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Analysis for Science Librarians of the 2018 Nobel Prize in Physiology or Medicine: The Life and Work of James P. Allison and Tasuku Honjo

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On October 1, 2018, James P. Allison and Tasuku Honjo were awarded the 2018 Physiology or Medicine Nobel for their work leading to Immune Chekpoint Inhibition (ICI). ICI is the fourth pillar of cancer treatment and has been used to treat previously un-treatable cancers. Allison discovered that the protein CTLA-4 acts as a T cell brake while Honjo discovered another T cell brake, PD-1. Releasing these brakes allows the immune system to attack tumors, sometimes leading to complete elimination. While there is still more research to be done, Allison's and Honjo's work is a breakthrough in cancer immunotherapy.

KEYWORDS: 2018 Nobel Prize in Physiology or Medicine, Immune Checkpoint, immuno-oncology, CLTA-4, PD-1

Introduction

Towards the end of 1895, Alfred Nobel signed his will dictating that his fortune be used to award prizes in five fields, the third of which is that of physiology or medicine (NobelPrize.org). Since 1901, there have been 216 laureates for the 109 Physiology or Medicine Nobels awarded (Nobelprize.org). Of the twelve new laureates among all the prizes this year, James P. Allison and Tasuku Honjo were awarded the physiology or medicine award "for their discovery of cancer therapy by inhibition of negative immune regulation" (Nobelprize.org 2018a). Both of these scientists have discovered and/or made advancements with T cell proteins and their ligands which, when manipulated, allow immune systems to better attack tumor cells. While there is still a great deal of research to be done in this area, their work has laid the foundation and already led to the development of therapeutics that improve outcomes, sometimes even

curing, previously therapy resistant cancers. Allison's award-winning work focused on cytotoxic T lymphocyte antigen 4 (CTLA-4), its homolog CD28, and their ligand B7, while Honjo's focused on Programed Death 1 (PD-1) and its ligands PDL-1 and PDL-2 (PDLs).

The work of this year's Physiology or Medicine Laureates is important enough to warrant the award partly due to the widespread and devastating effects of cancer and the difficulties in treatments. The basic definition of cancer from the National Cancer Institute (NCI) is "diseases in which abnormal cells divide without control and can invade nearby tissues" (National Cancer Institute Dictionary of Cancer Terms). There are over a hundred different types of cancers and, according to the World Health Organization, cancer as a whole led to an estimated 9.6 million deaths in 2018, or about 1 in 6 deaths, making it the second leading cause of death globally (National Cancer Institute ; World Health Organization). In the United States in 2015, cancer held the same standing of the second leading cause of death, killing 595,930 Americans. As of 2016, 9.4% of adults (22.9 million) in the USA had been diagnosed with cancer (National Center for Health Statistics) and the American Cancer Society estimates 1,735,350 new cancer diagnoses and 609,640 cancer deaths in the United States in 2018 (American Cancer Society 2018).

According to the American Cancer Association, there are currently four cancer treatments and a handful of other procedures and techniques used to fight cancer. The four treatments listed are: surgery, radiation, chemotherapy (including targeted therapy), and immunotherapy (American Cancer Society). During the Nobel in Physiology or Medicine prize announcement, Dr. Klas Kärre emphasized that for decades there were three pillars of cancer treatment: removing with surgery, eliminating with radiotherapy, and attacking with anti-cancer drugs (chemotherapy). The work leading to these standard treatments resulted in previous Physiology or Medicine Nobel Prizes, including the 1966 award to Huggins for the discovery of methods for hormone treatment for prostate cancer, the 1988 award to Elion and Hitchins for the development of drugs including on for leukemia, and the 1990 award to Thomas for the development of bone marrow transplantation methods for treatment of diseases such as leukemia

(NobelPrize.org 2018b). The work of this year's laureates fortified the fourth pillar of treatment, immunotherapy, with immune checkpoint therapy (Nobelprize.org 2018a). This treatment is often referred to as either Immune Checkpoint Inhibition (ICI), Checkpoint Inhibition (CPI), blockade therapy, or some combination of those words, and according to the NCI, is defined as "a type of drug that blocks certain proteins made by some types of immune system cells, such as T cells, and some cancer cells… Examples of checkpoint proteins found on T cells or cancer cells include PD-1/PD-L1 and CTLA-4/B7-1/B7-2" (Lamichhane et al. 2018; National Cancer Institute Dictionary of Cancer Terms ; Wei, Duffy, and Allison 2018). These therapies have been in place since 2011 and, perhaps most famously, were used to cure President Jimmy Carter of his metastatic melanoma that had spread to his brain and liver. Three months of treatment with pembrolizumab, an anti-PD-1 drug, resulted in his tumors disappearing (Cancer Research Institute).

The science

Immune systems

Simply put, immune systems recognize self from non-self in order to protect against and remove the non-self from an organism (Baumgarten and Polley 2018). This protection is provided largely by lymphocytes, a type of white blood cell, which consist of those that mature in the bone marrow (B cells) and those that mature in the thymus (T cells), as well as Natural Killer Cells and lymphoid dendritic cells. The work of this year's Physiology or Medicine Nobel Laureates focused on T cells which are further broken down into helper T cells ($CD4^+$) that stimulate B cells, and cytotoxic T cells ($CD8^+$) that kill identified cells (Loftus 2014). Both types of T cells release interleukins, a type of cytokine, which are proteins that enable cell-cell or cell-self communication, specifically released from lymphocytes (Clark 2014).

In order for T cells to release these cytokines that stimulate B cells, proliferate their own activation, and attack cells with non-self antigens, T cells themselves need to be switched on. Under normal conditions,

T cells are in an off state and multiple proteins and substances are needed to activate them. Both major histocompatibility complexes (MHC), which act as intercellular identifiers of self, and antigens need to be present in an antigen-MHC complex (Goldsby, Kindt, and Osborne 2000). T cell receptors (TCRs) that allow for the recognition of these complexes were long thought to exist but proved difficult to identify; it wasn't until 1982 that the TCR was identified by James P. Allison (Allison, McIntyre, and Bloch 1982; Graeber 2018). In addition to the antigen-MHC complex, costimulation by antigen presenting cells (APCs) and TCRs is also required for the activation of T cells (Allison, Hurwitz, and Leach 1995). One such T cell costimulatory receptor is Cluster of Differentiation 28 (CD28), which binds with APCs via B7 ligands (Krummel and Allison 1995; Loftus 2014).

The immune system and cancer

Cells naturally divide and grow in order to replace themselves or to produce new, necessary cells. This cell growth is controlled by various proteins that either prevent excess growth or lead to the death of unneeded cells. When the genes coding for these proteins are altered, cell growth can get out of control leading to a tumor. Cancer results if the cells that produce the tumor are malignant and able to travel to other parts of the body to continue growing. Sometimes, the immune system can recognize these rogue cells as non-self and eliminate them before they get out of control (Carson-DeWitt et al. 2013). However, this natural ability of the immune system to recognize and remove cancerous cells is not enough to prevent the prevalence of the disease. Understanding the role that the immune system plays on health, and how it protects against some excessive growth of cells, has made scientists wonder if it could play a more substantial role in cancer control. One of the first to see this connection was a physician in the late nineteenth century, Dr. William B. Coley, who noticed that a cancer patient's recurrent and inoperable sarcoma seemed to be cured after suffering from a case of Erysipelas, a *Streptococcus* bacterial skin infection (Coley 1910). Between 1891 and 1893, after this initial observation, Coley inoculated ten patients who had inoperable tumors with various cultures of Erysipelas bacterium. In all cases, the tumors

decreased or “softened” with the inoculations. These inoculations did not always lead to a symptomatic occurrence of the disease, but when they did the effect on the tumor was greater, with substantial and sustained decreases in tumor size (Coley 1893). In his 1893 article discussing these ten patients, Coley also mentions thirty-eight additional cases of cancer patients who also had an Erysipelas outbreak. Of these thirty-eight, 17-41% of the patients were cured while the majority of the rest had temporary improvements. While Coley’s work did not lead to a mainstream treatment for various reasons, his work is now seen as the basis of current cancer immunotherapy (McCarthy 2006). Coley’s daughter is the founder of the Cancer Research Institute, which awards the annual William B. Coley Award for Distinguished Research in Basic and Tumor Immunology. Both of this year’s Medicine or Physiology Laureates have received the Coley award in different years (Cancer Research Institute).

Coley’s initial work suggested that there might be ways to boost the immune system to get it to recognize more of the rogue cells that lead to cancer. This proved difficult to figure out and the question of why cancer is not recognized and stopped by immune systems had remained unsolved for nearly a century. In the late 1970s some believed that interferon, another cytokine released by immune system cells, could be a miracle molecule used to stimulate the immune system to attack tumor cells. However, it proved to be too dangerous and has not become a widespread treatment (Piore 2017). Allison (1995) postulated that the lack of immune system response to cancer is due to a lack of costimulatory ligands in addition to the antigen-MHC complex. By the early 1990s, it was understood that it was necessary for CD28 to bind to the ligand B7 in order to fully engage the immune system by activating T cells. Townsend and Allison (1993) showed the necessity of this costimulatory binding, and identified that it was specifically $CD8^+$ T cells, and not $CD4^+$, that were being activated. With the recognition of the necessity of costimulation, some researchers began to look at it as a way to boost the immune system to treat cancer. However, along with the excitement this possibility presented, it was also recognized that manipulating the immune system had its own dangers. Allison himself acknowledged this but believed that the science would prove

successful despite the obstacles (Allison, Hurwitz, and Leach 1995). By March 1996, his lab published findings that pushed the science even further and overcame at least some of the obstacles (Leach, Krummel, and Allison 1996). Around this same time, Honjo was making similar discoveries and observations with PD-1 and its ligands (Ishida et al. 1992; Agata et al. 1996; Nishimura et al. 1998).

CTLA-4

Even before the identification of TCR in 1987, it was understood that there were multiple steps needed to activate T cells. It was also understood that some sort of mechanism was necessary to keep T cells in check and shut them down when their job was done. One such mechanism is CTLA-4, which acts as a T cell brake. It was identified in 1987 and classified as belonging to the immunoglobulin superfamily (IgSF), which is a family of proteins that facilitates cell-cell communication and also includes both MCHs and TCRs (Brunet et al. 1987). Early studies of CTLA-4 showed it to be homologous to CD28 in both mice and humans, and this homologous nature initially led to the suggestion that the proteins are also analogous (Lafage-Pochitaloff et al. 1990; Gross, St John, and Allison 1990; Howard, Rochelle, and Seldin 1991). However, in 1994 scientists discovered that while CTLA-4 and CD28 shared about 30% amino acid identity, and both bind to B7 ligands, their roles are antagonistic; CD28 acts as a costimulator of T cells while CTLA-4 acts more as a brake (Walunas et al. 1994). Krummel and Allison (1995) published the results of their work confirming this finding a year later.

In addition to the discovery of the antagonistic nature of CTLA-4, more was also learned about the protein's proliferation and affinity. The activation of T-cells via the binding of CD28 to B7 (and TCR binding to MHC) produces cytokines, including interleukin-2 (IL-2), which not only drive further T cell proliferation, but also upregulates the presence of CTLA-4 on the T cell surface (Walunas et al. 1994). The expression of CTLA-4 mRNA increases a few hours after T cells become activated. The mRNA then transcribes the CTLA-4 protein which beings to show on the T cell surface, becoming prolific by 48 hours after T cell activation

(Krummel and Allison 1995). Once on the cell surface, CTLA-4 begins to compete with CD28 for binding to B7. Although the maximum CTLA-4 expression is less than half of that of CD28, it binds with B7 with about a 20-fold greater affinity than CD28 does and thus outcompetes the costimulator protein easily (Krummel and Allison 1995; Linsley et al. 1992). As CTLA-4 begins to bind, blocking the ability of CD28 to bind, the proliferation and activation of the T cell diminishes and eventually stops. In addition to slowing down and stopping T cell activation, CTLA-4 has also been shown to have a role in T cell apoptosis, or cell-death (Gribben et al. 1995). One way that CTLA-4 is regulated so that the brakes are not pressed on the immune system too soon, is by phosphorylation of CTLA-4 which stabilizes it. When un-phosphorylated, CTLA-4 has a half-life of about two hours and is relatively quickly removed from the T cell surface. T cells also require a certain amount of TCR-antigen-MHC binding signal in order for CTLA-4 for be activated and mobilized to the surface, preventing CTLA-4 from becoming prominent on the cell surface before needed (Egen, Kuhns, and Allison 2002).

As the knowledge that CD28 acts as accelerant and CTLA-4 acts as a brake became better understood, adding to the overall evidence of how T cells work, researchers began seeing possibilities for immune suppressing drugs for autoimmune disorders. Allison, however, began thinking about how the new understanding of T cells could be used to turn the immune system against cancer (Piore 2017; NobelPrize.org 2018b). In the winter of 1995, Allison's lab performed a quick and simple experiment of administering anti-CTLA-4 antibodies to mice injected with tumors. The results were so surprising that they immediately repeated the experiment, replicating the same astonishing results of CTLA-4 leading to a complete rejection of tumor growth (NobelPrize.org 2018b).

Allison's experiments consisted of mice being injected with tumor cells of a colon carcinoma variant and then either anti-CTLA-4, anti-CD28, or left untreated. Mice that were injected with the full dose of tumors (4×10^6 tumor cells) and then either left untreated or treated with anti-CD28 all developed tumors and were euthanized by the 35th day. These same results were seen in mice that were injected with a half

dose (2 x 10^6) of cells and left untreated. However, the mice that were treated with anti-CTLA-4 after receiving a full dose of tumor cells had some initial tumor growth that soon ceased, eventually decreasing until they were ultimately rejected. These cured mice were then re-injected with related, but not identical, wild-type tumor cells. Most of these re-exposed mice did not have any tumor growth, and in those that did, the growth was slow. Mice that were treated with a lower dose of tumor cells (1 x 10^6) were also examined. This group developed persistent tumors even after anti-CTLA-4, but at a slower rate than the non-anti-CTLA-4 mice. Additional experiments with mice being treated with anti-CTLA-4 at different intervals following an injection of 2 x 10^6 wild-type cells showed that while all mice benefited, those with a delayed anti-CTLA-4 treatment had a greater benefit in tumor control. Similar results were seen with mice injected with different doses of rapidly growing fibrosarcoma tumor cells, rather than the colon carcinoma cells (Leach, Krummel, and Allison 1996).

It was unclear exactly how anti-CTLA-4 was having these effects, but Allison and his team had two ideas. The first was that preventing CTLA-4 from binding with B7 (because it was blocked with the anti-CTLA-4) allows more time for those T cells that would remain unreactive in normal circumstances to become active, leading to more activated T cells that could detect and remove the tumor cells. Another hypothesis was that blocking CTLA-4 allowed tumor-specific T cells to remain active, and proliferate, for longer. Whatever was actually happening, these experiments showed that CTLA-4 blockade results in an increased immune response to tumors, even un-manipulated wild-type tumors. (Some previous work had been done on manipulated tumor cells, which benefited understanding but would prove unsuitable for in-vivo treatment.) The results also suggested that CTLA-4 blockade works best when there are more tumor cells (Leach, Krummel, and Allison 1996). This may be due to tumors not expressing B7 in sufficient amounts to sustain T cell activation. The more tumor cells, and thus pieces of tumor cells present, the better the chances were of T cells finding and responding to the tumors (Egen, Kuhns, and Allison 2002).

Around 2000, studies of CTLA-4 blockade on fourteen patients dying of metastatic melanoma saw three of them have their tumors decrease (Piore 2017). According to a 2002 review of research done on anti-CTLA-4, it has been shown to be effective in mice affected with lymphoma as well as prostatic, renal, and colon carcinomas, in addition to melanoma. However, it is not always effective. Studies have shown it to be ineffective against a specific melanoma and mammary carcinoma. Additional studies have shown that when combined with other drugs including tumor vaccines, the effect is greater (Egen, Kuhns, and Allison 2002). In early 2010, the results of a phase II clinical trial involving 155 advanced melanoma patients was published. The results of the study were positive even though over a quarter of the participants withdrew from the study due to adverse events, mostly immune related (O'Day et al. 2010). In March of 2011, ipilimumab, brand name Yervoy, was approved by the FDA as a CTLA-4 blocking drug for use against melanoma (Keegan 2018). Ipilimumab binds to CTLA-4, releasing the brake and allowing T cells to be more active. While it does boost the immune response against cancer cells, it also leads to an overactive immune system for normal self-cells which leads to side effects, sometimes quite serious ones. It is primarily currently used against melanoma of the skin (American Cancer Society). As of October 17, 2018 there are clinical trials examining the use of ipilimumab to treat non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), and metastatic hormone-refractory prostate cancer (SEER*Rx).

PD-1

Another brake for the T cell system is Programmed Death-1 (PD-1), which was isolated in 1992 by Tasuku Honjo's lab from an apoptosis-induced T cell and found to be another member of the immunoglobulin gene superfamily (Ishida et al. 1992). Early research of PD-1 suggested that it contributes to inducing cell-death, but it does not do it on its own (Ishida et al. 1992; Agata et al. 1996). Along with the hypothesis that PD-1 induces cell death, other possibilities considered were that it could be a phagocytosis marker, a cell rescuer, or that it just happens to be expressed around the same time as cell death (Ishida et al. 1992).

Later research from the same lab suggests that PD-1 plays a role in the helper or cytotoxic functions of T cells, or in the actual development of thymocytes (immature T cells), rather than, or in addition to, having an inhibitory effect on proliferation (Agata et al. 1996).

By 1997, PD-1 had been identified in humans, but its role in cell death was still questionable with research instead suggesting its role is actually in T cell activation and differentiation (Vibhakar et al. 1997). Around this same time however, Honjo's lab found, that at least with B cells, PD-1 does act as a negative regulator (Nishimura et al. 1998). The following year they proposed that a deficiency in PD-1 leads to lupus-like diseases, most likely due to the removal of a T cell inhibitor allowing the immune system to get out of control and start attacking the self (Nishimura et al. 1999). These later results correlate with the earlier suggestion that PD-1 does negatively regulate activated T cells by binding with a ligand on the APCs that stimulated the T cells.

The APC ligand that PD-1 binds to, PD-L1, is in the same gene family as B7, downregulates immune responses, and has been shown to lead to autoimmune disorders when lacking (Freeman et al. 2000; Nishimura et al. 1999). Contrary to CTLA-4 which is only found on activated mature T cells, PD-1 is found on thymocytes, activated mature T cells (as well as marginal amounts on unstimulated), activated B cells, and myeloid cells (Agata et al. 1996; Freeman et al. 2000). It was found that the most immature T cells, those that are still $CD4^-$ $CD8^-$, have a high amount of PD-1 (34%) and that they lose some of their PD-1 as they mature to double positives ($CD4^+$ $CD8^+$). Eventually the cells either die or fully mature to single positive (either $CD4^+$ or $CD8^+$) where their unstimulated PD-1 expression stabilizes around 3-5% (Nishimura et al. 1996). PD-1 mRNA is only found in the thymus, where T cells are created, while CTLA-4 mRNA is found in T cells throughout the body (Agata et al. 1996). Another difference from CTLA-4 is that PD-1 seems to come into play at a later stage of the immune response, perhaps leading to the less severe outcomes in mice that lack PD-1 compared with those that are deficient in CTLA-4. Additionally, while B7 ligands are not found in organs, PD-L1 is found in various organs including the heart, placenta, skeletal

muscle, and lung. It can also be expressed on some tumors, suggesting that some cancers may inhibit antitumor immune responses via PD-L1 (Freeman et al. 2000). Latchman (2001) found that T cell proliferations is inhibited by either PD-L1 or PD-L2 binding with PD-1. PD-L1 has a stronger effect than PD-L2, but both lead to decreased activation of T cells, and neither seems to lead to an increase in T cell death. This inhibition is partly a result of decreasing the synthesis of cytokine mRNA, including IL-2 whose lower levels are not able to stimulate additional T cells. Hamanishi (2007) expands on the previous research showing that tumor cells are able to avoid immune response in part due to expressing PD-Ls. In this research, the PD-L1 levels of ovarian cancer patients were compared and the results show that tumors with higher levels of the ligand have poorer prognosis than those with lower ligand expression. Less drastic, to the point of not being scientifically significant, but similar results were seen when comparing PD-L2 expression on the tumor cells. The differences in prognosis was suggested to be related to the additional finding that the higher the level of PD-L1 expression, the fewer number activated $CD8^+$ T cells were present, and vice versa.

A study published in early 2012 discusses significant findings in the effectiveness and related adverse effects of anti-PD-L1 against various types of advanced cancers. Patients in this study had advanced cases of melanoma or non-small cell lung, renal-cell, ovarian, colorectal, pancreatic, gastric, or breast cancers. By the end of the nearly three-year study, 6% to 17% of patients had positive results in regards to both tumor regression and prolonged stabilization of their disease. The success rates varied by types of cancer and researchers noted the unexpected success rate (10%) in patients with non-small cell lung cancer, which had previously proven to have a poor response to immunotherapy treatments. While investigators attributed adverse effects due to treatment in 61% of the participants, most of these effects were mild with around eleven patients (5%) suffering from serious treatment related adverse effects. Over the period of the trial, 6% of patients ended up withdrawing from the study due to adverse effects of the treatments and forty-five, or 22%, of the enrolled patients died, mostly from progression of their disease.

Most of the adverse effects fell into the categories of fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus, and headaches (Brahmer et al. 2012). Overall, this study adds to the research suggesting that immunotherapy that works on PD-1 and/or its ligands could be effective against a wide range of cancers and have fewer, and more mild, adverse effects than other current immunotherapy treatments.

Starting in 2014, the FDA began approving anti-PD-1 and anti-PD-L1 drugs. Opdivo, the brand name of nivolumab, was approved in December 2014 for the treatment of patients with unresectable or metastatic melanoma. Opdivo was further approved for metastatic squamous non-small cell lung cancer with progression on or after platinum-based chemotherapy in March 2015. In September 2015, it was approved as a combination therapy with Yervoy for another specific type of metastatic melanoma. In the year between November 2016 and December 2017, Opdivo was further approved for various other treatments including advanced renal cell carcinoma as well as specific cases of recurrent or metastatic squamous cell carcinoma of the head and neck, classical Hodgkin lymphoma (cHL), locally advanced or metastatic urothelial carcinoma, mismatch repair deficient (dMMR) and microsatellite instability high (MSI-H) metastatic colorectal cancer, and melanoma with involvement of lymph nodes. These specific cases usually involve failure to improve after standard treatments including chemotherapy, radiation, and surgery. Opdivo was also recently approved for combination treatment with Yervoy for previously untreated advanced renal cell carcinoma in April 2018 (SEER*Rx).

According to the American Cancer Society, PD-1 is a checkpoint protein that acts as a brake on T-cells by binding to its ligands, PD-Ls, which prevents T-cells from attacking other cells. PD-L1 is on normal and some cancer cells in differing levels which alters the natural resistance to an anti-tumor immune response. Some drugs in this category block PD-1 and some block PD-L1. The PD-1 inhibitors include pembrolizumab (Keytruda), nivolumab (Opdivo), and cemiplimab (Libtayo), and are currently FDA approved for use against melanoma, non-small cell lung cancer, kidney cancer, bladder cancer, head and neck cancers, and Hodgkin lymphoma. The PD-L1 inhibitors include atezolizumab (Tecentriq), avelumab (Bavencio), and durvalumab

(Imfinzi) with current FDA approval for bladder cancer, non-small cell lung cancer, and Merkel cell skin cancer (Merkel cell carcinoma). Both types, as well as CTLA-4, are currently being studied individually, and in combination, for a variety of other cancers. The end result of either anti-PD-1 or anti-PD-L1 drugs is that PD-1 and PD-L1 cannot bind together and thus the T-cells never get the feedback to slow down, leaving them to attack the tumor cells. However, this inability to slow can also lead to T-cells attacking normal self-cells resulting in immunologic adverse effects. These adverse effects include fatigue, coughs, nausea, loss of appetite, skin rash, and itching, as well as some more serious, although less frequent, problems in organs including the lungs, intestines, liver, kidneys, and hormone-making glands (American Cancer Society).

Current and future science/significance

Allison's and Honjo's discoveries led to immune checkpoint therapies for a small variety of hard-to-treat cancers. Their research has made significant contributions to the fight against cancer and helped to lay the foundation for additional significant discoveries. There are three primary areas that current research is focusing on. Anti-CLTA-4, anti-PD-1, and anti-PD-Ls all work by releasing the brakes on the immune system and sending it into overdrive, which is effective but also releases the immune system on normal self-cells leading to autoimmune adverse effects. Additional research may figure out ways to focus the overactive immune system on tumor cells, reducing the adverse effects of the treatments. Additionally, the current treatments in place are for a select number of cancers, and often only used after the other treatments of surgery, radiation, and/or chemotherapy have already failed. With the variety and severity of cancers affecting humans, additional research that identifies ways to use these brakes to set the immune system against more cancers is needed. Finally, there is still a lot to be understood about T cell activation and the mechanisms of action of checkpoint blockades. It is possible, even likely, that a better

understanding of how these mechanisms work could lead to treatments for more cancers with fewer adverse effects.

Adverse effects

Current research suggests that there is wide range of types and severity of adverse effects to ICI, including autoimmune disorders such as type 1 diabetes and myocarditis which can be serious and even fatal (Wei, Duffy, and Allison 2018). At the beginning of 2018, researchers collected data from Vigilyze-Vigibase, the World Health Organization (WHO) database of individual safety case reports and adverse drug reactions, and from clinical trials found by searching PubMed. Analysis of this data found a broad range of effects that ended up causing fatalities in 0.3%-1.3% of treated patients, most often early in the treatment phase (with a median of 40 days for monotherapy and 14.5 days for combination immunotherapy). Particular regiments seem to elicit different adverse effects. Some of the more common effects observed include colitis/diarrhea, hepatitis, pneumonitis, neurologic events (mostly encephalitis and myasthenia gravis), and myocarditis. Some of the even rarer effects include dermatologic events (such as toxic epidermal necrolysis), hematologic events (including hemophagocytic lymphohistiocytosis, hemolytic anemia, and idiopathic thrombocytopenic purpura), and endocrine toxic effects (hypophysitis and adrenal insufficiency). As use of ICI treatments has increased over the years, the reporting of the fatal effects has also increased with myocarditis resulting in the highest risk of deaths. Out of all the anti–PD-1/PD-L1 and combination therapy deaths reported, 65% of them were reported in 2017 and January of 2018 alone (Wang et al. 2018). Although the fatal toxic effects are rare, the diversity of causes, the high rates of cancer, and the continued increased use of ICI indicates that research looking into these effects could have a substantial impact.

Since his early discoveries, Allison has been concerned with the adverse effects of ICI and he personally continues to work on possible solutions. Along these lines, Allison and fellow researchers have expressed frustration towards the lack of preclinical animal models that would aid in the understanding of the

adverse effects. The current animal models do not reliably recreate immune related adverse events in a way that allows investigations into the actual mechanisms of the events (Wei, Duffy, and Allison 2018).

More cancers

Although Yervoy, the anti-CLTA-4 drug, was the first ICI drug approved, its use has not been as widely adopted as others have. This is due to its effectiveness being so far limited to just a couple types of cancer and to its adverse effects being more pronounced and sustained than those with anti-PD-1 or anti-PD-L1 drugs (NobelPrize.org 2018b). Checkpoint therapy against PD-1 or PD-L1 is used against more cancers than CTLA-4, yet the number of cancers currently treatable by these regiments, and the number of patients that actually benefit, remains relatively low. Allison, among others, is currently researching why treatment is not as effective on different cancers, or even different individuals who suffer from the same cancers (Piore 2017).

In April 2018, the FDA approved the combination therapy of Yervoy and Opdivo against renal cell carcinoma. By July 2018, the combination treatment had been additionally approved for specific types of colorectal cancers that have progressed despite treatment with chemotherapy (SEER*Rx). Currently, there are numerous clinical studies looking at combination therapies that target both CTLA-4 and PD-1/PD-L1. This combination treatment has been shown to be more effective against melanoma than monotherapy, and researchers are looking to see if similar results can be seen in other cancers. In addition to these combination trials, there are other checkpoint therapy trials targeted towards a wide range of cancers and new proteins are also being examined as potential targets (NobelPrize.org 2018b).

Researchers are also looking at combining ICI therapies with other anti-cancer treatments, not just each other. A phase-3 clinical trial recently published their results showing that treating metastatic triple-negative breast cancer, an aggressive treatment-resistant cancer, with a combined regimen of immunotherapy and chemotherapy improved patient survival. In the trial, the anti-PD-L1 immunotherapy drug atezolizumab was coadministered with nab-paclitaxel, a chemotherapy drug. The results showed

that this combination treatment extended the survival and improved the prognosis of patients. The study also showed that both PD-L^- and PD-L^+ tumor cells were affected, indicating that even tumor cells containing the T cell stopping ligand can be suppressed. Although almost 16% of the patients stopped treatment due to adverse effects, none of these effects were due to the combination of the drugs; the effects were all those that would be expected with monotherapy of either drug (Schmid et al. 2018). Similar work has been done looking at combining radiation and ICI as it is thought that radiotherapy may result in a "tumor microenvironment" that would allow blockade therapies to have a greater effect. Both preclinical and clinical studies have been done looking at this combination of treatments and results have shown outcomes overcoming monotherapy resistance. Radiotherapy seems to boost the immune system in a way that allows blockade treatments to further delay tumor growth. This may be due to radiation breaking double-stranded DNA, which causes tumor cell death and the release of tumor antigens as well as increased expression of MHCs, chemokines, and other molecules that further activate and prime T cells. Further research is needed to explore this treatment combination, including exploring different tumors, dosages, types of blockade therapy, radiotherapy fractionation, and timing intervals of the two treatments (Lamichhane et al. 2018).

Another area of research that is being examined, both for efficacy with various cancers as well as to improve adverse effects of the treatment, is the use of nanoparticles and liposome-mediated delivery of checkpoint inhibitors. It is thought that certain obstacles such as T cells' ability to get to the tumor, low expression of receptors and/or ligands, and high expression of immunosuppressive ligands may be overcome by using nanoparticles or liposomes to deliver treatment directly to the tumors. This targeted delivery may also result in a decrease of adverse effects since self-cells would avoid the T cells (Lamichhane et al. 2018).

According to a search of clinicaltrials.org on November 7, 2018 there are 1,938 trials looking at checkpoint therapies as cancer treatments. The search entered was: Cancer as condition or disease, and (checkpoint

OR CTLA-4 OR PD-1 OR PD-L1 OR PD-L2) as other terms. Clinicaltrials.gov expanded the search to broaden the terms, and these broadened terms were checked to ensure only trials on cancer with the selected treatments were found. When the search was expanded to also include (immunotherapy OR immuno-oncology) as other terms, the number of relevant trials jumped to 3,783, with 2,234 taking place in the USA. These nearly four thousand studies are looking at 789 different cancer conditions. Of all of these trials, ninety-seven of them had no participants enroll and have the current status of withdrawn. Another 2,067 of them are either currently recruiting or not-yet recruiting, suggesting that they are fairly new trials.

Mechanism of action

The numerous clinical trials looking at a variety of possible ICI treatments are mostly working off the current and limited knowledge of T cell reactions to tumor cells. In a 2018 review of immunotherapies for cancer, it was found that of the 940 immuno-oncology agents in clinical trials, 164 target PD-1 or PDL-1, showing that there is considerable duplication in the clinical trials. The same antibodies are being tested against a wide range of targets leading to a "fragmented and uncoordinated" path to discovering new treatments. The reason for this is likely the excitement of the possibilities and potential for breakthroughs, but it is leaving behind other research that could be just as, if not more, impactful. For example, even with all the ongoing studies there are still not any that directly compare anti-PD-1 and anti-PD-L1 to see if there may be differences in efficacy or safety (Tang, Shalabi, and Hubbard-Lucey 2018). Gregory B. Lesinski, Associate Professor and Co-Director of the Translational GI Malignancy Program at Winship Cancer Institute of Emory University, stated in an interview that "the pace of the clinical applications of the science is much faster than understanding mechanisms in the lab" (Piore 2017).

Allison is another researcher who believes that additional essential research needs to be done in order to understand the underlying biological mechanisms of the immune system and its response to tumor cells. In a recent review, he and his colleagues discuss how many aspects of checkpoint blockades, including T

cell activation itself, remain largely unknown. In addition to CD28 (CTLA-4's costimulatory homolog) there are many proteins, from different superfamilies, that act as T cell costimulatory molecules and some of these are currently being examined in preclinical and clinical trials for their therapeutic use even though their biological functions are not fully understood. Allison believes that there is still much to know about how these molecules, including CTLA-4 and PD-1, work with T cells on molecular, cellular, and physiologic levels and that there are likely other unknown variables to be discovered (Wei, Duffy, and Allison 2018). One way his lab is working towards a better understanding of the mechanisms is by utilizing an "immunotherapy platform" which analyzes patient tumor samples pre and post ICI treatment on a molecular level in order to identify why, and how, the treatments work or fail. One recent discovery from the platform is that prostate tumors do not have much immunologic activity, a discovery which has led to a clinical trial of Yervoy and Opdivo combination treatment (Piore 2017).

As with most things in science, the current Immune Checkpoint Inhibition therapies have come about due to the work of many researchers, not just Allison and Honjo. It will take more research done by more scientists to further advance cancer immunotherapies. A very recent immunotherapy article, published November 2018, is an example of this as it discusses a way to identify what specific antigens are present on a patient's particular tumor cells so that the needed TCR can be added to the patient's cells, allowing T cells to be activated and attack the tumor cells (UCI News 2018; Segaliny et al. 2018).

The scientists

James P. Allison

Life, education, and career

James (Jim) P. Allison, born on August 7, 1948 in Alice, Texas, is the youngest of three sons born to Albert and Constance Allison. Allison, whose father was a doctor, had an interest in science from an early age, having been given a chemistry set by his father and creating a science lab in his garage (Cavallo 2014; Park

2017; Kärre 2018). This interest was further cultivated by his eighth grade math teacher and later by his high school guidance counselor who encouraged him to take a biology course at the University of Texas (UT) during his senior year of high school when he refused to take the high school's biology class due to it not covering evolution. (At Allison's high school, creationism was taught in favor of evolution.) Allison was already familiar with the UT system as the summer before his senior year he attended a University of Texas at Austin (UT Austin) science-training program, which was funded by the National Science Foundation (Cavallo 2014). After graduating high school at age sixteen, Allison continued at UT Austin earning a BS in microbiology in 1969 and a PhD in biological sciences in 1973. He was then a postdoctoral fellow at Scripps Clinic and Research Foundation in their department of molecular immunology from 1974 - 1977 (MD Anderson Cancer Center ; Cornwall 2016, 67).

After his postdoctoral work, Allison returned to Texas where he was one of the first scientists at the new University of Texas MD Anderson science research center in Smithville, Texas. He worked there as a biochemist from 1977 - 1983 (MD Anderson Cancer Center 2018). In addition to his biochemist role, he was an Assistant, then Associate, Professor in the department of biochemistry at the Smithville Cancer Center, and Adjunct Assistant Professor in the department of Zoology at UT Austin. It was during his time in Smithville that he made his discovery of T cell receptors in 1982. With this discovery came a move to Stanford University School of Medicine, where he was a Visiting Scholar in the department of pathology from 1983 - 1984, and then to University of California, Berkeley where he was a Professor in the department of molecular and cell biology, division of immunology from 1985 - 2004. While at Berkeley, Allison was also a University of California, San Francisco School of Medicine Professor in the department of medicine, division of rheumatology (MD Anderson Cancer Center). It was at Berkeley where the Nobel winning work on CTLA-4 started, leading to the drug ipilimumab. Clinical trials of ipilimumab began taking place in New York in the early 2000's and Allison moved there in order to be closer to the trials, and to gain further credibility in the field (Piore 2017; Cavallo 2014). During this time, he worked as a Professor

at Weill Cornell Medicine as well as an Attending Immunologist (2004 - 2012) and Director of the Ludwig Center for Cancer Immunotherapy at Memorial Sloan-Kettering Cancer Center (2006 - 2012). Allison was also a Howard Hughes Medical Institute Investigator from 1997 - 2012 (MD Anderson Cancer Center).

In 2012, Allison returned to Texas where he continues to hold many positions at MD Anderson Cancer Center in Houston. He is the interim Chief Scientific Advisor, Vivian L. Smith Distinguished Chair in Immunology, Director of the Parker Institute for Cancer Immunotherapy, Chair of the Department of Immunology in the Division of Basic Science Research, and Deputy Director of the David H. Koch Center for Applied Research of Genitourinary Cancers (MD Anderson Cancer Center). Allison is also leading the SU2C-CRI Cancer Immunology Translational Research Dream Team, which is performing comprehensive experiments aimed at improving the ability of immunotherapy to improve patient outcomes (Cancer Research Institute). In 2013, Allison and others started Jounce Therapeutics, a company that works to further develop cancer immunotherapy approaches (Piore 2017). Another role Allison holds is that of Executive Director of the Immunotherapy Platform, which is a platform created by his longtime collaborator Padmanee Sharma to track immune responses to various cancers, working with over 100 MD Anderson immunotherapy clinical trials and collaborating with pharmaceutical companies (MD Anderson Cancer Center 2018). It was in discussions of the platform when Allison first told Sharma he loved her (Piore 2017). They married in 2014, after he proposed by saying “nobody else can stand either one of us - we might as well get married” and Sharma agreed, noting “I hate it when he's right” (Ackerman 2015). The platform is also part of the National Cancer Moonshot Initiative whose Blue Ribbon Panel is made up of twenty-eight individuals, including Allison, representing various scientific areas, clinical trial and cancer health disparities experts, cancer advocacy groups, and pharmaceutical and biotechnology companies (MD Anderson Cancer Center 2018; National Institutes of Health 2016). After the announcement of the Nobel, Vice President Joe Biden, initiator of the Cancer Moonshot, called Allison directly to congratulate

him (Lopez 2018). Allison's Nobel is the first to be awarded to a MD Anderson scientist (MD Anderson Cancer Center 2018).

Allison initially thought that he would follow in his father's footsteps and become a doctor. Upon realizing that doctors cannot make mistakes, he changed his focus to become a science researcher, where mistakes and testing hypotheses are part of the job. However, his interest was in basic immunology, not in cancer. This interest in immunology, and the then little-known T cells, led to his two major discoveries of TCR and CTLA-4's role as a brake. These discoveries ended up being highly personal as he had seen his mother, two uncles, and brother all suffer and die from cancer (Cavallo 2014). He himself has been diagnosed with both prostate cancer and melanoma (Park 2017). Allison often mentions the benefits of scientific research, believing that basic research is key to future growth, success, and major discoveries and requires the past and future work of many scientists, including his own graduate students, postdoctoral fellows, and colleagues (MD Anderson Cancer Center 2018; Lopez 2018). Allison, who has referred to himself as a mouse guy, is humble, determined, refuses to just go with the conventional wisdom, and motivated by pushing "the frontiers of knowledge" (Cavallo 2014; MD Anderson Cancer Center 2018). He continues to work on basic and focused science, digging deeper into immune checkpoint inhibitors and various cancers including melanoma, lymphoma, and lung, breast, gastric, kidney, and prostate cancers (Cavallo 2014).

Allison's work has earned him numerous awards. According to his MD Anderson faculty page, Allison has received fifty-five awards between 1986 and 2018, with most years since 2001 receiving at least one. He received at least five awards a year in 2011, 2014, 2015, 2016, and 2018. A few of these awards, in addition to the 2018 Nobel in Physiology or Medicine, have been shared with Tasuko Honjo. In 2014, Honjo and Allison were among the first scientists to be awarded a Tang Prize when they received the first Tang Prize for Biopharmaceutical Science. They also share the 2016 Fudan Zhongzhi Science Award and both earned a William B. Coley Award for Distinguished Research in Basic and Tumor Immunology, in different years. Other selected awards that Allison received include: 1986 National Institutes of Health Merit Award, 2008

Dana Foundation Award in Human Immunology Research, 2011 American Association of Immunologists Lifetime Achievement Award, 2011 Roche Award in Cancer Immunology and Immunotherapy, 2013 Economist Innovations Award in BioScience, 2014 Association of American Medical Colleges Distinguished Research in the Biomedical Sciences Award, 2014 National Foundation for Cancer Research Szent-Gyorgyi Prize for Progress in Cancer Research, 2015 Society for Melanoma Research Lifetime Achievement in Melanoma Research Award, 2015 American Cancer Society Medal of Honor for Basic Research, 2015 Lasker-DeBakey Clinical Medical Research Award, and two Honorary Doctorates in 2016 from Rockefeller University and Icahn School of Medicine at Mount Sinai (MD Anderson Cancer Center). In 2013, Science magazine cited Allison's work as crucial when naming cancer immunotherapy as the Breakthrough of the Year (Cavallo 2014). Allison was also named one of Time Magazine's 100 most influential people in 2017, under the category of Titan (Time Magazine 2017).

Beyond his love of science, Allison also has a love of music, especially the blues. He plays the harmonica with the Checkpoints, a national band made up of other doctors and scientists, and the Checkmates, a local band. He once played with Willie Nelson at a local bar and has since been invited to play with him again (Ackerman 2015).

Bibliometrics

An Author Search in Web of Science (WoS) on November 14, 2018 found 435 items indexed for James P. Allison since 1983 (which is as far back as the WoS analysis currently goes). Allison has published multiple times every year since 1983, often publishing over ten, and even twenty, articles a year. In 2013, he had 22 articles published (see Figure 1). His 435 items have been cited 50,271 times, averaging over 1,396 citations a year and over 115 citations per item (see Figure 2). When self-citations are removed, the number of total citations is still high at 48,818. These citations come from 32,472 citing articles, the majority of which are by researchers besides Allison (32,220 citing articles without self-citations). Allison's h-index is 109.

[Figure 1 - Allison publications per year]

FIGURE 1 James P. Allison, Number of Published Items in Each Year. Data source: Web of Science. Retrieved 14 November 2018.

[Figure 2 - Allison citations per year]

FIGURE 2 James P. Allison, Number of Times Cited in Each Year. Data source: Web of Science. Retrieved 14 November 2018.

Many of Allison's articles have been cited multiple times and eight of them have been cited over a thousand times each. His most cited article, having been cited over two-thousand times, is "Restoring function in exhausted CD8 T cells during chronic viral infection" published in *Nature* in 2006. The other articles that make up his five most highly cited articles have all been cited at least 1,460 times (see Table 1). While he often publishes in Nature, he most often publishes his works in *The Journal of Immunology*, with almost 12% (51 publications) of his articles being published in the journal. It was the new *Journal of Immunology* where Allison first published his identification of TCR. The other journals he has published in the most all have least twenty of his publications (see Table 2).

TABLE 1. Top Five Most Highly Cited Papers by James P. Allison.

[Table 1 - Allison top five cited articles]

TABLE 2. Top Five Journals Published in by James P. Allison.

[Table 2 - Allison top journals published in]

Tasuku Honjo

Life, education, and career

Tasuku Honjo was born in Kyoto, Japan in 1942 (NobelPrize.org 2018b). As a young child, he was curious about many things including science and from an early age he was fascinated by the story of Hideyo

Noguchi, the Japanese bacteriologist who discovered the cause of syphilis, among other things. The story of Noguchi's life and achievements was a strong motivator for Honjo (Honjo 2012). Although he was smart and curious, Honjo was not very studious as a child. It was not until junior high that he began to focus on his studies as he debated between studying to be a doctor, lawyer, or diplomat (Honjo 2003). Honjo decided to pursue becoming a doctor, partly due to his father being a doctor, and took premedical and medical courses at Kyoto University Faculty of Medicine from 1960 - 1966 (Honjo ; Cornwall 2016, 148).

While an undergraduate, Honjo read the book *The Revolution in Biology* by Atsuhiro Shibatani which introduced him to molecular biology and the future of DNA (Honjo 2003). This book added to his conviction that the way to discover more about the molecular mechanisms of life was through molecular biology (Honjo 2012). In 1966, Honjo earned his MD from Kyoto University then did a year internship at Kyoto University Hospital followed by returning to Kyoto University to study Medical Chemistry from 1967-1971 (Honjo). During this time, Honjo made his first major discovery, which gave him both the confidence and opportunity to further his studies. This discovery was that the protein synthesis factor EF-2 is inactivated when it undergoes ADP-ribosylation catalyzed by diptheria toxin (Honjo 2012).

After his graduate studies, Honjo visited the United States as a Fellow at Carnegie Institution, Department of Embryology from 1971-1973 and then as a Visiting Fellow and Associate at the National Institute of Child Health Laboratory of Molecular Genetics from 1973-1974. After his time in the United States, Honjo returned to Kyoto earning his PhD in 1975 and working as an Assistant Professor in the Department of Physiological Chemistry and Nutrition, Faculty of Medicine at University of Tokyo in Tokyo, Japan until 1979. From 1979-1984 he was a Department of Genetics Professor at the Osaka University School of Medicine in Osaka, Japan. In 1984, Honjo returned to Kyoto University where he continues to do research. From 1984-2005 he was a Professor in the Department of Medical Chemistry while also filling other roles including Director of the Center for Molecular Biology and Genetics (1988-1997) and Dean of Kyoto University Faculty of Medicine (1996-2000 and 2002-2004). During this time he was also a Professor in the

Department of Molecular Genetics at the Institute of Neurological Disease Hirosaki University School of Medicine in Aomori, Japan (1989-1997), Science Adviser at the Ministry of Education, Culture, Sports, Science and Technology (1999-2003), and Director of Japan Society for the Promotion of Science Research Center for Science Systems (2004-2006). Since 2005, he has been a Professor in the Department of Immunology and Genomic Medicine at Kyoto University Faculty of Medicine, and since 2006 has been an Executive Member of The Council for Science and Technology Policy Cabinet Office (Honjo).

Honjo has been described as a quiet man who is fascinated by immunology and molecular biology (Kyoto University). He has also been described as determined, using his ninety-minute train commute to collate data from the day's work. This determination led to numerous important discoveries prior to and since his Nobel winning PD-1 discovery. One of these discoveries was the identification of a class switch model for antibodies that is mediated by DNA recombination and deletion of DNA segments. He went on to find the molecule responsible for this, Activation Induced cytidine Deaminase (AID), identifying it as a master gene for both class switch recombination as well as somatic hypermutation (Honjo 2012). He says his key to success is being able to closely examine the problem he is trying to solve (Honjo 2003). Honjo also remains humble despite his numerous accomplishments. He often states his luck with timing and fellow researchers he has the opportunity to work with, often mentioning the great work of other researchers (Honjo 2012, 2018). He credits two of his major discoveries (AID and PD-1) as coming from a curiosity about basic biology. Honjo encourages young scientists to look at basic questions, saying that the curiosity of scientist is what takes research into unknown areas and that curiosity-derived science is critical (Honjo 2012).

Honjo's dedication to science has earned him numerous awards. There are the ones he has in common with Allison: The first Tang Prize for Biopharmaceutical Science in 2014, the 2016 Fudan Zhongzhi Science Award, and a William B. Coley Award for Distinguished Research in Basic and Tumor Immunology. He has also been awarded the 1981 Noguchi Hideyo-Memorial Award for Medicine, 1981 Asahi Prize, 1984 Osaka

Science Prize, 1984 Kihara Prize of the Japanese Genetics Society, 1985 Erwin von Baelz Prize, 1988 Takeda Medical Prize, 1992 Behring-Kitasato Prize, 1994 Uehara Prize, 1996 Imperial Prize and Japan Academy Prize, 2000 Persons of Cultural Merit Award by the Japanese Government, 2001 Foreign Associate of U.S. National Academy of Sciences, 2004 Thomson Leading Japanese Scientists in Emerging Research Fronts, 2012 Robert Koch Prize, 2013 Order of Culture, 2014 Japanese Cancer Association and Chugai Academy for Advanced Oncology (JCA-CHAAO) Award, 2015 Richard V. Smalley MD Memorial Award, 2016 Kyoto Prize, 2016 Keio Medical Science Prize, 2016 Pharmaceutical Society of Japan Award, and 2017 Warren Alpert Foundation Prize. Honjo was also a Fogarty Scholar-in-Residence at the National Institutes of Health from 1991-1996 and is an Honorary Member of the American Association of Immunologists, as well as belonging to other organization including the Human Genome Organization, The Japan Academy, and The German Academy of Natural Scientists (Honjo).

Besides being dedicated to science, Honjo has a strong interest in art and is an avid golfer, mentioning that he may become a professional golfer when he retires from the lab (Kärre 2018; Kyoto University).

Bibliometrics

A WoS Author Search on November 14, 2018 found 608 items indexed for Tasuku Honjo since 1983. Honjo's h-index is 118, having been cited 56,563 times (53,746 times without self-citations). These citations come from 33,587 articles (33,126 without self-citations). Every year between 1983 and 2011 he has published over ten articles a year, often times publishing over twenty per year and publishing thirty articles in 1988. He continues to publish multiple articles a year (see Figure 3). Being cited over fifty-thousand times, he averages over 1,571 citations times a year. Since 1985, he has been cited over two-hundred times a year, quickly jumping to over eight-hundred citations a year by 1988. After the year 2000, he is cited over a thousand times a year (see Figure 4).

[Figure 3 - Honjo publications per year]

FIGURE 3 Tasuku Honjo, Number of Published Items in Each Year. Data source: Web of Science. Retrieved 14 November 2018.

[Figure 4 - Honjo citations per year]

FIGURE 4 Tasuku Honjo, Number of Times Cited in Each Year. Data source: Web of Science. Retrieved 14 November 2018.

Honjo's 608 items have an average citation of over ninety-three each, with many having well over a hundred each. His most highly cited article is "Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme," published in *Cell* in 2000. The topic of this article is on one of the major discoveries Honjo made before PD-1 and it has been cited 2,108 times. His second most highly cited article was published in the same year and is on PD-1. "Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation" published in *The Journal of Experimental Medicine* has been cited 2,090 times. His other most highly cited articles are all on either AID or PD-1 (see Table 3). Honjo published in *Proceedings Of The National Academy Of Sciences Of The United States Of America* the most of any other journal, publishing fifty-seven, or over 9%, of his articles in the journal. He has published at least twenty articles in all of his other most published in journals (see Table 4).

TABLE 3. Top Five Most Highly Cited Papers by Tasuku Honjo.

[Table 3 - Honjo top five cited articles]

TABLE 4. Top Five Journals Published in by Tasuku Honjo.

[Table 4 - Honjo top journals published in]

Conclusion

As the rates and types of cancers continue to grow and cause suffering and death, researchers have continued looking for ways to fight the disease. The immune system has been investigated to this end for over a hundred years, but it was not until Allison's and Honjo's discoveries that the cancer immunotherapy field really began. Checkpoint therapy has provided new treatments for a variety of cancers and the research behind it has laid a foundation for further understanding on how to use the immune system to attack, and prevent, cancer. Getting to this point was not easy. One of the first individuals to look at the immune system and cancer, William Coley was mostly ignored or argued against for decades. Allison himself had many struggles getting his immune-oncology work to be taken seriously. Prior to his CTLA-4 findings, he struggled to get his TCR discovery published and noticed (Graeber 2018). He also remembers submitting his article discussing his discovery and receiving peer-reviewer recommendation for the journal to reject the article because "we all know that immunotherapy's crap. It's never worked" (Piore 2017). Then, even though there was strong research support, Allison had difficulties finding a pharmaceutical company that would be willing to work with him on taking his work beyond mice into humans (Cornwall 2016, 73).

The drug that came from Allison's work, ipilumimab, was the first checkpoint inhibitor and it and the drugs from Honjo's work have set the stage for others as well as for additional combination treatments. Opdivo (nivolumab) itself has recently been approved for treatment of eight different cancer situations (Piore 2017). These drugs are being tested independently, together, and in combination with other cancer treatments against a wide variety of cancers and patients. The future of cancer treatment will likely consist of combination therapies of one or more immunotherapy together or with other cancer treatments such as chemotherapy or radiation, in order to treat a wider range of tumors (Wei, Duffy, and Allison 2018). One of the exciting things about using the immune system against cancer cells is that the immune system has a memory and, once trained, will be able to recognize those cancer cells. It also will not develop a resistance to the treatment so it will continue working as long as the patient needs it (Cancer Research

Institute). The negative side of using the immune system is that it currently is not focused, sometimes causing negative side effects. However, the research started, and continued, by Allison and Honjo provides a starting point for further research on how to focus these treatments so the rest of the body is not affected.

Both Allison and Honjo have expressed the importance of continuing research in basic immunology in order to further their work and to make additional discoveries. They both also have thanked their luck and expressed gratitude at being able to have freedom in their research and ability to work on what they were interested in (Honjo 2012; Piore 2017; Graeber 2018). Their determination and hard work, combined with the freedom to explore, led to the discoveries resulting in this year's Physiology or Medicine Nobel. They continue to work, and encourage their fellow scientists to work, on making more discoveries to improve and expand immunotherapy cancer treatments.

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