ОГЛЯД

REVIEW

Protection of T-lymphocytes via PD-1 receptor: New molecular mechanism of cancer immunotherapy*

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After briefly reviewing major forms of cell death and discovery of programmed (cell) death 1 (PD-1) protein and its ligands (PD-L1 and PD-L2), this overview is focused on how PD-1 and PD-1 ligands are involved in a new form of in immunosuppression that represents a new approach to cancer therapy. Namely, tumor cells often kill cytotoxic T lymphocytes through PD-1 mechanisms – hence, PD-L1 inhibitors represent a new molecular mechanism of cancer immunotherapy. Recent translational clinical research also revealed a substantial influence of gut microbiome on response to PD-1-based cancer immunotherapy. The initial discovery was that cancer patients treated with antibiotics for various infections have diminished response to anti-PD-1 therapy. Comparing the fecal microbiota of responders to non-responders revealed increased abundance of *Akkermansia muciniphila, Faecalibacteria and Bifidobacteria* in patients showing favorable outcomes to anti–PD-1 treatment.

Conclusions: 1. Recent studies revealed how inhibition of certain cell death (e.g., cytotoxic T cells) via PD-1 leads to increased death of tumor cells. 2. The effectiveness of PD-1-based cancer immunotherapy is greatly influenced by gut microbiome (e.g., firmicutes and clostridia have positive, while bacteroidia exert negative effects). 3. Nevertheless, checkpoint inhibitors (via PD-1, PD-L1) represent a new and effective cancer immunotherapy, even in metastatic melanoma. 4. Thus, the discoverers of PD-1 and PD-L1 rightly shared the 2018 Nobel Prize in Physiology or Medicine.

Keywords: Cell death, apoptosis, programmed cell death, T-lymphocytes, PD-1 receptor, PD-1 ligands, tumor cells, cancer, immunotherapy, gut microbiome

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Захист Т-лімфоцитів за допомогою рецептора PD-1: новий молекулярний механізм імунотерапії раку**

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Після короткого огляду основних форм загибелі клітин та виявлення ролі першого білка (PD-1) у запрограмованій загибелі клітини та його лігандів (PD-L1 і PD-L2) у даній статті описано, як PD-1 та ліганди PD-1 беруть участь у новій формі імуносупресії, що є новітнім підходом до лікування раку. Зокрема, клітини пухлини часто вбивають цитотоксичні Т-лімфоцити через механізми PD-1. Отже, інгібітори PD-L1 є новим молекулярним механізмом для імунотерапії раку.

Нещодавні міждисциплінарні клінічні дослідження також виявили значний вплив мікрофлори кишки у відповідь на імунотерапію раку за допомогою PD-1. Первинно було виявлено, що онкохворі, які приймали антибіотики від різних інфекцій, мали менш виражену реакцію на PD-1 протиракову терапію. Порівняння фекальної мікробіоти пацієнтів із терапевтичним ефектом із пацієнтами без реакції виявило збільшення кількості бактерій *Akkermansia muciniphila, Faecalibacteria and Bifidobacteria* у пацієнтів із позитивними результатами лікування проти PD-1.

Висновки. 1. Недавні дослідження показали, як пригнічення загибелі певних клітин (наприклад, цитотоксичних Т-клітин) за допомогою PD-1 призводить до загибелі більшої кількості клітин пухлини. 2. Кишкова мікрофлора значно впливає на ефективність імунотерапії раку за допомогою PD-1 (наприклад, фірмікути та клостридії мають позитивний, а бактероїди - негативний впливи). 3. Утім, інгібітори імунних контрольних точок (через PD-1, PD-L1) є новою й ефективною імунотерапією проти раку, навіть у випадку метастатичної меланоми. 4. Відтак, першовідкривачі PD-1 і PD-L1 справедливо отримали Нобелівську премію з фізіології чи медицини у 2018 році.

Ключові слова: загибель клітин, апоптоз, програмована загибель клітин, Т-лімфоцити, рецептор PD-1, ліганди PD-1, клітини пухлини, рак, імунотерапія, мікрофлора кишки

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Reversible and irreversible cell injury (i.e., cell death), until recently, has been described as a spontaneous physiologic (e.g., apoptosis) or pathologic (e.g., necrosis) developments, without any connection to specific receptors. Mechanistically, cell membrane injury or mitochondrial or nuclear membrane damage (esp., in irreversible cell injury) has been described – but again, without any connection to receptors. It was then a surprise to the research community when the discovery of programmed (cell) death protein 1 (PD-1), also known as CD279 (cluster of differentiation 279), was announced in 1992 (1, 2). The discovery was regarded by many investigators as just another example of useless basic science research…

This assessment started to change when PD-1 ligands were discovered, especially after it has been established that PD-L1, the ligand on PD-1 is highly expressed on several cancer cells (2-4). Namely, when PD-L1 on tumor cells is attached to PD-1 on T-lymphocytes that normally infiltrate malignant tumors in attempt to kill the abnormal (cancer) cells, the lymphocytes become inactive and eventually die (5, 6). This is a very clever way of cancer to evade immune surveyance. i.e., the cancer cells kill the natural killer T-lymphocytes… (Fig. 1).

The unleashing of the natural immune system to attack cancer cells have been in the mind of many biomedical researchers, without much success. Even my research team (Fig. 2) that has been focused on the mechanisms of cell injury and death related to ulcerative and inflammatory diseases in the gastrointestinal (GI) tract (7, 8), started to investigate the possibilities of cancer immunotherapy, especially for the very aggressive non-small cell lung carcinoma (9). Nevertheless, our research has come to an abrupt halt when the project team leader and relatively young Martin Jadus, PhD was discovered to have an advanced lower esophageal carcinoma… Unfortunately, Martin passed away less than two years after the initial diagnosis… Because of his rapid demise, I would like to dedicate this short review article to the memory of our very creative, focused investigator Dr. Martin Jadus.
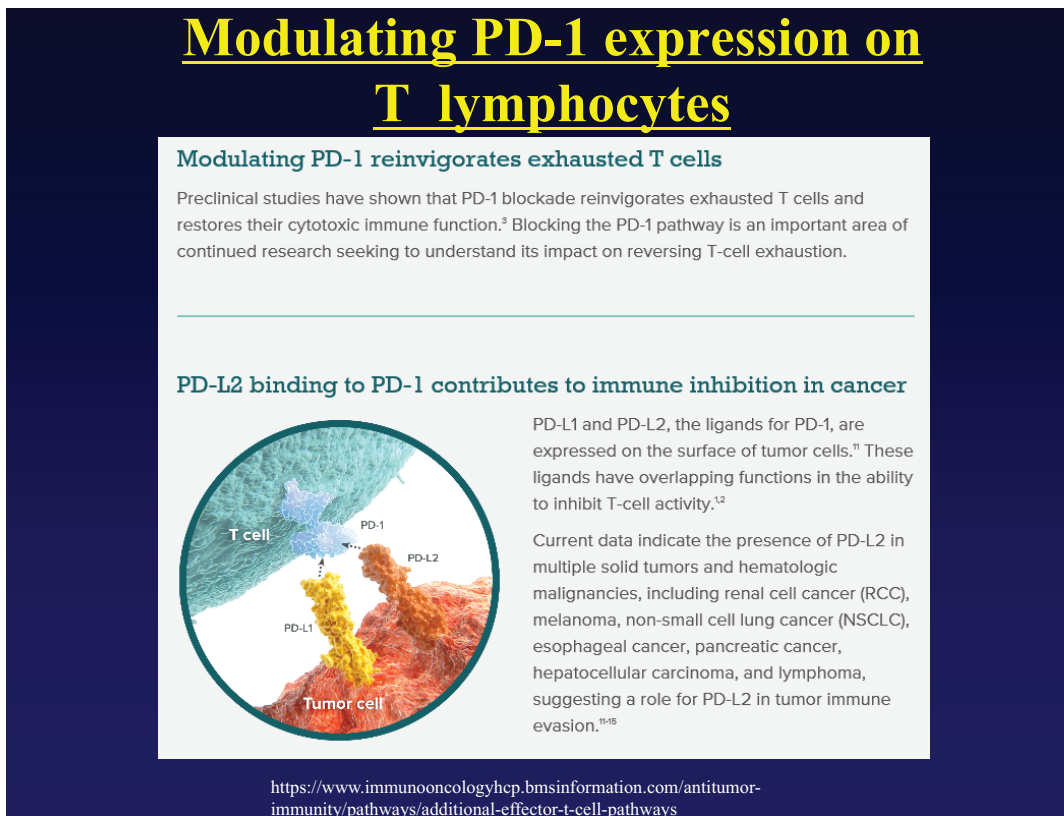

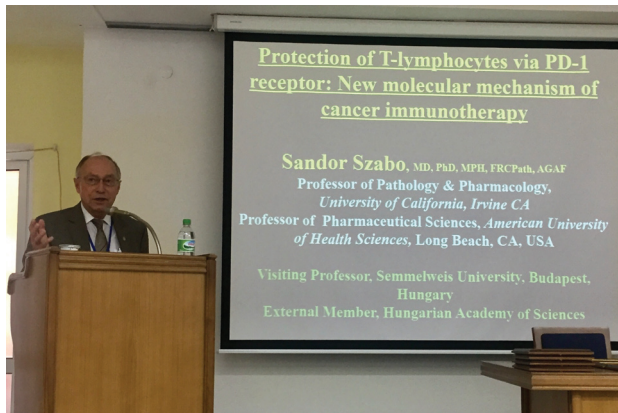Fig. 1. Modulating PD-1 expression on lynphocytes

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Fig. 2. Sandor Szabo during lecture, Lviv, 19.9.2019

The molecular and cellular mechanisms of lymphocyte-cancer cell interaction via PD-1
T- lymphocyte function and PD-1 expression is excellently illustrated in a video that is extracted and exemplified with four pictures in Fig. 3 (10). Namely, as T-lymphocytes in the vicinity of malignant solid or hematological tumor cells express more and more PD-1, they become increasingly exhausted and unable to exert their normal function, i.e., kill the cancer cells. Furthermore, in addition to increased expression of PD-1, other inhibitors of immune functions are also upregulated, essentially paralyzing the function of killer T-lymphocytes.

The Nobel Prize winners in Physiology or Medicine in 2018 James P.Allison and Tasuku Honjo (Fig. 4) realized that if we inhibit this interaction between PD-1 on T-lymphocytes and PD-L1 on tumor cells, natural killer lymphocytes will be allowed to exert their normal function, i.e., kill the invading cancer cells. Hence, the importance of checkpoint inhibitors and the justly awarded Nobel Prize for this major discovery (Fig. 4). The 2018 Nobel Prize also illustrates the importance of basic research that is often considered is not worth of major financial support. A recent analysis by Nature makes a strong point about this, i.e., that 2018 Nobel awards reinforce the continuous support for basic research – both intellectually and financially (11).

There is another important cellular element in the mechanisms of cancer immunotherapy, i.e., the antigen presenting cells (Fig. 4). Namely, although the surface of normal T-lymphocytes contains many antigen receptors that aim
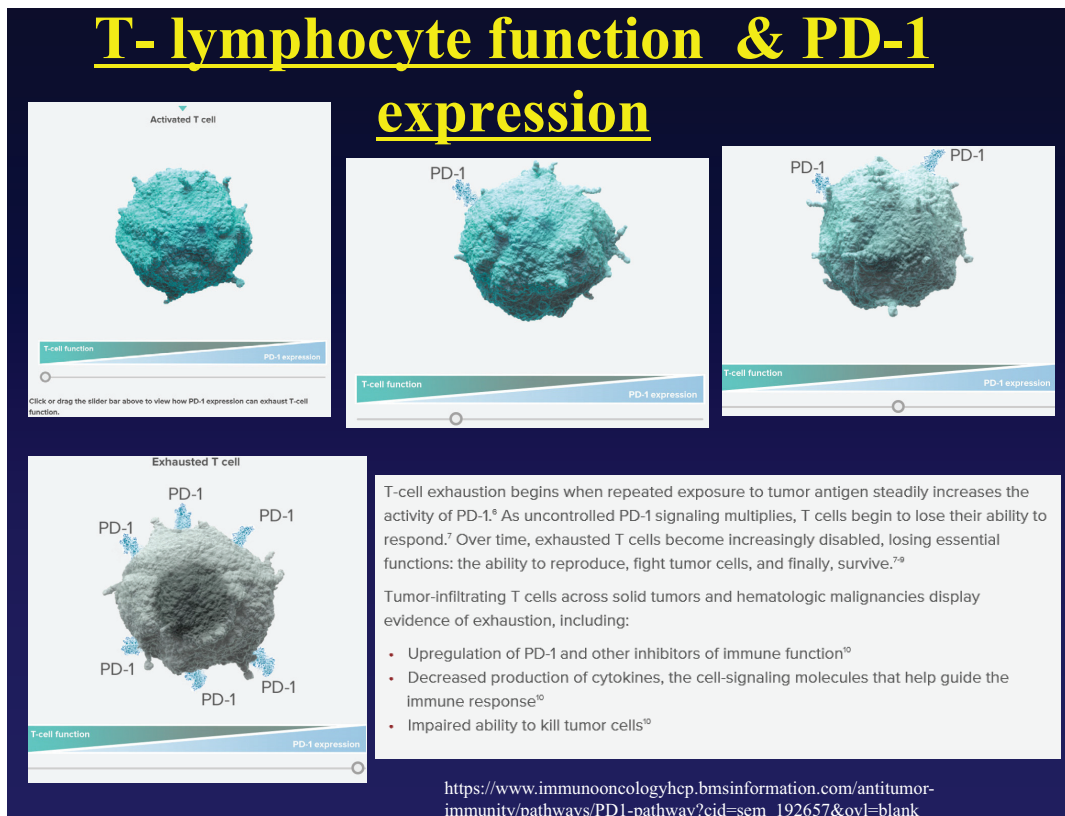

Fig. 3. T-lymphocyte function and PD-1 expression

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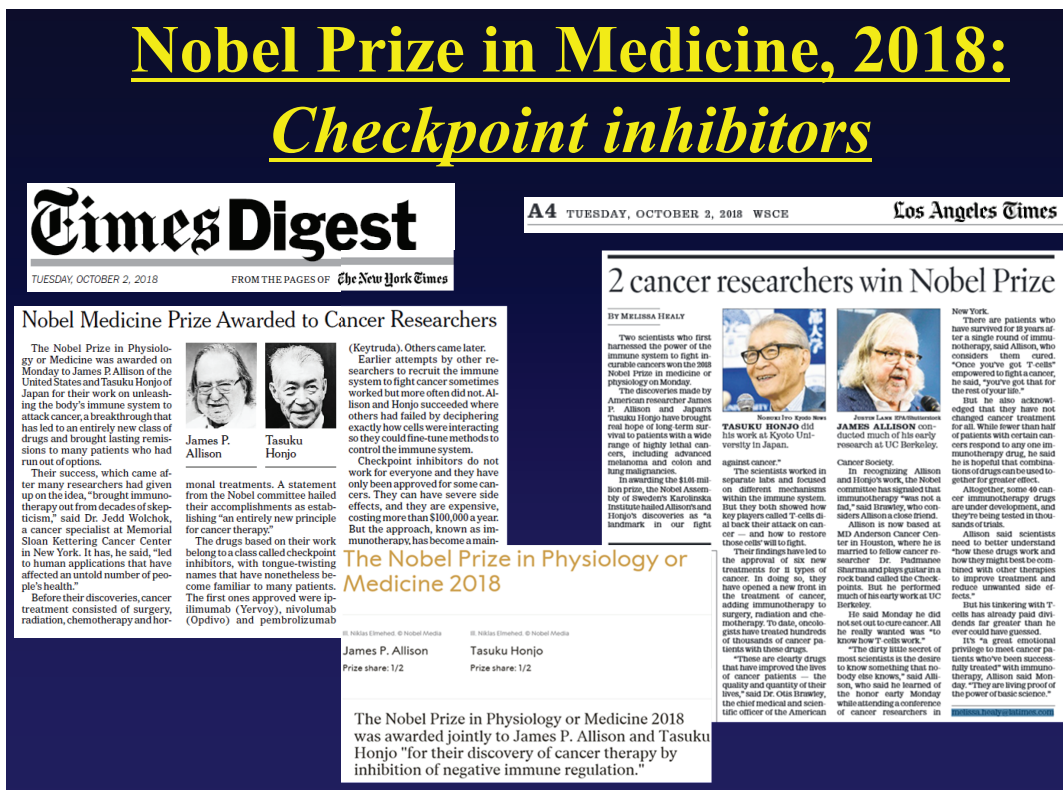ISSN 1563-3950   2019, Vol. 57, 2
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Fig. 4. Public media about the Nobel Prize winners in 2018 James P.Allison and Tasuku Honjo

to recognize foreign proteins, fragments of infected or cancer cells, killer T-lymphocytes cannot perform these functions without being activated by antigen presenting cells (12). These cells present the T-lymphocytes a cancer cell antigen, along with a costimulator and then the killer T cells start looking cells bearing this pathologic antigen and killing those cells.

Cancer cells are also very 'shrewd' and may avoid destruction by turning on the checkpoint inhibitor on T-cells that will essentially shut down, inactivate the killer T-lymphocytes, and cancer cells can proliferate and the malignant tumor grow undisturbed (Fig. 5). The new drugs, known as checkpoint inhibitors, can block these checkpoints, and then the immune system is free to destroy cancer cells. It is estimated that an activated T-call can kill thousands of malignant tumor cells.

Thus, the discovery of PD-1 receptor, its ligands and the checkpoint inhibitors unleashed our very powerful immune system to fight not only infections, but the survival and and proliferation of malignant tumor cells. This is essentially the triumph of human mind, ingenuity and basic

research over one of the most important medical problems of the 20th and 21st century. Namely, with the growth of ageing population, especially in Europe, Americas and Japan, cancer incidence increases with age, and we badly needed such a breakthrough in medical research.

Examples of effectiveness and challenges of the new cancer immunotherapy
One of the first examples of the clinical efficacy of checkpoint inhibitors, as related to PD-1 and its ligands came from clinical trials with one of the deadliest tumors of our times, i.e., metastatic malignant melanoma (13). Namely, the usually small malignant melanoma often remains undetected on skin surface and very often patients present with widely metastatic tumor disease when conventional chemotherapy and radiation therapy are ineffective and cannot be used. It's, unfortunately, not surprising that the 3-year survival of these patients is about 20% and the 5-survival drops to about 10%. In contrast, metastatic melanoma patients treated with checkpoint inhibitors the 3-years survival rates reached 50-60% and many patients remained free of cancer beyond 5 years. One of the most publicized cases is the dramatic
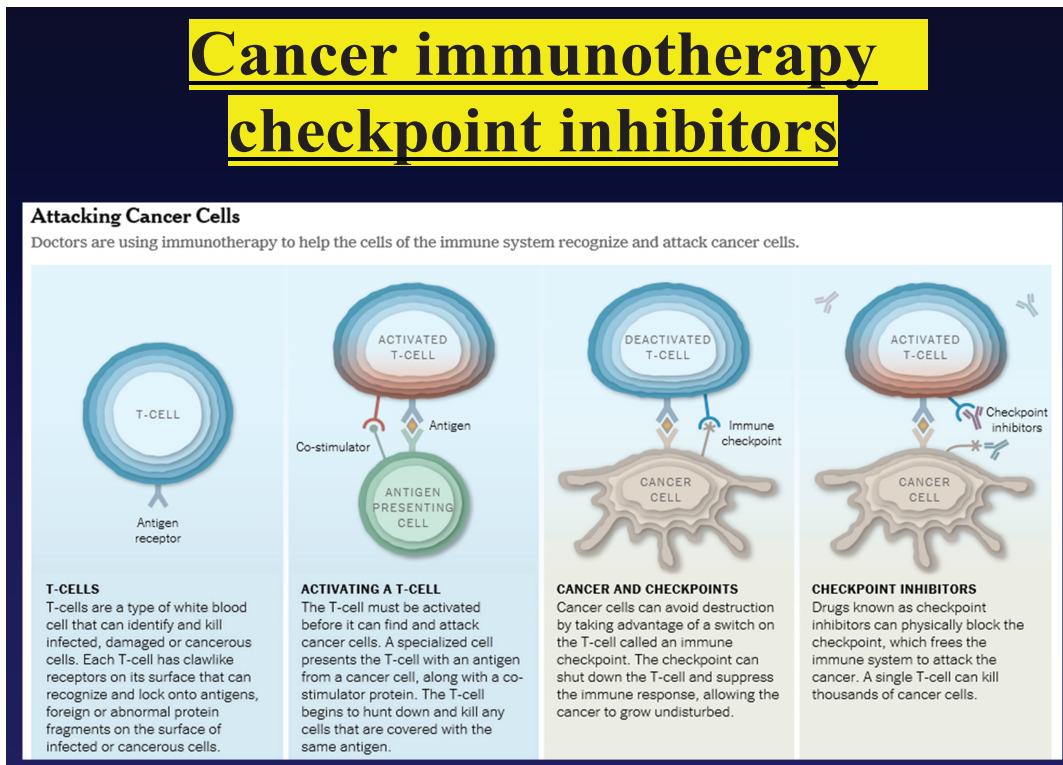
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Cancer immunotherapy checkpoint inhibitors

Fig. 5. Role of the antigin presinting cells in the mechanisms of cancer immunotherapy

effectiveness of new cancer immunotherapy is former USA president Jimmy Carter who was successfully treated for metastatic melanoma in 2015 and still remains not only healthy, but also very active 5 years later (14) in his "Habitat for Humanity" home/house building projects.

Other positive results with checkpoint inhibitors came from another equally deadly tumor which is also often diagnosed too late, i.e., non-small cell lung carcinoma (NSCLC), which was also the subject of our landmark review article with the late Dr. Martin Jadus (9). The outlook for this tumor was very dismal, about 10 years ago, but the new results with the novel forms of cancer immunotherapy are much more encouraging: the success rate in these clinical trials reached 60-70%.   Furthermore, PD-1 blocked was effective even in patients with NSCLC who didn't respond to other forms of cancer therapy (13).

Renal cell carcinoma (RCC) is also and frequently diagnosed late, often recognized just because of the manifestations and signs of malignant spread, usually to the lungs, liver and other organs.   Namely, RCC is retroperitoneal in origins and does not even

metastasize until it reaches about 2-4 cm in diameter (based on our clinicopathological observations). Fortunately, in the early clinical trials of RCC being treated with checkpoint inhibitors, the response and survival rates were also impressive, reaching about 60-80%.

If the reader of this article recognizes that even in the very promising clinical trials with checkpoint inhibitors in patients with metastatic melanoma, or NSCLC or RCC, the success rate never reached 90 or 100% - it's not a coincidence… This is not only because, despite all the standardizations in clinical trials, some patients have more advanced form of disease than others, - but as it was relatively early recognized, some patients just didn't respond or respond poorly, partially to PD-1 blockade. One of the main reasons for the no- or poor-response has been the composition of intestinal microbiota is certain patients. Two reports, published back-to-back in the same issue of *Science* in 2018 revealed that "gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients", and that "commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients" (15, 16) (Fig. 6). The analysis of
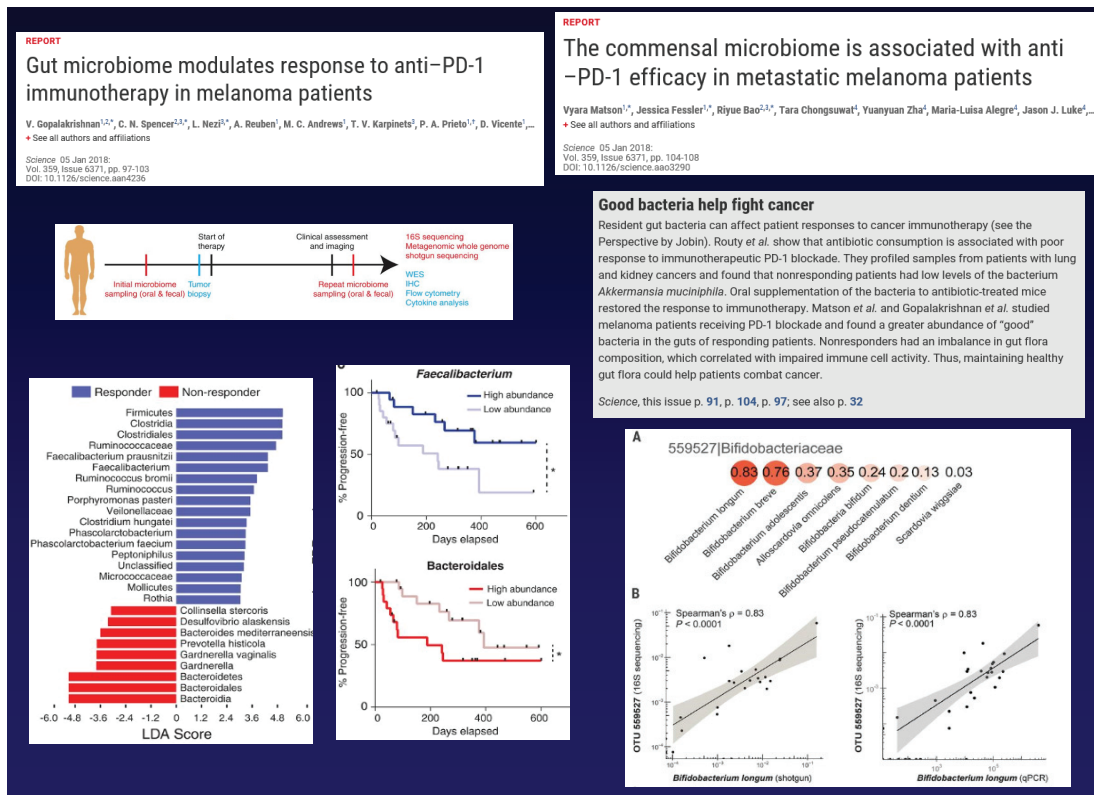
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Fig. 6. The role of out microbionie on modulation response to anti — PD-1 immunotherapy

gut microbiome I these patients revealed that some patents wit certain type of bacteria (e.g., Firmicutes, Clostridia, Clostridiales) responded well to anti-PD-1 therapy, while those patients who had predominantly other bacteria, like Gardnerella, Bacteroidetes or Bacteroidia didn't respond to this type of cancer immunotherapy.

These studies essentially demonstrated that "good bacteria help fight cancer" (15, 16). Examples of "good bacteria" for this purpose being Bifidobacteriaceae and similar bugs that showed a very strong positive correlation, as reveled by the Spearman's correlation coefficients, one of the strictest statistical analyses, with responders to cancer immunotherapy. And the influence gut microbiome on the efficacy of anti-PD-1 therapy is ot limited to malignant melanoma – it has been also demonstrated with cases of epithelial tumors, i.e., carcinomas (17).

The clinical trials that demonstrated these surprising results with the gut microbiome came from almost accidental observations… Namely, it was found that some patients treated with certain antibiotics responded better to anti-PD-1 therapy than others, implying and

essentially proving that eradication of certain bacterial special species leads to abundance of other bacteria. The altered balance of these gut bacteria may facilitate or blunt the efficacy of new forms cancer immunotherapy. Subsequently, this was proven by animal experiments: fecal microbiota transplantation (FMT) from nonresponding patients to PD-1 blockade to mice with experimental tumors had a very poor response to the to the new anti-PD-1 therapy in these animals (Fig. 6) (18). On the other hand, FMT from treated patients who responded to their anti-PD-1 therapy to mice with experimental cancer, confirmed that these animals responded very positively to PD-1 blockade. These basic and clinical investigations (15-19) were deemed to be so important that the *Science* magazine devoted its front cover illustration to this topic (Fig. 7).

The value of PD-1 pathways cannot be better summarized than the conclusion of a recent review article on this topic: "The PD-1/PD-L1 pathway in cancer is implicated in tumors escaping immune destruction and is a promising therapeutic target. The development of anti–PD-1 and anti–PD-L1 agents marks a new era in

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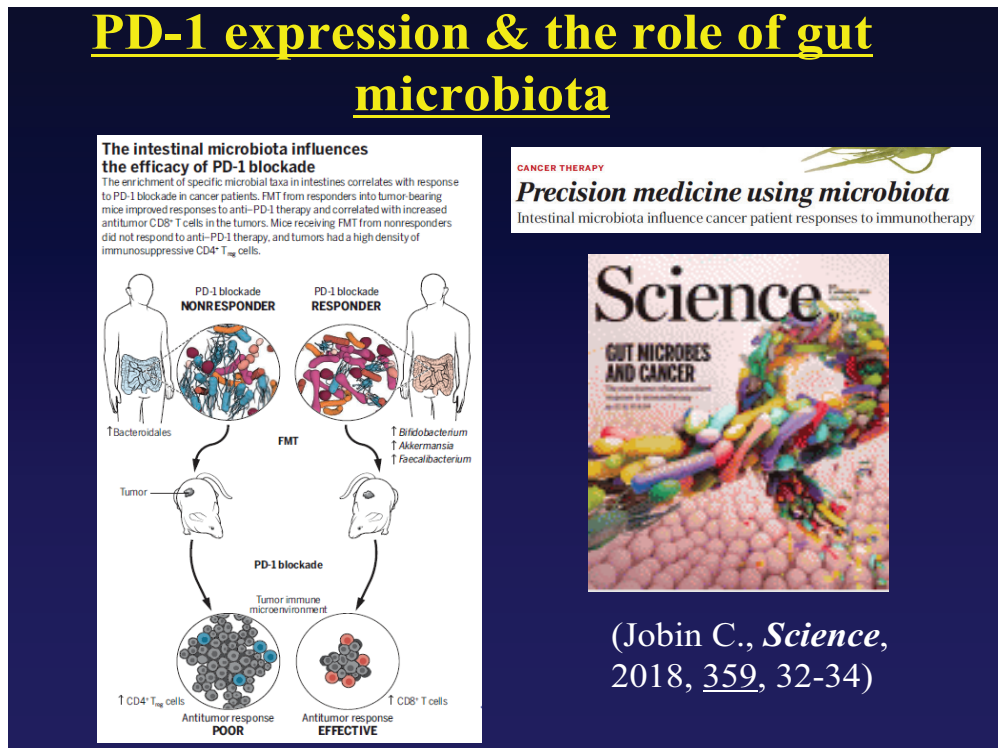ISSN 1563-3950   2019, Vol. 57, 2
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Fig. 7. Cover page of science, 2018, 359

the treatment of cancer with immunotherapies. Early clinical experience has shown encouraging activity of these agents in a variety of tumors, and further results are eagerly awaited from completed and ongoing studies" (19).

Discussion and conclusions: Translation medicine at best
Translation medicine is a very popular term nowadays, often used as 'lip service', without real and deep meaning… However, the story of the discovery of PD-1 receptor and its ligands as well as their rapid progression to clinical use are a really good example of translation medicine. Initially, investigating the mechanisms of cell and tissue injury seemed to be an unnecessary expense doing basic research. The discovery of PD-1 receptor on lymphocytes was also labelled as a 'so what – one more receptor'… Similar criticism and ignorance faced the discovery of PD-1 ligands on cancer cells… These attitudes suddenly changed when the 2018 Nobel Prize winners realized that these discoveries can be exploited in cancer immunotherapy. Hence, basic research is not an unnecessary expense, since it often leads to translational medicine and novel therapeutic interventions. Thus, it is not surprising that probably the oldest and

most prestigious scientific journals in the world, i.e., *Nature* used this 'from bench to bedside' discovery to emphasize the need and stimulate respect for basic research (11). A very recent issue of *Nature* also emphasizes the challenge of finding good targets in cancer therapy in the era after the success of anti- PD-1 success, and calls to attention that "many cancer drugs aim at the wrong targets" (20), adding that analysis using CRISPR gene-editing technology suggests that drugs' mechanism of action are often misunderstood.

The other obvious lesson and conclusion is that basic bench research, at least in biomedical sciences, needs to be followed up by clinical investigations, eventually by clinical drug trials. This is what often referred to as "reduce to practice" – that is, obviously, not always possible, as the early years after the PD-1 discovery illustrates. Nevertheless, this is one of the tenants of obtaining a high recognition, such as the Lasker award in USA and Nobel prize in Sweden. In our recent book (21) on the discovery of biologic stress by Hans Selye and on why he didn't get the Nobel prize (despite dozens of nominations), we also listed the three major requirements needed to get the highest

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recognition in medical sciences: a) original and creative discovery (i.e., not only following the next obvious and most likely step or pathway), b) which preferably should be linked to a new molecule or microbiol entity (e.g., new virus or bacterium), and c) the importance and practical use of the discovery should be proven by the results of clinical or epidemiologic investigations (i.e., as it was with the case with checkpoint inhibitors), not necessarily being performed by the original basic science investigators.

Besides the originality of initial discovery, the subsequent clinical investigations may also provide surprising new, original results, i.e., not only if the new drug is effective or not. The case in point is the recognition of the role gut microbiome in the efficacy of anti-PD-1 therapy, where – thanks to a few astute clinicians, who recognized that lack of response to the new cancer immunotherapy may be due to the fact that some patients were treated with antibiotics (presumably for an unrelated infectious disease) that affected the composition of their bacterial flora in their guts. This also symbolizes the unity of basic and applied medical research; one is not more important than the other and usually, one cannot success without the other.

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