PD-1 expression on tumor-specific T cells: Friend or foe for immunotherapy?
Sylvain Simon, Nathalie Labarrière

To cite this version:
Sylvain Simon, Nathalie Labarrière. PD-1 expression on tumor-specific T cells: Friend or foe for immunotherapy?. OncoImmunology, Taylor Francis, In press, <10.1080/2162402x.2017.1364828>. <inserm-01592666>

HAL Id: inserm-01592666
https://www.hal.inserm.fr/inserm-01592666
Submitted on 25 Sep 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
PD-1 expression on tumor-specific T cells: Friend or foe for immunotherapy?

Sylvain Simon & Nathalie Labarriere

To cite this article: Sylvain Simon & Nathalie Labarriere (2017): PD-1 expression on tumor-specific T cells: Friend or foe for immunotherapy?, OncoImmunology, DOI: 10.1080/2162402X.2017.1364828

To link to this article: http://dx.doi.org/10.1080/2162402X.2017.1364828

© 2017 The Author(s). Published with license by Taylor & Francis Group, LLC

Accepted author version posted online: 22 Aug 2017.
Published online: 22 Aug 2017.

Submit your article to this journal

Article views: 38

View related articles

View Crossmark data
PD-1 expression on tumor-specific T cells: Friend or foe for immunotherapy?

Sylvain Simon and Nathalie Labarriere

ABSTRACT
Inhibitory properties of PD-1 receptor engagement on activated T cells are well established in physiologic and pathological contexts. In cancer, the use of checkpoint blockade, such as anti-PD-1 antibodies, becomes progressively a reference treatment of a growing number of tumors. Nonetheless, it is also established that PD-1 expression on antigen-specific T cells reflects the functional avidity and anti-tumor reactivity of these T cells. We will discuss this dual significance of PD-1 expression on tumor-specific T cells, due to a complex regulation and the opportunity to exploit this expression to define, monitor and exploit tumor-reactive T cells for immunotherapy purposes.

Introduction
PD-1 inhibitory receptor (CD279) has been discovered in 1992, by the group of Pr Honjo. Initially identified for its ability to induce apoptosis, its role in the maintenance of peripheral immune tolerance has been further documented using PD-1−/− murine models. PD-1 is expressed at the cell surface of activated T cells, NK cells, B cells, macrophages and several subsets of DCs. PD-1 expression on naïve T cells is induced upon TCR activation. This transient expression decreases in absence of TCR signaling but is maintained upon chronic activation with a persisting epitope target such as in chronic viral infections and in cancer. PD-1 ligation to its ligands PD-L1 and PD-L2 impairs TCR signaling and CD28 co-stimulation. Constitutive PD-1 expression by tumor-specific T cells was initially described as associated with the expression of additional inhibitory receptors such as Tim-3, LAG-3 or TIGIT, leading to impaired T cell functions and to tumor escape, upon ligation to its ligand PD-L1 expressed by tumor cells or immune infiltrating cells within tumor microenvironment. Constitutive PD-1 expression constitutes a form of immune adaptation to chronic stimulation, leading to a physiologic limitation of immune responses limiting auto-immune phenomena. This system is thus hijacked by tumor cells to favor peripheral tolerance and in this context PD-1 can be considered as a marker of dysfunctional T lymphocytes.

However, results from different groups highlighted the ambiguous role of PD-1 in defining efficient or ineffective immune T cell responses. Indeed, although leading to an inhibition signal upon ligation with its ligands, it is now clear that PD-1 expression is first a marker of T cell activation, allowing the identification of the tumor-reactive CD8+ T cell fraction in melanoma tumors, and of high avidity CD8 T-cells specific for Melan-A or neoantigens. Indeed, the level of PD-1 expression is related to the strength of TCR signaling, and thus to the functional avidity of specific T cells, underlying the complex significance of PD-1 expression on tumor-specific T cells, also finely regulated by genetic and epigenetic dynamic mechanisms.

In this line of thoughts, the inability to identify exhausted or activated T cells based on the sole expression of inhibitory/co-stimulatory-receptors has been recently demonstrated. Transcriptomic analyses raised the possibility to uncouple activation and exhaustion gene programs in CD8+ T cells and provided new insight in the understanding of molecular mechanisms of CD8+ T cell dysfunction. Thus PD-1 expression can also be considered as a marker of activated tumor-reactive T cells.

Nonetheless, immune tumor escape is a dynamic process involving the induction of an immunosuppressive microenvironment in which PD-1/PD-L1 signaling pathway plays definitively a multilayered role. In a growing numbers of solid tumors, targeting this pathway with PD-1 or PD-L1 specific antibodies progressively transformed patient’s management and led to unprecedented clinical responses in a large spectrum of advanced human cancers. In US and Europe, anti-PD-1 immunotherapy is used as a first line of treatment of metastatic melanoma patients. Despite its undisputed superior clinical efficacy compared with chemo- or radiotherapy, anti-PD-1 monotherapy remains inefficient on more than 60% of cancer patients. Acute toxicities are less common (around 14% of treated patients) than those reported for anti-CTLA-4 therapy but remain an appreciable risk for patients. For these reasons, defining early and robust predictive markers of clinical response is crucial both to improve patient’s management and to reduce treatment costs. Furthermore, the comprehensive analysis of PD-1 regulation and signaling will also have a considerable impact on the optimization of other immunotherapies such...
as T-cell-based immunotherapies. Indeed, it could help
define the best T cell subset to be used in adoptive cell trans-
fer treatment in combination with check-point blockade.

This review will focus on this dual role of PD-1 as a reflect of
efficient T cell activation and also of T cell dysfunction, and
how these properties could be further exploited to improved
the clinical efficacy of T-cell-based therapies.

Regulation of PD-1 expression
PD-1 expression on T cells is intricately regulated by different
genetic and epigenetic programs adapted to either transient or
chronic antigenic stimulations. PD-1 is rapidly induced on T cells
following TCR-mediated activation and this expression decreases
with antigen clearance. In contrast, PD-1 expression is
maintained on antigen-specific T cells in chronic disease settings
and has been associated with a progressive loss of T-cell functions.

Oestreich and coll. described, in mice, 2 regulatory regions
in Pdcd1 promoter, CR-C and CR-B, important crucial for
Pdcd1 gene transcription. These regions, highly conserved in
humans, contained numerous potential binding sites for tran-
scription factors and CR-C, a CpG island, was mandatory for
PD-1 expression. In naïve T cells, CR-B and CR-C are highly
methylated whereas following first antigen encounter, both
regions are demethylated and concomitant coinciding with
PD-1 expression. After antigen clearance, Pdcd1 promoter is
progressively remethylated in effector or memory cells while
Pdcd1 locus remained largely demethylated in hyporesponsive
T cells during chronic antigen exposure. This Pdcd1 gene
demethylation was suggested as an active process, sensitive to
TCR-mediated T-cell activation.

NFAT transcription factors were recently proposed as key
modulators of this effector versus hyporesponsive T-cell
states. Martinez and coll. described NFATs as an early tran-
scriptional checkpoint progressively driving exhaustion. NFATs are quickly activated in T cells following TCR stimula-
tion. In effector T cells, NFATs form a protein complex with
AP-1 (c-Fos and Jun proteins) induced by appropriate co-stim-
ulation signaling and therefore regulate effector genes and
T-cell functions. In exhausted T cells, NFATs are predomi-
nantly "partnerless" thus binding to monomeric NFAT1 binding
elements and promoted the activation of a transcriptional program
associated with T cell dysfunction.

NFAT1 and NFAT2 were also described previously to
directly bind Pdcd1 promoter and activate its transcription. The
mutation of NFAT1 binding site on the CR-C region
completely abrogated PD-1 expression in a mouse model.
NFAT1 rapidly activates PD-1 expression following TCR stim-
ulation and this is via NFAT activation and nuclear transloca-
tion that PD-1 expression reflected the strength of TCR stimulation integrated by T cells. In absence of further activa-
tion signals, Blimp-1 actively repressed NFAT1 expression and
modified the chromatin structure at PD-1 locus therefore
down-regulating PD-1 expression.

Recent studies described, exclusively in exhausted T cells, a
transcription enhancer in Pdcd1 gene implicated in PD-1 sus-
tained expression. This activation region is equally accessible in
cells genetically modified to express a NFAT1 protein unable to
interact with AP-1 suggesting a role for the partnerless NFAT1
in the maintenance of PD-1 expression in hyporesponsive T cells.
FoxO1 transcription factor was also accumulated in
turn of PD-1/PD-L1 signalisation and FoxO1 directly sustained
PD-1 expression. Furthermore, PD-1 inhibitory signaling was
shown to upregulate BATF expression and to inhibit CD28
positive co-stimulation. These 2 mechanisms notably reduce
AP-1 availability within the nucleus and favor T cell loss of
function illustrating the feed-forward loop regulated by PD-1
upon chronic antigen stimulation.

This complex regulation of PD-1 expression clearly shows
that PD-1 expression status alone cannot discriminate between
exhausted and activated T cells, that are the result of distinct
genetic and epigenetic programs, dictated by TCR signaling
strength and microenvironment.

PD-1 expression and anti-tumor response
Although inhibiting T cell responses upon ligation to its
ligands, PD-1 expression is the reflect of T cell activation.
In HPV-positive head and neck cancers, it has been document-
ted that a favorable clinical outcome was associated with a strong infiltration of activated PD-1+ T cells, able to
get reinvigorated upon PD-1 blockade. Same results have
also been reported from non virus-induced solid tumors. In
melanoma, PD-1 expression identified tumor reactive CD8+ T
cells, within tumor infiltrating lymphocytes (TIL) derived from
melanoma patients. Furthermore, only this PD-1 positive fraction contains T lymphocytes specific for neoan-
tigens, potentially expressing a high affinity TCR. These T
lymphocytes are able to eliminate autologous tumor cells,
despite their PD-1 expression. PD-1 expression is propor-
tional to the strength of TCR signaling to compensate T
activation and to control immune response. Furthermore,
PD-1+ T-cell fraction was largely pauciclonal (TCR repertoire)
suggesting an antigen-driven amplification of those PD-1+ T-cell clonotypes within the tumor. In addi-
tion, expression of PD-1 on circulating T cells also identifies
patient-specific antitumor T cell response, similar to that
detected within TIL.

In this line, we demonstrated the correlation between the
expression of PD-1 by human CD8+ T cell clones specific for
the shared melanoma antigens Melan-A, MELOE-1 and their high functional avidity. We described the exist-
ence of melanoma-specific T cell clones (isolated from the
blood of patients and healthy donors or from the TILs)
unable to induce PD-1 after T cell stimulation. This absence of PD-1 expression was due to the persistent methylation of
Pdcd1 promoter. These PD-1neg/T cell clones were not suscept-
tible to PD-L1 negative signaling but they exhibited lower anti-tumor reactivity than their PD-1pos counterparts in the
absence of PD-L1 signaling. Logically, PD-1pos T cell clones exhibited greater functional avidity than PD-1neg T cell clones suggest their interest for adoptive cell transfer (ACT) pro-
tocols. In this study, we also modulated PD-1 signaling dur-
ing the melanoma-specific T cells production procedures
with the addition of anti-PD-1 blocking antibody. Recovered
specific-T cell Vβ repertoire, was different from that obtained
in the control condition with the preferential expansion of
clonotypes exhibiting high functional avidities that could
potentially lead to a better anti-tumor activity in vivo, in combination with PD-1 blockade or gene inactivation in these T cells. Another recent study, deciphering the kinetics of TCR binding to peptide-major histocompatibility molecules (pMHC) with NTAmers resonates with these results. Indeed, this work documents that TCR-pMHC off-rate, associated with CD8⁺ T cell potency, closely follows costimulatory/coinhibitory receptor expression in activated CD8⁺ T cells, among them PD-1 expression.37

The relevance of PD-1 expression to identify highly reactive specific T cells has also been underlined in vaccination studies. In mice engrafted with epithelial tumors expressing HPV-associated E7 protein, vaccination with E7 protein induced the proliferation of PD-1⁺ specific CD8⁺ T cells, partly inhibiting tumor growth. In this setting, PD-1 blockade synergized with vaccine in eliciting antitumor efficacy.33 Another recent study demonstrated that vaccination with altered peptide ligands with optimized CMH or TCR affinity led to a strong but inefficient specific CD8 T cell response, impaired by a sustained expression of PD-1 (and other inhibitory receptors) by stimulated specific T cells.38 The anti-tumor efficiency of such vaccines could thus be improved through a combination therapy targeting PD-1/ PD-L1 signaling pathway.

All these studies concurred to demonstrate the complex significance of PD-1 expression on tumor-specific T cells, particularly because of its dynamic and fine regulation by genetic and epigenetic mechanisms. Globally, the expression of inhibitory receptors (among them PD-1) is a common feature of activated and exhausted T cells and recent single cell transcriptomic studies demonstrated that the sole expression of these inhibitory receptors does not allow discriminating these 2 functional status. The exhausted status of T lymphocytes can be documented on the basis of specific gene expression profile, notably involved in zinc metabolism.18,39

Thus, setting up combination strategies associating PD-1 blockade and ACT with PD-1⁺ high avidity specific T-cells or vaccination strategies inducing such a CTL response would be a relevant approach to improve anti-tumor responses.

**PD-1 expression by CD8 T lymphocytes in patients treated by anti-PD-1 therapy**

The vast majority of studies about anti-PD-1 therapy, mainly documented immune markers related to the mode of action of PD-1 in the regulation of anti-tumor T cell response.40 PD-1 regulates tumor-specific T cell response presumably on the tumor site, through the inhibition of specific T cell activation (Chen 2015). Therefore, the presence of a pre-existing CD8⁺ T cell infiltrate,41 along with an increased infiltration by CD8⁺ T cells upon anti-PD-1 therapy have been associated with therapeutic responses. Indeed, several studies demonstrated that regressive lesions were densely infiltrated by cytotoxic CD8⁺ T cells.42,43 More precisely, it has been documented that therapeutic responses were associated with the presence of PD-1 expressing CD8⁺ T cells at the tumor margin, before therapy, co-localized with PD-L1 expressing tumor cells.41 Following anti-PD-1 treatment, in responding patients, cytotoxic CD8⁺ T cells accumulated within the tumor. This CD8⁺ T cell infiltrate (pre-existing or accumulating in tumors upon treatment) exhibited a low TCRβ diversity, suggesting a clonal expansion of tumor-specific T cells. In addition, IFN-γ associated gene expression was also documented in patients responding to anti-PD-1 therapy.44

These different studies concurred to define an “active” tumor microenvironment, associating the accumulation of PD-1⁺ CD8⁺ activated effector T cells, the presence of IFN-γ, the activation of IFN-γ responsive genes and thus the expression of PD-L1 by tumor cells. Anti-PD-1 therapy could reinvigorate pre-existing tumor-specific T cell by removing the inhibition induced by the activation of PD-1/PD-L1 axis, and finally induce tumor rejection. This active microenvironment (present in about 38% of cancer patients) defines a sub-group able to respond to anti-PD-1 therapy.45 A recent study confirmed the importance of this active microenvironment in melanoma tumors, identified by the expression of class II HLA on melanoma cells, and correlating with the expression of genes associated with T cell activation, notably PD-1 gene.46 Thus, class II HLA expression, detected by immunohistochemistry on
melanoma tumors could be a relevant immune marker for therapeutic decision support, associated with PD-1 expression on effector T cells.

In addition to the significance of PD-1$^{+}$ tumor infiltrating CD8$^{+}$ T cells in predicting anti-PD-1 therapeutic response, the presence of activated PD-1$^{+}$ CD8$^{+}$ T cells in patient blood was also associated with therapeutic efficacy. Indeed, most non-small cell lung cancer (NSCLC) patients receiving PD-1 therapy exhibited an early increase in circulating Ki67$^{+}$ PD-1$^{+}$ CD8$^{+}$ T cells. These effector-memory T cells are most likely recirculating tumor specific T cells, reinvigorated upon PD-1 blockade. Interestingly, this T cell subset was preferentially detected in responding patients, after the first cycle of anti-PD-1 administration, and could represent an immune biomarker of anti-PD-1 therapeutic efficacy.

**PD-1 expression as a marker identifying efficient CD8 T cells for T-cell-based immunotherapy**

The adoptive transfer of tumor-specific T lymphocytes is a therapeutic strategy that demonstrated a relative efficacy, notably for metastatic melanoma. This strategy aims at transferring high amounts of tumor-reactive lymphocytes, amplified and selected *ex-vivo*, to circumvent local tolerance mechanisms and eradicate tumor cells. The therapeutic efficacy of ACT relies both on antigenic specificity of infused T cells and on their functional properties (tumor reactivity, persistence, migration...). Several selection strategies of tumor-reactive T cells have been tested based on the expression of activation receptors (CD25, PD-1, 4-1BB...), the anti-tumor reactivity (cytokine secretion), or their specificity (HLA-peptide multimer sorting). In the periphery the initial frequency of circulating tumor-specific T cells could represent a limitation for the production of high amounts of specific T cells, with preserved properties of expansion and *in vivo* persistence. The genetic transfer of specificity in primary T cells or other T cell subsets through the use of TCR or CAR transduction allows bypassing this limitation. Whatever the approach for the production of tumor-specific T cells for T-cell-based therapy, a crucial issue is to identify the most relevant T-cell subset to select for *ex-vivo* amplification. In this line of thoughts, 2 recent studies investigated the therapeutic potential of T-cell-based therapy using PD-1-selected TIL, and came to the same conclusion. They documented, in myeloma and melanoma mouse models, that only T cells from the PD-1 positive fraction exhibited tumor reactivity and that their adoptive transfer to tumor-bearing mice resulted in tumor control, in contrast to the adoptive transfer of PD-1 negative T lymphocytes from the same TIL population. Furthermore, PD-1$^{+}$ transferred T cells were able to persist *in vivo* and to mount an adaptive memory immune response against the tumor. In both studies, anti-tumor efficacy was further enhanced upon combination with PD-1 blockade. In the same line, and starting from the vast T cell repertoire specific for Melan-A antigen, we recently showed that PD-1 expression identified a peculiar specific T cell repertoire, exhibiting higher functional avidity and functional properties of melanoma specific effector T cells. We further documented *in vitro* that PD-1 blockade during a GMP-compliant procedure to produce Melan-A specific T cells for ACT, favored the amplification of peculiar TRCß subfamilies, with better functional avidity against their cognate antigen. All these studies concur to strengthen the rationale for the use of PD-1 selected T lymphocytes for T-cell-based therapy. However, a major drawback for the use of PD-1 positive T cells for ACT purposes could be their impaired functions *in vivo*. To preserve the functions of these T cells after transfer, therapeutic combinations with PD-1 or PD-L1 blockade are explored, together with the possibility to inactivate PD-1 gene in selected T cells, before transfer. This latter perspective would offer twin benefits: first, the autoimmune adverse

![Figure 2](image-url)  
**Figure 2.** PD-1 based selection and inactivation for optimal T-cell based therapies. 1/ High avidity tumor specific T cells can be expanded from the CD8$^{+}$ PD-1$^{+}$ TIL fraction or after antigen stimulation of PBMC upon PD-1 blockade that favors the amplification of highly reactive CD8$^{+}$ T cells. 2/ PD-1$^{+}$ recovered high avidity T cells, CAR-T cells or TCR-transduced T cells can be further inactivated for PD-1 expression (and other inhibitory receptors) by genome editing, to bypass immunosuppression mechanisms. 3/ High avidity tumor specific T cells inactivated for PD-1 expression can be infused to cancer patients, alone or in combination with other therapies such as radioimmunotherapy.
effects of the systemic infusion of anti-PD-1 would be avoided and the cost of the treatment would be significantly reduced. On this topic, TALEN or CRISPR-Cas9 genome editing tools provided an unprecedented and promising technological breakthrough by modifying selected human T cell subsets to improve the anti-tumor efficiency of ACT treatments. The successful use of this technology has been first reported for the silencing of CCR5 gene in HIV-1-susceptible human CD4+ T cells. The feasibility of PD-1 inactivation following CRISPR/CAS9 edition has been initially reported in human primary T cells, and resulted in a significant decrease of PD-1 expression on edited T cells, associated with enhanced IFN-γ production and cytotoxicity, without affecting the viability of these T cells. The edition of PD-1 gene with the TALEN technology has also been tested in polyclonal tumor-reactive T cells, from mice engrafted with fibrosarcoma cells. The adoptive transfer of these PD-1 edited T cells in tumor-bearing mice resulted in tumor rejection and long-term protective memory. Thus, the edition of genes coding PD-1 and other inhibitory receptors could be done in high avidity tumor-specific T cells, selected on the basis of PD-1 expression, but also in the highly reactive CAR- or TCR transduced-T cells (Fig. 2). Indeed, a recent study illustrated the feasibility to inactivate PD-1 gene in human CD19-CAR-T cells. This inactivation led to an enhanced cytotoxicity of CAR-T cells against tumor cells in vitro, and an improved tumor reactivity in vivo against tumor xenografts.

Furthermore, the transfer of genome-edited T cells to humans recently passed a key safety review in the USA, by the Recombinant DNA Advisory Committee (RAC) at the US. National Institutes of Health. This decision will accelerate the clinical transfer of new immunotherapy approaches, combining the selection of highly tumor-reactive T cell subset (based on the expression of PD-1 and potentially of other co-stimulation molecules) and genome engineering to counteract the tumor microenvironment driven immunosuppression.

**Conclusion**

In conclusion the fine regulation of PD-1 expression on tumor-specific T cells makes this molecule a valuable marker to select high avidity-specific T lymphocytes, for immunotherapy purposes. Furthermore, to prevent autoimmune adverse events related to anti-PD-1 systemic therapy, genome edition of specific T lymphocytes targeting immune checkpoint inhibitors appears a promising option, while leaving the possibility to associate another combination therapy.

**Disclosure of potential conflicts of interest**

The authors declare no conflicts of interest.

**Acknowledgments**

This work was performed in the context of the LabEx IGO program supported by the National Research Agency via the investment of the future program ANR-11-LABX-0016-01. Sylvain Simon is supported by a specific funding from the LabEx IGO program. This work was also supported by grants from the «Ligue contre le Cancer, Comite 44», the canceropole Grand-Ouest and the DHU Onco-greffe.

**ORCID**

Sylvain Simon http://orcid.org/0000-0001-5985-3946

**References**

1. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J 1992;11:3887-95. PMID:1396582


