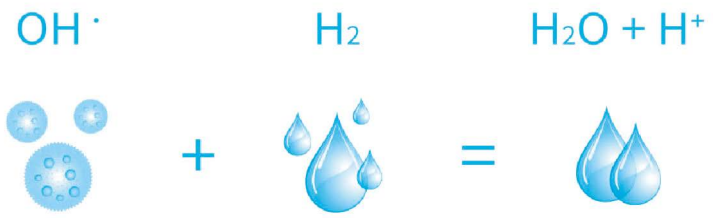

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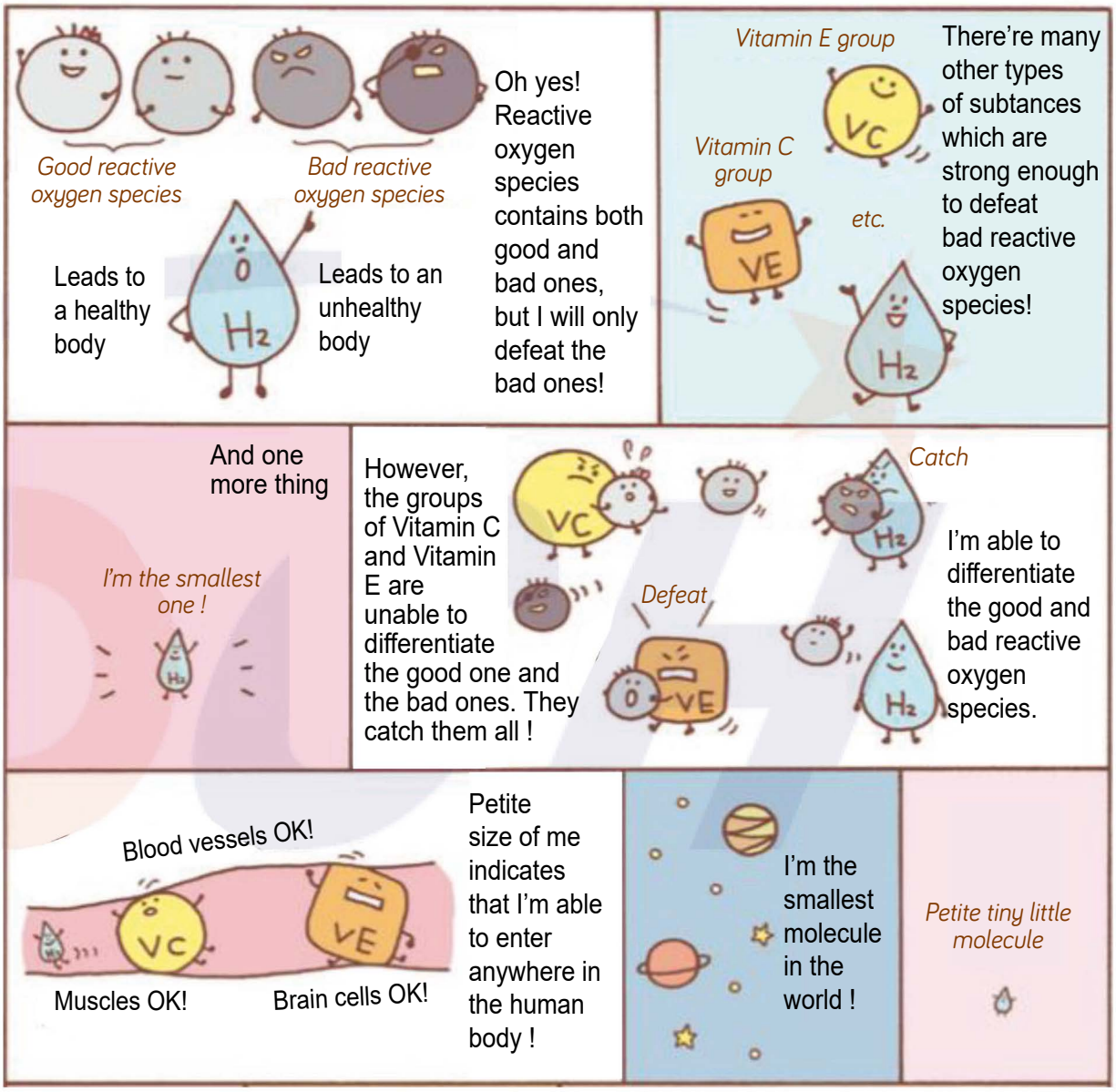
CONTENTS

The benefits & uses of hydrOxygen

Selective Antioxidant

Only targeting the most toxic free radicals: OH Hydroxyl, nitrous acid (HNO2).
Water (H20) is produced when the hydrogen molecules are combined with free radicals, and they're from the body via metabolism.

H2 + OH. → H20 + H+



Images Sources from The Power of Hydrogen

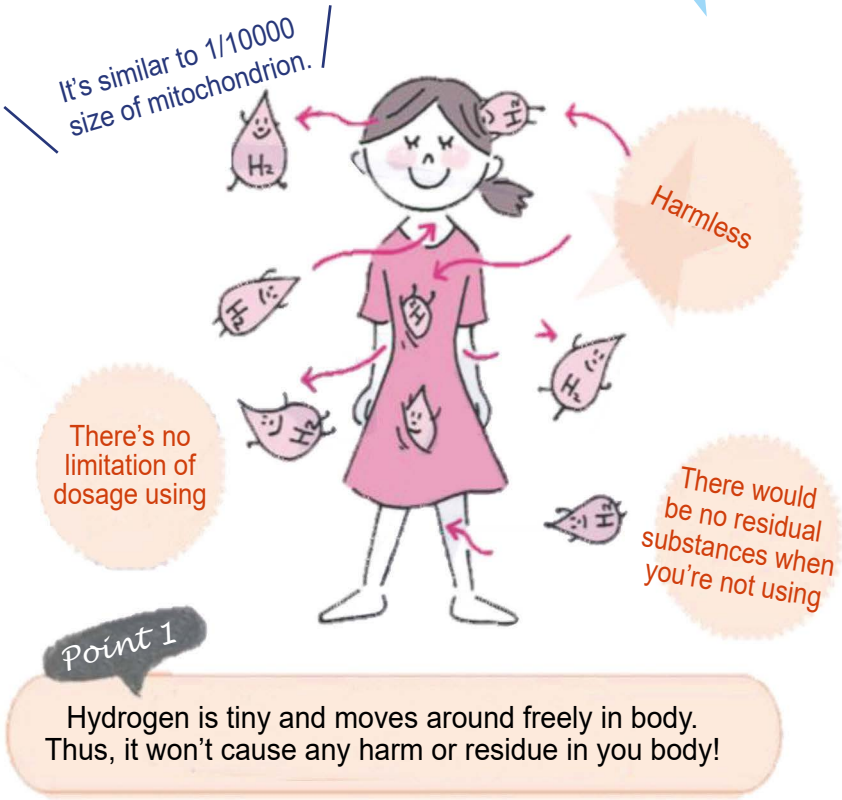
The benefits & uses of hydrOxygen

- **The penetrating power and diffusion force of hydrogen are very strong**

It diffuses over the whole body rapidly, thus it might provide a good impact to health-threatening diseases such as cancer and aging.

- **Hydrogen penetrates blood-brain barrier easily**

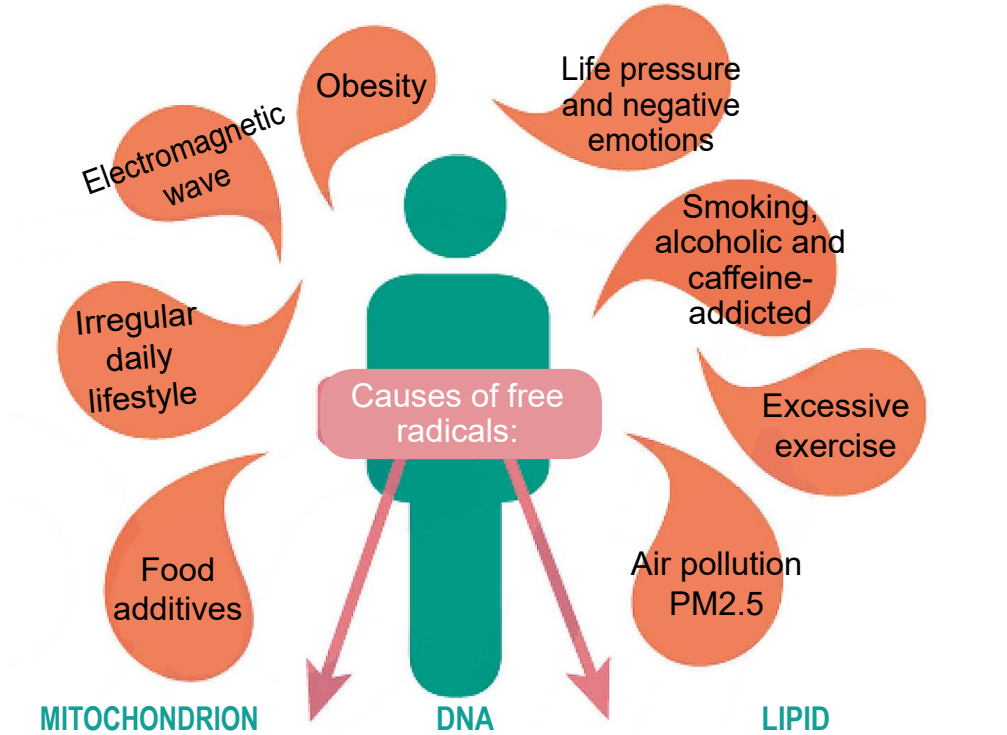
It improves senile dementia and Parkinson's disease. There's no other antioxidant that could make such health improvement.



Images Sources from The Power of Hydrogen

The benefits & uses of hydrOxygen

- Hydrogen neutralizes malignant free radicals
- Hydrogen is a good antioxidant
- Hydrogen has an anti-inflammatory properties
- Hydrogen enables cell-activation and improves immunity system



MITOCHONDRION	DNA	LIPID
Damages in competent cells	**Immunologic injury**	**Damages in blood vessels and vascular force**
Wrinkles White hair Cataract Freckles Ulcer Diabetes	Cancer Imflammation Allergic condition	High blood pressure Stroke Atherosclerosis Myocardial infarction Cold body Body numbness Dementia

Immuno-oncology OPDIVO (Anticarcinogen)

+

Hydrogen Oxygen Therapy

Immuno-oncology OPDIVO (Anticarcinogen)

Tasuku Honjo

The winner of Nobel prize in physiology of medicine in year 2018

The father of OPDIVO

- **1982 – Asahi Prize**
- **1984 – Kihara Prize, Genetics Society of Japan**
- **1988 – Takeda Medical Prize**
- **1994 – Uehara Prize**
- **1996 – Imperial Prize of the Japan Academy**
- **2012 – Robert Koch Prize**
- **2013 – Order of Culture**
- **2014 – William B. Coley Award**
- **2016 – Keio Medical Science Prize**
- **2016 – Thomson Reuters Citation Laureates**
- **2017 – Warren Alpert Foundation Prize**
- **2018 – Nobel Prize in Physiology or Medicine**

In 1992, Professor Tasuku Honjo first identified PD-1 as an inducible gene on activated T-lymphocytes and he continued his studies for about 22 years. In 1993, OPDIVO is legalized by the Japanese government. In 2015, OPDIVO had gained 7 types of certifications from FDA. In 2018, he won a Nobel Prize in Physiology or Medicine.

Professor Honjo said :" Whenever OPDIVO works on certain kind of patients, the medical outcome is very impressive! But, it sometimes doesn't work on some kind of patients."

The current cancer treatment including surgery, radiation therapy and chemotherapy, but such types of cancer treatment has their limitations and barriers. For instance, surgery and radiation therapy are only designed for local treatment. Besides, chemotherapy contains severe side effects which could cause death, although it helps to carry anticarcinogenic drugs all over the body in order to defeat cancer cells.
OPDIVO enables immune cell activation to defeat cancer cells. Hence, it poses less side effects and it could be used in any kind of cancer diseases. Also, it might still be helpful in terminal stage cancer as when OPDIVO works on you once you apply it, it will work continuously.
Professor Honjo predicts that the current anticarcinogenic drugs will be less popular in the near future and OPDIVO turns out to be the best cancer therapy.

Even though the price of maintaining is high, the selling price in Japan had lowered from 73000yen to 27000yen per bottle in four years and the selling price will furture drop to 17000yen in coming November, hoping to reduce the financial burden of patients. Moreover, there're some cases showing that the current mainstream treatments are ineffective for certain patients, but more studies will be carried out for this problem...
(China Times)

News from: https://www.chinatimes.com/realtimenews/20181009005236-260410

Professor Honjo : " This therapy is not effective for some patients "

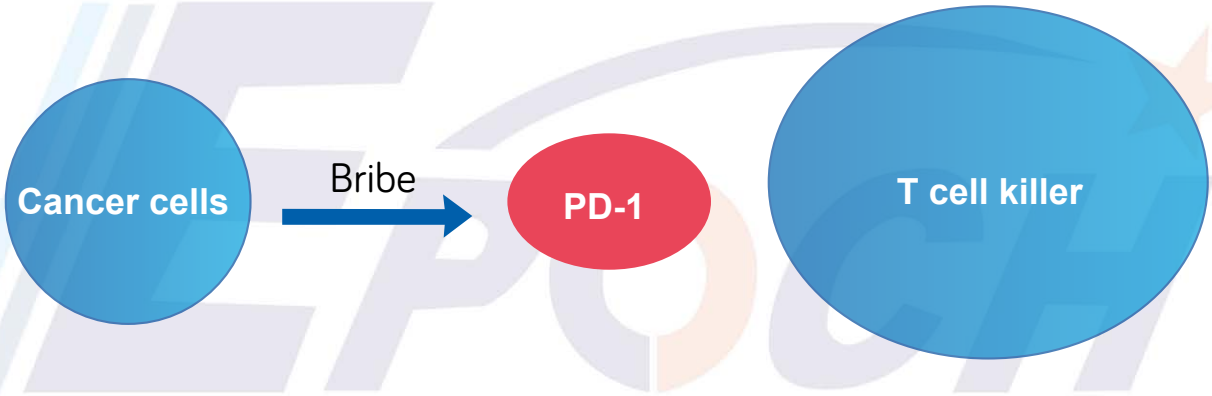
Professor Honjo claims that there're still some problems leave to be solved for the PD-1 anticarcinogenic drug, e.g. firstly, the therapy is not effective for some patients and cancer diseases, thus the future needs to be oriented towards a comprehensive anti-cancer strategy. Secondly, the efficiency of taking the medicine needs to be diagnosed precisely and quickly, so that they can grasp the anticarcinogenic opportunity and this has entered the cooperation stage with international pharmaceutical companies.

The medicine is only effective for 2-3% of the population

News from: https://udn.com/news/story/7241/3398007

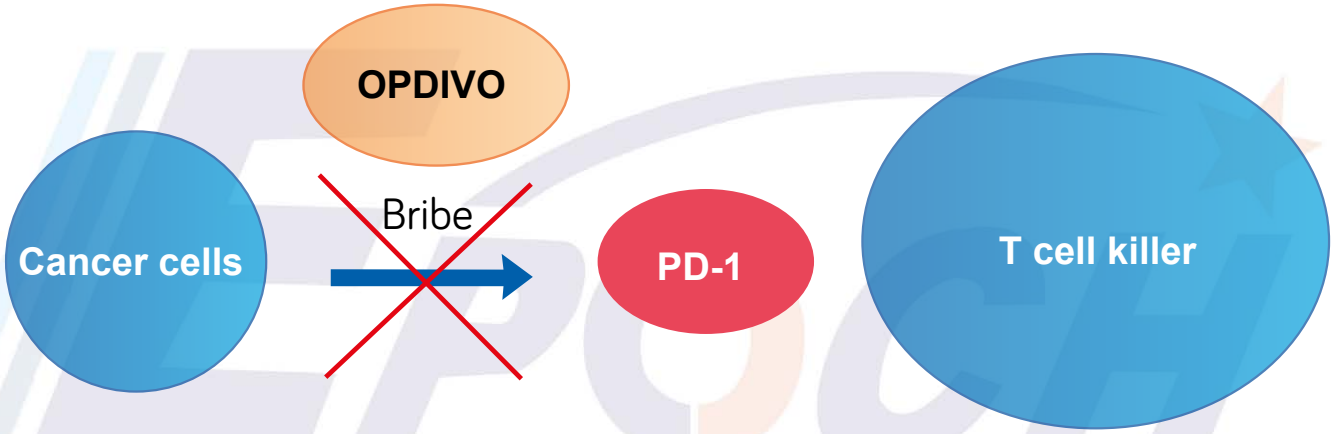
What's the medical purpose of OPDIVO?

PD-1 is the identification system in our immune system, to recognize our health enemy, such as cancer cell, viruses, bacteria, etc. After the identification, PD-1 will call the immune system to attack our health enemy!



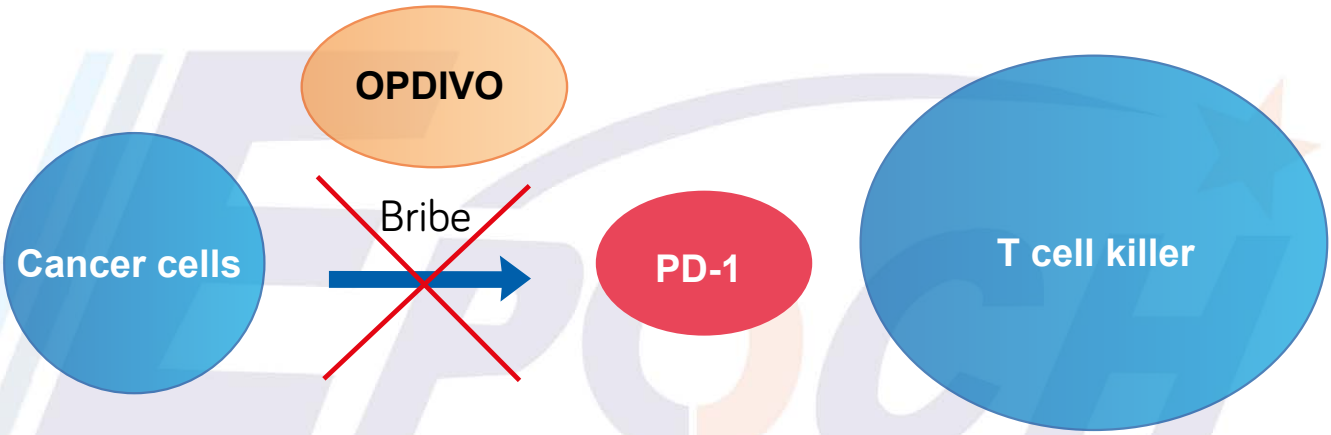
In medical science, cancer cells will try to "bribe" PD-1, to let PD-1 accept the existence of cancer cells as well as to let the cancer cells escape from immune system attacks.

What's the medical purpose of OPDIVO?



OPDIVO is designed for preventing the "bribery" between cancer cells and PD-1, so that the cancer cells can't escape from the attack of immune cells!

But why does OPDIVO got its Pharmacodynamic effect limitation?



How many cell warriors left?

Professor Honjo's medical concept is
To stop cancer cells from "bribing" PD-1, and to let our own body immune system to attack the cancer cells!
However, the crucial part that we should take notice regarding the treatment is, how many capable immune cells are left to fight with those cancer cells?

Thus, the key factors in this battle against cancer cells would be the CAPABLE IMMUNE CELLS in your body !

Hydrogen Oxygen Therapy combines with anticarcinogenic immune drug

Doctor Junji Akagi

赤木 純児（アカギ ジュンジ）

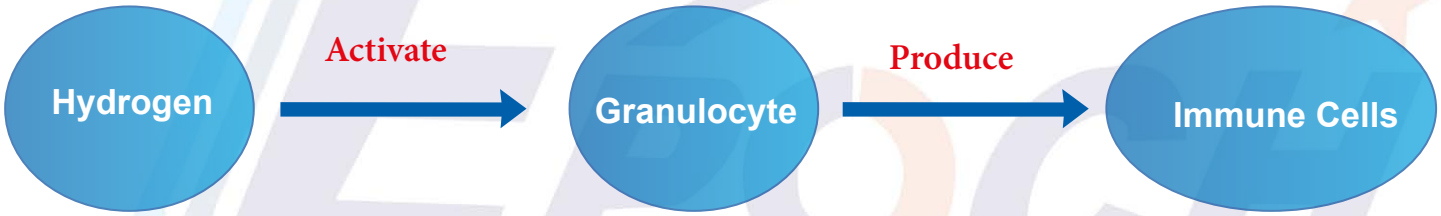
[Profile]

March, Year 1983	Graduated from Miyazaki Medical College
April, Year 1983	Kumamoto University School of Medicine Hospital second surgical entry
October, Year 1984	Kumamoto Municipal Hospital (Surgery, Anesthesis)
March, Year 1989	Kumamoto University medical doctorial courses completed
April, Year 1989	National Miyazaki Hospital
July, Year 1991	Kumamoto University School of Medicine Hospital Second Affiliated Hospital
November, Year 1992	US NIH (NCI National Cancer Institute, Immunology) (Tumor Immunolgy & iology (Lab) Dr Schlom)
April, Year 1995	Tamana Regional Health & Medical Center, Minister of surgery
July, Year 1998	Kumamoto University Tsukinama Regional Health and Medical Director of Center Surgery
June Year 2000	National Hospital Institute Kumamoto South Hospital Ministry of Regional Health Care
April, Year 2010	Tamana Regional Health & Medical Center Director

Doctor Junji Akagi Clinical Research

Doctor Junji Akagi research:

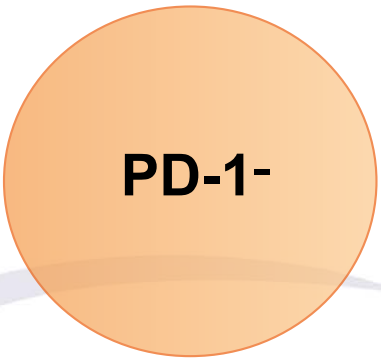
" Create a large number of combatable immune system cells for the body with Hydrogen and oxygen activated cell "



Doctor Junji Akagi's research main argument

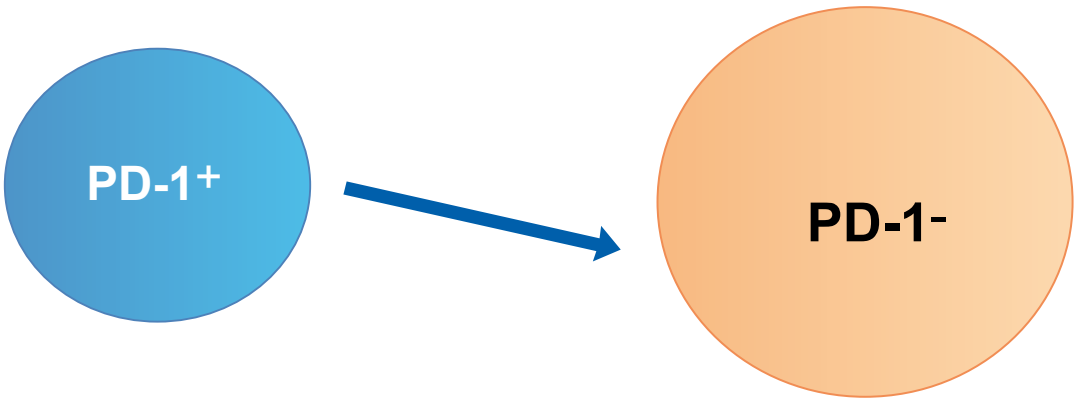
PD-1 + is a kind of "fatigued T-cell", without any combat ability.

Based on Doctor Akagi's research studies, he argued that the existence of PD-1 + in cancer patients' body will affect their recovery rate.



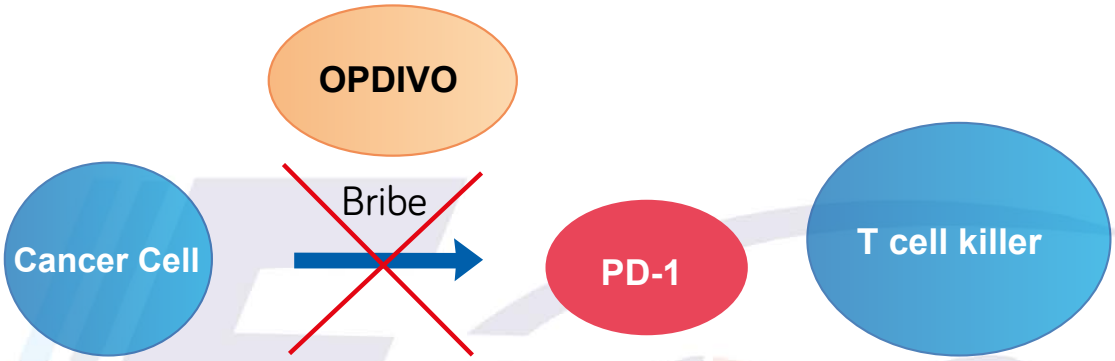
PD-1 − is a kind of healthy immune T-cell, which is able to fight against cancer cells, and also eliminate, control and decrease cancer cells.

Doctor Junji Akagi's research main argument

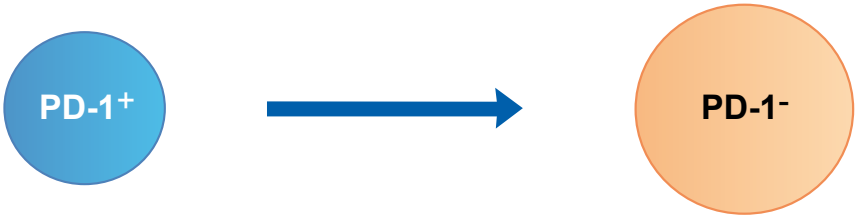


The inhalation of hydrogen will let PD-1 + restore to PD-1 -, causing the cells to activate and able to fight against cancer cells.
On the other words, this therapy will transform the cancer cells or so called "fatigued T-cells" into "fighting T-cells".

The relevance of Professor Junji Akagi and Professor Honjo



OPDIVO prevents cancer cells from skipping the identification stage by the immune system and also allowing the immune system to fight against cancer cells.
"The success healing rate will be based on how many immune combat cells left in the cancer patient's body."



Dr. Akagi claims that to raise the "success healing rate", OPDIVO should be combined with hydrogen and oxygen, in order to build up the identification ability of immune system and to create huge amount of immune cells to fight against cancer cells.

OPDIVO prevents the combination of PD-1 and cancer cells and helps the immune system to attack cancer cells. However, the success rate of the therapy only remains about 2%-3% if is not backed by a healthy immunity system.

To increase OPDIVO cancer therapy success rate, Dr. Akagi suggests the importance of activating human body cells and creating huge amount of cancer cell killers by using OPDIVO with hydrogen and oxygen.

$OH^{\cdot}$ H_2 $H_2O + H^+$

/ OPDIVO + The application of hydrogen oxygen /

Hydrogen gas restores exhausted $CD8^+$ T cells in patients with advanced colorectal cancer to improve prognosis

『已發表於美國腫瘤醫學期刊』

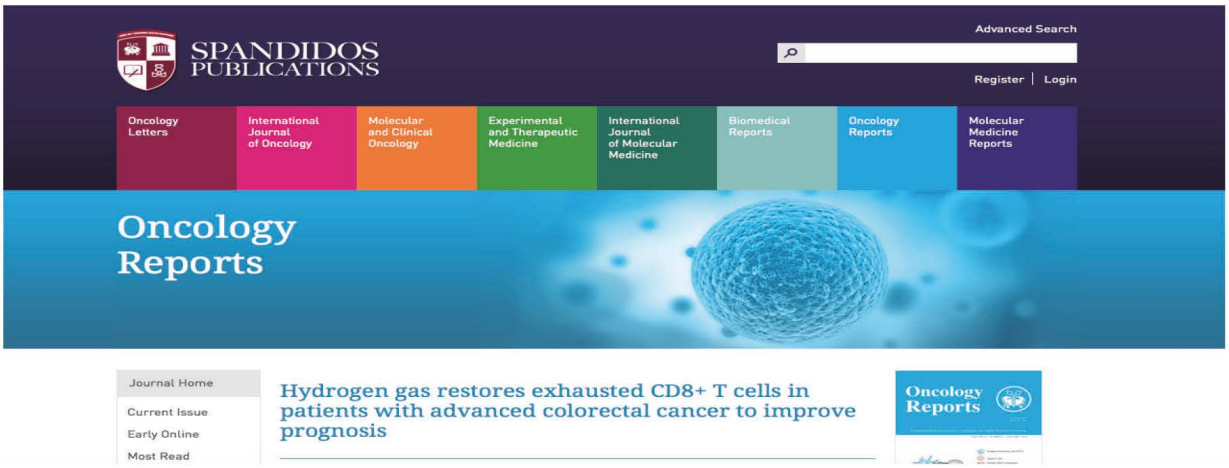
Journal of Clinical Oncology®
An American Society of Clinical Oncology Journal

Effects of hydrogen gas on exhausted $CD8^+$ T cells (PD-1-positive, terminally differentiated $CD8^+$ T cells).

Background: $CD8^+$ T cells progress from an early-differentiated (early-$CD8^+$ T cells) to a terminally differentiated state (terminal-$CD8^+$ T cells). PD-1 expression is rapidly upregulated in antigen-activated T cells, and rapidly downregulated when the antigen is cleared. In the presence of persistent antigen stimulation, such as that in cancer patients, PD-1 expression is not downregulated and terminal-$CD8^+$T cells become exhausted. **Methods:** Using flow cytometry, we investigated PD-1 expression in $CD8^+$T cells in the peripheral blood of 37 patients with stage IV cancer, before and after they received hydrogen gas treatment. We analyzed associations between treatment and progression-free survival (PFS) using Cox and Kaplan-Meier survival analyses. **Results:** Treatment lead to partial response (PR) in 12 patients and stable disease (SD) in 14 patients, with response rates of 32.4% and 37.8%, respectively (with an overall clinical response rate of 70.3%), and reduced the number of PD-1-positive, terminal-$CD8^+$ T cells in 9 of 12 patients with PR (75%) and in 8 of 14 patients with SD (57%). However, in 8 of 11 patients with progressive disease (PD) (72.7%), these cell counts increased. Multivariate analyses demonstrated that higher PD-1-positive, terminal-$CD8^+$ T cell counts were associated with shorter PFS periods, both before (hazard ratio [HR] = 3.23; 95% confidence interval [CI], 1.475–7.070; $p = 0.003$) and after (HR = 2.75; 95% CI, 1.273–5.951; $p = 0.01$) treatment, although the hazard ratio was decreased by treatment. Additional analysis showed that patients with higher PD-1-positive, terminal-$CD8^+$ T cell counts had shorter survival rates than those with lower counts, both before and after treatment (log rank test, $p = 0.001$ and $p = 0.005$, respectively). The 20-month survival rates for patients with higher PD-1-positive, terminal-$CD8^+$T cell counts improved from 0% to 36.7% after treatment. **Conclusions:** These results suggest that PD-1-positive, terminal-$CD8^+$ T cell counts are highly associated with PFS duration in patients with stage IV carcinomas, and hydrogen gas treatment contributes to clinical outcomes by reducing PD-1-positive, terminal-$CD8^+$ T cell counts.

http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.15_suppl.e23071

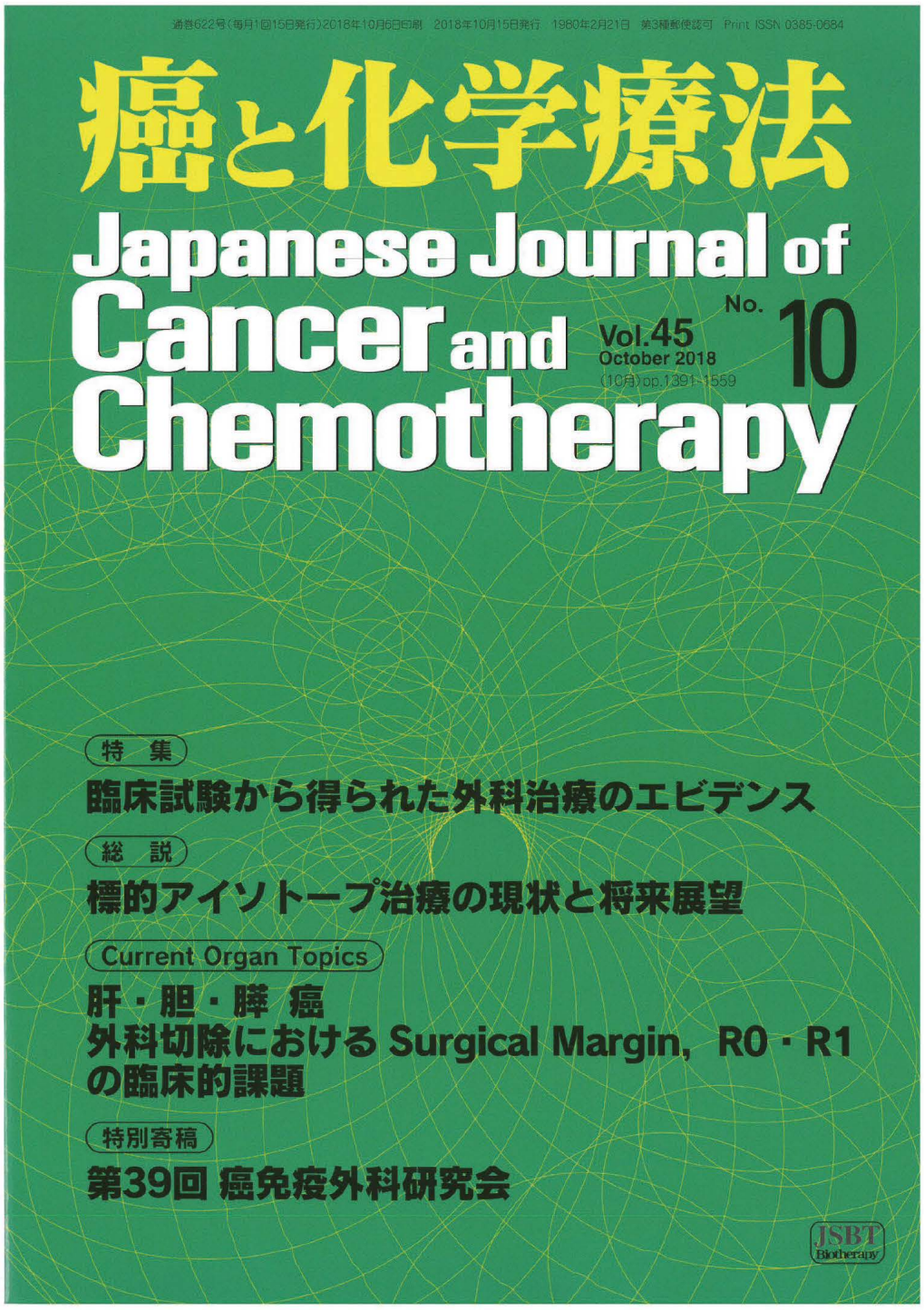
Published in British Journal of Oncologists



Abstract

Exhausted cluster of differentiation (CD)8+ T cells lose immunological activity due to mitochondrial dysfunction caused by peroxisome proliferator–activated receptor γ coactivator 1α (PGC–1α) inactivation, resulting in a poor prognosis in patients with cancer. As hydrogen gas was recently reported to activate PGC–1α, the present study investigated whether it restores exhausted CD8+ T cells to improve prognosis in patients with stage IV colorectal cancer. A total of 55 patients with histologically and clinically diagnosed stage IV colorectal carcinoma were enrolled between July 2014 and July 2017. The patients inhaled hydrogen gas for 3 h/day at their own homes and received chemotherapy at the Tamana Regional Health Medical Center (Tamana, Kumamoto, Japan). The CD8+ T cells were isolated from the peripheral blood and their phenotype was analyzed by flow cytometry. It was found that exhausted terminal programmed cell death 1 (PD–1)+ CD8+ T cells in the peripheral blood are independently associated with worse progression–free survival (PFS) and overall survival (OS). Notably, hydrogen gas decreased the abundance of exhausted terminal PD–1+ CD8+ T cells, increased that of active terminal PD–1– CD8+ T cells, and improved PFS and OS times, suggesting that the balance between terminal PD1+ and PD1– CD8+ T cells is critical for cancer prognosis. Therefore, a novel system for patient classification (category 1–4) was developed in the present study based on these two indices to assist in predicting the prognosis and therapeutic response. Collectively, the present results suggested that hydrogen gas reverses imbalances toward PD–1+ CD8+ T cells to provide an improved prognosis.

Japanese Journal of Cancer & Chemotherapy



特別寄稿

水素ガスの免疫学的効果
—Nivolumabの臨床効果増強効果—

赤木 純児*

〔*Jpn J Cancer Chemother* **45**(10): 1475-1478, *October*, 2018〕

Immunological Effect of Hydrogen Gas—Hydrogen Gas Improves Clinical Outcomes of Cancer Patients: Junji Akagi (*Tamana Regional Health Medical Center*)

Summary

It has been reported that PD-1-expressing $CD8^+$ T cells in the peripheral blood of cancer patients are associated with poor cancer prognosis. In addition, these cells are in a state of energy shortage caused by mitochondrial dysfunction with a low level of PGC-1α. Recently, hydrogen gas was reported to activate PGC-1α, leading to the enhancement of mitochondrial activity. In the present study, we investigated whether hydrogen gas influences the proportion of PD-1^+ $CD8^+$ T cells in the peripheral blood of 55 Stage Ⅳ colorectal carcinoma patients. We found that the proportion of terminal PD-1^+ $CD8^+$ T cells was an independent factor for poor prognosis. We also found that the proportion of terminal PD-1^+ $CD8^+$ T cells was reduced in 35 out of 55 patients (63.6%) and was increased in 39 out of 55 patients (70.9%) after treatment with hydrogen gas. The ratio of the terminal PD-1^+ $CD8^+$ T cells after hydrogen gas treatment to that before hydrogen gas treatment (terminal PD-1^+ $CD8^+$ T cell ratio) was found to be an independent factor predicting PFS and OS. Out of another 26 patients treated with nivolumab, 14 patients treated with a combined therapy of hydrogen gas and nivolumab showed a significantly longer OS than the remaining 12 patients who were treated with nivolumab alone. These results suggest that hydrogen gas improves the prognosis of cancer patients by reducing the proportion of terminal PD-1^+ $CD8^+$ T cells. **Key words**: Hydrogen, PD-1, Prognosis

要旨 がん患者で増加するPD-1を発現する$CD8^+$ T細胞はexhausted $CD8^+$ T細胞と呼ばれていて，がん患者の予後不良に関与していることが報告されている。exhausted $CD8^+$ T細胞はエネルギー代謝的にはPGC-1αの発現低下によるミトコンドリア機能不全に起因するエネルギー枯渇の状態にあると考えられている。最近，水素はミトコンドリアの活性にかかわるPGC-1αを活性化することが報告された。今回われわれは，Stage Ⅳがん患者において水素ガスを吸入させることで，末梢血中のPD-1^+ $CD8^+$ T細胞の割合が変化するのかどうか，その変化と患者のprogression free survival（PFS）とoverall survival（OS）との関連について検討した。Stage Ⅳの直腸大腸癌患者55例で検討したところ，terminal PD-1^+ $CD8^+$ T細胞はPFSとOSの両者において独立予後不良因子であった。水素ガス吸入により，55例中35例（63.6%）でterminal PD-1^+ $CD8^+$ T細胞が減少し，逆にterminal PD-1^- $CD8^+$ T細胞は39例（70.9%）で増加した。水素ガス吸入前のterminal PD-1^+ $CD8^+$ T細胞の割合に対する吸入後の割合の変化（terminal PD-1^+ $CD8^+$ T cell ratio）がPFS，OSに関して独立予後予測因子であった。また，nivolumabを使用した別の26症例中14例は水素ガスを併用したが，水素ガス併用群のOSはnivolumab単独群に比して有意に良好であった。これらの結果は，水素ガスがterminal PD-1^+ $CD8^+$ T細胞を減少させることにより，がん患者の予後を改善していることを示唆している。

はじめに

活性酸素には，スーパーオキシド，ヒドロキシラジカル，過酸化水素，一重項酸素の4種類があるが，水素はこのうちの悪玉活性酸素であるヒドロキシラジカルのみを除去することが報告されている[1]。水素ガスは，感染症[2]，糖尿病[3]，化学療法の副作用[4]，放射線障害[5]などの酸化ストレスを逓減するのに使用されている。最近，水素がperoxisome proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α) を活性化すること

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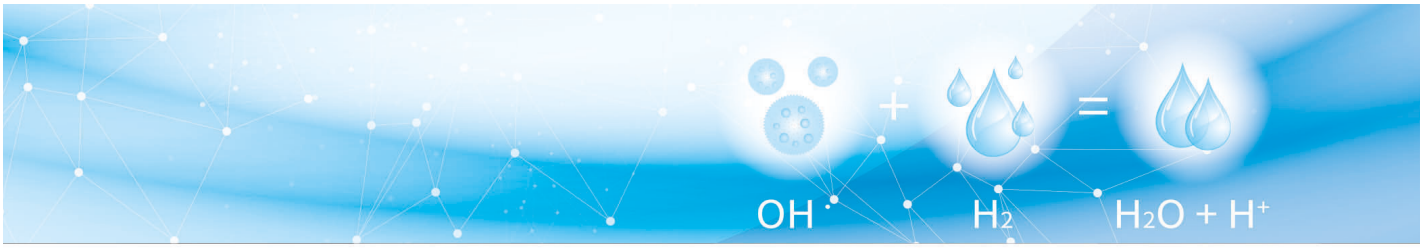
連絡先: 〒865-0005 熊本県玉名市玉名2172 地方独立行政法人くまもと県北病院機構 玉名地域保健医療センター 赤木 純児



が報告された[2]。PGC-1α は転写因子 PPARγ に結合する転写コアクチベーターとして同定された分子であり，ミトコンドリア機能の調整に中心的役割を果たしている[3]。

PD-1 を発現する $CD8^+$ T 細胞は，exhausted $CD8^+$ T 細胞とも呼ばれていて，cytotoxic $CD8^+$ T 細胞としての機能，細胞障害活性化，サイトカイン分泌能，細胞増殖能を失っている。exhausted $CD8^+$ T 細胞では PGC-1α の発現が低下し，その結果ミトコンドリア機能不全によるエネルギー枯渇の状態になっており[4,5]，PD-1 などの免疫チェックポイント分子が発現している[4,5]。がん患者の TIL 中や末梢血中で増加した $PD\text{-}1^+$ $CD8^+$ T 細胞は患者の予後不良に関与することが報告されてきた[6,7]。

今回われわれは，水素ガスが $CD8^+$ T 細胞上の PD-1 の発現にどのような影響を与えるか，それががん患者の progression free survival (PFS) や overall survival (OS) にどのように関連するのかを検討した。

Ⅰ．患者と方法

1．試験参加者

2017 年 1 月～2018 年 12 月までに玉名地域保健医療センターで，Stage Ⅳと診断された 55 例の直腸大腸癌（男性 21 例，女性 34 例）の患者で，他院で third-line まで化学療法を行い無効と判定された患者である。また，これとは別に nivolumab を使用した 26 例も（男性 16 例，女性 10 例），すべて切除不能または再発の Stage Ⅳで，他院で third-line まで化学療法を行い，無効と判定された患者である。この 26 例の原発巣は，頭頸部 3 例，肺 7 例，食道 1 例，胃 2 例，肝胆 4 例，膵臓 3 例，大腸 2 例，腎臓 1 例，子宮/卵巣 3 例であった。この 26 例中 14 例は水素ガスを併用した。これらの患者の末梢血より水素ガス吸入前と吸入開始 3 か月後に 2 回 10 mL の採血を行い，FACScan を行った。また，3 か月に一度ほど，治療効果判定のために画像診断を行った（CT，PET-CT など）。

2．フローサイトメトリー

使用した抗体は，anti-CD57 conjugated to FITC (NK-1，part No. 347393)，mouse anti-human CD27 conjugated to APC (M-T271，part No. B09983)，anti-PD-1 conjugated to PE (EH12.1，part No. 557946)，and mouse anti-human CD8 conjugated to PerCP (SK1，part No. 347314) (BD Pharmingen, San Diego, CA, USA) である。リンパ球は BD FACSCalibur で解析し，そのデータの解析には BD CellQuest software を使用した。これらの検査は，すべて SRL Inc. (Tokyo, Japan) で行った。

3．水素ガスの投与方法

患者は水素ガス発生器である Hycellvator ET 100 (Helix Japan, Co., Ltd., Tokyo, Japan) につないだカニューレあるいはマスクを用いて，1 日に 3 時間毎日水素を吸入してもらった。Hycellvator ET 100 は水の電気分解により，1 分間に 1.67 L/min 発生させることができる。ガスクロマトグラフィーによる測定では (Kureha Special Laboratory Co., Ltd., Iwaki, Fukushima, Japan)，この器械から発生するガスは，680,000 vol. ppm の水素ガスと 320,000 vol. ppm の組成であった。これまでのところ，水素ガスの副作用は認められていない[3]。

水素ガスを治療に使用するに当たって当院の倫理審査委員会を行い，承認された（玉名地域保健医療センター倫理審査委員会 No. 16-1，2016 年 7 月 7 日）。

4．統 計 処 理

得られたデータは，SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL, USA) を用いて統計解析を行った。生存率の解析には Kaplan-Meier method，生存率の有意差の検定には logrank test を用いた。多変量解析には Cox regression model を使用した。

Ⅱ．結　　果

1．末梢血中の terminal $PD\text{-}1^+$ $CD8^+$ T 細胞は直腸大腸癌患者の予後に深く関与

通常の $CD8^+$ T 細胞の分化（early-diff. $CD8^+$ T 細胞から，intermediate-diff. $CD8^+$ T 細胞を経て，terminal $CD8^+$ T 細胞[注]，end $CD8^+$ T 細胞への分化）の過程では，PD-1 は early-diff. $CD8^+$ T 細胞で最も多く発現しており，その後分化に伴って減少していくが，がん患者ではそれが遷延し，それが患者の予後不良に関与している[6,7]。各分化型での PD-1 の発現が PFS と OS に関与するかを単・多変量解析にて検討し，多変量解析の結果，terminal $PD\text{-}1^+$ $CD8^+$ T 細胞が PFS と OS ともに独立予後不良因子であった（Table 1, 2）。

2．水素ガス吸入による末梢血中の terminal $PD\text{-}1^+$ $CD8^+$ T 細胞割合の変化と予後

terminal $PD\text{-}1^+$ $CD8^+$ T 細胞は 55 例中 35 例 (63.6%) で低下し，逆に terminal $PD\text{-}1^-$ $CD8^+$ T 細胞は 39 例 (70.9%) で増加した。水素ガス吸入前の各分化型 $CD8^+$ T 細胞の割合に対する吸入後の割合の変化（たとえば terminal $PD\text{-}1^+$ $CD8^+$ T cell ratio＝吸入後 terminal $PD\text{-}1^+$ $CD8^+$ T 細胞割合/吸入前 terminal $PD\text{-}1^+$ $CD8^+$ T 細胞割合）を求め，これらの比と PFS と OS との関連を単・多変量解析で調べた。単変量解析では，唯

[注]: terminal $CD8^+$ T 細胞: $CD8^+$ T 細胞は early-diff. $CD8^+$ T 細胞から，intermediate-diff. $CD8^+$ T 細胞を経て，terminal-diff. $CD8^+$ T 細胞に分化する。terminal $CD8^+$ T 細胞が effector $CD8^+$ T 細胞と考えられている。

Table 1　Univariate and multivariate analysis of PD-1$^{\pm}$CD8^{+} T cells

Univariate analysis (progression free survival)

	HR	HR 95%CI	p value
Early-CD8^{+} T cells			0.468
PD-1^{+}-early-CD8^{+} T cells			0.447
PD-1^{-}-early-CD8^{+} T cells	**0.957**	**0.922-0.993**	**0.021**
Intermediate-CD8^{+} T cells			0.528
PD-1^{+}-intermediate-CD8^{+} T cells			0.167
PD-1^{-}-intermediate-CD8^{+} T cells			0.154
Terminal-CD8^{+} T cells			0.144
PD-1^{+}-terminal CD8^{+} T cells	**1.178**	**1.108-1.254**	**<0.0001**
PD-1^{-}-terminal CD8^{+} T cells	**0.967**	**0.937-0.996**	**0.029**
End-CD8^{+} T cells	**1.137**	**1.058-1.223**	**<0.0001**
PD-1^{+}-end-CD8^{+} T cells	**1.303**	**1.108-1.532**	**0.001**
PD-1^{-}-end-CD8^{+} T cells			0.124

Multivariate analysis (progression free survival)

	HR	HR 95%CI	p value
PD-1^{+}-terminal CD8^{+} T cells	**1.178**	**1.108-1.254**	**<0.0001**

Table 2　Univariate and multivariate analysis of PD-1$^{\pm}$CD8^{+} T cells

Univariate analysis (overall survival)

	HR	HR 95%CI	p value
Early-CD8^{+} T cells			0.723
PD-1^{+}-early-CD8^{+} T cells			0.737
PD-1^{-}-early-CD8^{+} T cells	**0.953**	**0.921-0.986**	**0.005**
Intermediate-CD8^{+} T cells			0.723
PD-1^{+}-intermediate-CD8^{+} T cells			0.369
PD-1^{-}-intermediate-CD8^{+} T cells			0.093
Terminal-CD8^{+} T cells			0.734
PD-1^{+}-terminal CD8^{+} T cells	**1.150**	**1.083-1.222**	**<0.0001**
PD-1^{-}-terminal CD8^{+} T cells			0.217
End-CD8^{+} T cells	**1.137**	**1.060-1.220**	**<0.0001**
PD-1^{+}-end-CD8^{+} T cells	**1.335**	**1.135-1.570**	**<0.0001**
PD-1^{-}-end-CD8^{+} T cells	**1.039**	**1.003-1.075**	**0.031**

Multivariate analysis (overall survival)

	HR	HR 95%CI	p value
PD-1^{+}-terminal CD8^{+} T cells	**1.150**	**1.083-1.222**	**<0.0001**

一 terminal PD-1^{-} CD8^{+} T cell ratio が OS の改善に寄与していた（Table 3）。多変量解析の結果，terminal PD-1^{+} CD8^{+} T cell ratio が PFS と OS に関する独立予後予測因子であった（Table 3, 4）。

3．水素ガスによる nivolumab の治療効果増強

nivolumab に水素ガスを併用した患者の OS は nivolumab 単独群に比して有意に良好であった（Fig. 1）。

Ⅲ．考　　察

Stage Ⅳの直腸大腸癌患者において，末梢血中の terminal PD-1^{+} CD8^{+} T 細胞が PFS，OS に関して独立予後不良因子であった。水素ガス吸入によって，55 例中 35 例で terminal PD-1^{+} CD8^{+} T 細胞の割合は低下し，また水素ガス吸入前後の terminal PD-1^{+} CD8^{+} T 細胞の割合の比（terminal PD-1^{+} CD8^{+} T cell ratio）が PFS と OS に関しての独立予後予測因子であった（Table 3, 4）。これらの結果は，水素ガス吸入の最大の効果は末梢血中の terminal PD-1^{+} CD8^{+} T 細胞割合の低下であり，それががん患者の PFS，OS の改善に寄与していることを示している。

水素は，最近，PGC-1α を活性化することが報告され[2]，PGC-1α はミトコンドリア機能を高めることが報告されている[3]。がん患者の末梢血中で PD-1^{+} CD8^{+} T 細胞が増加すると予後不良になることが数多く報告されており[6,7]，今回の研究でも Stage Ⅳの直腸大腸癌患者では PD-1^{+} CD8^{+} T 細胞のなかでも，terminal PD-1^{+} CD8^{+} T 細胞が独立予後不良因子であった。PD-1^{+} CD8^{+} T 細胞は疲弊 T 細胞とも呼ばれており，代謝的にみるとミトコンドリア機能不全によるエネルギー枯渇の状態であり，その結果として PD-1 などの免疫チェックポイント分子を発現するようになる[4,5]。本研究では，水素ガス吸入による terminal PD-1^{+} CD8^{+} T 細胞の減少が認められ，それが PFS，OS に深く関与していた。このことは，水素ガスが PGC-1α の活性化を介してミトコンドリア機能を回復させた結果，terminal PD-1^{+} CD8^{+} T 細胞上の PD-1 の発現が減少し terminal PD-1^{-} CD8^{+} T 細胞が増加して予後改善に寄与したと考えられる。

最近，nivolumab の臨床効果を増強するにはミトコンドリアの活性化が重要であるという報告がなされており[8]，水素ガスも同様に PGC-1α を介してミトコンドリア機能を活性化することで nivolumab の臨床効果を増強した可能性が考えられた（Fig. 1）。

Table 3 Univariate and multivariate analysis of the rate of variability of PD-1$^{\pm}$CD8^{+} T cells before and after hydrogen gas treatment

Univariate analysis after treatment (overall survival)

	HR	HR 95%CI	p value
Early PD-1^{+}CD8^{+} T cell ratio			0.219
Early PD-1^{-}CD8^{+} T cell ratio	**2.398**	**1.384-4.156**	**0.002**
Intermediate PD-1^{+}CD8^{+} T cell ratio	**2.398**	**1.384-4.156**	**0.002**
Intermediate PD-1^{-}CD8^{+} T cell ratio	**2.398**	**1.384-4.156**	**0.002**
Terminal PD-1^{+}CD8^{+} T cell ratio	**4.158**	**1.718-10.06**	**0.002**
Terminal PD-1^{-}CD8^{+} T cell ratio	**0.002**	**0.000-0.131**	**0.004**
End PD-1^{+}CD8^{+} T cell ratio			0.539
End PD-1^{-}CD8^{+} T cell ratio			0.212

Multivariate analysis after treatment (overall survival)

	HR	HR 95%CI	p value
Terminal PD-1^{+}CD8^{+} T cell ratio	**2.957**	**1.185-7.378**	**0.020**

Table 4 Univariate and multivariate analysis of the rate of variability of PD-1$^{\pm}$CD8^{+} T cells before and after hydrogen gas treatment

Univariate analysis after treatment (progression free survival)

	HR	HR 95%CI	p value
Early PD-1^{+}CD8^{+} T cell ratio			0.179
Early PD-1^{-}CD8^{+} T cell ratio			0.248
Intermediate PD-1^{+}CD8^{+} T cell ratio	**2.286**	**1.284-4.070**	**0.005**
Intermediate PD-1^{-}CD8^{+} T cell ratio	**2.286**	**1.284-4.070**	**0.005**
Terminal PD-1^{+}CD8^{+} T cell ratio	**6.459**	**2.384-17.50**	**<0.0001**
Terminal PD-1^{-}CD8^{+} T cell ratio			0.060
End PD-1^{+}CD8^{+} T cell ratio			0.229
End PD-1^{-}CD8^{+} T cell ratio			0.711

Multivariate analysis after treatment (progression free survival)

	HR	HR 95%CI	p value
PD-1^{+}-terminal CD8^{+} T cell ratio	**6.459**	**2.384-17.50**	**<0.0001**

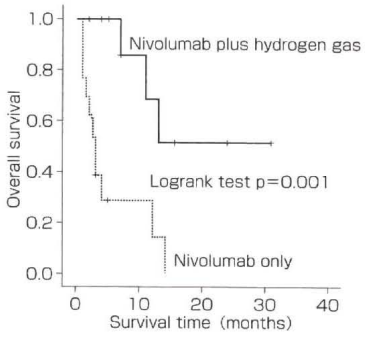


Fig. 1 Comparison of overall survival between the patients treated with nivolumab plus hydrogen gas and nivolumab only

結　語

このように，水素ガスはがん患者での疲弊したT細胞を再活性化することでがん患者の予後改善に寄与していると考えられる。

謝辞　Hycellvator ET 100を提供していただいた株式会社ヘリックスジャパンに謝辞を表します。

文　献

1) Ohsawa I, Ishikawa M, Takahashi K, *et al*: Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nat Med* **13**(6): 688-694, 2007.
2) Kamimura N, Ichimiya H, Iuchi K, *et al*: Molecular hydrogen stimulates the gene expression of transcriptional coactivator PGC-1α to enhance fatty acid metabolism. *NPJ Aging Mech Dis* **2**: 16008, 2016.
3) Mäkelä J, Tselykh TV, Kukkonen JP, *et al*: Peroxisome proliferator-activated receptor-γ (PPARγ) agonist is neuroprotective and stimulates PGC-1α expression and CREB phosphorylation in human dopaminergic neurons. *Neuropharmacology* **102**: 266-275, 2016.
4) Scharping NE, Menk AV, Moreci RS, *et al*: The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell metabolic insufficiency and dysfunction. *Immunity* **45**(3): 374-388, 2016.
5) Gros A, Parkhurst MR, Tran E, *et al*: Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients. *Nat Med* **22**(4): 433-438, 2016.
6) Ahmadzadeh M, Johnson LA, Heemskerk B, *et al*: Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood* **114**(8): 1537-1544, 2009.
7) Zarour HM: Reversing T-cell dysfunction and exhaustion in cancer. *Clin Cancer Res* **22**(8): 1856-1864, 2016.
8) Chamoto K, Chowdhury PS, Kumar A, *et al*: Mitochondrial activation chemicals synergize with surface receptor PD-1 blockade for T cell-dependent antitumor activity. *Proc Natl Acad Sci USA* **114**(5): E761-E770, 2017.

本論文の要旨は第39回癌免疫外科研究会において発表した。

Hydrogen gas restores exhausted CD8$^+$ T cells in patients with advanced colorectal cancer to improve prognosis

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Received June 20, 2018; Accepted October 26, 2018

DOI: 10.3892/or.2018.6841

Abstract. Exhausted cluster of differentiation (CD)8$^+$ T cells lose immunological activity due to mitochondrial dysfunction caused by peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) inactivation, resulting in a poor prognosis in patients with cancer. As hydrogen gas was recently reported to activate PGC-1α, the present study investigated whether it restores exhausted CD8$^+$ T cells to improve prognosis in patients with stage IV colorectal cancer. A total of 55 patients with histologically and clinically diagnosed stage IV colorectal carcinoma were enrolled between July 2014 and July 2017. The patients inhaled hydrogen gas for 3 h/day at their own homes and received chemotherapy at the Tamana Regional Health Medical Center (Tamana, Kumamoto, Japan). The CD8$^+$ T cells were isolated from the peripheral blood and their phenotype was analyzed by flow cytometry. It was found that exhausted terminal programmed cell death 1 (PD-1)$^+$ CD8$^+$ T cells in the peripheral blood are independently associated with worse progression-free survival (PFS) and overall survival (OS). Notably, hydrogen gas decreased the abundance of exhausted terminal PD-1$^+$ CD8$^+$ T cells, increased that of active terminal PD-1$^-$ CD8$^+$ T cells, and improved PFS and OS times, suggesting that the balance between terminal PD1$^+$ and PD1$^-$ CD8$^+$ T cells is critical for cancer prognosis. Therefore, a novel system for patient classification (category 1-4) was developed in the present study based on these two indices to assist in predicting the prognosis and therapeutic response. Collectively, the present results suggested that hydrogen gas reverses imbalances toward PD-1$^+$ CD8$^+$ T cells to provide an improved prognosis.

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Abbreviations: FACS, fluorescence-activated cell sorting; PD-1, programmed cell death 1; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; PFS, progression-free survival; OS, overall survival

Key words: exhausted CD8$^+$ T cells, peroxisome proliferator-activated receptor γ coactivator 1α, colorectal cancer, hydrogen gas, prognosis

Introduction

In patients with cancer, the treatment strategy, outcome and prognosis are strongly dependent on immune status, which should thus be monitored, ideally via markers that are easily and non-invasively measurable in the peripheral blood. Cytotoxic effector cluster of differentiation (CD)8$^+$ T cells (equivalent to terminal CD8$^+$ T cells) become exhausted by persistent stimulation with tumor antigens, resulting in cessation of proliferation, cytokine production and immune activities (1,2). Programmed cell death 1 (PD-1) has been proposed as a marker of exhausted T cells (1). PD-1 is abundantly expressed in circulating CD8$^+$ T cells in patients with cancer, as well as in tumor-infiltrating lymphocytes, and is associated with a poor prognosis in various cancer types, including breast, pancreatic and gastric cancer (3-6). As recently reported, exhausted tumor-infiltrating (7) and circulating (8) CD8$^+$ T cells exhibit metabolic insufficiency, characterized most prominently by persistent loss of mitochondrial function and mass (7), typically following progressive loss of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) (7).

Molecular hydrogen, that is dihydrogen or H_2, was previously reported to efficiently neutralize hydroxyl radicals (•OH), but not other reactive oxygen species, including superoxide anions ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO•) (9). Accordingly, hydrogen is now believed to reduce oxidative stress and ischemia-reperfusion injury in the brain, spinal cord (10), myocardium (11), intestinal epithelium (12), retina, testes (13) and kidneys (14). Hydrogen has been used to treat various states associated with oxidative stress, including trauma (15), neurodegenerative disease (16), inflammatory disease (17), organ transplantation, metabolic syndrome (18), diabetes mellitus (19), sepsis (20), burns (21), adverse reactions to chemotherapy (22), radiation injury (23), hearing disorders and preeclampsia (24). There are several studies on the preventive and therapeutic effects of hydrogen on various diseases, including cancer (25-27). Notably, it was reported that molecular hydrogen activates PGC-1α (28), which is a positive regulator of mitochondrial biogenesis and respiration, adaptive thermogenesis, gluconeogenesis and a number of

other metabolic processes (29), suggesting that it may rescue exhausted $CD8^+$ T cells with mitochondrial dysfunction.

Therefore, the present study investigated whether PD-1 expression in circulating $CD8^+$ T cells in 55 patients with colorectal carcinoma was associated with progression-free survival (PFS) and overall survival (OS), and whether hydrogen gas impacts prognosis via influencing PD-1^+ $CD8^+$ T lymphocytes.

Materials and methods

Patients, sample collection and processing. All participants provided written informed consent prior to enrollment, and the Institutional Review Boards at the Tamana Regional Health Medical Center (Tamana, Kumamoto, Japan) approved the study protocol. All methods and procedures were consistent with Good Clinical Practice, the Declaration of Helsinki and local laws. In total, 55 patients with histologically and clinically diagnosed stage IV colorectal carcinoma, based on the unified Tumor-Node-Metastasis criteria (30), were enrolled at Tamana Regional Health Medical Center between July 2014 and July 2017. The specific inclusion and exclusion criteria were a performance status of ≥2 and <2, respectively. Among the patients with colorectal carcinoma, there were 21 men and 34 women, who ranged in age from 28 to 96 years, with a mean age of 65.7±14.8 years (Table I). The patients were treated with chemotherapy using XELOX (CapeOX) (1,200-1,800 mg capecitabine for 14 days continuously, followed by a 7-day rest period, and 85 mg/m^2 oxaliplatin at a 3-week interval) + bevacizumab (7.5 mg/m^2 at a 3-week interval). A total of 3 weeks was regarded as one cycle. Treatment was repeated until the cancer progression was confirmed by imaging. The patients inhaled hydrogen gas for 3 h daily at their own homes through a cannula or mask, rented or purchased by themselves, connected to a Hycellvator ET 100 (Helix Japan, Co., Ltd., Tokyo, Japan) (Fig. 1A). None of the patients reported any complaints regarding the daily 3-h hydrogen gas inhalation. Peripheral blood (10 ml) was collected from the patients prior to and 3 months after treatment with hydrogen gas.

Hydrogen gas treatment. The Hycellvator ET 100 (Helix Japan, Co., Ltd.) generates 1.67 l/min hydrogen gas (hydrogen purity, 99.99%) by electrolysis. As measured by gas chromatography at Kureha Special Laboratory (Iwaki, Fukushima, Japan), the gas generated consisted of 680,000 ppm hydrogen gas and 320,000 ppm oxygen gas. Recently, hydrogen gas inhalation was used in patients with post-cardiac arrest syndrome, and adverse events were not observed (31). Furthermore, no adverse events were observed in the 55 patients who inhaled hydrogen gas for 3 months in the present study.

Antibodies and fluorescence-activated cell sorting (FACS). Briefly, Ficoll-Hypaque solution (20 ml) was placed into a 50-ml conical centrifuge tube using a sterile pipette. Anti-coagulated blood (10 ml) mixed with an equal volume of PBS was then slowly layered over the Ficoll-Hypaque solution by gently pipetting down the side of the tube. Subsequently, samples were centrifuged for 40 min at 400 x g and 22˚C for 30-40 min with no braking. Mononuclear cells that accumulated at the interface between the plasma (upper) and Ficoll-Hypaque layers (bottom) were carefully recovered using a Pasteur pipette and transferred to a 15-ml conical tube. Cells were then analyzed on a BD FACSCalibur (Nippon Becton Dickinson, Tokyo, Japan) with BD CellQuest software (version 5.1), using anti-CD57 conjugated to fluorescein isothiocyanate (clone NK-1; cat. no. 347393; Nippon Becton Dickinson), mouse anti-human CD27 conjugated to APC (clone M-T271; cat. no. B09983; Beckman Coulter, Tokyo, Japan), mouse anti-human PD-1 conjugated to PE (clone EH12.1; cat. no. 557946; Nippon Beckton Dickinson) and mouse anti-human CD8 conjugated to PerCP (clone SK1; cat. no. 347314) (BD Pharmingen, San Jose, CA, USA), incubated at 4˚C for 30 min, following blocking with 1% γ-globulin for 15 min at 4˚C. To determine the independent contributions of each marker to PFS and OS, FACS data were used to stratify patients based on the proportion of early, intermediate, terminal, and end PD-1^+ and PD-1^- $CD8^+$ T cells. All blood samples obtained from the patients were transferred to SRL, Inc. (Tokyo Japan), where lymphocyte separation and flow cytometry were performed. Therefore, the status of the laboratory data, reliable protocols and flow cytometry assays were certified by SRL, Inc., one of the most reliable clinical laboratory centers in Japan. The flow cytometry data was analyzed using SPSS version 19.0 for Windows (IBM Corp., Armonk, NY, USA).

Study endpoints and assessments. The primary endpoints were PFS and OS time, which were measured from the date of randomization to the first recurrence and mortality regardless of cause, respectively. Patients were monitored by dynamic computed tomography or magnetic resonance imaging every 3 months from the baseline up to 60 months and every 3-6 months thereafter. Two independent and blinded radiologists, each with >5 years of experience, reviewed all scans at each site. In cases of discord, the two radiologists reviewed the images to reach the same conclusion following discussion. Adverse events were classified and graded every 2 months according to the Common Terminology Criteria for Adverse Events version 3.0 (National Cancer Institute, Bethesda, MD, USA) (32) from the day of consent until the end of the study at least 30 days after the treatment. Multiple events were counted once for each patient, of which the most severe was noted.

Statistical analysis. Testing of the significance of the differences between groups was performed using the χ^2 test. In case of persistent abnormal distribution, the linear correlation between two continuous variables was tested with the Spearman correlation coefficient. Receiver operating characteristic (ROC) analysis was used to determine optimal cut-off values for continuous variables. The ROC curve shows 1-specificity on the x-axis and sensitivity on the y-axis. The optimal cut-off value is calculated by maximizing the sensitivity and specificity across various cut-off points on the ROC curve. The probability of survival was estimated by the Kaplan-Meier method, and differences in survival were evaluated by the log-rank test. Prognostic factors were tested by univariate and multivariate Cox regression. All statistical analyses were performed using SPSS version 19.0 for Windows (IBM Corp.). Differences were considered statistically significant at $P<0.05$.

Table I. Comparison of clinicopathological data between patients with high- and low-terminal PD-1$^+$ CD8$^+$ T cells.

	Terminal PD-1$^+$ CD8$^+$ T cells (PFS)			Terminal PD-1$^+$ CD8$^+$ T cells (OS)		
Characteristic	High >8.18%	Low <8.18%	P-value	High >6.81%	Low <6.81%	P-value
Age, years	66.1±14.5	65.4±14.9	NS	66.1±14.3	65.2±15.6	NS
Sex, n			NS			NS
Male	10	11		12	9	
Female	14	20		17	17	
T factor, n			NS			NS
T1	1	0		1	0	
T2	5	3		5	3	
T3	8	9		9	8	
T4	4	6		5	5	
Tx	6	13		9	10	
N factor, n			NS			NS
N0	3	5		5	3	
N1	6	5		7	4	
N2	3	5		4	4	
N3	4	5		4	5	
Nx	8	11		9	10	
M factor, n			NS			NS
M0	0	0		0	0	
M1	24	31		29	26	
Histology, n			NS			NS
Tub1	6	9		8	7	
Tub2	10	11		11	10	
Poor	8	11		10	9	

NS, not significant; T factor, primary tumor; N factor, regional lymph node; M factor, distant metastasis; Tub1, well-differentiated adenocarcinoma; Tub2, moderately-differentiated adenocarcinoma; Poorly, poorly-differentiated adenocarcinoma; PD-1, programmed cell death 1; CD, cluster of differentiation; OS, overall survival; PFS, progression-free survival.

Results

Circulating terminal PD-1$^+$ CD8$^+$ T cells are critical for prognosis in colorectal cancer. In the normal state, early CD8$^+$ T cells abundantly express PD-1, which gradually diminishes with differentiation into terminal CD8$^+$ T cells. However, loss of PD-1 is delayed in patients with cancer and may result in a poor prognosis. Hence, Cox proportional-hazards regression analysis was used to identify PD-1$^{+/-}$ CD8$^+$ T cell subsets (Fig. 1B) and clinicopathological factors [age, sex, primary tumor (T), regional lymph nodes (N), distant metastasis (M) and histology] associated with PFS and OS in patients with stage IV colorectal cancer. By univariate analysis of 18 factors, including 6 clinicopathological factors, terminal PD-1$^+$ CD8$^+$ T cells were found to be significantly associated with poorer PFS [hazard ratio (HR), 1.239; 95% confidence interval (CI), 1.106-1.389; P<0.0001] and OS (HR, 1.183; 95% CI, 1.066-1.314; P=0.002), as were end PD-1$^+$ CD8$^+$ T cells (PFS: HR, 1.296; 95% CI, 1.053-1.595; P=0.015; OS: HR, 1.333; 95% CI, 1.103-1.610; P=0.003). By contrast, early PD-1$^-$ and end PD-1$^-$ CD8$^+$ T cells were associated with better (HR, 0.961; 95% CI, 0.926-0.997; P=0.036) and worse (HR, 1.044; 95% CI, 1.005-1.084; P=0.025) OS, respectively. The univariate analysis data of the other 14 factors for PFS and OS, respectively, were as follows: Age, P=0.270 and P=0.886; sex, P=0.894 and P=0.398; T factor, P=0.332 and P=0.664; N factor, P=0.080 and P=0.150; M factor (the univariate analysis could not be performed as all patients had distant metastasis); histology, P=0.503 and P=0.184; early CD8$^+$ T cells, P=0.953 and P=0.273; early PD-1$^+$ CD8$^+$ T cells, P=0.757 and P=0.560; intermediate CD8$^+$ T cells, P=0.434 and P=0.560; intermediate PD-1$^+$ CD8$^+$ T cells P=0.799 and P=0.505; intermediate PD-1$^-$ CD8$^+$ T cells P=0.137 and P=0.099; terminal CD8$^+$ T cells, P=0.453 and P=0.595; terminal PD-1$^-$ CD8$^+$ T cells, P=0.681 and P=0.886; and end CD8$^+$ T cells, P=0.285 and P=0.566.

Based on multivariate Cox regression, terminal PD-1$^+$ CD8$^+$ T cells were more strongly associated with PFS (HR, 1.239; 95% CI, 1.106-1.389; P<0.0001) and OS (HR, 1.136; 95% CI, 1.019-1.266; P=0.022) than others. The multivariate data of the other 3 factors were as follows: Early PD-1$^-$ CD8$^+$ T cells, P=0.677 for PFS and P=0.352 for OS; end PD-1$^+$ CD8$^+$ T cells, P=0.274 for PFS; HR, 1.247; 95% CI, 1.007-1.543; P=0.043;

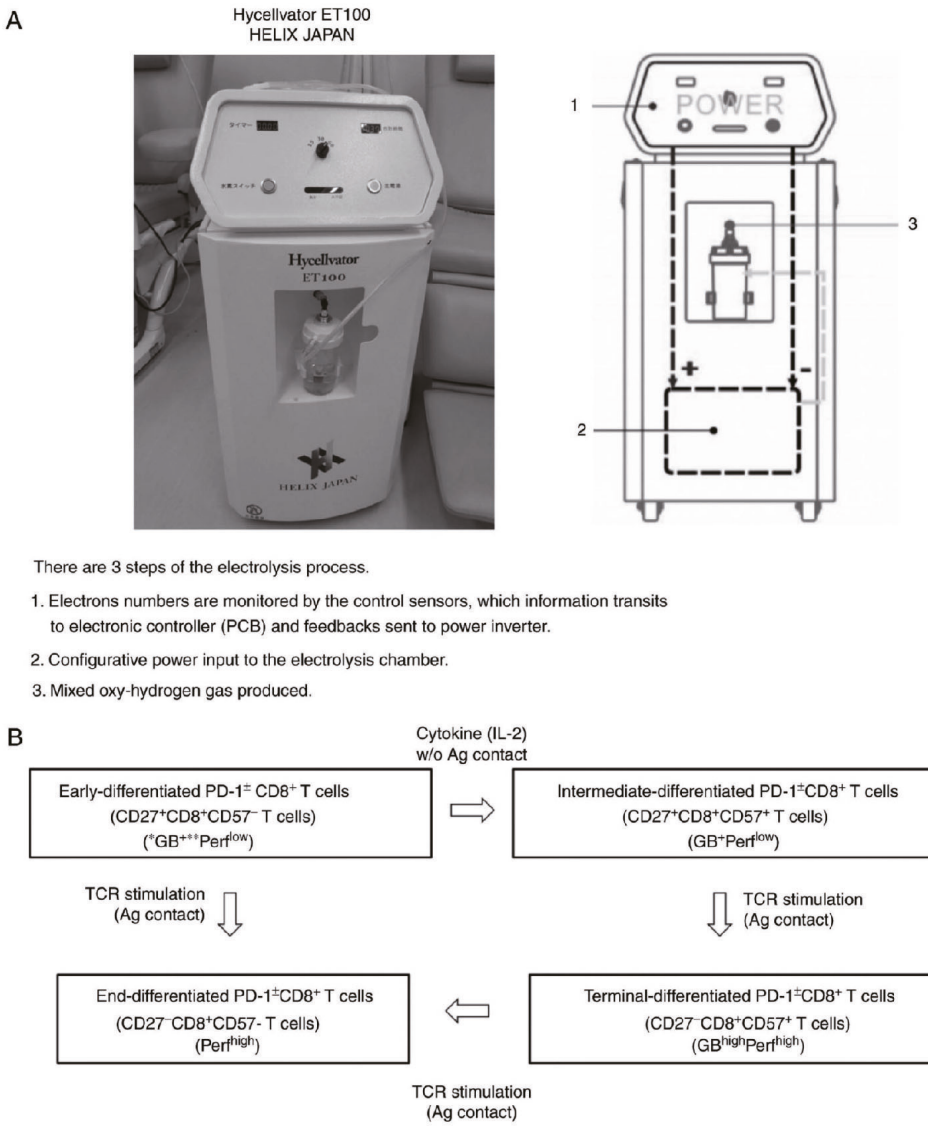


Figure 1. (A) Hycellvator ET100 (Helix Japan, Co., Ltd.), which generates hydrogen gas. (B) Differentiation pathway of CD8+ T cells from early-differentiated to end-differentiated CD8+ T cells (via intermediate- and terminal-differentiated CD8+ T cells). PCB, printed circuit board; CD, cluster of differentiation; GB, granzyme; Perf, perforin.

and end PD-1- CD8+ T cells, P=0.561 for PFS and P=0.206 for OS). Accordingly, patients were stratified based on the abundance of terminal PD-1+ CD8+ T cells, using cut-off values of 8.18 and 6.81% for PFS and OS, respectively, as determined from receiver operating characteristic curves (Fig. 2A and B). There were no significant differences in clinicopathological factors between the patients with high and low terminal PD-1+ CD8+ T cells (Table I). The resulting stratified Kaplan-Meier survival curves revealed that PFS (log-rank test, P=0.001) and OS (log-rank test, P=0.008) were significantly worse for patients with terminal PD-1+ CD8+ T cells higher than the cut-off (Fig. 2C and D). The median PFS time was 18 months for the patients with a high-terminal PD-1+ CD8+ T cell ratio, but was not reached (>50% of patients remained alive) for those with a low-terminal PD-1+ CD8+ T cell ratio, while OS was 18 months for the patients with a high-terminal PD-1+ CD8+ T cell ratio and 46 months for those with a low-terminal PD-1+ CD8+ T cell ratio.

Hydrogen gas reduces the proportion of PD-1+ CD8+ T cells and improves prognosis. Molecular hydrogen was reported to activate PGC-1α (28), which enhances mitochondrial activity (29), thereby rescuing exhausted CD8+ T cells with inactive mitochondria following progressive loss of PGC-1α (15). Therefore, the present study investigated whether hydrogen gas alters the proportion of PD-1+/- CD8+ T cell subsets and whether alterations, if any, are associated with prognosis in patients with stage IV cancer. Notably, hydrogen gas reduced the proportion of early, intermediate, terminal and end PD-1+ CD8+ T cells in 27 (49.1%), 28 (50.9%), 35 (63.6%) and 32 (58.2%) out of 55 patients, respectively. Conversely, hydrogen gas enhanced the proportion of early, intermediate,

Table II. Comparison of clinicopathological data between patients with high- and low-terminal PD-1$^+$ CD8$^+$ T cell ratio (the ratio of terminal PD-1$^+$ CD8$^+$ T cells following the hydrogen treatment to prior to it).

	Terminal PD-1$^+$ CD8$^+$ T cell ratio (PFS)			Terminal PD-1$^+$ CD8$^+$ T cell ratio (OS)		
Characteristic	High >0.87%	Low <0.87%	P-value	High >0.77%	Low <0.77%	P-value
Age, years	64.1±17.4	66.6±13.2	NS	65.3±15.8	66.0±14.0	NS
Sex, n			NS			NS
Male	10	11		13	8	
Female	10	24		13	21	
T factor, n						NS
T1	0	1		1	0	
T2	1	3		2	2	
T3	3	10	NS	5	8	
T4	3	8		3	8	
Tx	13	13		15	11	
N factor, n			NS			NS
N0	0	5		1	4	
N1	1	6		2	5	
N2	4	4		5	3	
N3	1	5		2	4	
Nx	14	15		16	13	
M factor, n			NS			NS
M0	0	0		0	0	
M1	20	35		26	29	
Histology, n			NS			NS
Tub1	5	15		8	12	
Tub2	7	11		9	9	
Poor	8	9		9	8	

NS, not significant; T factor, primary tumor; N factor, regional lymph node; M factor, distant metastasis; Tub1, well-differentiated adenocarcinoma; Tub2, moderately-differentiated adenocarcinoma; Poorly, poorly-differentiated adenocarcinoma; PD-1, programmed cell death 1; CD, cluster of differentiation; OS, overall survival; PFS, progression-free survival.

terminal and end PD-1$^-$ CD8$^+$ T cells in 32 (58.2%), 27 (49.1%), 39 (70.9%) and 31 (56.4%) patients, respectively. Univariate analysis showed that the ratio of the proportion of intermediate PD-1$^+$ CD8$^+$ T cells following treatment with hydrogen gas to that prior to treatment (intermediate PD-1$^+$ CD8$^+$ T cell ratio) was significantly associated with shorter PFS (HR, 2.286; 95% CI, 1.284-4.070; P=0.005) and OS (HR, 2.398; 95% CI, 1.384-4.156; P=0.002) times. Similar associations were observed for the intermediate PD-1$^-$ CD8$^+$ T cell ratio (PFS: HR, 2.286; 95% CI, 1.284-4.070; P=0.005; OS: HR, 2.398; 95% confidence interval, 1.384-4.156; P=0.002) and terminal PD-1$^+$ CD8$^+$ T cell ratio (PFS: HR, 6.459; 95% CI, 2.384-17.50; P<0.0001; OS: HR, 4.158; 95% CI, 1.718-10.06; P=0.002). By contrast, the early PD-1$^-$ CD8$^+$ T cell ratio was predictive of worse OS time (HR, 2.398; 95% CI, 1.384-4.156; P=0.002), whereas the terminal PD-1$^-$ CD8$^+$ T cell ratio was significantly associated with increased overall survival time (HR, 0.002; 95% CI, 0.000-0.131; P=0.004). For the multivariate analysis, the terminal PD-1$^+$ CD8$^+$ T cell ratio was found to be an independent predictor of poor PFS (HR, 6.459; 95% CI, 2.384-17.50; P<0.0001) and OS (HR, 2.957; 95% CI, 1.185-7.378; P=0.020). Based on these results, patients were stratified as having a high and low terminal PD-1$^+$ CD8$^+$ T cell ratio using cut-off values of 0.87 and 0.77% for PFS and OS, respectively (Fig. 3A and B). There were no significant differences in clinicopathological factors between the patients with high and low terminal PD-1$^+$ CD8$^+$ T cell ratio (Table II). The resulting stratified PFS and OS curves are plotted in Fig. 3C and D, and show that patients with a low terminal PD-1$^+$ CD8$^+$ T cell ratio have significantly increased PFS (P<0.0001) and OS (P=0.004) times compared with those with high ratios. The median follow-up time was 13 months for the patients with a high-terminal PD-1$^+$ CD8$^+$ T cell ratio, but was not reached (>50% of patients remained alive) for those with a low-terminal PD-1$^+$ CD8$^+$ T cello ratio, while that of OS was 15 months for the patients with a high-terminal PD-1$^+$ CD8$^+$ T cell ratio and 46 months for those with a low-terminal PD-1$^+$ CD8$^+$ T cell ratio.

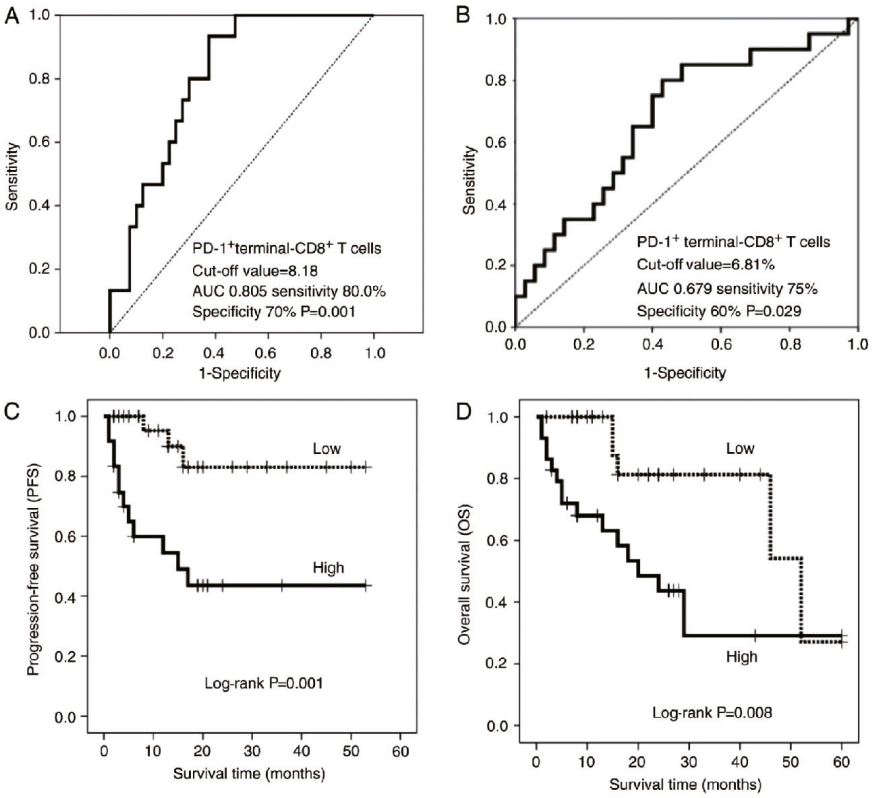


Figure 2. Receiver operating characteristic curves of (A) PFS and (B) OS based on terminal PD-1$^+$ CD8$^+$ T cells. The cut-off values are 8.18 and 6.81%, respectively. (C) PFS and (D) OS in patients with a high- (solid line) and low- (dotted line) percentage of terminal PD-1$^+$ CD8$^+$ T cells. PFS, progression-free survival; OS, overall survival; AUC, area under the curve; PD-1, programmed cell death 1; CD, cluster of differentiation.

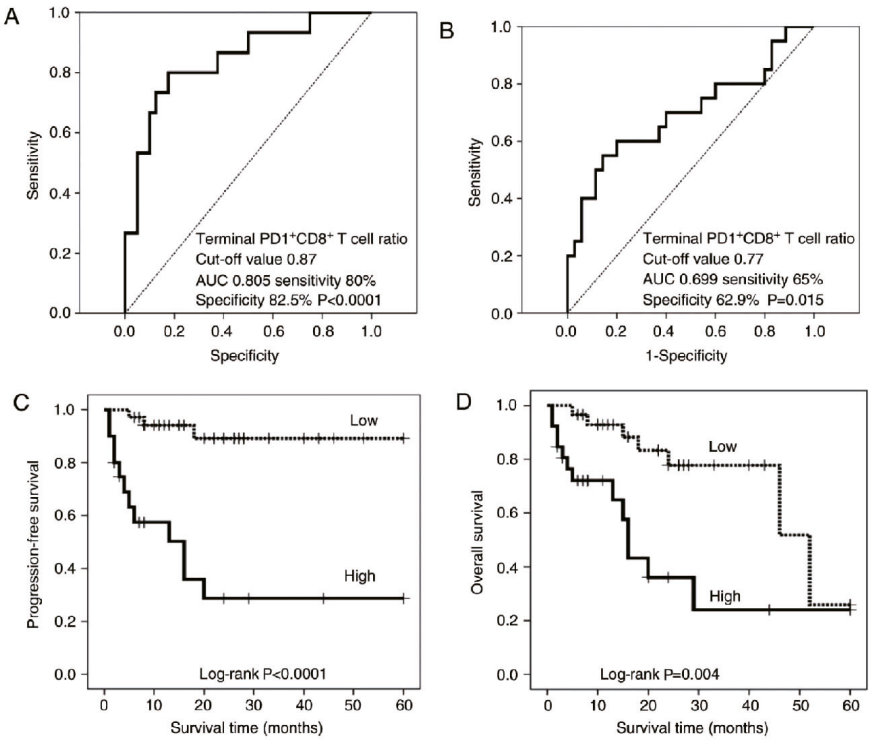


Figure 3. Analysis of the ratio of terminal PD-1$^{+/-}$ CD8$^+$ T cells prior to and following treatment with hydrogen gas (terminal PD-1$^{+/-}$ CD8$^+$ T cell ratio). Receiver operating characteristic curve of (A) PFS and (B) OS based on the terminal PD-1$^+$ CD8$^+$ T cell ratio. The cut-off values are 0.87 and 0.77%, respectively. (C) PFS and (D) OS in patients with a high-(solid line) and low-(dotted line) terminal PD-1$^+$ CD8$^+$ T cell ratio. PFS, progression-free survival; OS, overall survival; AUC, area under the curve; PD-1, programmed cell death 1; CD, cluster of differentiation.

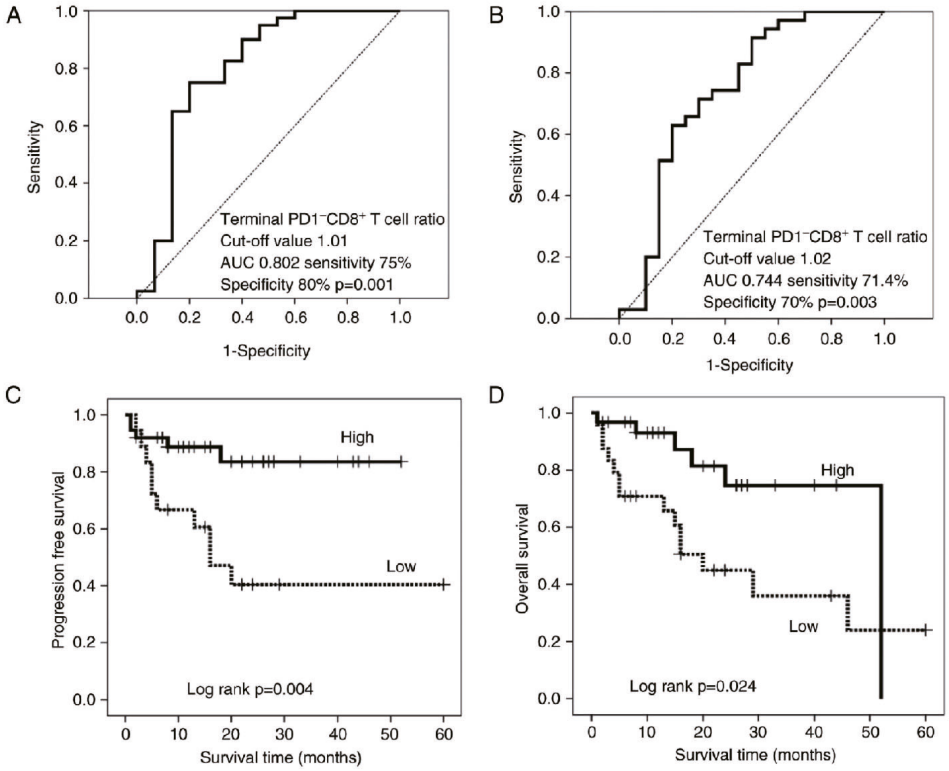


Figure 4. Receiver operating characteristic curve of (A) PFS and (B) OS based on the terminal PD-1$^-$ CD8$^+$ T cell ratio, with cut-off values of 1.01 and 1.02%, respectively. (C) PFS and (D) OS in patients with a high- (solid line) and low- (dotted line) terminal PD-1$^-$ CD8$^+$ T cell ratio. PFS, progression-free survival; OS, overall survival; AUC, area under the curve; PD-1, programmed cell death 1; CD, cluster of differentiation.

Hydrogen-induced accumulation of terminal PD-1$^-$ CD8$^+$ T cells is associated with an improved prognosis. Based on the hypothesis that hydrogen gas may activate mitochondrial function and thereby convert exhausted terminal PD-1$^+$ CD8$^+$ T cells into active terminal PD-1$^-$ CD8$^+$ T cells, the present study investigated whether the change in the abundance of the latter impacts prognosis in patients treated with hydrogen gas. Thus, patients were stratified by the terminal PD-1$^-$ CD8$^+$ T cell ratio based on cut-off values of 1.01 and 1.02 for PFS (Fig. 4A) and OS (Fig. 4B), respectively. There were no significant differences in clinicopathological factors between the patients with high- and low-terminal PD-1$^-$ CD8$^+$ T cell ratios (Table III). The resulting stratified survival curves are plotted in Fig. 4C and D, respectively, and indicate that patients with high ratios have significantly increased PFS (P=0.004) and OS (P=0.024) times compared with those with low ratios. The median follow-up time was 15 months for the patients with a low-terminal PD-1$^-$ CD8$^+$ T cell ratio, but was not reached (>50% of patients remained alive) for those with a high-terminal PD-1$^-$ CD8$^+$ T cell ratio, while that of OS was 16 months for the patients with a low-terminal PD-1$^-$ CD8$^+$ T cell ratio and 52 months for those with a high-terminal PD-1$^-$ CD8$^+$ T cell ratio. Furthermore, hydrogen gas treatment resulted in a significantly longer PFS time (P=0.014) and a generally longer, but non-significant, OS time (P=0.165) in patients with a high level of terminal PD-1$^-$ CD8$^+$ T cells compared with that in patients with a low level, although there was no significant difference between the groups prior to treatment (Fig. 5A and C).

Serum terminal PD-1$^{+/-}$ CD8$^+$ T cells are important immune indices in patients with advanced cancer. As aforementioned, hydrogen gas reduces the proportion of terminal PD1$^+$ CD8$^+$ T cells, but increases the abundance of terminal PD1$^-$ CD8$^+$ T cells, and the magnitude of these changes is strongly associated with the prognosis. Notably, the terminal PD1$^+$ CD8$^+$ T cells were significantly and inversely correlated with the terminal PD1$^-$ CD8$^+$ T cells (Fig. 6A and B), suggesting that the dynamic balance between these subsets contributes strongly to prognosis in patients with advanced colorectal carcinomas. Therefore, patients were stratified based on all possible high/low combinations of these subsets [category (Cat) 1-4; Cat 1: Patients with low PD1$^+$ and high PD1$^-$ terminal CD8$^+$ T cells; Cat 2: Patients with high PD1$^+$ and high PD1$^-$ terminal CD8$^+$ T cells; Cat 3: Patients with low PD1$^+$ and low PD1$^-$ terminal CD8$^+$ T cells; Cat 4: Patients with high PD1$^+$ and high PD1$^-$ terminal CD8$^+$ T cells; Fig. 6A and B]. Kaplan-Meier analysis revealed that Cat 1 patients experienced significantly longer PFS times than all other groups, whereas Cat 3 patients experienced significantly longer OS times than the others. By contrast, Cat 4 patients experienced significantly worse PFS (Fig. 6C) and OS (Fig. 6D) times than others. Hydrogen gas also increased the number of patients with low PD1$^+$ terminal CD8$^+$ T cells (Cat 1 and 3), but decreased the number of patients with high PD1$^+$ terminal CD8$^+$ T cells (Cat 2 and 4), leading to an improved prognosis in patients with stage IV colorectal carcinoma (Fig. 6A and B; Table IV).

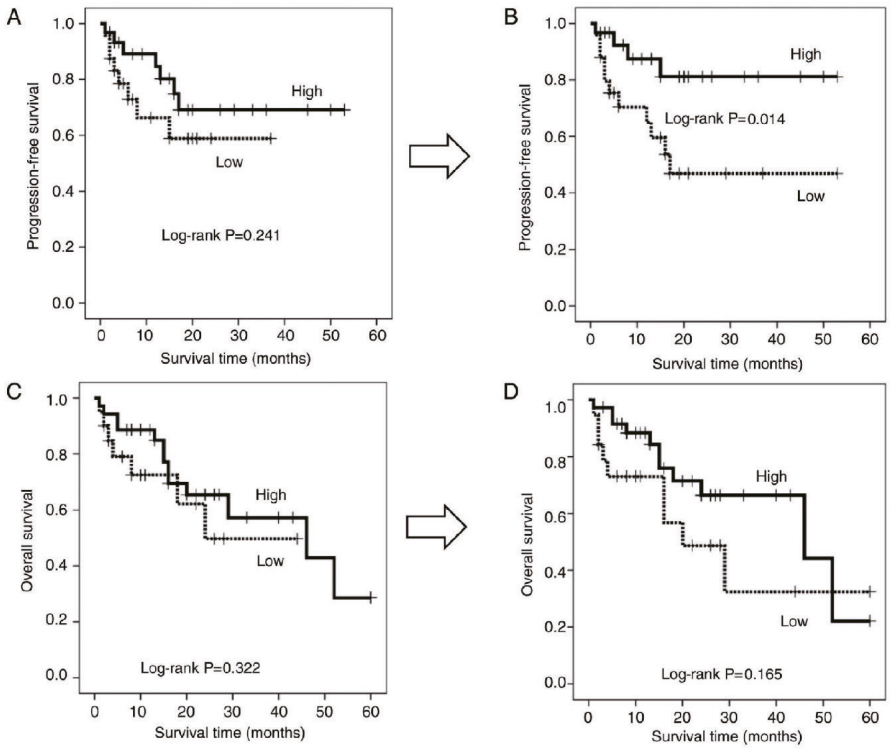


Figure 5. Progression-free survival in patients with a high-(solid line) and low-(dotted line) terminal PD-1$^-$ CD8$^+$ T cell ratio (A) prior to and (B) following treatment with hydrogen gas. Comparison of overall survival between patients with a high-(solid line) and low-(dotted line) terminal PD-1$^-$ CD8$^+$ T cell ratio (C) prior to and (D) following treatment with hydrogen gas. PD-1, programmed cell death 1; CD, cluster of differentiation.

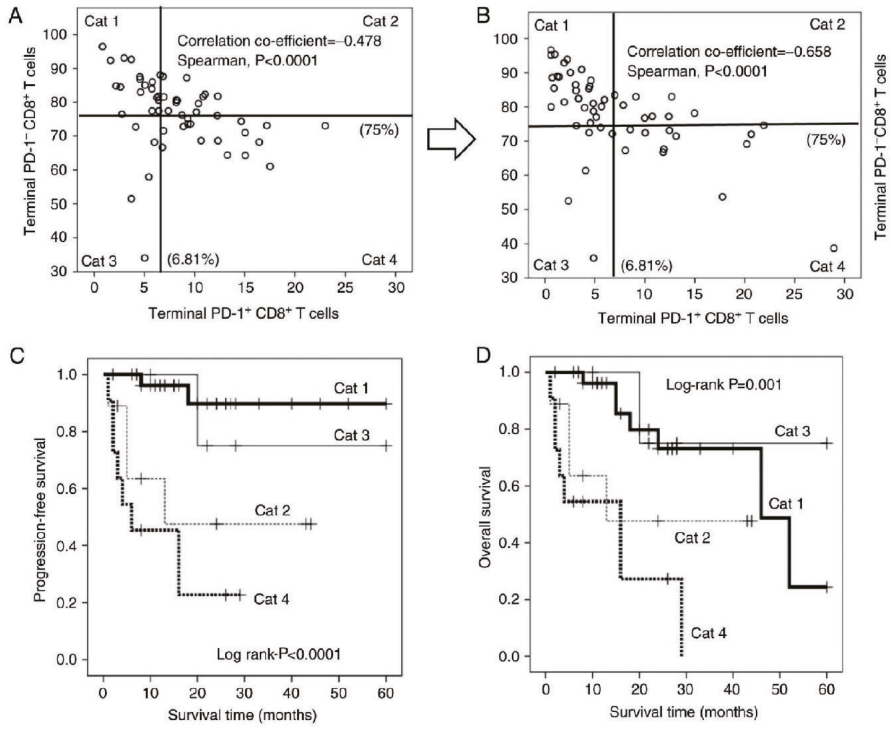


Figure 6. Point diagram of the correlation between terminal PD-1$^+$ (x-axis) and PD-1$^-$ CD8$^+$ T cells (y-axis) (A) prior to and (B) following treatment with hydrogen gas. The x-axis of terminal PD-1$^+$ and the y-axis of PD-1$^-$ CD8$^+$ T cells are divided into two sections with cut-offs of 6.81 and 75%, respectively. Cat 1: Low-terminal PD-1$^+$ CD8$^+$ T cells and high-terminal PD-1$^-$ CD8$^+$ T cells; Cat 2: High-terminal PD-1$^+$ CD8$^+$ T cells and high-terminal PD-1$^-$ CD8$^+$ T cells; Cat 3: Low-terminal PD-1$^+$ CD8$^+$ T cells and low-terminal PD-1$^-$ CD8$^+$ T cells; Cat 4: High-terminal PD-1$^+$ CD8$^+$ T cells and low-terminal PD-1 CD8$^+$ T cells. (C) Progression-free survival and (D) overall survival in Cat 1 (bold line), Cat 2 (thin dotted line), Cat 3 (thin line) and Cat 4 (bold dotted line) patients. PD-1, programmed cell death 1; CD, cluster of differentiation; Cat, category.

Table III. Comparison of clinicopathological data between patients with high- and low-terminal PD-1$^-$ CD8$^+$ T cell ratio.

Characteristic	Terminal PD-1$^-$ CD8$^+$ T cell ratio (PFS)		P-value	Terminal PD-1$^-$ CD8$^+$ T cell ratio (OS)		P-value
	High >1.01%	Low <1.01%		High >1.02%	Low <1.02%	
Age, years	65.8±13.8	65.3±17.0	NS	64.7±13.8	66.9±16.2	NS
Sex			NS			NS
Male	13	8		10	11	
Female	24	10		21	13	
T factor			NS			NS
T1	1	0		1	0	
T2	2	2		2	2	
T3	12	1		11	2	
T4	8	3		6	5	
Tx	14	12		11	15	
N factor			NS			NS
N0	5	0		4	1	
N1	5	2		5	2	
N2	4	4		3	5	
N3	5	1		5	1	
Nx	18	11		14	15	
M factor			NS			NS
M0	0	0		0	0	
M1	37	18		31	24	
Histology			NS			NS
Tub1	14	6		13	7	
Tub2	13	5		12	6	
Poor	10	7		6	11	

NS, not significant; T factor, primary tumor; N factor, regional lymph node; M factor, distant metastasis; Tub1, well-differentiated adenocarcinoma; Tub2, moderately-differentiated adenocarcinoma; Poorly, poorly-differentiated adenocarcinoma; PD-1, programmed cell death 1; CD, cluster of differentiation; OS, overall survival; PFS, progression-free survival.

Table IV. Change of category following hydrogen gas treatment.

Category classification	Hydrogen gas treatment		Rate of variability
	Prior to treatment	Following treatment	
Cat 1	14	21 ↑	7/14=50% ↑
Cat 2	13	9 ↓	4/13=31% ↓
Cat 3	14	18 ↑	4/18=22% ↑
Cat 4	14	7 ↓	7/14=50% ↓

Cat 1, low PD1$^+$ and high PD1$^-$ terminal CD8$^+$ T cells; Cat 2, high PD1$^+$ and high PD1$^-$ terminal CD8$^+$ T cells; Cat 3, low PD1$^+$ and low PD1$^-$ terminal CD8$^+$ T cells; Cat 4, high PD1$^+$ and low PD1$^-$ terminal CD8$^+$ T cells; PD-1, programmed cell death 1; CD, cluster of differentiation; Cat, category.

Discussion

Persistent stimulation by carcinoma cells renders cytotoxic CD8$^+$ T cells into an exhausted state without proliferation, cytokine production or cytotoxic capabilities. Accumulation of exhausted CD8$^+$ T cells in the peripheral blood, as well as at the tumor site, has been reported to result in a poor prognosis in patients with cancer (3-6). In the present study, terminal PD-1$^+$

$CD8^+$ T cells were an independent poor prognostic factor in the patients with stage IV colorectal carcinoma. It was recently reported that exhausted $CD8^+$ T cells exhibit mitochondrial dysfunction, caused by the inactivation of PGC-1α, to express immune checkpoint inhibitors such as PD-1 and Tim-3, but return to the active effector state following exposure to certain stimulants (7). Hydrogen was recently reported to stimulate PGC-1α (28), which enhances mitochondrial function (29) and thus may rescue exhausted $CD8^+$ T cells. The present study found that hydrogen gas reduced the proportion of all four $PD\text{-}1^+$ $CD8^+$ T cell subsets and increased the proportions of all four $PD\text{-}1^-$ $CD8^+$ subsets. Univariate and multivariate analyses indicated that loss of terminal $PD\text{-}1^+$ $CD8^+$ T cells is the most significant contributor to improved prognosis. Furthermore, reduction of terminal $PD\text{-}1^+$ $CD8^+$ T cells and accumulation of terminal $PD\text{-}1^-$ $CD8^+$ T cells following hydrogen gas treatment were significantly associated with improved PFS (Figs. 3C and 4C) and OS (Figs. 3D and 4D) times. Moreover, treatment with hydrogen gas significantly extended PFS time in patients with abundant $PD\text{-}1^-$ $CD8^+$ T cells compared with time in others (Fig. 5B) and slightly improved OS, although this result was not significant (Fig. 5D). Collectively, these results suggested that hydrogen gas converts exhausted terminal $PD\text{-}1^+$ $CD8^+$ T cells into active terminal $PD\text{-}1^-$ $CD8^+$ T cells, thereby improving prognosis.

The present study found that circulating terminal $PD\text{-}1^+$ $CD8^+$ T cells in patients with stage IV colorectal cancer were strongly and independently associated with short PFS and OS times, in agreement with recent reports that circulating and tumor-infiltrating $PD\text{-}1^+$ $CD8^+$ T cells contribute to poor prognosis in various cancer types, including breast, pancreatic and gastric cancer (7-10). These results suggested that prognosis and therapeutic response in patients with cancer may be easily and non-invasively predicted based on terminal $PD\text{-}1^+$ $CD8^+$ T lymphocytes in the peripheral blood, consistent with data demonstrating that circulating $CD8^+$ $PD\text{-}1^+$ lymphocytes could provide a window into determining the characteristics of tumor-resident antitumor lymphocytes (8). The abundance of terminal $PD\text{-}1^+$ $CD8^+$ T cells was inversely correlated with that of terminal $PD\text{-}1^-$ $CD8^+$ T cells (Fig. 6A and B), and a novel classification system composed of four quadrants was created (Fig. 6). The patients belonging to the first (Cat 1) and third (Cat 3) quadrants, in whom the proportion of terminal $PD\text{-}1^+$ $CD8^+$ T cells was below the cut-off value, had an improved prognosis (Fig. 6C and D). By contrast, the patients belonging to the second (Cat 2) and fourth (Cat 4) quadrants, who had a higher level of terminal $PD\text{-}1^+$ $CD8^+$ T cells than the cut-off value had a poorer prognosis, particularly the patients of Cat 4, with the worst PFS and OS times (Fig. 6C and D). These results suggested that the balance between terminal $PD\text{-}1^+$ and $PD\text{-}1^+$ $CD8^+$ T cells is critical for cancer prognosis and that the novel patient classification system based on terminal $PD\text{-}1^+$ and $PD\text{-}1^-$ $CD8^+$ T cells is useful to predict therapeutic effects, as well as prognosis.

In the future, in order to confirm that hydrogen gas can restore exhausted $CD8^+$ T cells ($PD\text{-}1^+$ $CD8^+$ T cells) into active $CD8^+$ T cells ($PD\text{-}1^-$ $CD8^+$ T cells) by the activation of the mitochondria, we will investigate whether the aforementioned conversion ($PD\text{-}1^+$ $CD8^+$ T cells → $PD\text{-}1^-$ $CD8^+$ T cells) is associated with measured values of coenzyme Q_{10} (CoQ10) and growth differentiation factor 15 (GDF15), which are supposed to reflect actual mitochondrial function. Tim-3, a member of the recently discovered T cell Immunoglobulin and mucin domain family, is supposed to be expressed on senescent $CD8^+$ T cells, of which mitochondrial function may be irreversible (33). CoQ10 and GDF15 will also be measured on senescent $CD8^+$ T cells ($Tim\text{-}3^+$ $CD8^+$ T cells). Furthermore, future studies will investigate whether hydrogen gas actually converts $PD\text{-}1^+$ $CD8^+$ T culture cells into $PD\text{-}1^-$ $CD8^+$ T culture cells *in vitro*.

In conclusion, hydrogen gas decreases the number of terminal $PD\text{-}1^+$ $CD8^+$ T cells, i.e., exhausted $CD8^+$ T cells, possibly by activating mitochondria via PGC-1α, thereby increasing the number of terminal $PD\text{-}1^-$ $CD8^+$ T cells and improving the patient prognosis. Thus, terminal $PD\text{-}1^+$ and $PD\text{-}1^-$ $CD8^+$ T cells are critical immune parameters in patients with cancer and are conveniently measurable in the peripheral blood. A novel system for patient classification (Cat 1-4) based on these indices was also developed in the present study in order to assist in predicting prognosis and therapeutic response.

Acknowledgements

The authors would like to thank Helix Japan, Co., Ltd. (Tokyo, Japan) for providing the Hycellvator ET 100 hydrogen gas generator.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JA was involved in the analysis and interpretation of the data, and HB was involved in the analysis of the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were approved and in accordance with the ethical standards of Kumamoto University (Kumamoto, Japan) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Patient consent for publication

Approval was obtained from all patients for the publication of the study.

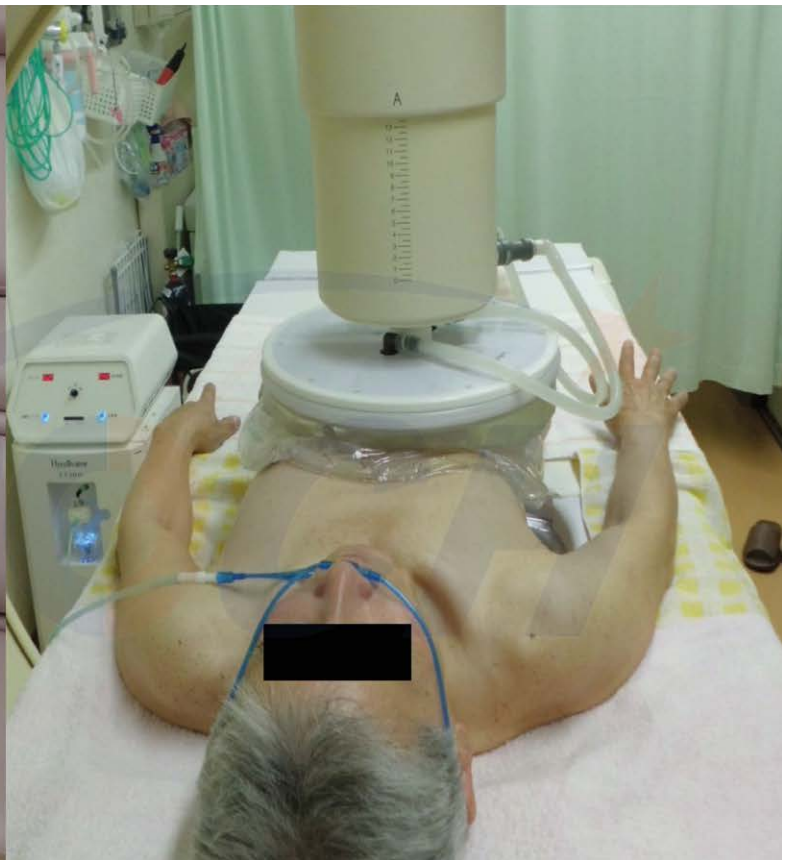
Competing interests

The authors declare that they have no conflicts of interest.

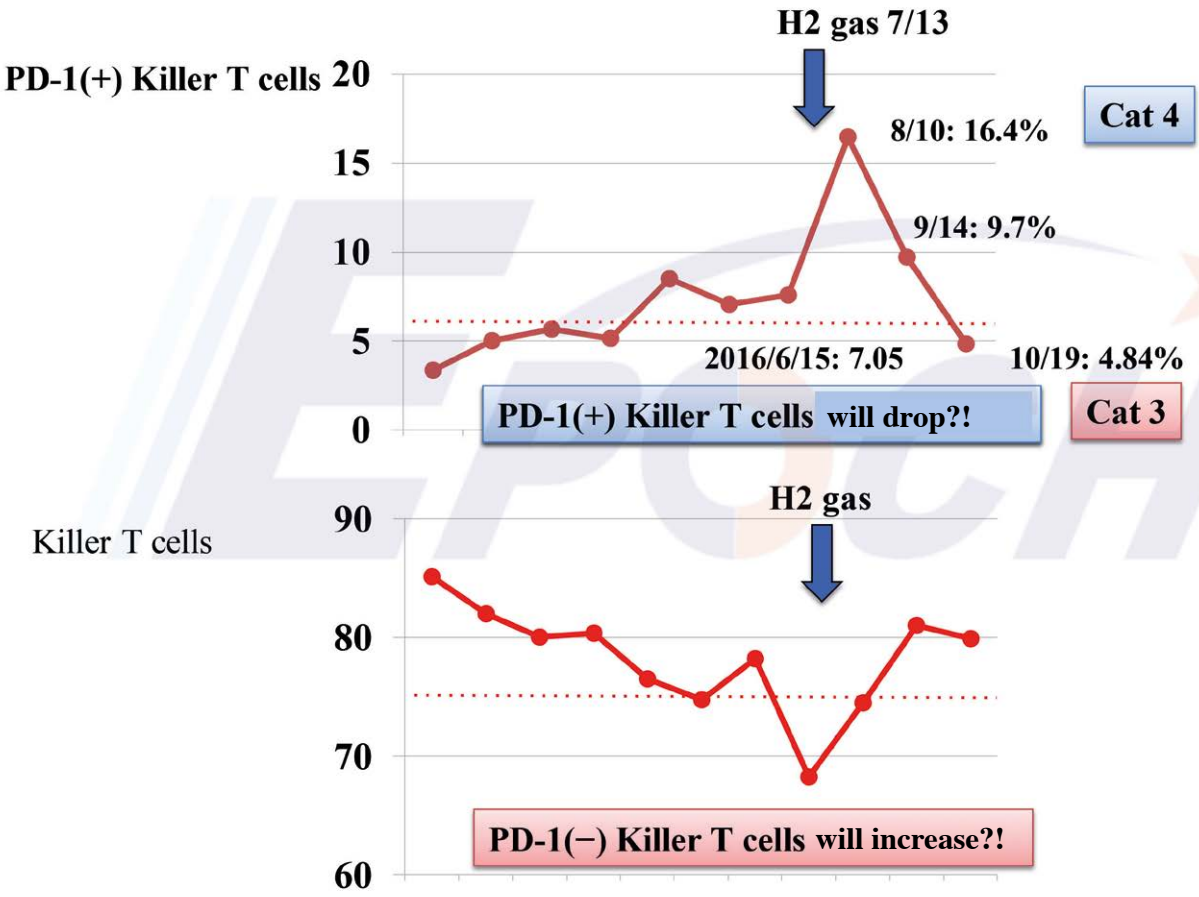
References

1. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ and Ahmed R: Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 439: 682-687, 2006.
2. Wherry EJ: T cell exhaustion. Nat Immunol 12: 492-499, 2011.
3. Ahmadzadeh M, Johnson LA, Heemskerk B, Wunderlich JR, Dudley ME, White DE and Rosenberg SA: Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood 114: 1537-1544, 2009.
4. Sun S, Fei X, Mao Y, Wang X, Garfield DH, Huang O, Wang J, Yuan F, Sun L, Yu Q, *et al*: PD-1^{+} immune cell infiltration inversely correlates with survival of operable breast cancer patients. Cancer Immunol Immunother 63: 395-406, 2014.
5. Zarour HM: Reversing T-Cell dysfunction and exhaustion in cancer. Clin Cancer Res 22: 1856-1864, 2016.
6. Lu X, Yang L, Yao D, Wu X, Li J, Liu X, Deng L, Huang C, Wang Y, Li D, *et al*: Tumor antigen-specific CD8^{+} T cells are negatively regulated by PD-1 and Tim-3 in human gastric cancer. Cell Immunol 313: 43-51, 2017.
7. Scharping NE, Menk AV, Moreci RS, Whetstone RD, Dadey RE, Watkins SC, Ferris RL and Delgoffe GM: The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell metabolic insufficiency and dysfunction. Immunity 45: 374-388, 2016.
8. Gros A, Parkhurst MR, Tran E, Pasetto A, Robbins PF, Ilyas S, Prickett TD, Gartner JJ, Crystal JS, Roberts IM, *et al*: Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients. Nat Med 22: 433-438, 2016.
9. Ohsawa I, Ishikawa M, Takahashi K, Watanabe M, Nishimaki K, Yamagata K, Katsura KI, Katayama Y, Asoh S and Ohta S: Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nat Med 13: 688-694, 2007.
10. Zhou L, Wang X, Xue W, Xie K, Huang Y, Chen H, Gong G and Zeng Y: Beneficial effects of hydrogen-rich saline against spinal cord ischemia-reperfusion injury in rabbits. Brain Res 1517: 150-160, 2013.
11. Hayashida K, Sano M, Ohsawa I, Shinmura K, Tamaki K, Kimura K, Endo J, Katayama T, Kawamura A, Kohsaka S, *et al*: Inhalation of hydrogen gas reduces infarct size in the rat model of myocardial ischemia-reperfusion injury. Biochem Biophys Res Commun 373: 30-35, 2008.
12. Zheng X, Zheng X, Mao Y, Cai J, Li Y, Liu W, Sun P, Zhang JH, Sun X and Yuan H: Hydrogen-rich saline protects against intestinal ischemia/reperfusion injury in rats. Free Radic Res 43: 478-484, 2009.
13. Lee JW, Kim JI, Lee YA, Lee DH, Song CS, Cho YJ and Han JS: Inhaled hydrogen gas therapy for prevention of testicular ischemia/reperfusion injury in rats. J Pediatr Surg 4: 736-742, 2012.
14. Wang F, Yu G, Liu SY, Li JB, Wang JF, Bo LL, Qian LR, Sun XJ and Deng XM: Hydrogen-rich saline protects against renal ischemia/reperfusion injury in rats. J Surg Res 167: e339-e344, 2011.
15. Ji X, Tian Y, Xie K, Liu W, Qu Y and Fei Z: Protective effects of hydrogen-rich saline in a rat model of traumatic brain injury via reducing oxidative stress. J Surg Res 178: e9-e16, 2012.
16. Chen T, Tao Y, Yan W, Yang G, Chen X, Cao R, Zhang L, Xue J and Zhang Z: Protective effects of hydrogen-rich saline against N-methyl-N-nitrosourea-induced photoreceptor degeneration. Exp Eye Res 148: 65-73, 2016.
17. Ren JD, Ma J, Hou J, Xiao WJ, Jin WH, Wu J and Fan KH: Hydrogen-rich saline inhibits NLRP3 inflammasome activation and attenuates experimental acute pancreatitis in mice. Mediators Inflamma 2014: 930894, 2014.
18. Nakao A, Toyoda Y, Sharma P, Evans M and Guthrie N: Effectiveness of hydrogen rich water on antioxidant status of subjects with potential metabolic syndrome-an open label pilot study. J Clin Biochem Nutr 46: 140-149, 2010.
19. Amitani H, Asakawa A, Cheng K, Amitani M, Kaimoto K, Nakano M, Ushikai M, Li Y, Tsai M, Li JB, *et al*: Hydrogen improves glycemic control in typel diabetic animal model by promoting glucose uptake into skeletal muscle. PLoS One 8: e53913, 2013.
20. Li GM, Ji MH, Sun XJ, Zeng QT, Tian M, Fan YX, Li WY, Li N and Yang JJ: Effects of hydrogen-rich saline treatment on polymicrobial sepsis. J Surg Res 181: 279-286, 2013.
21. Guo SX, Jin YY, Fang Q, You CG, Wang XG, Hu XL and Han CM: Beneficial effects of hydrogen-rich saline on early burn-wound progression in rats. PLoS One 10: e0124897, 2015.
22. Kikkawa YS, Nakagawa T, Taniguchi M and Ito J: Hydrogen protects auditory hair cells from cisplatin-induced free radicals. Neurosci Lett 579: 125-129, 2014.
23. Watanabe S, Fujita M, Ishihara M, Tachibana S, Yamamoto Y, Kaji T, Kawauchi T and Kanatani Y: Protective effect of inhalation of hydrogen gas on radiation-induced dermatitis and skin injury in rats. J Radiat Res 55: 1107-1113, 2014.
24. Ushida T, Kotani T, Tsuda H, Imai K, Nakano T, Hirako S, Ito Y, Li H, Mano Y, Wang J, *et al*: Molecular hydrogen ameliorates several characteristics of preeclampsia in the Reduced Uterine Perfusion Pressure (RUPP) rat model. Free Radic Biol Med 101: 524-533, 2016.
25. Ge L, Yang M, Yang NN, Yin XX and Song WG: Molecular hydrogen: A preventive and therapeutic medical gas for various diseases. Oncotarget 8: 102653-102673, 2017.
26. Runtuwene J, Amitani H, Amitani M, Asakawa A, Cheng KC and Inui A: Hydrogen-water enhances 5-fluorouracil-induced inhibition of colon cancer. PeerJ 3: e859, 2015.
27. Wang D, Wang L, Zhang Y, Zhao Y and Chen G: Hydrogen gas inhibits lung cancer progression through targeting SMC3. Biomed Pharmacother 104: 788-797, 2018.
28. Kamimura N, Ichimiya H, Iuchi K and Ohta S: Molecular hydrogen stimulates the gene expression of transcriptional coactivator PGC-1α to enhance fatty acid metabolism. NPJ Aging Mech Dis 2: 16008, 2016.
29. Handschin C and Spiegelman BM: Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. Endocr Rev 27: 728-735, 2006.
30. Sobin LH and Wittekind CH (eds): UICC TNM Classification of malignant tumors. John Wiley and Sons, New York, 1997.
31. Tamura T, Hayashida K, Sano M, Suzuki M, Shibusawa T, Yoshizawa J, Kobayashi Y, Suzuki T, Ohta S, Morisaki H, *et al*: Feasibility and safety of hydrogen gas inhalation for post-cardiac arrest syndrome-First-in-Human Pilot Study. Circ J 80: 1870-1873, 2016.
32. Cancer therapy evaluation program, common terminology criteria for adverse events, Version 3.0, DCTD, NCI, NIH, DHHS. Int J Clin Oncol 9 (Sup PIII): S1-S82, 2004.
33. Crespo J, Sun H, Welling TH, Tian Z and Zou W: T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. Curr Opin Immunol 25: 214-221, 2013.

The application of hydrogen oxygen



KH 68y.o., After breast cancer operation + Metastatic tumor of bone + Liver metastases



PD-1+CD8+ T cells in poor prognosis

PD-1 + Case studies of cancers

TIL (Tumor Infiltrating Lymphocytes)

1. Mojgan Ahmadzadeh, Laura A. Johnson, and Steven A. Rosenberg. Tumor antigen–specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood 114: 1537-1544, 2009. Cancer TIL
2. Sun S, Fei X, Mao Y,et al. PD-1(+) immune cell infiltration inversely correlates with survival of operable breast cancer patients.Cancer Immunol Immunother. 63(4):395-406, 2014. Cancer TIL
3. Hassane M. Zarour. Reversing T-cell Dysfunction and Exhaustion in Cancer. Clin Cancer Res. 22(8): 1856–1864, 2016. Cancer TIL
4. Lu X, Yang L, Yao D, et al. Tumor antigen-specific CD8+ T cells are negatively regulated by PD-1 and Tim-3 in human gastric cancer. Cell Immunol. 313:43-51, 2017. Gastric Cancer TIL

Peripheral blood

Shuichi Takano, Hiroaki Saito, and Masahide Ikeguchi. An increased number of PD-1+ and Tim-3+ CD8+ T cells is involved in immune evasion in gastric cancer. Surgery Today 46: 1341–1347, 2016. Gastric Cancer, Peripheral blood

Peripheral blood and TIL

1. Tao Shen, Liangjing Zhou, Hua Shen, et al. Prognostic value of programmedcell death protein 1 expression on CD8+ T lymphocytes in pancreatic cancer. Scientific Reports 7: 7848, 2017. Pancreatic Cancer, Peripheral blood
2. Giraldo NA, Becht E, Vano Y, et al. Tumor-Infiltrating and Peripheral Blood T-cell Immunophenotypes Predict Early Relapse in Localized Clear Cell Renal Cell Carcinoma. Clin Cancer Res. 23(15): 4416-4428, 2017.Gastric Cancer, TIL Peripheral blood

Peripheral Blood Lymophocytes ≒ TIL

= Peripheral blood lymphocytes is shown TIL

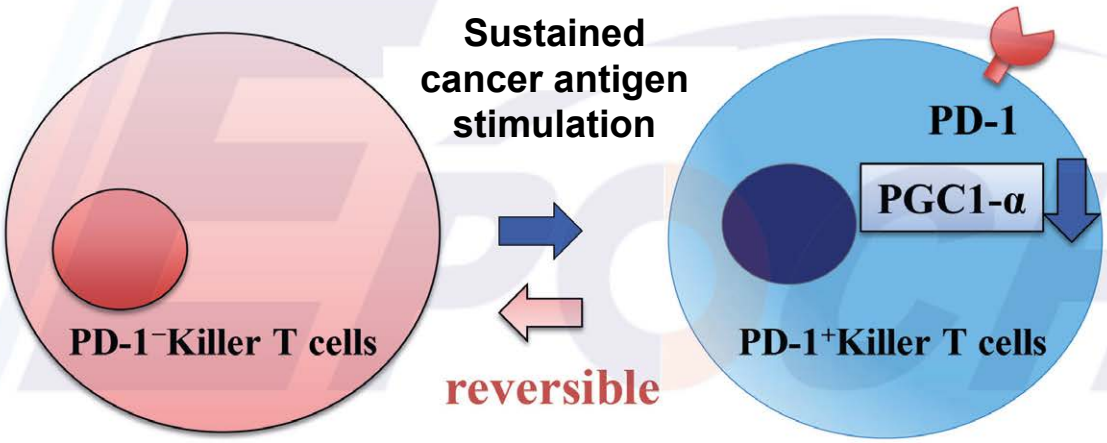
Circulating CD8+PD-1+ lymphocytes could provide a window into the tumor-resident antitumor lymphocytes.
末梢血のCD8+PD-1+リンパ球は、
腫瘍周辺に存在する抗腫瘍リンパ球のウインドウである

Alena Gros, Maria R Parkhurst, Eric Tran, et al. Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients. ***Nature Medicine.*** **22, 433–438 (2016)**

Detection Method: Extract lymphocyte from blood to examine index number of PD-1(+-)

PD-1⁺ - killer CD8⁺ What is T Cells (Fatigue T Cells)?

PD-1⁺ - killer CD8⁺ T Cells = exhausted CD8⁺ T Cells
=①Active cell damage ↓ ② 2 types of Cytokine ↓ ③Cell proliferation ability ↓
= Participate in poor prognosis



ACTIVE T CELLS
Mitochondrial activation

Exhausted CD8+ T Cells
= Fatigue T Cells
=PGC1-α Reduced
→ Mitochondrial inactivation
= Energy consumption status

npj | Aging and Mechanisms of Disease

氫氣提升PGC-1α再活化粒線體！

ARTICLE OPEN

Molecular hydrogen stimulates the gene expression of transcriptional coactivator PGC-1α to enhance fatty acid metabolism

npj Aging and Mechanisms of Disease (2016) 2, 16008.

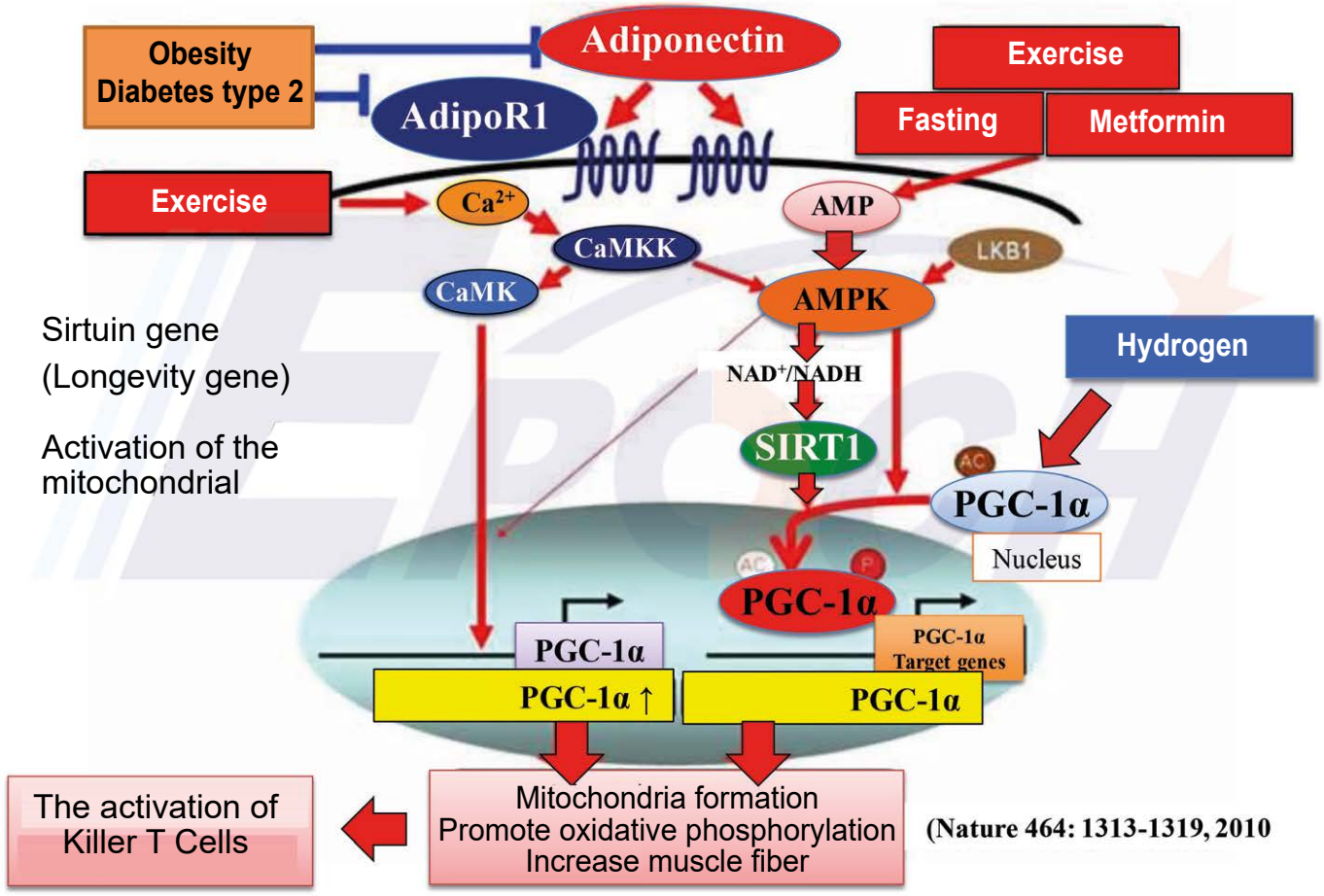
Naomi Kamimura[1], Harumi Ichimiya

水素ガス→PGC1-α増強
→ミトコンドリアの活性化

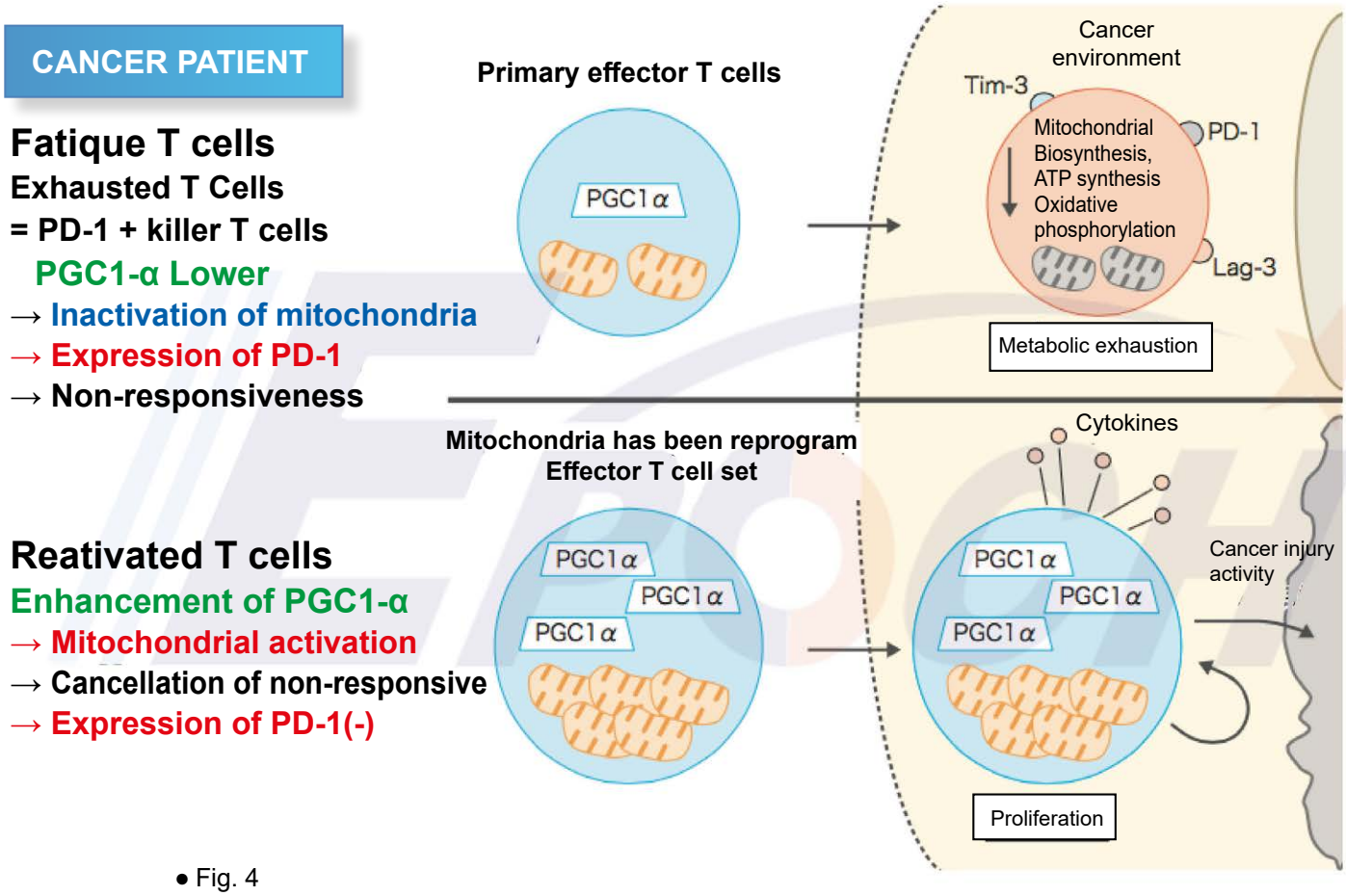
We previously reported that mole multiple functions. Moreover, long-term drinking of H_2-water (water infused with H_2) enhanced energy expenditure to improve obesity and diabetes in *db/db* mice accompanied by the increased expression of fibroblast growth factor 21 (FGF21) by an unknown mechanism. H_2 was ingested by drinking of H_2-water or by oral administration of an H_2-producing material, MgH_2. The comprehensive gene expression profile in the liver of *db/db* mice was analyzed by DNA microarray. The molecular mechanisms underlying the gene expression profile was investigated using cultured HepG2 cells. Moreover, the effects on lifespan of drinking H_2-water were examined using wild-type mice that were fed a fatty diet. Pathway analyses based on comprehensive gene expression revealed the increased expression of various genes involved in fatty acid and steroid metabolism. As a transcription pathway, the PPARα signaling pathway was identified to upregulate their genes by ingesting H_2. As an early event, the gene expression of PGC-1α was transiently increased, followed by increased expression of *FGF21*. The expression of *PGC-1α* might be regulated indirectly through sequential regulation by H_2, 4-hydroxy-2-nonenal, and Akt/FoxO1 signaling, as suggested in cultured cell experiments. In wild-type mice fed the fatty diet, H_2-water improved the level of plasma triglycerides and extended their average of lifespan. H_2 induces expression of the *PGC-1α* gene, followed by stimulation of the PPARα pathway that regulates FGF21, and the fatty acid and steroid metabolism.

npj Aging and Mechanisms of Disease (2016) **2,** 16008; doi:10.1038/npjamd.2016.8; published online 28 April 2016

Exercise produces PGC-1 α and activates mitochondrion.
Hydrogen water provides the same function too!



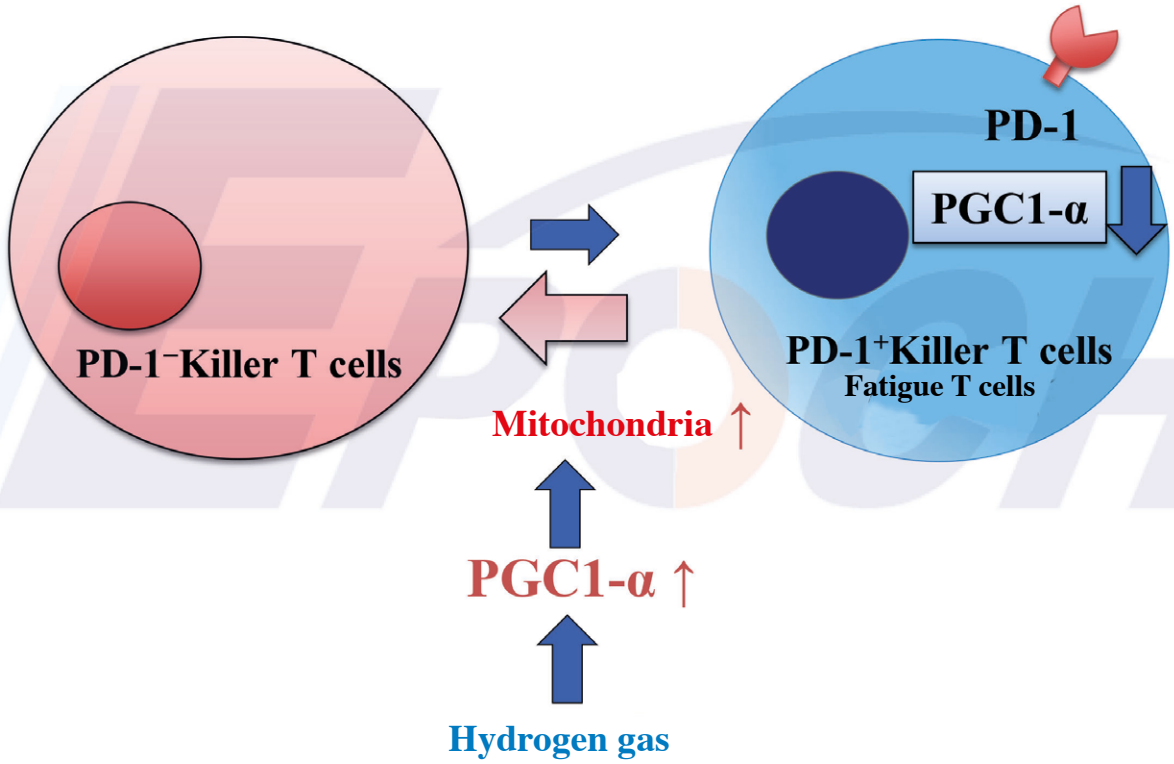
(Nature 464: 1313-1319, 2010



● Fig. 4

Recovery which invading of killer T Cell unresponsiveness by mitochondrial reprogramming. CD8-T cells invading the cancer microenvironment are mitochondria. By enhancing PGC-1α expression, mitochondria can be reactivated to release unresponsiveness

Hypothesis: Hydrogen activates mitochondria via PGC1-α and Reactivates exhausted T Cells



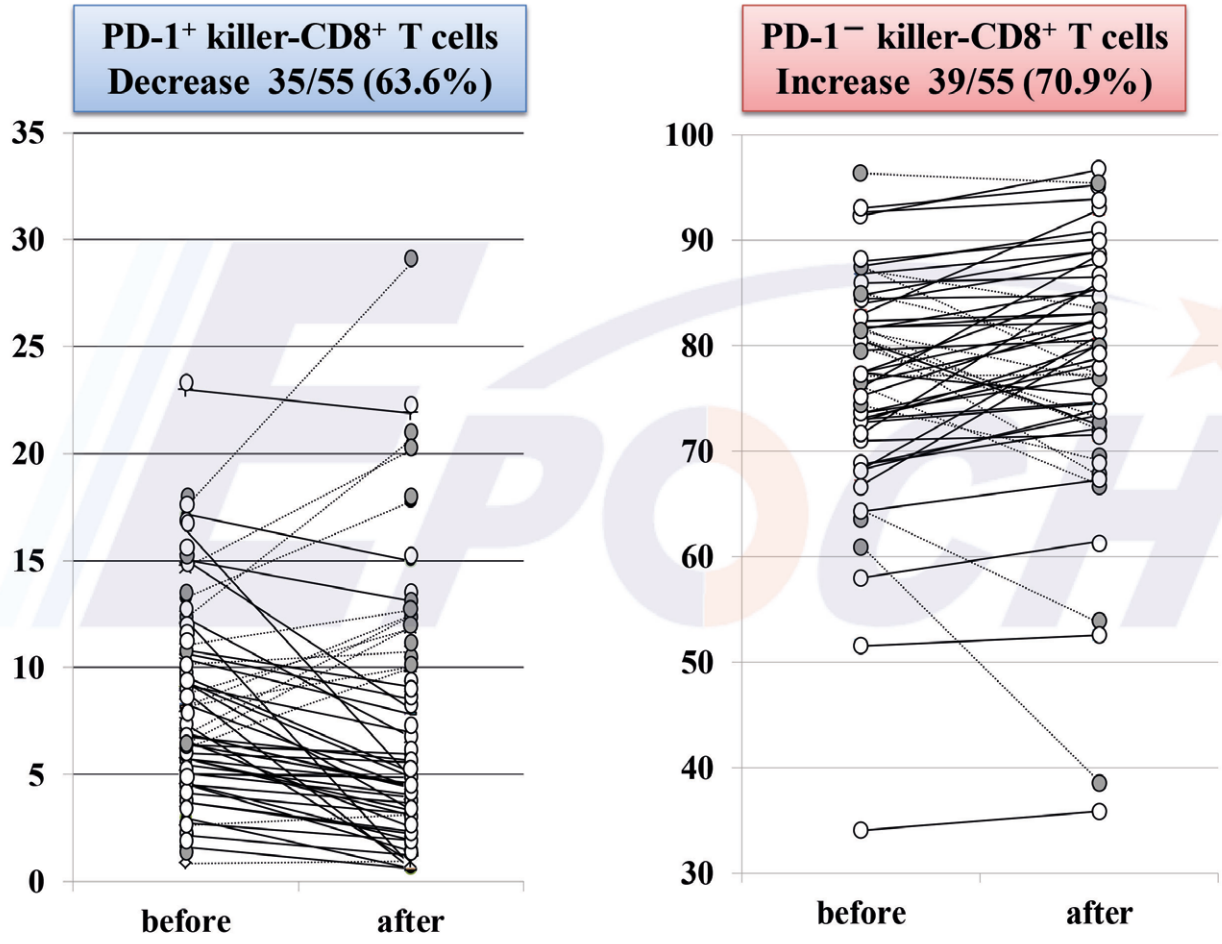
Hydrogen increases PGC-1α and activates mitochondrion. It activates PD-1+ into PD-1⁻

Stage IV in peripheral blood of 55, stage cancer patients with advanced cancer, we measured the expression of PD-1 on CD8+ T cells using flow cytometry and analyzed the relationship with prognosis.

Stage IVの進行癌患者 55例の末梢血で、
フローサイトメトリーを用いてCD8+ T細胞上の
PD-1の発現を測定し予後との関連を解析した

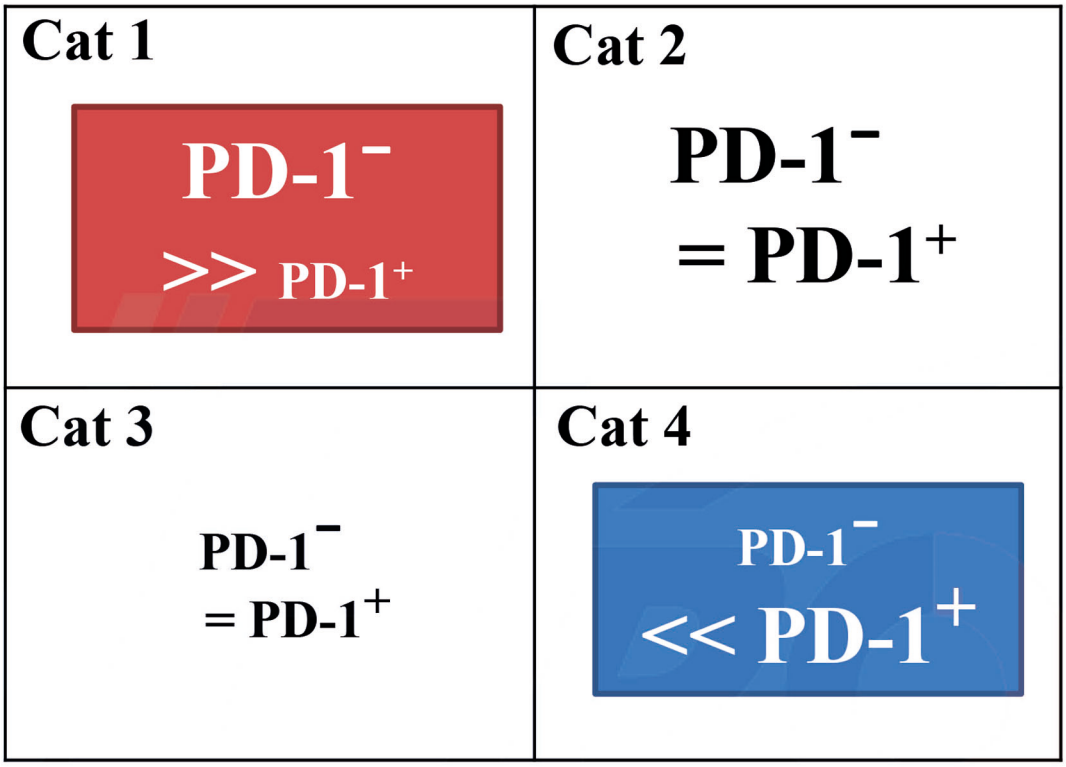
PD-1(-+) level of 55 terminal stage cancer patients have changed after inhaling hydrogen.

Changes after hydrogen inhalation



The PD-1+ in 33 out of 55 patients decrease, and the PD-1- in 39 out of 55 patients increase after inhaling hydrogen.

Category



PD-1⁻= PD-1⁻-killer-CD8⁺ T cells (killer)

PD-1⁺=PD-1⁺killer-CD8⁺ T cells (PD-1⁺killer)

Dr. Akagi divides PD-1- into 4 categories according patients level of PD-1(-+).

1. Own the most of PD-1- and the least of PD-1+
2. Both share the same amount of PD-1-
3. Both share the same amount of PD-1+
4. Own the most of PD-1+ and the least of PD-1-

According to scientific studies, healthy body cells enable OPDIVO to provide better result

CrossMark click for updates

PNAS

Mitochondrial activation chemicals synergize with surface receptor PD-1 blockade for T cell-dependent antitumor activity

ベザフィブラートがオプジーボの効果を増強する

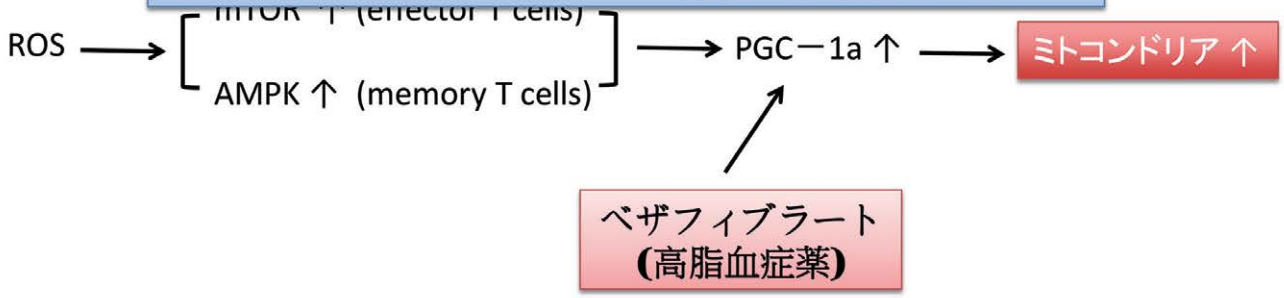
Kenji Chamoto[a,1], Partha S. Chowdhury[a,1], Alok Kumar[a], Kazuhiro Sonomura[b,c], Fumihiko Matsuda[b], Sidonia Fagarasan[d], and Tasuku Honjo[a,2]

本庶佑氏

「私はリンパ球内のミトコンドリアを活性化することで、オプジーボの治療効果が上がるとみている。」

2016; reviewed by Hiroyoshi Nishikawa and Hiroshi Shiku)

水素ガスはオプジーボの効果を増強する ?!



Opdivo case before using hydrogen

Only consume **OPDIVO** before inhaling hydrogen

Case of illness	Age	Gender	organ	Stage	Result	cat	Hydrogen	PD-1 KT	Outcomes
1.YN	60	Y	Stomach	Temporal ovarian metastases	SD →PD	Cat 1	(—)	1.42	Dead (14)
2.NH	79	M	Bile duct	Resection of intrahepatic cholangiocarcinoma	PR →PD	Cat 4 →Cat 1 →Cat 2	(—)	17.14 →5.46 →14.2	Dead (3)
3.SY	59	M	Lung	Cancerous pleurisy	SD →PD	Cat 1	(—)	5.05	Dead (12)
4.KY	88	M	Lung	Resection can not do on lung cancer	SD	Cat 1	(—)	6.06	Dead (3)
5.OK	67	M	Colon	Lung metastases, bone metastases	PD	Cat 4 →Cat 3	(—)	11.66 →4.49	Dead (1.5)
6.MH	67	M	Lung	Bone metastases, brain metastases	SD →PD	Cat 4 →Cat 3	(—)	11.98 →2.81	Dead (2.5)
7.FY	60	M	Lung	Bone, brain, lung metastases	SD →PD	Cat 3	(—)	3.52	Dead (4)
8.SY	70	Y	Panc	Mutiple lung metastases	PD	Cat 4	(—)	16.78 →11.56	Dead (1)

Case of illness	Age	Gender	organ	Stage	Result	cat	Hydrogen	PD-1 KT	Outcomes
9. MK	50	F	Ovarian	Ovarian cancer + Peritoneal seeding	PD	Cat 3	(—)	6.89	Dead (2)
10. KM	49	F	Uterus	Uterus cancer + lung seeding	PD	Cat 4	(—)	26.84	Dead (1)
11. EE	63	F	Ovarian	Ovarian cancer + Peritoneal seeding + liver seeding	PD	Cat 4	(—)	21.19	Dead (1)
12. HK	58	M	Neck & head	Lung seeding	SD	Cat 4	(—)	17.17	Alive (5)
13. MM	81	M	Liver	Peritoneal seeding	SD	Cat 2	(—)	28.14	Dead (3)

Survival rate 2/13 (15.4%) if only consume anticancer medicine

Opdivo case with combination of hydrogen

Hydrogen + OPDIVO

Case of illness	Age	Sex	Organ	Stage	Result	CAT	Hydrogen	PD-1 KT	Outcomes
1.TY	50	M	Lung	Lung cancer after surgery	SD	Cat 1	(+)	12.26	Alive (31)
2. KK	68	M	Urinary System	Lung metastases	PR	Cat 4 →Cat 1	(+)	9.26	Alive (15)
3. OM	81	M	Subgingival gland	Local recurrence	SD	Cat1	(+)	0.84	Dead (11)
4. US	70	F	Panc	Partial immersion	SD	Cat 4	(+)	13.93	Dead (7)
5. KS	61	M	Lung	Pleural seeding	SD	Cat 2	(+)	11.84	Alive (5)
6. HS	62	F	Lung	Cancerous-pleurisy	PR	Cat1	(+)	2.98	Alive (24)
7. TT	84	Y	Colon	Local recurrence	SD	Cat 2	(+)	8.88	Dead (13)
8. NH	56	M	Head & neck cancer	Arterial infiltration	SD	Cat 4	(+)	10.15	Alive (5)
9. SK	50	M	Esophagus	Lymph mode metastases	SD	Cat 1	(+)	4.8	Alive (4)
10. SR	77	F	Panc	Partial immersion	SD	Cat 1	(+)	5.9	Alive (4)
11. MM	81	M	Liver	Peritoneal seeding	SD	Cat 2	(+)	28.14	Dead (3)

Case of illness	Age	sex	organ	Stage	Result	cat	Hydrogen	PD-1 KT	Outcomes
12.TK	48	M	Bile duct	Lung metastases	SD	Cat 3	(+)	7.49	Alive (2)
13. TH	66	F	Stomach	Peritoneal seeding	PD	Cat 2	(+)	10.71	Alive (4)
14. HK	68	M	Bile duct	Liver metastases	SD	Cat 3	(+)	5.41	Alive (7)

Survival rate 10/14 (71.4%) if consume hydrogen with anticancer medicine

Category

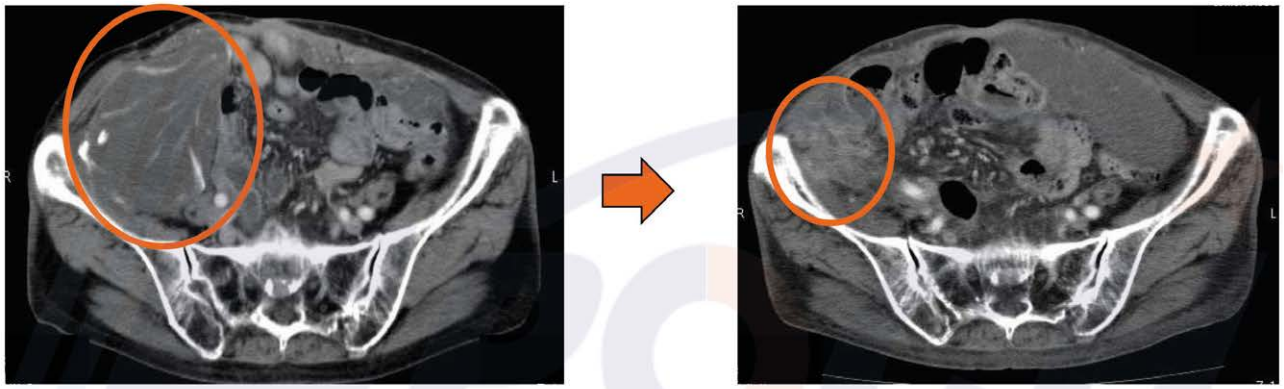
Cat 1 $PD\text{-}1^-$ >> $PD\text{-}1^+$	**Cat 2** $PD\text{-}1^-$ = $PD\text{-}1^+$
Cat 3 $PD\text{-}1^-$ = $PD\text{-}1^+$	**Cat 4** $PD\text{-}1^-$ << $PD\text{-}1^+$

$PD\text{-}1^-$= $PD\text{-}1^-$-killer-$CD8^+$ T cells (killer)

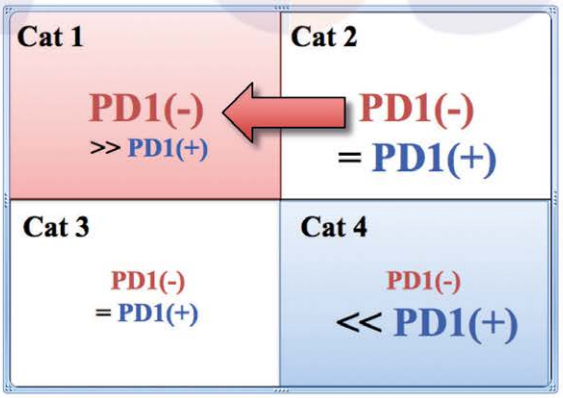
$PD\text{-}1^+$=$PD\text{-}1^+$killer-$CD8^+$ T cells ($PD\text{-}1^+$killer)

Local recurrence after colon cancer
Low-dose chemotherapy combined with hyperthermia
+ opdivo + hydrogen

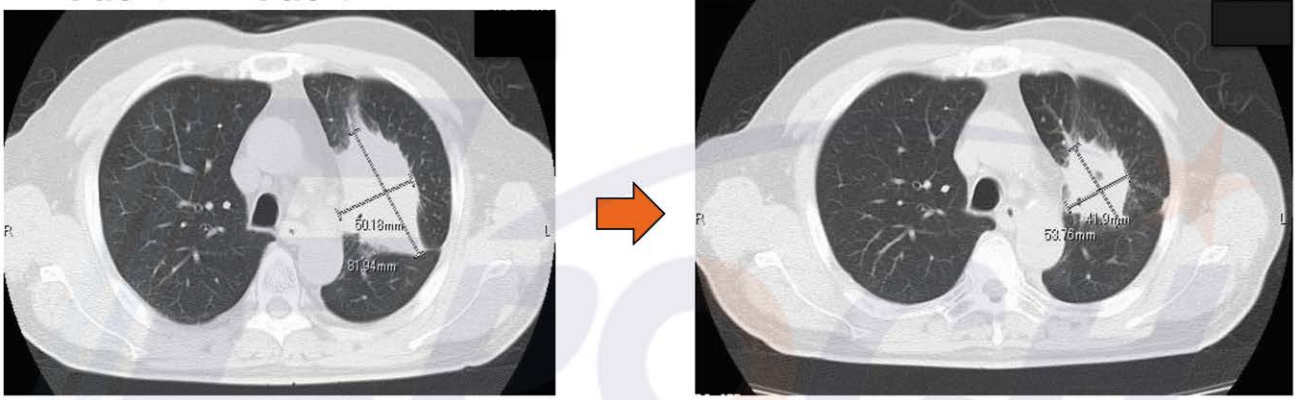
Cat 2 → Cat 1



Patient's PD-1 category has changed after inhaling hydrogen



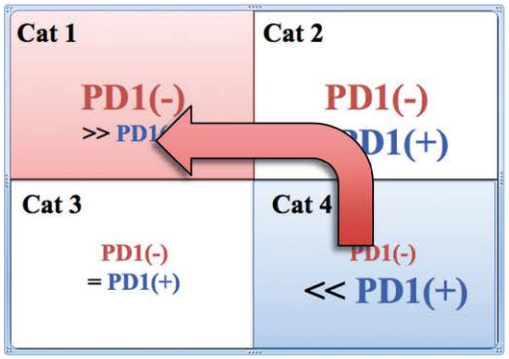
尿管癌肺転移
低用量化学療法併用ハイパーサーミア＋オプジーボ＋水素ガス
Cat 4 → Cat 1



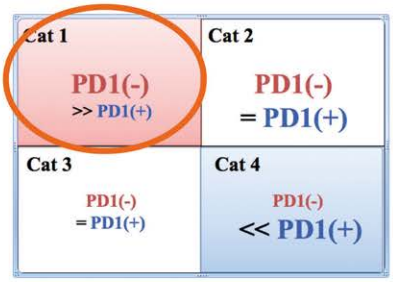
81.94x50.18mm

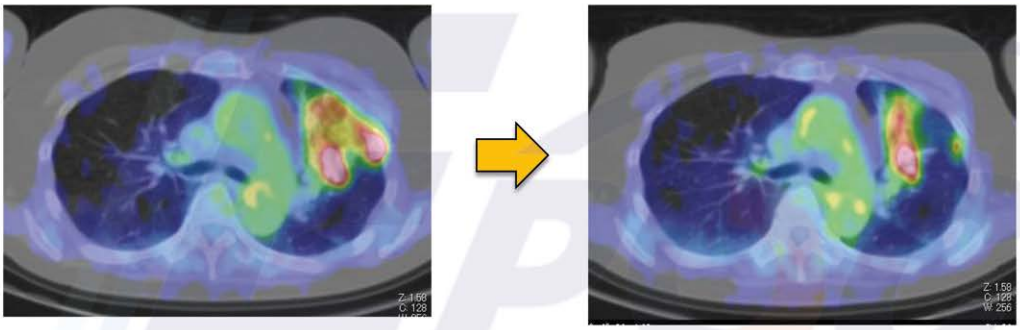
53.76x41.90mm
RECIST 34.4%

Patient's PD-1 category has changed from cat 4 to cat 1 after inhaling hydrogen

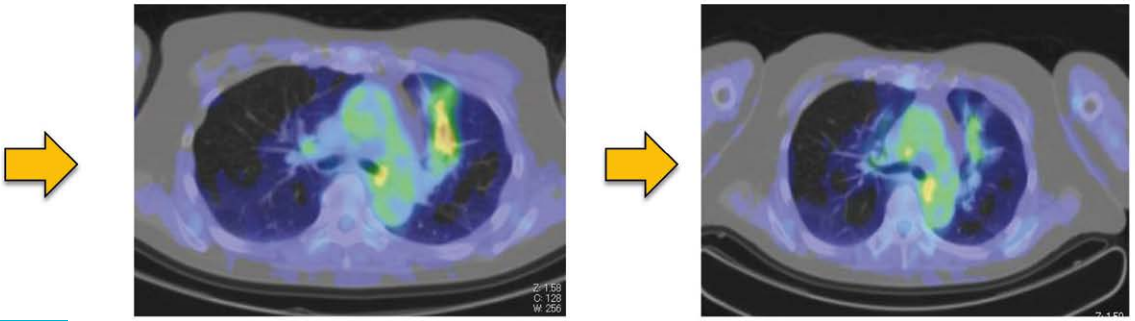


PR症例：H.S. 62y.o. female 非小細胞肺癌 (cat 1)
Stage IV 癌性胸膜炎、癌性胸水
ハイパーサーミア併用低用量化学療法
＋オプジーボ＋水素ガス
平成26年6月～平成30年1月 3年7ヶ月経過





The treatment effect of Cat 1 is the best !



Conclusion:

OPDIVO blocks cancer cells by hiding the attack of immune cells.

Hydrogen oxygen (HO) activates cells to produce immune cells and attack cancer cells.

It increases the success rates for cancer treatments!

Hydrogen → increase PGC-1a → activate mitochondrion → increase $PD\text{-}1^-$ and decrease $PD\text{-}1^+$

Hydrogen is a decent source to increase anticancer cells $PD\text{-}1^-$ and body self-healing ability.

$OH^{\cdot}$ H_2 $H_2O + H^+$

/ HydrOxygen Chi Therapy Machine /
Japan Helix JP- ET-100

Netturul HO series

本格的水素吸入マシン

Hycellvator®

ハイドレックス

HELIX JAPAN
MADE IN Japan

ET100

ガス発生方法	電気分解方式
水素酸素ガス発生量	水素1.200ml/m 酸素600ml/m
水素純度	99.99%
寸法/重量	800×360×425mm 39kg
吸入時間設定	15分/30分/60分
連続使用時の待機時間	2分
オートオフ機能	使用 5分後　電源自動オフ
専用水の使用	本機専用に開発した高純度蒸留水を使用
メンテナンス	使用時間1000hまたは年1回

HO MACHINES ARE CURRENTLY WIDELY USED IN MORE THAN HUNDREDS OF MEDICAL CENTERS IN JAPAN.

01 location

Saitama
Otomo Surgery Orthopedic Surgery

02 location

Arakaki
Plastic Surgery Clinic

03 location

Matsuhashi
Ear, nose and throat · Internal Medicine Clinic

04 location

Aichi
Medical University Hospital

05 location

Nomura
Clinic

●総合病院

大阪府

新井クリニック

熊本県

玉名地域保健医療センター

●外科・整形外科

埼玉県

大友外科整形外科

●消化器内科・免疫療法

鳥取県

よろずクリニック

●心療内科

東京都

まいんずたわーメンタルクリニック

●美容・泌尿器科

神奈川県

統合医療研究所 T-LAB

●内科・循環器科・皮膚科

茨城県

くらのクリニック

●内科・婦人科

東京都

ひめのともみクリニック

●内科・免疫療法

東京都

クリニック真健庵

三重県

飛鳥メディカルクリニック

大阪府

田中クリニック

東京都

新宿溝口クリニック

熊本県

わかばクリニック

●外科・免疫療法

沖縄県

新垣形成外科

●内科・小児科

神奈川県

おおり医院

●介護・ケアステーション経営

香川県

（有）ケアステーション

医療コンサルタント

熊本県

㈱ＢＫＮ

●耳鼻咽喉科・内科

熊本県

松橋耳鼻咽喉科

内科

宮崎県

野村循環器内科クリニック

東京都

馬場クリニック

●歯科・口腔外科

島根県

真理渡部歯科クリニック

●老人ホーム（養老院）

神奈川県

グループホーム観音崎

●薬局

大阪府

あおぞら薬局

●医療機器販売

香川県

高松医療器株式会社

●整体

埼玉県

堀内整骨院

東京都

国立駅前カイロプラクティック

東京都

千歳烏山北口カイロプラクティック

東京都

RENATUS

●マッサージ店(按摩)

鳥取県

手もみ屋本舗　米子旗ケ崎店

岡山県

手もみ屋本舗　水江店

●東洋医学関連、調剤

群馬県

㈲ホリスティク

●サロン(沙龍)

埼玉県

癒しの空間　心

●美容健康スペース

東京都

トゥエイボア ヒルトンホテル東京Ｂ１Ｆ

●介護施設

愛知県

くらし応援ネットワーク

●水素サロン(氫沙龍)

長崎県 佐世保市

本格的水素吸入サロン 水セレブ

神奈川県

サロンR

東京都

サントップ水素サロン

東京都

元代々木サロン

埼玉県

水素健康ステーション　浦和

●スポーツトレーナー（體育教練）

京都府

㈱リーチ

●野球部

東京都

早稲田大学

●陸上部

神奈川県

プレス工業株式会社

●レーシングドライバー(賽車手)

埼玉県

坪井　翔様

東京都

石浦宏明様

●水泳部

東京都 中央大学

埼玉県 自衛隊体育学校

神奈川県 日本体育大学

●美容院

茨城県

ココデメル

大阪府

スペースアーツ心斎橋

東京都

ハイドロジェニック

●鍼灸

神奈川県

愈楽

スポーツ関係・美容・サロン

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The trend of hydroxygen health care therapy in Japan

There’re many hydroxygen health care therapy centers in Japan, it becomes a trendy beauty and health care therapy

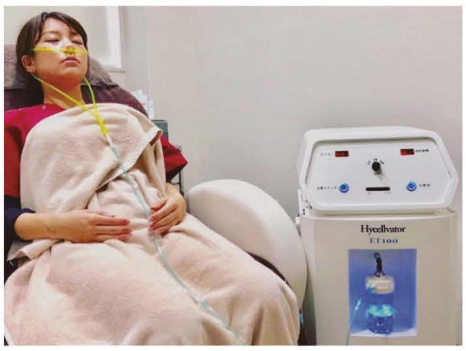
#水素サロン#アンチエイジング#万能らしい#水素サーバー#水素サプリメント#水素風呂#欲しい#欲しい#ぁあ#欲しい（笑）#パパ（笑）#育毛に良いらしい#ダイエットに良いらしい#良い#良い#良い#良い#良い

ファブ#都賀#千葉#水素吸入#むくみ解消#美肌#鼻炎#冷え性改善#

こんにちは
スカルクイックには水素吸入マシンも導入しています!ヘッドスパと水素吸入を一緒に行うことで脳のリフレッシュに
水素吸入は悪玉活性酸素を水素で身体の外へ。エイジングケアや二日酔い、倦怠感幅の改善が期待できます。

サロン内の一部をご紹介致します。
足をしっかり伸ばせるリクライニングソファでお友達とご一緒に吸引して頂くこともできます。
日差しも程よく、明るいのでついつい寝てしまいます　アットフォームな空間を意識している為、お一人で友人宅に行くかのようにふらっと立ち寄るのも良し、お友達とランチに行く感覚で是非お立ち寄り下さい。

高濃度水素吸入サロン
水素は飲む→吸う時代です *水素業界No1の高濃度水素の吸
入器(flow)を取り扱っております
flowの水素吸入は、たった1分間で水素水100ℓ以上の摂取が出来るので30分の吸入で12トンの水素水を飲んだのと同じ効果です



#江守クリニック#江守クリニック皮膚科#なつこクリニック#皮膚科#美容皮膚科#女医#皮膚科専門医#金沢#北陸#エイジングケア#スキンケア#美肌#美白#シミ#シワ#たるみ#肝斑#毛穴#ニキビ#ニキビ跡#肌荒れ#活性酸素#フリーラジカル#水素吸入#水素#水素水#疲労回復#ダイエット#モデル#Hycellvator

There're many famous hydroxygen therapy centers,

using HO machines by Epoch Energy Technology Corp in Japan.

からだの水素専門店
超高濃度
水素吸入サロン
水素発生量業界第一位
1分間に2500cc

HO Series

FLOW-100/HB-33

Specifications

Gas production (L/hr)	70 - 75
Size (mm)	480*300*460
Weight (kg)	28
Power consumption(w)	400
Power supply (v)	110/220
Number of users (person)	1

Hydrogen (cc/min)	913
Oxygen (cc/min)	337
Purity of Hydrogen (%)	99.99

EPOCH ENGERGY TECHNOLOGY CORP.

No.3, Beigong 5th Rd., Environmental Science and Technology Park, Gangshan District, Kaohsiung City, 82050, Taiwan (R.O.C.)
Tel: 07-6235588 Fax: 07-6221133
http://www.oxy-hydrogen.com

CERTIFICATE FOR EXCLUSIVE SOLE DISTRIBUTOR (MALAYSIA & SINGAPORE)

Issuing party	Epoch Energy Technology Corp.		
Representative	Brian Aiello	Title	Vice President
Address	No.3, Beigong 5th Road, Gangshan Dist., Kaohsiung City, Taiwan		
Authorized party	Netturul Resources Sdn Bhd (903910)		
Representative	Jasmine Ng	Title	Director
Address	16-2 – 16-5, Setia Avenue, Jalan Setia Prima SU13/S, Setia Alam, 40170 Shan Alam, Selangor Darul Ehsan, Malaysia		
Sales with scopes:	Marketing & Sales, Maintenance, Spare Parts Sales and Technical Supports.		
Products for authorization	Oxy Hydrogen for Health & Beauty (HB Series & Accessories)		
Duration of authorization	2018/10/01 to 2021/12/31		

Authorized by

Brian Aiello

1. 友聚科技實業股份有限公司 EPOCH Energy Technology Corporation
2. This certificate of dealership shall not be deleted or modified. If any discrepancy, the authorized party has responsibility to follow the authorized correction and signature.

Certificate of Exclusive Sole Distributor

HB-E1

Specifications

Gas production (L/hr)	35 - 40
Size (mm)	485*233*410
Weight (kg)	12.5
Power consumption(w)	200
Power supply (v)	110/220
Number of users (person)	1

Hydrogen (cc/min)	450 ± 10
Oxygen (cc/min)	155 ± 10
Purity of Hydrogen (%)	99.99

HB-133

Specifications

Gas production (L/hr)	70 - 75
Size (mm)	370*340*670
Weight (kg)	38.5
Power consumption(w)	400
Power supply (v)	110/220
Number of users (person)	1

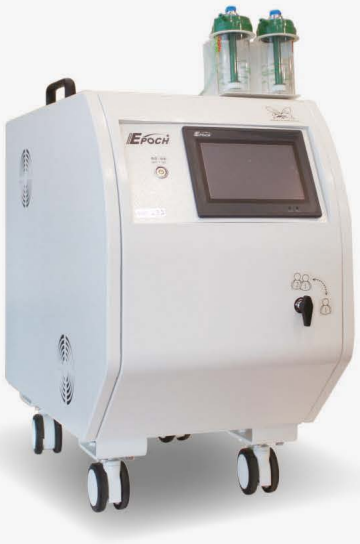
Hydrogen (cc/min)	913
Oxygen (cc/min)	337
Purity of Hydrogen (%)	99.99

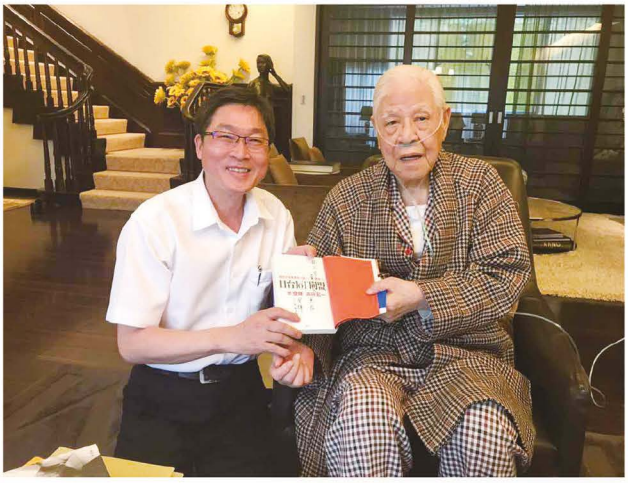
HB-233

Specifications

Gas production (L/hr)	145 - 150
Size (mm)	480* 510*600
Weight (kg)	78
Power consumption(w)	700
Power supply (v)	110/220
Number of users (person)	2

Hydrogen (cc/min)	1826
Oxygen (cc/min)	674
Purity of Hydrogen (%)	99.99





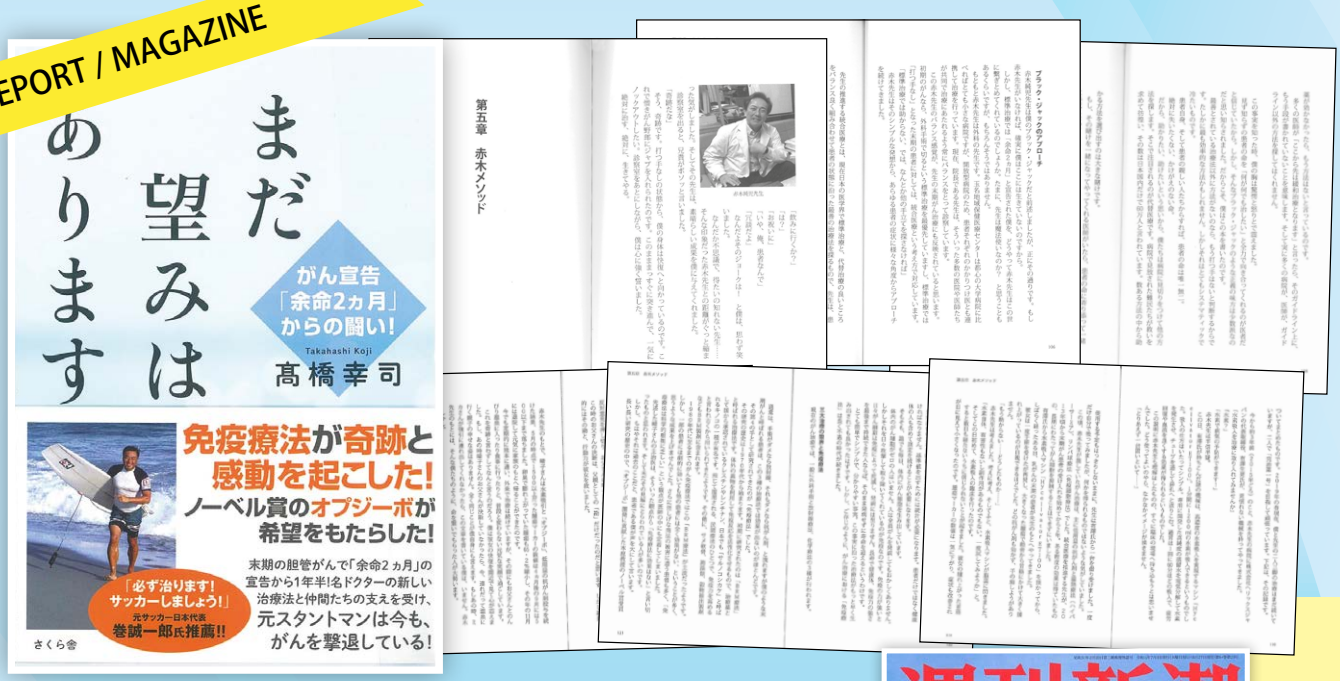
The most natural hydroxygen health care therapy

Recover your beauty, health and fatigue by the power of hydrogen

MEDICAL RESEARCH

SCAN FOR FULL RESEARCH

REPORT / MAGAZINE



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HO
HydrOxygen Chi Therapy

HO HydrOxygen creates a healthy body

The health and beauty industry has recently become more aware of the antioxidant capacity of HydrOxygen. The medical field also studied the application of HydrOxygen treatment for the prevention of various diseases.

Without Oxygen, people cannot live

Without Hydrogen, people cannot live well

Hydrogen Atomizer

The atomizer for the hydrogen beauty machine works through ultrasonic vibration, the spray of water is rich in hydrogen and oxygen, and can be in direct contact with the skin, to achieve cosmetic results.

1. Steaming face
   - Nourishing & repair skin cells
2. Eye treatment
   - Eliminate eye soreness and fatigue
3. Cleansing
   - Decontamination of dust mites

HO MAKES PEOPLE HEALTHIER

Sore shoulders, colds, swelling and other signs of physical discomfort are a precursor to disease. In addition to helping to reduce these discomforts, HO also helps to eliminate fatigue and reduce the effects of hangovers.

HydrOxygen Water Generator

Comparison

Characteristic	HYDROXY HYDROGEN	GENERATOR FEATURES
	* The purest HydrOxgen enriched water * The water does not contain any chemical or metallic substances.	The general market for hydrogen cups to electrolysis or chemical production of hydrogen
Made in	Taiwan	China or other manufacturers
Capacity	1500cc	Specifications (300-500cc)
Hydrogen Content	measured 0.8 - 1.2ppm	0.2 - 1.0ppm (with chemical composition)
Hydrogen Production Rate	3-5 minutes to reach saturation	3-5 minutes (water oxygen unchanged)
Hydrogen Production mechanism oxygen water	HydrOxygen gas pressure to refinement, producing hydrogen water while producing	By metal electrolysis by adding magnesium, calcium chemicals hydrogen

Sleep | Sensitive skin | Enhance physical strength | Whitening moisturizing | Healthy maintenance

HydrOxygen is popular in Japan for health care

The HydrOxygen gas produced by pure electrolysis is used in human health care and beauty, and is of high-tech medical-grade quality. With regular use of HO, users can breathe through the cannula tube to inhale natural hydrogen and oxygen, and is the best way for the body to absorb hydrogen molecules, combine with drinking HydrOxygen enriched water to even improve our health.

Fresh HydrOxygen water means drinking within 2 hours after produce

The User can read book, enjoy relaxation music. Purification of the body, mind and spirit.

UP

Self-Healing Effect

DOWN

Free Radical