

Immune checkpoint drug goes viral: developing new treatment paradigm for chronic viral infection

Abstract

During chronic viral infection, antigen-specific T cells are functionally exhausted, characterized by over expression of multiple co-inhibitory molecules. Recently advances in immune-oncology, experimental and early clinical virology studies have demonstrated sound rationale and early promise of breaking virus-mediated immune tolerance by using immune checkpoint blockage antibodies against CTLA4, PD-1, and PD-L1. If safety concern is appropriately addressed, this novel therapeutic approach may deliver suppression of viral replication and reduction in latency reservoirs, resulting in functional cure for HIV infection and clinical cure for chronic HBV infection.

Keywords: Immune checkpoint, T cell exhaustion, immune-oncology, immunovirology, inhibitory molecule, CD80, CD28, CTLA4, PD-1, PD-L1, LCMV, HBV, HCV, HIV, hepatitis, hepatocellular carcinoma

Volume 1 Issue 1 - 2014

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Received: May 01, 2014 | **Published:** June 02, 2014

Abbreviations

GWAS, Genome-Wide Association Study; IBD, Inflammatory Bowel Diseases; IFN, Type 1 Interferon; HCV, Hepatitis C Virus; HBV, Hepatitis B Virus; SLE, Systemic Lupus Erythematosus; TCR, T Cell Receptor; MHC, Major Histo Compatibility Complex; APCs, Antigen-Presenting Cells; CTLA4, Cytotoxic T-Lymphocyte-Associated Antigen 4; PD-1, Programmed Cell Death-1; BTLA, B and T Lymphocyte Attenuator; KIR, Killer Immunoglobulin-Like Receptor; LAG3, Lymphocyte Activation Gene 3; TIM3, T Cell Membrane Protein 3; LCMV, Lymphocytic Choriomeningitis Virus; HIV, Human Immunodeficiency Virus; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; HCC, Hepato Cellular Carcinoma; DAAs, Direct Acting Anti Viral; ETV, Entecavir; ORR, Objective Response Rate; cART, Combination Antiretroviral Therapy; SIV, Simian Immunodeficiency Virus; ACTG, AIDS Clinical Trial Group; CHB, Chronic Hepatitis B; cccDNA, Covalently Closed Circular DNA

Introduction

Across the history of mammalian evolution, selective pressure on the genes encoding the molecules that regulate immune responses has resulted in one intriguing consequence with two opposite aspects. The overall positive selection favors mutants with a survival advantage against pathogenic microbes. However, these alleles also inevitably contribute to the susceptibility of autoimmune diseases. Thus adaptation to infection underlies the maintenance of autoimmune risk alleles. One good example is that network analysis based on RA susceptibility genes from genome-wide association study (GWAS) indicated the enrichment of two pathways, Measles and Intestinal Immune network for IgA production in RA patients.¹ Another study also suggested that there is considerable overlap between GWAS susceptibility loci for inflammatory bowel diseases (IBD) and those for mycobacterial infection, reflected by shared pathways between host responses to mycobacterial and those predisposing to IBD.² Surprisingly, a great majority of these selected mutations occur in regulatory components that control transcriptional expression rather

than in protein coding region. To counter balance the advantage of alleles favoring immune activation, a plethora of inhibitory pathways (aka immune checkpoints) have evolved to maintain self tolerance. These no redundant checkpoint proteins seem to have distinctive functions as they put immune activation on a brake through different mechanisms.

They are necessary in terms of modulating the extent of immunological responses in peripheral systems to minimize collateral damage to the host tissues, at least by evidence generated from knockout mice model. Given the nature of immune regulation, not surprisingly, druggable immuno-modulation on the same target/pathway aiming from different angles has been successfully applied in virologic, oncologic, and autoimmune diseases. A perfect example is type 1 interferon (IFN). Interferon alfa-2a or -2b (pegylated or non-pegylated) are licensed medicines for treating chronic infections of hepatitis C virus (HCV) and hepatitis B virus (HBV). In addition, they are also used in certain tumors (e.g. hairy cell leukemia, melanoma). Furthermore, interferon beta-1a and -1b have been used in relapsing-remitting multiple sclerosis and secondary progressive multiple sclerosis to slow down disease progression and activity. Lastly, to shut down IFN-triggered pathogenesis, antibodies targeting interferon alfa (rotalizumab by Roche/Genentech and sifalimumab by Medimmune) or its receptor (anifrolumab/MEDI-546 by Medimmune) are being developed for systemic lupus erythematosus (SLE). The downstream pathway of type 1 IFN is multi-dimensional, including transcriptional modulation at JAK-STAT pathway in a complexed human system.

T cells stand at the center stage of rendering effective yet specific immune responses and maintaining immune homeostasis. To become activated, T cells require the stimulation from two types of signals. The first signal is mediated through the engagement of T cell receptor (TCR) by antigens presented on major histo compatibility complex (MHC) molecules from antigen-presenting cells (APCs). The second stimulation arises from the interaction between CD28 on T cells and a co-stimulatory ligand such as B7.1 (CD80) or B7.2 (CD86) on APCs. Without the additional signal from CD28:B7 crosslink, TCR

ligation will lead to energy instead of activation. In the past two decades, many B7/CD28 family molecules and TNF family receptors/ligand have been identified with either co-stimulatory or co-inhibitory function. At the immune synapse, they fine tune the amplitude and duration of the immune response by regulating the balance between stimulatory and inhibitory effects. In general the immune checkpoint proteins specifically refer to those co-inhibitory molecules. One of the key checkpoint proteins on T cells is the cytotoxic T-lymphocyte-associated antigen 4 (CTLA4). The binding of CTLA4 to either CD80 or CD86 delivers a strong negative signaling cascade that counteracts the activating engagement of CD28:B7 interaction, resulting in tolerance. Another major brake for immune activation, the programmed cell death-1 (PD-1) is expressed on T cells upon activation. The interaction between PD-1 and either of its two ligand, PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273) results in inhibition of the T cell activation kinases. The inhibitory effect is possibly through activating phosphatase SHP2 when a PD-1 negative micro cluster is formed inside the cell surface.³ Human PD-L1 can also be inducibly expressed on T cells and interacts with CD80. However less physiology is known though it appears a negative signal.⁴ The inhibitory roles of PD-1 and CTLA4 are rather distinctive. Whereas Ctl4-knockout in mice is lethal with systemic hyper activation of immune system,⁵ PD1-deficient mice (Pcd1^{-/-}) spontaneously develop various autoimmune diseases, indicating that this receptor plays a critical role in the maintenance of peripheral tolerance.⁶ To summarize, CTLA4 dampens T cell response at the stage of initial activation, whereas PD-1 attenuates the immune response of effectors T cells by clumping down the extent of T cell-APC or T cell-target cell contact, thus minimizing self tissue damage during immune activation. Other T cell inhibitory molecules, such as BTLA (B and T lymphocyte attenuator), KIR (Killer Immunoglobulin-like receptor), LAG3 (lymphocyte activation gene 3), TIM3 (T cell membrane protein 3) were also identified as co-inhibitory molecules. Even through these molecules are less known than CTLA4 and PD-1, it is still very fascinating that through the evolution, many parallel inhibitory pathways have emerged in an effort to maintain self-tolerance and limit the scope of immunological responses in peripheral systems.

During chronic viral infection or in the presence of tumor micro environment, persistent high levels antigen exposure can trigger a status of T cell exhaustion. The hallmarks of T cell exhaustion are represented by poor antigen-specific effector cell function (e.g. lower cytokine production, decreased proliferation potential, as well as impaired target cell lytic capacity), sustained over expression of multiple inhibitory receptors and altered transcriptional signature distinct from that of functional effectors or memory T cells.^{7,8} T cell exhaustion has been well characterized in a murine model of lymphocytic choriomeningitis virus (LCMV) infection. While the LCMV Armstrong strain leads to a resolved acute infection, the clone 13 strain establishes a persistent infection. Intriguingly, during chronic infection by clone 13 strain, many LCMV-specific CD8⁺ T cells were functionally exhausted even though they expressed activation markers such as CD69 and CD44. In addition, these cells are classified by high levels of PD-1 expression as well as increased expression of other inhibitory receptors such as CD244 (2B4), CD160, CTLA4 and LAG3.⁹ Blocking the PD-1/PD-L1 interaction during the chronic phase of clone 13 infection efficiently rejuvenated these fatigued CD8⁺ T cells, ultimately leading to the clearance of the persistent viral infection. Similar exhaustion phenotype in human T cells has been found at the tumor microenvironment and during chronic viral

infections such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV).¹⁰

In recent years, there has been tremendous interest in T cell inhibitory molecules as druggable targets. Many research highlights have been generated from immune checkpoint blocking antibodies. In particular, the immuno-oncology area has been the brightest spot perhaps across the entire therapeutic areas of drug development with some virology data generated as an intentional byproduct. Backed by the regulatory approval of two active immunotherapies, sipuleucel-T (Provenge®) in 2010 and Ipilimumab (Yervoy®) in 2011 respectively, the confidence in the concept of immunotherapy has never been higher. Both drugs demonstrated statistically improved survival in randomized Phase 3 oncology trials. While sipuleucel-T (developed by Dendron) is the first cancer therapeutic vaccine, it is Ipilimumab (developed by BMS), the first CTLA4-targeting immune checkpoint blockade antibody that has materialized the concept of boosting tumor antigen-specific T cell response via the suppression of the T cell inhibitory signal. Although the development of a similar anti-CTLA4 drug, Pfizer's tremelimumab (now being developed by Medimmune) was prematurely terminated during Phase 3 trial in patients with advanced melanoma, data generated from a small Phase 2a study of tremelimumab stood out noteworthy.¹¹ The trial was conducted in a group of 20 patients with hepato cellular carcinoma (HCC) and chronic infection of HCV genotype 1b. All patients had inoperable HCC and 43% of them had a Child-Pugh score of grade B. Patients were given a dose of 15mg/kg on Day 1 of every 90 days for up to 4 cycles. In the presence of some modest antitumor activity, tremelimumab induced a significant drop in HCV RNA from 3.78×10^5 IU/ml (median value) at day 0 to 3.02×10^4 IU/ml (median value) at day 120 (n=11, p=0.011) and 1.69×10^3 IU/ml at day 210 (n=6, p=0.017). This antiviral effect was associated with an increase in IFN- γ producing T cells specific for HCV antigens. In addition, the overall safety profile was acceptable, without any severe immune-mediated adverse events that needed the systemic use of steroids. It was also notable that there were 45% patients who experienced a transient yet intense (\geq Grade 3) elevation of transaminases after the first dose. However the liver enzymatic flare seemed not associated with a parallel decline in liver function and did not recur following subsequent cycles. The results came as a surprise. Prior to this trial, the antiviral activity of CTLA4 blockade had never been demonstrated or ever tested in humans.

Additionally, in a study when 3 chimpanzees with persistent HCV infection were treated with multiple doses of anti-PD-1 antibody,¹² there was a significant reduction in viral load in one animal in the absence of apparent hepatic injury. Control of HCV replication was accompanied by restoration of intra hepatic CD4⁺ and CD8⁺ T cell immunity against multiple HCV antigens. HCV RNA rebounded when antibody treatment was stopped. The heterogeneous responses from these animals may indicate that the sufficient presence of pre-existing HCV-specific exhausted T cells in liver likely determines the success of PD-1 blockade.

In an SIV-infected rhesus macaque model, in vivo blockade of pd-1 led to rapid expansion and recovery of SIV-specific multi-functional CD8⁺ T cells and enhanced B cell responses. Furthermore, blocking PD-1 signaling also resulted in a significant decrease in viral load. Finally, it also prolonged the survival of infected macaques.¹³ These results are highly significant considering that the related co-inhibitory molecule CTLA4 failed to either expand virus-specific CD8⁺ T cells or reduce viremia in SIV macaque model.¹⁴ Similarly,

inhibition of PD-1 signaling by anti-PD-1 antibody also achieved a reduction in viral load in a humanized mouse model with chronic HIV-1 infection.¹⁵

Woodchuck with chronic hepadnaviral infection closely resembles to human chronic HBV infection. A recent study showed that when chronically infected animals received a combination therapy of nucleoside analogue entecavir (ETV), therapeutic DNA vaccination and woodchuck anti-PD-L1 antibody, the functions of virus-specific T cells were revived. Moreover, compared to treatments without anti-PD-L1 antibody, the combination therapy dramatically suppressed viral load, leading to sustained immune control of viral infection, anti-WHs antibody development as well as complete viral clearance in some woodchucks.¹⁶

Taken all information together, the evidence of viral inhibition by suppressing T cell co-inhibitory signaling has opened the new and promising therapeutic strategies that targeting immune checkpoint proteins can break immune tolerance triggered by chronic viral infection. The rationale of developing immunomodulation drugs targeting co-inhibitory molecules in chronic viral infection is scientifically sound. Although the utilization of disrupting immune tolerance strategy is no longer implementable due to the rapid advances in the combination of direct acting anti viral (DAAs) against HCV, there is still a very strong need that certain checkpoint blocking antibodies may have the potential to be developed for chronic HIV or HBV infections when the life-long treatment is not a preferred option.

In order to move immune checkpoint blockers into infectious diseases, several factors have to be considered as chronic viral infections have very different aspects on overall benefit/risk profile when compared to oncology indications. First, from safety side, mechanism-related non-specific immune activation may arise so risk management needs to be carefully incorporated into development plan and AEs should be cautiously evaluated as the investigative drugs moves along in clinical trials. Second, the effectiveness of immune checkpoint blockers is likely determined by the sufficient presence of antigen-specific, exhausted T cells. Thus identifying the biomarker-positive population becomes an important evaluation part during development to maximize the likelihood to observe incremental benefits from therapeutic intervention. Third, T cell exhaustion is a hierarch process of losing functionality, high level exposure to antigen or longer duration of chronic infection may lead to irreversible stage of severe exhaustion or clone depletion.¹⁰ Therefore combination therapy with other antiviral(s) may be considered or even necessary to bring down the antigen level first before the initiation of immune intervention.

Due to the nature of immune checkpoint blockers, nonspecific activation of self immune response is always a concern during clinical development. CTLA4 blockade antibodies seemed to have an overall higher incidence of autoimmune activation than PD1/PD-L1 blocking antibodies from existing clinical trials. While there is no direct comparison between anti-PD1 (nivolumab) and anti-PD-L1 (BMS-936559), the two Phase 1 studies by BMS may have generated some useful insights. Both studies had comparable trial size (n=296 for nivolumab vs. n=207 for BMS-936559) and patient disposition (35% melanoma, 41% non-small-cell lung cancer, and 24% other cancers for nivolumab vs. 27% melanoma, 36% with non-small-cell lung cancer, and 37% other cancers for BMS-936559). While these two studies suggested that the overall safety profiles of anti-PD1 and anti-PD-L1 compared favorably against ipilimumab. When nivolumab

vs. BMS-936559 was compared, both antibodies demonstrated solid anti-tumor activity. However, PD-L1 blocker appeared to have lower incidence of AEs, in particular fewer immune-activation related events (Grade 3 or 4 AEs in all patients: 14% for nivolumab vs. 9% for BMS-936559) while nivolumab seemed to deliver better cumulative objective response rate (ORR) (ORR in melanoma: 28% for nivolumab vs. 17% for BMS-936559; ORR in NSCLC: 18% vs. 10%). In the nivolumab Phase 1 trial, there were 3 deaths due to immune-related pneumonitis.^{17,18} Thus when the overall benefit/risk is considered, anti-PD-L1 antibody seemed a little better drug candidate warranting further exploratory investigation in chronic infection.

Because of the existing latency reservoir, combination antiretroviral therapy (cART) can efficiently decrease viral load to undetectable level but still fails to eradicate persistent HIV-1 infection. Viremia rebounds once cART stops. Although complete eradication of all latent HIV reservoirs remains extremely difficult to attain, functional cure through control of viremia in the absence of cARTs may be achieved by reducing latent reservoir and restoring antigen-specific immune function. Experimental study in treatment-naive, simian immunodeficiency virus (SIV)-infected rhesus macaques demonstrated that PD-1 blockade by anti-PD-1 antibody achieved transient suppression of viremia, revitalized both T and B cell function, and prolonged survival.¹³ In another treatment-experienced macaque model of SIV infection, BMS-936559 (anti-PD-L1) or isotype control antibody were administered to the infected animals (n=8 and n=4 respectively) with plasma viral load suppressed at <50 IU/ml on cART. Four out of eight anti-PD-L1 treated macaques had dramatically delayed viral rebound after cessation of cART. They had low SIV RNA (<1000 IU/ml) status for more than 8 weeks. In particular, two animals from these 4 responders achieved undetectable viral load for 3-4 weeks. While further analysis is ongoing, this finding is indicative of possible reduced latent reservoirs and host control of virus following the withdrawal of cARTs. Finally, repeated dosing appeared to be well tolerated.¹⁹ To summarize, macaque studies have provided strong scientific rationale that justifies further evaluation of anti-PD-L1 antibody in HIV patients. Currently, AIDS Clinical Trial Group (ACTG) is conducting a double-blind, placebo-controlled, single ascending dose Phase 1 study of BMS-936559 (ACGT 5326, Clinicaltrials.gov identifier: NCT02028403) in HIV-1 patients. Starting in the 1st quarter of 2014, 48 patients who take cART with suppressive viral load (<LOD of either the Abbott m2000 Real-time HIV-1 assay or the Roche HIV-1 CobasTaqman HIV-1 v2.0 Test) will be recruited in this study. The primary outcomes are related to safety (any AEs and >Grade 3 AEs), HIV-specific immune response, and latent HIV reservoir (via HIV-1 RNA single copy assay). Additionally, many exploratory biomarkers will also be evaluated as secondary outcomes. Another Phase 1 open-label, single ascending dose study (ACGT 5301) of Lambrolizumab (MK3475 by Merck) in 40 HIV-infected patients who have viral suppression by cARTs is being planned with the aim to evaluate the clearance of latency reservoir by PD-1 blockage.

Both HCV and HBV cause chronic hepatic infection. However, given the fact that combination of direct acting antiviral (DAAs) have achieved >90% cure rate in HCV treatment, there is no additional benefits to bring an immune checkpoint blocker into development. Nevertheless, the safety and efficacy data generated from both experimental and clinical HCV studies can add valuable information when the development of immune checkpoint blockers is considered for chronic hepatitis B (CHB). At the early clinical development of

nivolumab, Medarex (bought by BMS) initiated a Phase 1 single ascending dose study in 54 HCV patients.²⁰ Among 45 patients treated with MDX-1106 (renamed as BMS-936558 and then nivolumab), 3 out of the 20 patients in the high dose group had impressive $>4 \text{ Log}_{10}$ HCV RNA decline. One subject remained viremia free for more than 1 year post treatment which met the criteria of sustained virologic response in HCV treatment. Whereas the response was spotty, this is the first proof of concept in human that PD-1 blockade not only can transiently control viral replication but also can potentially clear the viral infection. Among drug-treated patients, six of them experienced immune-related adverse effects of mild-to-moderate intensity. While the therapeutic application of this approach in HCV infection is limited by the ongoing development of highly effective new treatments, the promising results still suggest the value of further exploring PD-1 pathway blockade in chronic hepatitis B, possibly in combination with other immune modulators or DAAs.

During chronic infection, HBV presumably have infected all hepatocytes. Therefore liver flare triggered by immune activation is a primary safety concern. In order to move immune checkpoint antibodies such anti-PD-L1 into CHB, there is a need to generate more bridging safety data. Currently BMS is conducting a Phase 1b multiple ascending dose study to investigate the safety, immunoregulatory activity, and preliminary antitumor activity of nivolumab in advanced hepato cellular carcinoma (HCC) patients (ClinicalTrials.gov Identifier: NCT01658878). These patients will include HCC without chronic viral hepatitis, with either HBV or HCV. While the primary outcomes are adverse events related including liver flare, the dynamic change of HCV RNA or HBV DNA during the study can be evaluated. Considering the fact that anti-PD-L1 may have a cleaner safety profile than anti-PD-1 due to its mode of action, additional anti-PD-1 safety data from HCC patients with chronic hepatitis C or with chronic hepatitis B could subsequently lower the development hurdle and better justify the rationale of putting a presumably safer molecule into patients with chronic HBV infection. Similar to HIV infection, in CHB infection the nuclear presence of covalently closed circular DNA (cccDNA) functionally serve as a reservoir for viral rebound if antiviral treatment is stopped in patients who have suppressed viral replication. Although pegylated interferon achieves clinical cure (HBsAg seroconversion) in some patients with a definite treatment, the response rate is very modest. In chronic HBV infection, virus-specific T cells were detected mainly in patients with lower levels of viremia. In vitro study suggested that both peripheral and intra hepatic HBV-specific CD8⁺ T cells expressed high levels of PD-1. Their antiviral function was improved by in vitro blocking PD-1/PD-L1 engagement.²¹ This hypothesis can be tested in CHB patients treated with antiviral or interferon. If viremia is lowered by antiviral or if both viremia and HBsAg are lowered by interferon, it will be interesting to see whether improved T cell and B cell function can trigger a decrease in cccDNA reservoirs and boost HBsAg seroconversion. While it is still possible that in treatment-naïve patients, PD-L1 blockade can lead to restored virus-specific T cell activities which can translate into a decrease in viral replication and potentially clinical cure of CHB (reflected by loss of HBsAg and presence of anti-HBsAg antibody), this scenario is less realistic.

Conclusion

Preclinical studies of T cell exhaustion and co-inhibitory molecules in chronic viral infection have fundamentally shaped the idea about

targeting immune checkpoint proteins to activate antigen-specific immune response and changed the perception about immuno-oncology. As we look forward to the next paradigm for cancer treatment, we are also excited to see that immune checkpoint blockers have moved into chronic viral infection, albeit in an extremely cautious fashion. With more proof of concept data generated from HCC with viral hepatitis, HIV infection, CHB, and better characterization of many of these co-inhibitory molecules, we are not only looking for deepening our understanding about immune-virology but also developing mechanistically novel molecules for treating chronic infection.

Acknowledgments

None.

Conflict of interest

Author declares that there is no conflict of interest.

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