

T-cells and cancer immunotherapy (immuno-oncology): the 2018 Nobel Prize in Physiology or Medicine

The 2018 Nobel Prize in Physiology or Medicine has been awarded to two scientists, James P. Allison and Tasuku Honjo for discovery of checkpoint molecules CTLA-4 and PD-1, which inhibit the T-cells of the immune system from attacking the cancerous tumours. They also discovered antibodies, which could be used for inhibiting the activity of these checkpoint molecules, thus relieving the T-cells from inhibitory activity of checkpoints, to be able to attack cancerous tumours. Such antibodies have already been converted into a number of immunotherapeutic drugs, which have been approved and are already being used for treatment of a variety of cancers.

The 2018 Nobel Prize in Physiology or Medicine has been awarded to James P. Allison, who is currently working at MD Anderson Cancer Center, Texas, USA, and Tasuku Honjo of Kyoto University, Japan¹. Allison studied a protein CTLA-4 (Cytotoxic T-Lymphocyte Associated Protein-4) that is associated with T-cells and functions as a brake on the immune system resulting in its failure to recognize the tumours and destroy them. In parallel, Honjo discovered protein PD-1 (Programmed Cell Death-1), which also controls the response of T-cells towards cancerous tumours. A brief outline of the functions of CTLA-4 and PD-1 and the corresponding antibodies is presented in Figure 1. The subject of research of the 2018 Nobel Prize has changed the whole area of cancer therapy forever and is thus indeed bringing about a revolution. In this article, a brief account of the contributions of the two 2018 Nobel Prize winners and other related research will be presented. An elementary knowledge of the immune system and the associated B-cells and T-cells will be required for an understanding of the research contributions of the two 2018 Nobel Laureates.

Major contributions of Allison

The research career of Allison started in the year 1971, when during his Ph D programme, he published his work on substrate-specificity of the enzyme L-asparaginase. This was followed by a period of more than 10 years of active research, during which Allison extensively worked on immune-system involving a variety of antigens and antisera. Initially, during 1980s, he and his group were the first to identify T-cell receptors (TCRs), which recognize the antigens present on the surface of foreign bodies. With the help of these TCRs, the T-cells

destroy the unwanted foreign objects like virus particles and other pathogens. However TCRs may often need co-stimulatory molecules for their activation. The first T-cell co-stimulatory molecule, CD28, was identified in 1986 and was found to enhance T-cell proliferation upon binding to ligands like B7 molecules that occur on APCs. Later, Allison's group, while working at University

of California, Berkeley, was also able to show that CD28 was necessary and sufficient to provide the second co-stimulatory signal required for full T-cell activation¹⁻³. A complete list of publications of James Allison is available as a [Supplementary Material file #1](#).

The work of Allison, for which he was awarded the Nobel Prize, however, started during 1990s, when he first (in

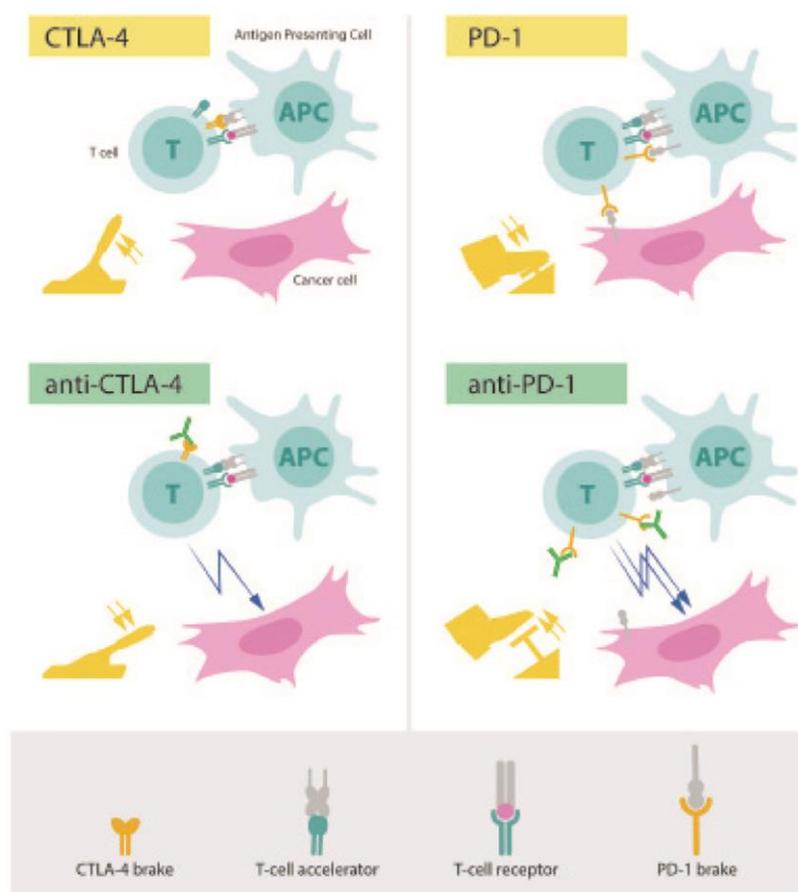


Figure 1. Activation of T-cells due to antibodies acting on checkpoints, (left) cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and (right) programmed death 1 (PD-1), both shown in yellow; in both panels, upper part shows inhibitory effect of checkpoints (CTLA-4 and PD-1), and the lower part shows the effect of corresponding antibodies (in green) in removing the effect of checkpoints leading to attack on cancer cells¹.

1995) identified the checkpoint molecule CTLA-4 (refs 4, 5), which besides other functions in immune system, acts as a brake on the function of T-cells in attacking self-tissues. Among the different effects of CTLA-4 on immune system, the prominent effects observed in initial studies included reduction of immunity⁵ and proliferation of lymphocytes, eventually leading to death of mice, used as a model system⁶. Its utility for treatment of mammary carcinoma was demonstrated during 1996 to 1998 (refs 7, 8). This was the beginning of an era of immunoncology (IO). Later, in 2003, Allison and his colleagues tested an anti-CTLA-4 antibody on human subjects carrying metastatic melanoma and found that the cancer regressed in 3 of the 14 patients⁹. In 2011, the Food and Drug Administration (FDA) approved the first anti-CTLA-4 antibody (ipilimumab) as a treatment for late-stage melanomas¹⁰.

Major contributions of Honjo

Initial work of Honjo for almost three decades was not directly related with the subject of Nobel Prize. He started his research career in 1964 with the publication of his first paper on enzymatic syn-

thesis of NAD (nicotine adenine dinucleotide). This was followed by a series of papers on metabolism of a variety of biological molecules. Later during 1970s and 1980s, he also worked extensively on origin, organization and diversity of genes encoding immunoglobulin and T-cell receptors. In 1978 he also proposed (and later proved) that the class switching in antibodies takes place due to recombination involving excision of genomic fragments¹¹. This class switching provides antibody diversity, a phenomenon, for which Tonegawa of Japanese origin (working in USA) received the 1987 Nobel Prize for Physiology or Medicine. Honjo is also known for identification and study of interleukins and for his work on RNA activation-induced cytidine deaminase (AID), which could be used for inducing mutations in antibody and the transcripts of proto-oncogenes (this work was done during 2000–2004). More recently (in 2017), these RNA AIDs have also been used for developing artificial DNA AIDs for the purpose of base editing (by D. R. Lui and his team at Harvard) ([see Supplementary Material file 2 for complete list of publications of Honjo](#)).

The Nobel Prize winning research of Honjo really started in 1992 with the dis-

covery of Programmed Cell Death 1 (PD1) protein¹², another checkpoint molecule, which controls the activity of T-cells. This was followed by reports on structure, chromosome assignment and expression of PD-1^{13,14}. The effects of deficiency and blockade of PD-1 was also examined in mice and it was observed that a loss of PD-1 could cause lupus-like auto-immune disease; implications of PD-1 functioning as negative regulator of the response of lymphocytes were also discussed^{15–19}. The mechanism of action of PD-1 was also examined and it was shown that a receptor of PD-1 is engaged by a B7 protein, which leads to negative regulation of lymphocyte activation, thus suggesting that PD-1 inhibitors can be used for treatment of cancers²⁰. PD-1 inhibitors were later developed and in 2008, a PD-1 inhibitor in the form of a humanized antibody (CT-001) was tested on patients with advanced stage of cancer²¹. By 2012, the drugs based on PD-1 inhibitors had shown remarkable effectiveness, with several patients experiencing long-term remission. The FDA approved the first PD-1 checkpoint inhibitor (pembrolizumab) to treat melanoma in 2014 (ref. 22) (the CTLA-4 inhibitor ‘ipilimumab’ was approved earlier in 2011). A number of other PD-1 inhibitors have since been approved for the treatment of at least nine different types of cancers.

CTLA-4 and PD-1 pathways

The pathways of checkpoints CTLA-4 and PD-1 discovered by Allison and Honjo are not very different²³. CTLA-4 is actually a homolog of the co-stimulatory molecule CD28 mentioned above, but with a much higher binding affinity for B7; however, instead of providing a stimulatory signal, CTLA-4 inhibits the activity of T-cells (Figure 2). The relative extent of CD28 : B7 binding versus CTLA4 : B7 binding determines whether a T-cell will undergo activation or anergy (‘anergy’ is a term that is used in immunology literature to describe immunologic self tolerance, in which T-cells become functionally inactivated after previous stimulation). CTLA-4 itself is also subjected to regulation, particularly by control on localization within the T-cell. In resting phase, CTLA-4 is located in the intracellular compartments of T-cells. Signals from both TCR and CD28 : B7

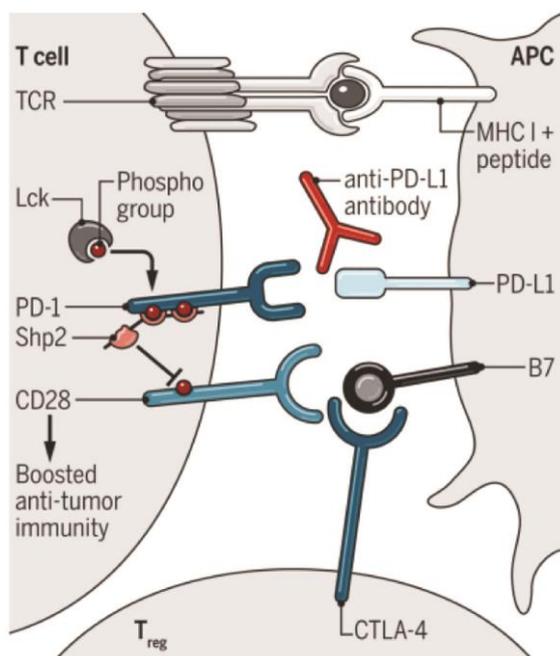


Figure 2. The mechanisms of CTLA-4 and PD-1 pathways. CTLA-4 regulates CD28 ligand binding, whereas PD-1 inhibits CD-28 signalling. CD-28 co-stimulation is required for anti-PD-L1 antibodies to enhance immunity²³. (Reproduced with permission from AAAS.)

Table 1. Comparison of CTLA-4 and PD-1

1. Similarities
(i) Both are members of B7 receptor family
(ii) Both are expressed in activated T-cells
(iii) Expression of both depends upon strength and/or duration of TCR signal
(iv) Both regulate the same set of intracellular T-cell signalling proteins
(v) Both reduce T-cell proliferation
2. Differences
(i) CTLA-4 limits T-cell responses early in an immune response, while PD-1 limits T-cell responses later in an immune response
(ii) Both expressed by T-cells, but PD-1 also expressed by T other immune cells
(iii) CTLA-4's ligand is B7, expressed on APC; the ligand for PD-1 are PDL-1 and PDL-2, expressed on tumour cells also
(iv) PD-1 interferes with many more T-cell signalling pathways
(v) CTLA-4 affects functioning of Tregs; role of PD-1 on Tregs is not clear

Table 2. Some drugs (designated as orphan drugs by FDA) under development (with their targets and status in 2017)

Target	Drug	Status in 2017	Company
CTLA-4	Tremelimumab	Phase II: lung cancer	MedImmune/AstraZeneca
PD-1	Pidilizumab	Phase II: kidney cancer and hematologic malignancies	CureTech/Medivation
PD-L1	Durvalumab	Phase III: head and neck/lung cancer	MedImmune/AstraZeneca
PD-L1	Atezolimumab	Phase II: colorectal cancer and glioblastoma Phase III: bladder cancer and lung cancer	Roche
PDL1	Avelumab	Phase II: kidney cancer Metastatic merkel cell carcinoma	Merck KGaA/Pfizer/Eli Lilly

binding induce exocytosis of CTLA-4-containing vesicles to the surface of T-cells. This process operates in a feedback loop, where a stronger TCR signalling elicits transport of more CTLA-4 to the cell surface, thus preventing the activity of T-cells.

Like CTLA-4, PD-1 is also a co-stimulatory receptor, belonging to B7/CD28 family. It regulates the activity of T-cells through binding with two ligands (PDL-1 and PDL-2), which often occur on cancerous cells (Figure 2). When both TCR and PD-1 occur together, signals from PD-1 prevent phosphorylation of molecules involved in TCR signalling, leading to termination of TCR signalling and reduction in the activity of T-cells. PD-1 gene also has several cis-elements that are involved in regulation of the expression of PD-1. Like CTLA-4, expression of PD-1 is also controlled by epigenetic modifications including DNA methylation and histone modifications²⁴.

Innate immune system and checkpoint inhibitors for treatment of cancers

As mentioned earlier, the immune system is naturally blocked by one or more checkpoints involving proteins like

CTLA-4 and PD-1, both having similar negative effects on T-cell activity, but there are also differences. The similarities and differences between CTLA-4 and PD-1 are summarized in Table 1. The immune system can be made to attack cancer cells through inhibition of these checkpoints²⁵. Such checkpoint inhibitors were actually developed and are being used as immunotherapy drugs. It has also been shown that a single T-cell can kill thousands of cancer cells. Immunotherapeutic drugs based on this principle have been developed and are being used for cancer treatment.

Individual checkpoint inhibitors (CTLA-4/PD-1) as cancer drugs

Three most important drugs (already mentioned above) developed by 2018 Nobel Laureates using two checkpoints (CTLA-4 and PD-1) and their checkpoint inhibitors were also approved by FDA for different cancers. These three drugs included the following: (i) ipilimumab (brand name Yervoy; approved in 2011) for metastatic melanoma; (ii) nivolumab (brand name Opdivo; approved in 2014), and (iii) pembrolizumab (brand name Keytruda; approved in 2014). Current status and future prospects of these three

drugs have been discussed²⁶. Later, other drugs also became available. Some other drugs, developed and approved later are listed in Table 2. According to Cancer Research Institute in New York City, ~2000 immunotherapeutic drugs are currently in different stages of development²⁷. Some cancer patients respond better than others, and many do not respond at all. Sometimes, tumours can also become resistant to drugs over time.

Combination therapies using both CTLA-4 and PD-1

The drugs developed through the use of antibodies, which function as checkpoint inhibitors, have also been used in combinations. The efforts, which are being made in using combinations of drugs to treat cancer patients have recently been reviewed²⁸. It has been shown that individual drugs for blocking an individual checkpoint (either CTLA-4 or PD-1) may not be enough. Therefore, combination therapy targeting both CTLA-4 and PD-1 checkpoints is already being used, which has proved to be more effective than individual drugs (but see later). New combinations have also been steadily increasing during 2009–2017, although mean size of individual trials has shown

a decline, perhaps because more targeted populations are being used for each combination (Figure 3). In some cases, three-way trials have also been conducted, where both pembrolizumab and nivolumab were combined with chemotherapy/radiotherapy or with other drugs that are routinely used for cancer treatment.

In the above review by Schmidt²⁸, a case of a 69-year-old male patient is described, who had undergone treatment at the University of California, San Francisco Medical Center in 2015. In this patient, a fairly good size tumour under his armpit disappeared, when subjected to treatment with a combination of nivolumab and ipilimumab. There may be similar other examples, which are not documented. According to some estimates, more and more patients are now reaching five-year survival due to immunotherapy, and the number of such cases with combination treatments will hopefully increase in future.

The use of antibodies against the two checkpoints, namely CTLA-4 and PD-1 has also been shown to be toxic²⁹. Also, in most cases, the improvement due to combined treatment (nivolumab + ipilimumab) over treatment with single drug (nivolumab) has been shown to be minimal. In this connection, the trials conducted by two companies, Astra Zeneca

(based in Cambridge, UK) and Bristol-Myers Squibb (based in New York in USA) are important. The results of phase III MYSTIC trial (conducted by Astra Zeneca) involving the use of durvalumab (a PD-1 inhibitor) and tremelimumab (a CTLA-4 inhibitor) for lung cancer have not been encouraging till 2017. Similar are the results of another advanced phase III trial (conducted by Bristol-Myers Squibb) for treatment of non-small-cell lung cancer (NSCLC), where nivolumab and ipilimumab were used. The strategies are being planned to deal with this problem of toxicity.

Challenges needing attention (development of biomarkers)

There are challenges (including toxicity discussed above), which need to be addressed. These challenges include the following: (i) unpredictable efficacy; (ii) acquired resistance against a drug; (iii) autoimmune reactions due to enhanced activity of T-cells; (iv) unaffordable cost of treatment, often ranging from US\$ 40,000 to 150,000 per year, depending upon the nature of cancer and the drug being used. These challenges have been discussed in a recent review³⁰. As discussed in this review and elsewhere,

one of the major emerging areas of research dealing with the major challenges is the development of biomarkers for cancer immunotherapy, which will be briefly discussed.

It is widely known now for some time that different patients suffering with the same disease respond differently to the same treatment. This is described as pharmacogenetics/pharmacogenomics, and holds good for cancer immunotherapy also. Therefore biomarkers have been developed, and are recommended for use while prescribing immunotherapy treatments. This is rather important keeping in view the high cost and toxicity of immunotherapy treatments. In this connection, expression of the ligand PDL-1 has been considered to be the most important approved biomarker (in the form of PD-L1 IHC 22C3 pharmDx). This can be used before prescribing a drug (e.g., pembrolizumab), which is a PD-1 inhibitor. Microsatellite instability (MSI-H) that is known to be caused by impaired DNA repair system, can also help to predict, whether a patient will respond to PD-1 inhibition. Tumour mutation burden (TMB), which is a measure of mutations carried by tumour cells and T-cell-inflamed gene expression profile (GEP) are two other good biomarkers for using the drug pembrolizumab³¹. The utility of TMB and GEP was recently evaluated using 300 patients across 22 tumour types showing poor correlations³². The above four biomarkers (PDL-1, MSI-H, TMB, GEP) can be used for designing clinical trials involving monotherapy and combination immunotherapy for a variety of cancers. This research area is currently in its infancy, and major efforts are being made in this direction. For instance, to support research involving development of biomarkers, which would predict which cancer patients are most likely to respond to immunotherapy treatment, a network has also been established by the National Cancer Institute (NCI), New York. The network consists of four Cancer Immune Monitoring and Analysis Centers (CIMACs) and a data storage center, the Cancer Immunologic Data Commons (CIDC).

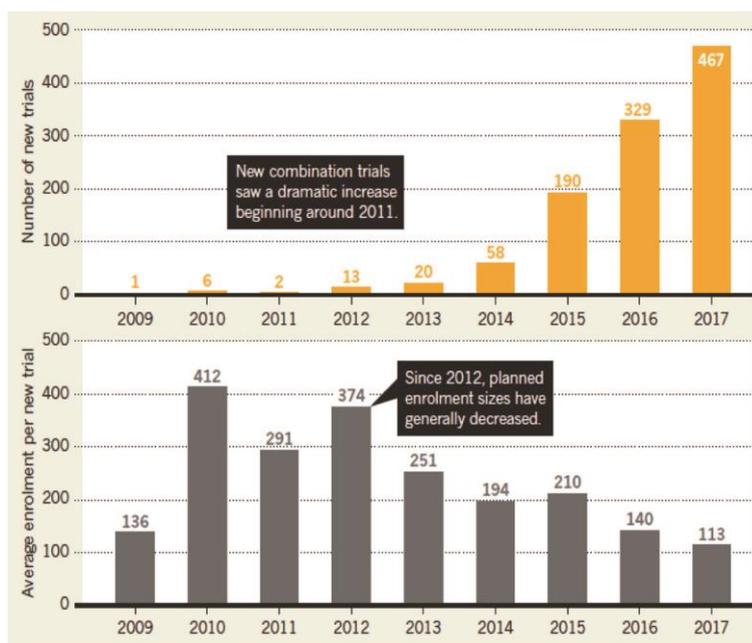


Figure 3. New combination trials (above) and size of new enrolment in trials using combination of drugs, better targeted: The number of new clinical trials that combine checkpoint inhibitors targeting PD-1 or PD-L1 with other treatments is soaring worldwide. At the same time, the average planned enrolment for each trial is dropping, because of more targeted study populations²⁸. (Reproduced with permission.)

Summary and conclusions

The 2018 Nobel Prize in Physiology or Medicine has been awarded to James P.

Allison and Tasuku Honjo for their outstanding research contributions in the field of cancer-immunotherapy. Their Nobel Prize winning research work involved identification of two checkpoints (CTLA-4, PD-1) of the T-cells of the immune system, and their inhibitors (antibodies), which were used for the development of immunotherapy drugs for the treatment of about a dozen different types cancers. The work of 2018 Nobel Prize has thus brought a revolution in the treatment of cancers. Follow up and future work involves development of biomarkers to be used for predicting the outcome of treatment of a patient suffering with a specific cancer type with a specific drug. The future of immunotherapy for treatment of a large variety of cancers thus seems to be bright, bringing about a change in the manner cancers will be treated in future.

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P. K. Gupta, Department of Genetics and Plant Breeding, CCS University, Meerut 250 004, India
e-mail: pkgupta36@gmail.com