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Review

Acquired and intrinsic resistance in cancer immunotherapy

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ABSTRACT

A number of immunotherapies, in particular immune checkpoint targeting antibodies and adoptive T-cell therapies, are starting to transform the treatment of advanced cancers. The likelihood to respond to these immunotherapies differs strongly across tumor types, with response rates for checkpoint targeting being the highest in advanced melanoma, renal cell cancer and non-small cell lung cancer. However, also non-responsiveness is observed, indicating the presence of intrinsic resistance or naturally acquired resistance. In addition, a subgroup of patients that do initially respond to immunotherapy will later recur, thereby also pointing towards a role of therapy-induced acquired resistance.

Here, we review our current understanding of both intrinsic and acquired resistance mechanisms in cancer immunotherapy, and discuss potential strategies to overcome them.

1. Introduction

For many tumor types, including melanoma, renal cell cancer, colon cancer, ovarian cancer, and some subtypes of breast cancer, the presence of lymphocytic infiltrates within the tumor is highly correlated with improved outcome (Alexe et al., 2007; Clemente et al., 1996; Erdag et al., 2012; Galon et al., 2006; Hwang et al., 2012; Mahmoud et al., 2011; Nakano et al., 2001; Zhang et al., 2003). These infiltrates mostly consist of $CD4^+$ and $CD8^+$ T-cells, and especially for melanoma it has been well established that part of these T-cells recognize tumor-associated antigens (Coulie et al., 1994; Kawakami et al., 1994). The fact that these cells can have direct tumoricidal potential is well illustrated by the clinical effects of adoptive transfer of *ex vivo* expanded tumor-infiltrating lymphocytes (TIL) in metastatic melanoma patients. In several small clinical trials, response rates varying from 40% to 70% have been observed in highly selected metastatic melanoma patients (Dudley et al., 2010, 2008). In a more recent intent-to-treat analysis in a TIL trial for melanoma, a response rate of 30% has been reported (Besser et al., 2013). Within these studies, the absolute numbers of $CD8^+$ T-cells infused is strongly correlated with response to treatment, suggesting an important role for MHC class I restricted, cytotoxic T-lymphocyte (CTL) mediated tumor killing (Besser et al., 2013; Radvanyi et al., 2012). Direct evidence in support of such a role has been obtained through the administration of TIL products enriched for $CD8^+$ T-cells, which showed a response rate comparable to that seen with unselected TIL products (Dudley et al., 2013). In addition to tumor-reactive $CD8^+$ T-cells, it is clear that TIL products can also contain $CD4^+$ T-cell populations that are tumor-reactive, and there is

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evidence for an anti-tumoral effect of such tumor-reactive $CD4^+$ populations in melanoma and cholangiocarcinoma (Hunder et al., 2008; Tran et al., 2014).

A second, much more widely used, group of immunotherapeutic strategies that target the same cellular compartment focuses on the administration of antibodies that bind to immune checkpoint molecules, thereby (re)activating an endogenous tumor-specific T-cell immune response. Administration of ipilimumab, an antibody that binds the inhibitory receptor cytotoxic T-lymphocyte antigen 4 (CTLA-4) on T-cells, has shown a four month increase in median overall survival in phase III trials, leading to FDA and EMA approval (Hodi et al., 2010; Robert et al., 2011). An analysis of a large cohort of melanoma patients treated following this registration shows a long-term survival in 20–25% of treated metastatic melanoma patients (Prieto et al., 2012), a number that compares favorably to the 8–10% seen previously in patients treated with chemotherapy. More recently, objective response rates up to 50% have been reported in phase I/II trials testing antibodies that target another checkpoint molecule, programmed cell death protein 1 (PD-1) or its ligand (PD-L1). Importantly, clinical responses upon PD-1 – PD-L1 targeting have been observed in malignancies other than melanoma, such as renal cell carcinoma (RCC) and non-small cell lung cancer (NSCLC) (Brahmer et al., 2012; Hamid et al., 2013; Topalian et al., 2012).

These encouraging clinical results have rightfully put immunotherapy at the forefront of oncological practice. Nevertheless, it is important to note that a substantial number of patients still derive no or only limited benefit for reasons largely unknown, sometimes at the cost of severe toxicities. The disparity in response rates observed between different immunotherapeutic treatment modalities, but also across tumor types strongly suggests a role for immune resistance. Further evidence for such resistance comes from patients treated with immunotherapy who experience an initial decrease in overall tumor burden but eventually succumb to disease recurrence. In the following sections we describe the relevance of different classes of immunotherapy resistance in oncology and contrast this with therapy resistance seen with targeted therapies. Furthermore, we describe the strategies that may be taken to obtain a better understanding of immunotherapy resistance, and how this knowledge can be used clinically.

2. Requirements for an optimal anti-tumor T-cell response

To understand at which levels resistance to T cell-based cancer immunotherapy may occur, it is important to first describe the key elements that are required for a successful T-cell response that leads to cancer regression. To do so, we subdivide this process into three discrete steps (Figure 1).

First, T-cells need to be properly activated by professional antigen-presenting cells (APCs) in peripheral lymphoid organs. For this to occur, two things are required: A). Dendritic cells (DCs) need to display tumor antigens (derived from apoptotic or necrotic tumor cells) in the context of MHC class I or II for which an antigen-specific T-cell repertoire is present. B). These DCs need to have received maturation signals that instruct the development of an effector T-cell response, rather than T-cell anergy or the expansion of regulatory T (Treg) cells.

Second, following priming in peripheral lymphoid organs, the activated T-cells need to home to the tumor, extravasate through the endothelium and infiltrate via the surrounding stromal tissue into the tumor before they can bind to their target. This both requires certain phenotypic characteristics, such as expression of chemokine receptors, on the T-cells and the expression of cell adhesion molecules/chemokines by the vascular endothelium for cells to pass the endothelial

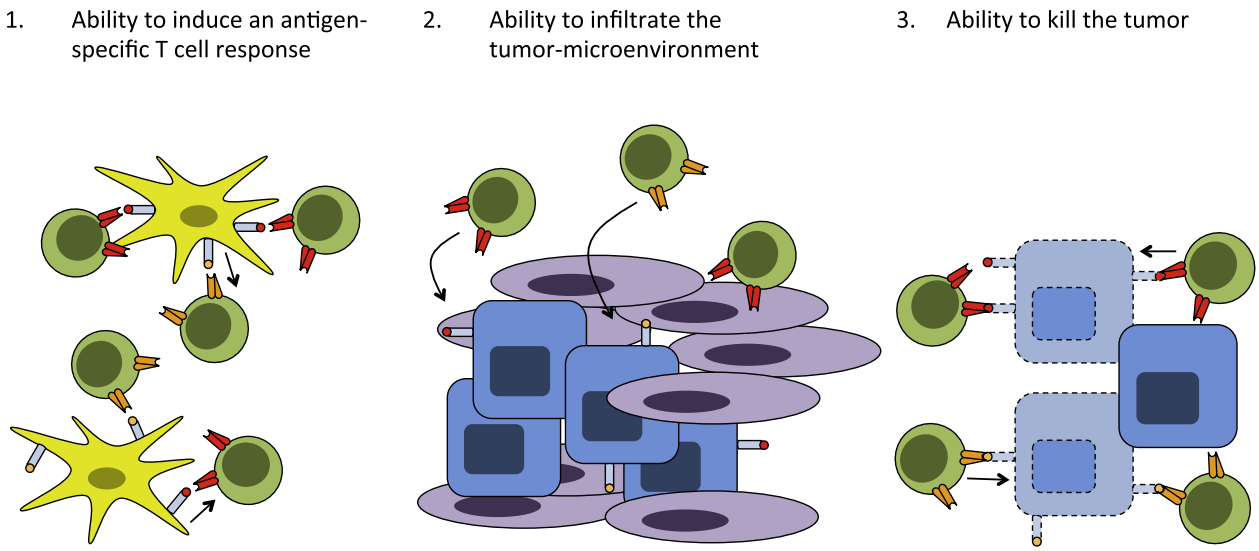
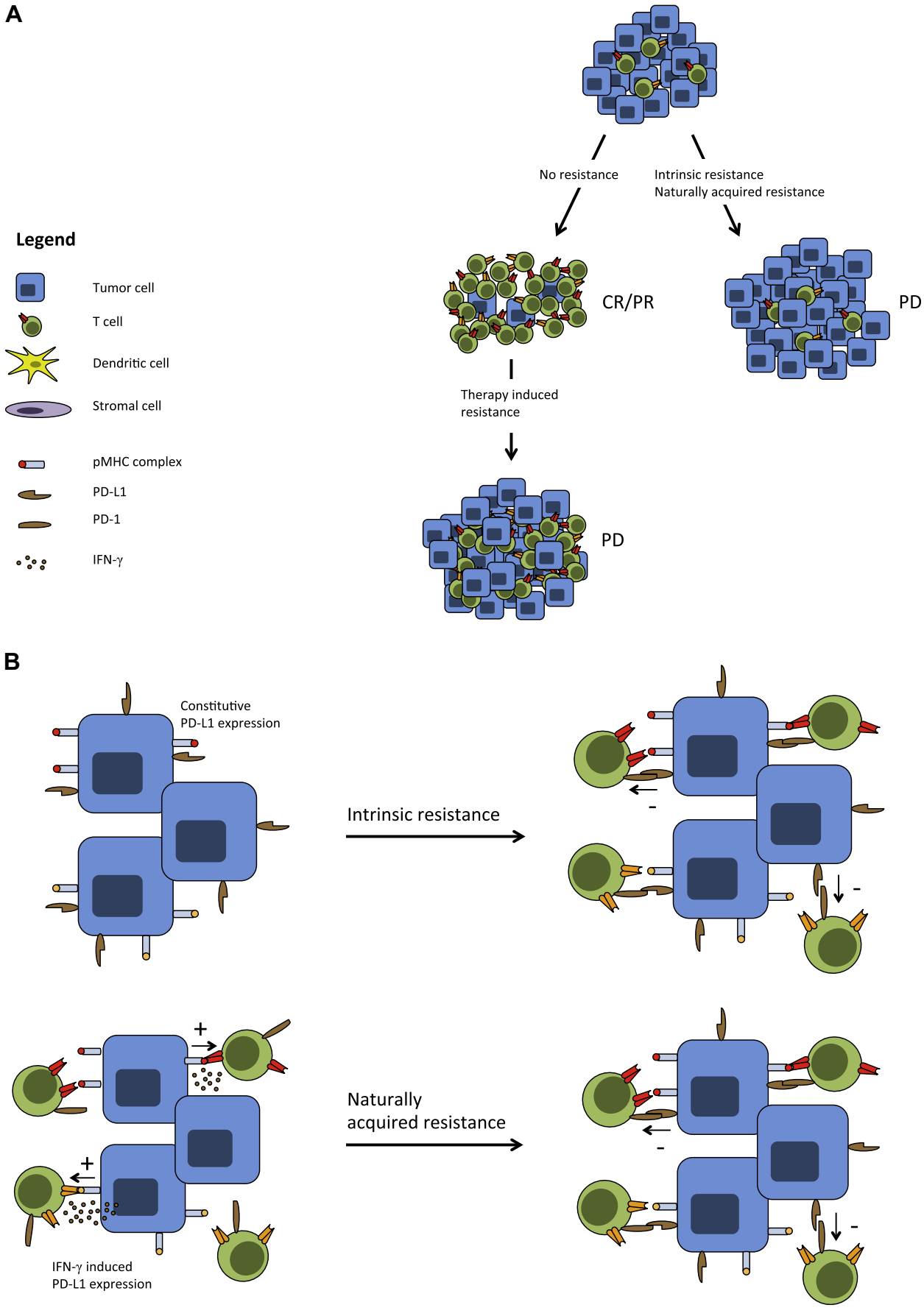


Figure 1 – Key elements for an effective anti-tumor T-cell response. The development of an effective anti-tumor T-cell response follows three distinct steps: 1.) Priming and activation of naïve antigen-specific T-cells; 2.) Migration and infiltration of activated T-cells through the vasculature and tumor-surrounding stroma; 3.) Recognition of cognate peptide in the context of MHC and release of cytolytic granules to mediate tumor cell killing.


A
No resistance
Intrinsic resistance
Naturally acquired resistance
CR/PR
PD
Therapy induced
resistance
PD
Legend
Tumor cell
T cell
Dendritic cell
Stromal cell
pMHC complex
PD-L1
PD-1
IFN-γ
B
Constitutive
PD-L1 expression
Intrinsic resistance
Naturally
acquired resistance
IFN-γ induced
PD-L1 expression

barrier and invade the tumor (Harlin et al., 2009). T-cells that have been inefficiently activated, because of lack of co-stimulatory molecule expression on APCs, or as a result of ineffective priming, can become anergic. By the same token, also when T-cell priming is efficient, but the tumor lacks the inflammatory signals to attract these cells, the tumor-specific immune response will be of little value.

Third, the T-cell receptors on the infiltrating T-cells need to contact peptide MHC complexes on the tumor cell surface, in the case of $CD8^+$ cells, to release lytic granules in the immune synapse thereby mediating tumor destruction. Furthermore, the environment that the T-cells encounter needs to permit such cytolytic activity. Negative feedback loops that regulate T-cell activity at effector sites are abundant and are essential to prevent run-away immune responses, but can also inhibit T-cell mediated tumor regression.

Having described the requirements for an optimal anti-tumor immune response, we can now make a subdivision of cancer immune resistance into three distinct classes (Figure 2A) that are further detailed below.

3. Intrinsic resistance

First, there are non-responding patients that lack anti-tumor immune activity and that also fail to elicit a T-cell response that has substantial tumoricidal potential upon immunotherapy, indicating *intrinsic resistance*. This form of resistance can be the result of a failing anti-tumor immune response either locally or systemically. A first class of systemic immune failure is observed in patients that are unable to elicit a potent immune response to a large variety of truly foreign antigens, such as viruses. This is for example observed in severely immune-compromised HIV patients or transplantation patients who carry an increased risk of virally induced neoplasias (Butel, 2000). Also elderly people may lose the capacity to mount a sufficiently strong systemic immune response upon foreign antigen exposure, possibly caused by a decrease in the diversity of the total T-cell pool (Akbar and Fletcher, 2005; Khan et al., 2004; Messaoudi et al., 2004). This effect is demonstrated in elderly patients who suffer from the reactivation of silent viruses, such as VZV that causes shingles, or infection with Merkel cell polyomavirus that can cause Merkel cell carcinoma (Feng et al., 2008). Although speculative, it is conceivable that such patient subgroups are less capable of mounting an anti-tumor immune response that is efficient enough to eradicate cancer cells. A second class of systemic intrinsic resistance is formed by tumors that express few antigens that can be seen as foreign by the immune system. Tumor antigens can be subdivided in distinct antigen classes that together form the antigenic landscape of a particular tumor. Specifically, many of the antigens that are (over-) expressed by tumors are also expressed on healthy tissues. For at least some of these 'self antigens', the avidity of the available T-cell repertoire will be low because of T-cell tolerance (Kvistborg et al., 2013). As a second class of antigens, human tumors can express epitopes that are truly foreign to the immune system, either derived from viral proteins or from mutant epitopes formed as a consequence of mutations. Recent evidence suggests that recognition of such neo-antigens may be of particular importance for tumor control (Brown et al., 2014; Champiat et al., 2014; Heemskerk et al., 2013; Robbins et al., 2013; van Rooij et al., 2013). Consequently, non-viral tumors with a low mutational load, such as pediatric and many of the liquid tumors, may be more likely to evade immune detection than tumors with a high mutational load, such as melanoma or smoking-related non-small cell lung cancer (NSCLC) (Alexandrov et al., 2013; Vogelstein et al., 2013).

Local intrinsic immune resistance may manifest itself in several ways. In some patients tumors may completely lack lymphocytic infiltrates. Assuming that in at least some of these patients, a systemic tumor-specific T-cell response was induced (data are presently lacking on this), this would signify the presence of a non-inflammatory tumor microenvironment that hampers infiltration of immune cells that would otherwise be able to recognize the tumor (Gajewski et al., 2010; Taube et al., 2012).

Assuming that tumor-specific T-cells are properly activated and capable of homing to the tumor, the tumor microenvironment can pose the last barrier for T-cells to exert their effector functions thereby giving rise to intrinsic resistance. It has been described that expression of PD-L1, which is the main ligand for the T-cell inhibitory molecule PD-1, can be induced upon loss of the tumor-suppressor gene PTEN and activation of the PI3K pathway in glioblastoma cell lines (Parsa et al., 2007) (Figure 2B). Additionally, the secretion of inhibitory molecules such as TGF-β, IL-10 and IDO can have a direct negative effect on T-cell function in the microenvironment (Braun et al., 2005; Geissmann et al., 1999; Pickup et al., 2013; Steinbrink et al., 1999), but also indirectly via the recruitment of tolerogenic immature DCs, myeloid derived-suppressor cells (MDSCs) or (inducible) regulatory $CD4^+$ T-cells (Gabrilovich et al., 2012; Lutz and Schuler, 2002; Strauss et al., 2007; Vukmanovic-Stejic et al., 2006). It is important to point out though that the presence of a T-cell infiltrate within a progressing tumor does not necessarily imply local inhibition of T-cell function as the mechanism of intrinsic resistance. Specifically, for most cancer types where T-cell infiltration is apparent we do not presently know to what extent this T-cell infiltrate consists of tumor-specific T-cells

Figure 2 – Categories of immune resistance. A. Several categories of immune resistance can be distinguished in either treatment-naïve or treatment experienced cancer patients. In the first group, tumor-infiltrating T-cells are either absent or scanty indicative of *intrinsic resistance* or *naturally acquired resistance*. These patients are unlikely to respond to immune modulatory treatments. In the second group of patients, the degree of immune infiltration is sufficient to establish tumor regression upon immunotherapy initiation. However, due to several potentially overlapping mechanisms the tumor becomes resistant to this immune pressure and *therapy-induced resistance* ensues. B. Mechanisms of intrinsic and naturally acquired resistance are exemplified by PD-L1 expression and subsequent effector function inhibition of antigen-specific T-cells. Upper panel shows tumor cells that constitutively express PD-L1 as a result of genetic alterations related to the oncogenic process. Lower panel shows induced expression of PD-L1 mediated by IFN-γ producing T-cells.

or of bystander cells, and only in the former case, local inhibition needs to be considered as a barrier to immune control.

Intrinsic resistance is not unique to immunotherapy but can also be observed in patients treated with targeted therapies. A well-described example of this is the different sensitivities of tumors that carry the BRAF V600E mutations to drugs such as vemurafenib that bind the mutant BRAF protein. Specifically, whereas the majority of melanoma patients with a BRAF V600E mutation show a rapid (albeit often transient, see below) tumor regression upon treatment with BRAF inhibitors, patients with BRAF V600E colorectal cancer are unresponsive to these drugs. Recent work has demonstrated that this intrinsic resistance is due to EGFR expression in the BRAF mutant colorectal tumors, and that sensitivity can be imposed by concomitant EGFR inhibition (Prahallad et al., 2012; Sun et al., 2014). While obtained in an entirely different therapeutic field, these data illustrate that intrinsic resistance can occur as a coincidental side effect of the oncogenic process, and can be overcome upon a better understanding of this process.

4. Naturally acquired resistance

Naturally acquired resistance is special in that it is unique to immunotherapy. This form of resistance is defined as a reduced sensitivity that is not induced by cancer immunotherapy but that develops as a consequence of naturally occurring immune pressure. In this group of patients, there will generally be signs of an ongoing immune response in peripheral blood or tumor tissue, but they will fail to derive benefit from immune modulatory treatment.

In the case of naturally acquired resistance, there is presently little evidence for altered T-cell activation or homing. Rather, this form of resistance may mostly manifest itself as mechanisms that interfere with T-cell activity within the tumor microenvironment. Multiple inhibitory feedback mechanisms can play a role here, including the expression of a variety of (potentially overlapping) checkpoint molecules that dampen the immune response, such as LAG-3, TIM-3 and BTLA (Pardoll, 2012). As an example, when tumor-infiltrating effector T-cells start to produce IFN-γ upon binding of cognate antigen, this will induce PD-L1 expression on the tumor cell surface, which serves to limit further T-cell effector function by engaging the immune checkpoint molecule PD-1 (Taube et al., 2012) (Figure 2B).

In addition, a naturally occurring immune response may select for tumor cell subpopulations with loss of MHC class I expression, or other defects in the antigen processing machinery, thereby cloaking the tumor cell from the immune system (del Campo et al., 2014; Khong et al., 2004; Restifo et al., 1996). A similar immune evasive effect may be achieved through selection of tumor subclones present within heterogeneous tumors lacking one or multiple antigens that are subject to strong Darwinian selection, a process called immune-editing (Dunn et al., 2002; Khong and Restifo, 2002; Matsushita et al., 2012). Strong evidence for immune-editing has been obtained in mouse model systems. However, other murine studies suggest that antigen loss may be less of an issue in cases in which the release of IFN-γ and TNF-α by CTLs leads to the destruction of tumor stroma (Zhang et al., 2008). Human data on this topic are at present lacking but may conceivably be obtained with the recently developed abilities to describe T-cell responses against (mutant) antigens within individual patients.

5. Therapy-induced resistance

A third class of resistance is observed when patients that initially respond to immunotherapy relapse, which we define as *therapy-induced resistance*. This type of resistance is well known in patients treated with classical cytotoxic agents or with targeted agents, such as BRAF inhibitors (Chapman et al., 2011). Natural resistance upon treatment with such targeted therapies, where virtually all patients eventually relapse, can be due to selection of resistant tumor clones already present at low numbers at the start of treatment, or of newly mutated resistant clones. This stands in stark contrast with immunotherapy-treated patients where durable complete responses are often already observed after a single-modality treatment. Although immunotherapy-induced clinical responses can last up to years, a subgroup of patients experiences only temporary disease regression (Di Giacomo et al., 2013; Prieto et al., 2012; Rosenberg et al., 2011). The general mechanisms of therapy-induced resistance will be very similar to those mentioned previously in the setting of naturally acquired resistance: When a properly activated T-cell pool with homing capacity is present, an equilibrium between effector T-cells and the tumor is reached locally, which at some point in time tips the balance in favor of renewed tumor growth.

6. Strategies to study resistance mechanisms

To increase our understanding of immunotherapy resistance, we suggest to analyze this process on the basis of the three different nodes that are involved in an effective anti-tumor immune response.

First, a diverse T-cell pool is required that can respond to a wide variety of tumor-associated antigens. The currently used immunotherapeutic strategies that exploit the activity of the endogenous T-cell compartment appear predominantly effective in tumors with median to high mutational loads, consistent with a role of neo-antigen recognition in tumor control. While the occurrence of neo-antigen reactive T-cells appears to be a common trait in human melanoma (Robbins et al., 2013; van Rooij et al., 2013), more direct evidence for their role in tumor control is still lacking. Longitudinal immune monitoring of neo-antigen-specific T-cells in a setting of cancer immunotherapy, using polychromatic flow cytometry or mass cytometry (Bendall et al., 2011) should be of value here. In a similar manner, assessment of immune competence (i.e. the ability to elicit a polyfunctional T-cell response) on a per patient basis could help guide eligibility for immunotherapeutic intervention.

Second, to study the homing capacity of endogenously activated T-cells in the context of immune escape, we need to address to what extent the tumor microenvironment is capable of triggering T-cell infiltration. To study this, pre-therapy tumor biopsies can be taken from patients included

in immunotherapy trials and predictive gene-expression signatures established that correlate with ongoing or subsequent T-cell infiltration or with clinical benefit (Ji et al., 2012).

The third and final step that needs to be analyzed is the ability of T-cells to release their effector functions at the site where it is needed. Several feedback mechanisms are at play here. Importantly though a hierarchy has not yet been determined, and such a hierarchy 1). Is likely to differ between tumor types; 2). Is within tumor types likely to differ depending on the specific genetic alterations; 3). May for a given tumor conceivably even vary depending on the site of metastasis. Recent work has emphasized the role of PD-L1 expression as an important regulator of local T-cell effector function. Using PD-L1 expression as a biomarker grouped responding patients in an anti-PD-1 phase I clinical study, although absence of expression did not exclude a response to therapy (Weber et al., 2013). These data underscore the value of biomarker discovery not only for the early phases of the endogenous immune response (e.g. local inflammation) but also for the later effector phase. Notably though, patients with colorectal cancer only infrequently show responses to PD-1 blockade, even though these tumors have high mutational loads and T-cell infiltrates within these tumors has been shown to form a prognostic factor superior to the standard TNM classification (Galon et al., 2014; Pages et al., 2009). These data are consistent with the hypothesis that within these tumors, another inhibitory pathway could be dominant.

7. Strategies to overcome resistance

The efficacy of many immunotherapeutic strategies is dependent on the strength of the endogenous T-cell response, including the level of tolerance towards the antigens recognized. Therefore, patients with an impaired capacity to mount immune responses, or who carry tumors that express few strong T-cell antigens may gain most clinical benefit from strategies that create the missing tumor-reactive T-cell pool. This may be achieved by the adoptive transfer of T-cells genetically modified to express an exogenous CAR or TCR capable of effective target killing (Morgan et al., 2006; Robbins et al., 2011) (Grupp et al., 2013; Kalos et al., 2011; Porter et al., 2011). In patients with a weak T-cell response against tumor antigens, low-frequency tumor-specific T-cell populations may be enriched from PBMNC or, perhaps preferable, from TIL, in order to steer reactivity towards predefined tumor-associated epitopes and thereby augment the anti-tumor response. Alternatively, expression of co-stimulatory and co-inhibitory markers in fresh tumor digest has been shown to define the tumor-reactive T-cell subset in melanoma lesions, offering a potential means to create TIL products that are enriched for tumor reactivity without the need for prior knowledge on antigen-specificity (Gros et al., 2014; Ye et al., 2014).

Patients that have the capacity to mount a systemic T-cell response but where tumors do not permit the infiltration of immune cells or prevent the initiation of a local endogenous immune response, are unlikely to benefit from treatment regimens that rely on such local immune responses. Such patients might benefit more from pre-conditioning regimens that promote an immune supportive tumor microenvironment by providing 'danger signals' and the establishment of an inflammatory signature (Gajewski, 2012). This may conceivably be achieved through the induction of immunogenic cell death either by chemotherapy, radiotherapy or even local injection of Toll-like receptor (TLR) agonists (Kroemer et al., 2013). For cancer types with a relatively low mutational load, such as ovarian, breast or pancreatic cancer, the use of DNA damaging agents could lead to an increase in the mutation frequency and as such broaden (albeit in a non-clonal manner) the epitope landscape (Segal et al., 2008; Zhang et al., 2014).

Patients that do exhibit an endogenous anti-tumor immune response in all the relative compartments discussed before are eligible for at least several immunotherapeutic interventions such as ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1). As with many classical cytotoxic agents, combination therapy could be of importance here in preventing escape from immune pressure. In this context, the nodes in the tumor immune interaction that are targeted should be as little overlapping as possible and preferably complementary in nature. The combination of ipilimumab (thought to be involved in the early priming phase of T-cell activation) and nivolumab (thought to be involved in the later effector phase of T-cell activation) has already shown higher response rates than either treatment modality alone (Wolchok et al., 2013). Presently, little is known about optimal timing or sequencing of available therapies but there is increasing evidence that patients failing one type of immunotherapy can respond to another, indicating independently operating resistance mechanisms than can be targeted accordingly. As an example, patients that have not benefitted from ipilimumab treatment still can have a meaningful objective response to anti-PD-1 treatment (Hamid et al., 2013) and vice versa (Weber et al., 2013). Patients failing ipilimumab treatment can likewise develop a durable complete remission upon TIL therapy (Besser et al., 2013).

8. Conclusion

In cancer immunotherapy, future rational treatment decision-making should be based on the specific node that is affected in the tumor immune system interaction: 1.) Are antigen-specific T-cells efficiently activated in the treatment-naïve host? 2.) Is there infiltration of those T-cells into the tumor? and 3.) Are the tumor-infiltrating T-cells able to exert their function at that site? To achieve this, efforts should be focused on the discovery and implementation of simple biomarkers at each stage in the immune response that can predict whether a patient is likely to respond to a specific type of immunotherapy or not. Considering the variation in response rates of immunotherapy within one tumor entity and between tumor types, immunotherapy finds itself at the point where a patient-specific approach is required in order to achieve durable tumor control in a larger group of patients.

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