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Mini Review

Resistance to PD1/PD-L1 Blockade

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Abstract

Monoclonal antibodies targeting PD-1/PD-L1 as a monotherapy are useful in varieties of tumors to provide durable response not seen with other therapies. However large majority patients do not respond. The major reason for lack of response is lack of inflammation. Even in tumors with inflammation other defects in cancer immune cycle are responsible for lack of response. Changes in cancer immune cycle are also seen at the time of relapse in those who responded to therapy initially. In this article current information about resistance mechanisms to anti PD-1/PD-L2 therapy are reviewed.

Keywords: PD-1; PD-L1; Primary Resistance; Acquired Resistance; Resistance Mechanism CD8 Cells; T Cells; Cancer Immune Phenotype; Check Point Inhibitors

Introduction

Cancer development and progression involve increasingly accumulating mutations. These mutations leads to expression of a diverse set of proteins (foreign antigens/neo antigens). Immune system uses them to recognise cancer cells as 'foreign' and destroys them (immune surveillance). This is seen in normal circumstances in all who do not develop cancer. The manifest tumor is associated with immune 'tolerance', wherein in tumor cells are not recognised as "foreign" and are not destroyed. Tolerance is associated with dampening of immune surveillance. Mechanisms involved in immune 'tolerance' include presence of regulatory immune cells, secretion of immunosuppressive cytokines, chemokines, and expression of immune checkpoint proteins on surface of tumor cells. Immune checkpoint proteins and associated system is in place to control activity of immune system and prevent collateral damage to normal cells. They behave as receptors and regulate the activation and effector functions of T lymphocytes. This system designed to prevent collateral damage is also found to induce immunosuppression in tumor and plays role in "tolerance" and prevent effective antitumor immune response. Program death receptor 1 (PD-1) and its ligand (PD-L1) are part of these immune check point system. Anti-PD1/PD-L1 therapies are designed to counter this by harnessing immune system to attack and destroy tumors. They have revolutionized management of cancer. They provide durable response in varieties of advanced Cancer. However response rate following their administration in management of solid tumours is not very high. They are approved for management of metastatic melanoma, renal-cell carcinoma (RCC), advanced non-small-cell lung cancer (NSCLC), classic Hodgkin's lymphoma (HL), bladder carcinoma, Merkel cell carcinoma, head and neck squamous cell cancer, solid tumors with microsatellite instability-high (MSI-H) or mismatch repair-deficiency, hepatocellular carcinoma, gastric cancer, cervical cancer, Primary Mediastinal Large B-Cell Lymphoma (PMBCL), metastatic Merkel cell carcinoma.

In spite of initial enthusiasm, they are not found to be effective across whole range of tumors e.g. pancreatic ductal adenocarcinoma and metastatic castration-resistant prostate cancer, are largely resistant to checkpoint inhibitor-based immunotherapy. When effective, the objective response rate (ORRs) with PD-1/PD-L1 blockade as monotherapy in management of relapse/recurrence solid tumors are variable across tumor types. The ORR is close to ~40% in skin cancers, ~20% in lung cancers, ~25% in renal cancer, 13 - 3% in bladder cancer, and 13 - 16% in HNSCC. Thus large number (more than two third patients) fail to respond to therapy. This is termed as primary resistance. Amongst those who respond, all do not have durable response with around one third relapsing. This is known as acquired resistance [1,2].

Identifying mechanisms of primary resistance at diagnosis, helps in preselecting persons who will respond to therapy and improve response rate as is done for targeted therapies in management of lung cancer. Identifying mechanism for acquired resistance is helpful in segregating patients in two groups, one with durable response and other with potential of response. The information about mechanism of primary and acquired resistance also help in developing better therapies. The possibility of extending long-term clinical benefit to more patients with advanced cancer using anti PD-1, PD-L1 therapy is an active area of research. In this article, available information related to resistance mechanism anti PD-1, PD-L1 therapy is reviewed.

Response to PD-1/PD-L1 blockade

Originally anti PD-1/PD-L1 therapies were developed as a checkpoint inhibitors to overcome tumor induced immunosuppression and induce regression of tumors. However as development progressed, more information became available about role of T cells. Currently, it is believed that successful anti-tumour immune responses following PD-1/PD-L1 therapy requires reactivation and clonal-proliferation of antigen-experienced T cells already present in the tumor microenvironment prior to initiation of therapy [1,2].

Killing of cancer cells by T cells typically follows seven steps of immunity cycle [3].

1. Cancer antigen release.
2. Presentation of antigen to immune cells (antigen presenting cells/dendritic cells.
3. Activation of immune cells.
4. Trafficking of immune cells to tumor
5. Infiltration of immune cells in tumor.
6. Recognition of cancer cells as foreign.
7. Killing of cancer cells.

In the context of anti-PD-1/PD-L1 therapy cancer antigens [step 1] comprises the release of neoantigens created by oncogenesis (presence of nonsynonymous mutations) from dying/dead cells. They are taken up by antigen presenting cell (APC) present within tumor. Following antigen uptake, APCs migrate to the draining lymph node to present processed antigen; in the context of major histocompatibility complex class I (MHC-I) molecules to CD8+ T cells [step 2]. This is followed by activation of T lymphocytes [step 3] leading to the clonal expansion of tumor-specific T cells and the expression of cell adhesion molecules and chemokine receptors necessary for T-cell trafficking [step 4] and tumor infiltration [step 5]. Clonal expansion of T cells is also associated with differentiation of T cells for survival, cytotoxic function, memory formation, and cytokine production. These T cells extravasate from tumor vasculature [step 4] and enter the tumor stroma and infiltrate the tumor parenchyma [step 5]. Expanded tumor specific T cells infiltrating the tumor now recognize cancer cells as foreign kills them [step 7] [4,5].

Efficacy of PD-1/PD-L1 blockade is seen only in tumors having pre-existing T cell specific immunity manifested by tumor neoantigen specific T cell infiltration [3-6].

Cancer immune-phenotypes

Evaluation of pre-treatment biopsies of patients enrolled in clinical studies evaluating anti-PD-1/anti PD-L1 for immune cells reveals two distinct immune profiles of tumors and are classified as immune-inflamed or immune- non-inflamed [4].

Immune-inflamed phenotype is characterized by the presence of both CD4 and CD8-expressing T cells in the tumour parenchyma in the proximity of tumor cells [6-8]. They may/may not be accompanied by other cells. Myeloid cells and monocytic cells are seen more frequently accompanying CD4 and CD8 cells compared to other immune cells. Inflamed tumours also contain pro-inflammatory cytokines that should provide a more favourable environment for T-cell activation and expansion, including type I and type II IFNs, IL-12, IL-23,IL-1β, tumour-necrosis factor (TNF)-α and IL-2 [4]. The description is suggestive of the antitumor immune response which was not adequate (suboptimal) for arresting tumor growth [4]. This phenotype can be found across varieties of cancer types but is more common in tumors responding anti-PD-1/PD-L1 therapy like melanoma, non-small lung cancer, bladder cancer etc.

In contrast to Immune-inflamed phenotype, non-inflamed phenotype is characterized by the absence of CD4- and CD8- expressing T cells in the tumour parenchyma. Non-inflamed tumours generally express cytokines that are associated with immune suppression or tolerance. They can also contain cell types associated with immune suppression like regulatory T cells, myeloid-derived suppressor cells and tumour-associated macrophages (M2 macrophages). Presence of regulatory T cells is not unique to non-inflamed tumours as they also accompany effector T cells [4].

This phenotype is divided into two subtypes; 1) immune-excluded and 2) immune-deserted.

Immune-excluded phenotype is characterized by the presence of abundant immune cells retained in the stroma that surrounds nests of tumour cells [6,9]. The stroma may be limited to the tumour capsule as seen in many tumors. Tumor stroma might penetrate the tumour itself mimicking as if immune cells are within tumor parenchyma and needs to be differentiated. In this pheno-

type the immune cells do not penetrate the parenchyma of tumours. Penetration of tumor parenchyma is the hallmark of immune-inflamed tumors. The description of this phenotype is suggestive of a pre-existing antitumor cell mediated immune response which failed to penetrate tumor stroma to reach parenchyma. Majority of pancreatic and colorectal cancer belong to this phenotype [3].

The immune-deserted phenotype, is characterized by a paucity of T cells in either the parenchyma or the stroma of the tumor [3]. Myeloid cells may be present but regulatory cells are not seen. This phenotype is indicative of lack of tumor specific immune response. Majority of prostate cancer belong to this phenotype [3].

PRIMARY RESISTANCE

Primary resistance is defined as failure of tumor to respond to anti-PD1/PDL1 monotherapy. Generally non-inflamed tumors like pancreatic cancer, colorectal cancer and prostate cancer demonstrate primary resistance and respond poorly to anti PD-1/PD-L1 therapy [3]. Even amongst inflamed tumors (melanoma, NSCLC) response rate is relatively low to anti PD-1/PD-L1 monotherapy and thus large number of patients have primary resistance to monotherapy.

There are two biomarkers identified for response to PD-1/PD-L1 therapy. Presence of PD-L1 is one of them.

Microsatellite instability arising from mismatch-repair deficiency is the second predictive biomarker to predict response to therapy approved by FDA [10]. Their presence in isolation and/or combination predicts higher chance of response rate to PD-1/PD-L1 blockade.

Following section reviews current knowledge about primary resistance.

Failure of cancer antigen release

Availability of tumor specific antigen is necessary for mounting tumor specific immune response for lasting shrinkage of tumor [durable response]. Tumor cells demonstrate heterogeneity and it is difficult to identify a single antigen for effective therapy. It is believed that some of the proteins released by dying/dead mutated cancer cells work as an antigen (neoantigen) [11]. Lack of sufficient or suitable neoantigens is identified as one of the key factor for primary resistance [1,11] and are seen:

a) Following genetic and epigenetic alteration tumor [12].

b) Low/reduced no synonymous mutation burden: Mutational load governs availability of neoantigen in inflamed cancer phenotype [4]. Higher synonymous mutation load is correlated with increased expression of genes and increases availability of neoantigen [3] probably from increased number of non-synonymous single nucleotide variants [11]. As a corollary, low nonsynonymous mutational load is associated with lack of sufficient or suitable antigens [3].

c) Mismatch repair deficiency also increases availability of neoantigens [13]. This is seen even in tumors like colorectal cancer known to be non-inflamed [14] and its absence is associated with lack of sufficient or suitable antigens.

Presentation of antigen to immune cells- (T cell priming)

Dendritic cells are main antigen presenting cells. They need to be present within tumor to capture antigens released by tumor and should be in a position to migrate to lymph node for its presentation to T cells to generate tumor specific immune response. There is a decreased frequency of dendritic cells within tumor as well as blood [15]. Dendritic cells present within tumor are known to be dysfunctional and do not possess capacity [maturation potential] to present captured antigen to T cells [15]. They fail to provide costimulatory signal to T cell required for activation and proliferation of T cells [15].

For antigen processing by antigen presenting cells and its presentation to immune cells, MHC class 1 molecules is required. Its loss and/or down regulation is associated with impaired antigen presentation to immune cells. Down regulation of MHC class I molecules in cancer is well known. Beta-2-macroglobulin is responsible for MHC class I molecule expression on APC. Its loss leads to impaired MHC class I expression. Beta 2 macroglobulin is responsible for expression of MHC class I expression. Loss of B2M expression is also described in cancer [12,16].

Tumors can also inhibit antigen presentation by:

a) Oxidative posttranslational modifications of HMGB1

b) Increased tumor derived soluble factors

   i. Vascular endothelial growth factor (VEGF),

   ii. Transforming growth factor β (TGF-β),

   iii. Prostaglandin E2,

   iv. Interleukin (IL) 10,

   v. Macrophage colony-stimulating factor,

vi. Adenosine [17,18] and

vii. Indoleamine 2,3-dioxygenase (IDO) [15,19]

c) Suppressive immune cell subsets in the TME,

i. Including regulatory T cells (Tregs) and

ii. Myeloid-derived suppressor cells (MDSCs)

d) Suppression through signaling pathway

i. Increased tumor intrinsic beta-catenin signaling reduces CCL4 and thereby suppresses CD103 dendritic cell recruitment [20].

ii. Loss of IFN-gamma signaling through alteration of JAK/stat pathway [16,21].

iii. Loss of PTEN [22].

e) Role of gut microflora:

Altered microflora [4].

They inhibit DC maturation and ultimately impair T-cell expansion and differentiation into IFN-γ producing cells by reducing the expression of MHC and costimulatory molecules, which reduces the production of inflammatory cytokines such as IL-12, and by catabolizing nutrients required for T-cell activation.

Activation of immune cells

Successful anti-tumor immune response to anti PD-1/PD-L1 therapy is dependent on reactivation and clonal-proliferation of antigen-experienced T cells already present in the tumor microenvironment prior to initiation of therapy [1,2]. Tumors with a higher no. of PD-1 high T cells provides relative resistance and do not respond well [23]. These cells also express other co-inhibitory receptors [23].

Following factors limit/inhibit activation of T cells and thereby contribute to resistance.

I. Adenosine, which is generated by the conversion of extracellular ATP through the enzymatic activity of the ectonucleotidases CD39 and CD73, acts to inhibit T-cell activation and expansion via the A2A adenosine receptor [3].

II. During T-cell priming, T cells express negative feedback regulators, such as cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) to limit T-cell activation and its overexpression may be responsible for primary resistance to PD-L1: PD-1 blockade. Improved efficacy with combination of PD-1 and CTLA-4 blockade strengthens this [24].

III. Defective T cell co-stimulation in cancer is seen due to immunosuppressive factors [25]. In the absence of appropriate co-stimulation, TCR activation leads to excessive calcium/nuclear actor of activated T-cell signaling and the subsequent expression of negative regulatory factors that result in T-cell energy or functional unresponsiveness [26]. Suboptimal co-stimulation during T-cell priming can be a barrier for PD-L1: PD-1 blockade [27]. Partial T-cell activation can also result in limited production of IL-2 [28], leading to impaired CD8+ T-cell responses [29].

IV. Impaired CD8 T cell activation.

a. Increased lactate levels within tumor [30].

b. Higher LDH levels also within tumor [31].

c. Partial T-cell activation following therapy can also result in limited production of IL-2 [28,29].

V. Increased expression of genes (AXL, TWIST2, WNT5A, LOXL2, ROR2, TAGLN, and FAP) involved in the epithelial-to-mesenchymal transition [32].

VI. Presence of fewer effector memory cells are associated with absence /low level generation of effector memory cells [1].

Trafficking of immune cells to tumor

Impaired trafficking leads to absence /suboptimal CD8 cells reaching tumor leading to primary resistance. For trafficking of CD8 cells from blood vessels to tumor, cell adhesion molecules and chemokine receptors are required. Tumor tissues may impair T-cell trafficking by

I. Down regulation of adhesion molecules such as ICAM and VCAM in local endothelium essential for T-cell extravasation [33]. E.g. by angiogenic factors (e.g. VEGF) down regulates intercellular adhesion molecule 1, intercellular adhesion molecule 2, and vascular cell adhesion.

II. Up-regulation of endothelin-B receptor by tumor endothelial cells, which has been associated with an absence of tumor-infiltrating T cells in multiple cancer types [34].

III. Inactivating chemokines via proteolytic cleavage or post-translational modification.

Infiltration of immune cells in tumor

Following trafficking (extravasation from tumor vessels) activated immune cells need to reach close to tumor cells i.e. within tumor parenchyma. Retention of CD8+ T cells in stroma and prevention of it from entering parenchyma is associated with

I. Low levels of CXCL9 or other CXCR3 ligands, such as CXCL10 and CXCL11 [35].

II. Presence of cleaved CXCR3 ligands can serve as receptor antagonists [35].

III. Presence of CCL4 is associated with absence of tumor infiltration [20].

IV. Immunosuppressive leukocytes [36]

V. Cancer-associated fibroblasts at the tumor margins [37-39].

   a. physically exclude T cells via the production of extracellular matrix proteins

   b. Actively prevent T-cell migration to the tumor center through chemokine-mediated repulsion and other unidentified mechanisms [33,37]. An imaging study of human lung tumors revealed that areas of dense accumulation of fibronectin or collagen in the tumor stroma prevented T cells from contacting tumor cells [9].

   c. Produce CXCL12, which has been shown to inhibit intratumoral T-cell infiltration in a pancreatic cancer model [38]. In multiple preclinical models, CAF depletion by targeting fibroblast activation protein inhibits tumor growth in a lymphocyte-dependent manner [39].

VI. Abundance of MDSC and TAM [40,41].

Recognition of cancer cells as foreign

Recognition of cancer cells as foreign by CD8 T cell is necessary for its killing.

Cancer cells evade T-cell recognition by the loss, down regulation, or alteration of the MHC-I protein on the surface of cancer cells [42]. Down-regulation of MHC-I has been reported in a variety of cancers and frequency of down regulation in tumor ranged from 16% to 50% [43].

Killing of cancer cells

Suppression /dysfunction of CD8 T cell prevents killing of cancer cells and is responsible for primary resistance to PD-1/PD-L1 blockade. Suppression/dysfunction of CD8 T cell is seen under following circumstances

a) Co-expression of other co-inhibitory receptors like lymphocyte-activation gene 3 protein (LAG-3), T-cell immunoglobulin domain, mucin domain-3 (TIM-3), and the recently described T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) [5,32,44].

b) Significant presence of immune suppressive cells

   a. M2-like macrophages, which produce IL-10 [45].

   b. Myeloid derived suppressor cells [46].

   c. Tregs. An increase in frequency of Treg or higher ratio of Treg to effector T cells are indicative of progression with anti-pd1 therapy [8].

c) Significant presence of immunosuppressive soluble factors in Tumor microenvironment.

   a. IDO [19]

   b. VEGF

   c. TGF-β [47]

   d. Prostaglandin E2 [48]

   e. Arginase [49]

   f. Adenosine [50].

d) Alteration in JAK/stat pathway rendering Tumor cells insensitive to IFN-gamma [16].

e) Severe exhaustion of T cells: PD-1 blockade is effective in reinvigorating CD8 T cells expressing low to intermediate amount of PD-1 but not those expressing high PD-1 [1].

Acquired resistance

Acquired resistance is defined as a clinical scenario in which a cancer initially responded to immunotherapy but after a period of time it relapsed and progressed. Acquired resistance to anti-PD-1/PD-L1 therapy is seen in approximately 25% to 35% of responders [16].

Acquired resistance can be due to alteration in tumor cells (change in antigen), immune cells or tumor microenvironment.

Alteration in Antigen (tumor cells)

Only neo-antigen expressing tumor cells recognised by CD8 T cells are killed following anti-PD-1 /PD-L1 therapy. Acquired resistance follows loss of recognised neoantigen expression by tumor cells. This can happen following outgrowth of tumour cell clones that never expressed the neo-Ag, associated with killing of all other cells expressing recognised neoantigens or mutational loss of key genes involved in immunotherapeutic responses.

Presentation of antigen to immune cells

Acquired resistance is associated with defects in antigen presentation by:

I. β2-microglobulin frameshift deletion leading to HLA class I loss [16],
II. Mutations in the MHC-I pathway [3,16]
III. Failure to upregulate TAP1 [16]

Activation of immune cell

JAK1/JAK 2 mutations seen at time of relapse, results in defective antigen presentation and activation of CD8 T cells [16].

Infiltration of immune cells in tumor

Prevention of T cells from entering parenchyma is seen on acquiring resistance to anti-PD-1 based therapy. Many tumors revert to immune excluded phenotype with CD8+ T cell infiltrate almost exclusively seen at margin of relapse. Mutation of JAK1/2 is one of the reasons for same [16].

Recognition of cancer cells as foreign

Loss of MHC class-1 molecules under immune pressure is known. This leads to loss of recognition of tumor cells as 'foreign' [16].

Killing of cancer cells

Following mechanism are known to prevent killing of tumor cells by T cells:

I. Up-regulation of other co-inhibitory receptors like CTLA-4, TIM-3, LAG-3, and VISTA [3].
II. An increase in immune suppressive cells may reinforce immunosuppression as a reaction to T-cell–dependent attack [3].
III. Mutation of JAK1 and JAK2 are seen with acquired resistance. They confer resistance to CD8 T cells against effect of IFN-alpha and IFN-gamma in spite of recognition of cancer cell [16].
IV. IFN-gamma induced increased expression of suppressive factors [12].
    a. IDO
    b. CECAM1-Carcinoembryonic antigen related cell adhesion molecule

Summary

Check point inhibitors like anti PD-1/PD-L1 therapy has improved cancer care by providing durable responses. They have limitations in their activity due to pre-existing as well as acquired changes in immune profile of tumor. Identification of them helps in selection of patients as well as improving outcome by combination therapy. Immune- inflamed phenotype is associated with response to therapy and immune-non inflamed phenotype is associated with lack of response. Other factors like defects in antigen presentation, T cell activation, trafficking, infiltration and T cell interaction with tumor are also responsible for lack of response.

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