strengthened and fulfilled. We should look into two factors before selection of ICI therapy: 1) mixed pattern of T cell infiltration as discussed previously, and 2) expression of PD1-negative T cells as part

of concomitant antitumor immunity [3]. On the other hand, we shall avoid the selection of ICI therapy for fear of depletion of antitumor T cells leading to hyper-progression when antitumor T cells are located in the interstitial space and most of them are PD1-positive. Further, the trigger effect of ICI therapy as demonstrated in Case 1and 2 has argued against repeated ICI antibody dosing, which may cause subsequent depletion of antitumor T cells as illustrated in Case 1 and 2.

The selective pressure through repeated anti-PD1 antibody may be helpful in some cases where PD1-nagative population may be preferentially expanded. But this is not sure so far by clinical evidence. Case 1 had probably selected PD1-nagative T cells eventually through repeated antibody dosing, but the depletion and hyper-progression before eventual establishment of a PD1-nagetive response was deadly and could be avoided if no repeated dosing of ICI antibody was carried out. Now days, we have always withheld repeated ICI antibody dosing. With these selection criteria and practice in place, we have totally avoided hyper-progression associated with ICI therapy. On the same time, we have achieved over 70% responses in cases we selected to receive ICI therapy. The 20% no responders seem to belong to a category of "depletion defective" situation in which T cells are simply not removed by ICI antibody. This is witnessed through monitoring lymphocyte reduction following antibody dosing. By the depletion model, this is probably caused by the lack of recognition of the Fc receptor sequence of the ICI antibody by the host macrophages that express certain subtypes of Fc receptor [26]. As such, no activation or depletion of antitumor immunity is observed. We will discuss this phenomenon in another article in the future.

If anti-PD1 antibody target PD1-positive antitumor T cells for its antitumor effect, what is the repeated use of such antibody in a durable response mediated by PD1-nagative T cells? Current clinical practice for ICI therapy is continued antibody dosing in every three weeks. Some durable responders received dozens of doses of antibody in 1-2 years. Was this necessary? There is certainly no proof from ICI therapy developer that continued dosing of anti-PD1 antibody is necessary. It's continued dosing is a natural thinking based on the blocking model. On the other hand, based on the depletion model, ICI therapy has trigger effect that only requires a single dosing of antibody to generate T cell activation and antitumor response. This was demonstrated by Case 1 and 2 following the initial treatment. And these two cases also demonstrated that repeated antibody dosing may reverse a previously antitumor response into a hyper-progression. We do not have an accurate account of how many such cases had taken places in the real-world clinic, but based on our own experiences, roughly 40% of ICI therapy-treated cases ended up with loss of tumor control. This high ratio of harm to benefit for ICI therapy may explain the low response ratio and lack of clear impact in real-world use of ICI therapy [1, 2]. On the other hand, since we have recognized the depletion model in the past 15 months, we have established a record of >90% accuracy in selecting potential responders and avoiding all harmful use of ICI therapy. In a few cases where ICI therapy was used without our knowledge and generated harm, there was no exception that had we evaluated the case for selection of ICI therapy, we would not have recommended it.

These clinical records indicate that the depletion model must be correct. If adapted by the mainstream medicine, many lives could be saved. After all, ICI therapy is a great development for cancer management, it is just that it is like a double-edged sword that may benefit or harm its users. By understanding its true working mechanism, we should be able to save the benefit while prevent the harm.

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