Drug delivery system with anaerobic bacteria for cancer therapy

2019/10/17

M2 Takahashi Kazuki

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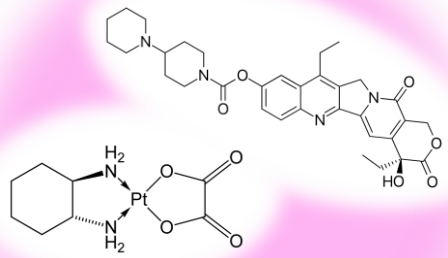
Orthodox method to treat cancers

Photo by JAFAR AHMED on Unsplash

Downleft: scienceblog.cancerresearchuk

Surgery

Radiotherapy

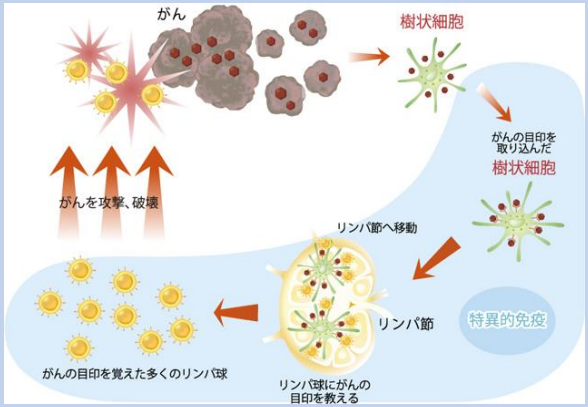


Chemotherapy

- Surgery is performed in the limited areas in the body.
- Some solid tumors are resistant to radio- & chemotherapy.

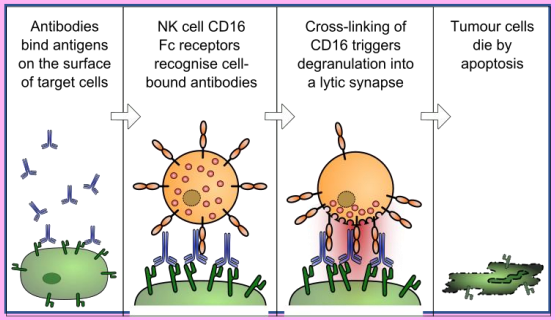
Immunotherapy

- Dendritic cell therapy
  - Cancer vaccine



https://serenclinic.jp/dc/vaccell/vacell02.php

- Antibody therapy
  - Antibody-dependent cellular cytotoxicity



https://en.wikipedia.org/wiki/Antibody-dependent_cellular_cytotoxicity

- Cytokine therapy
  - Interferon
  - Interleukin (IL-2)

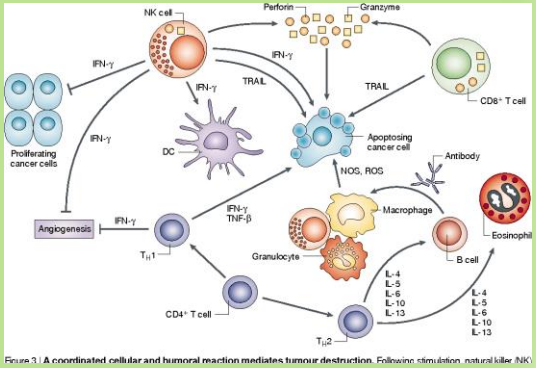
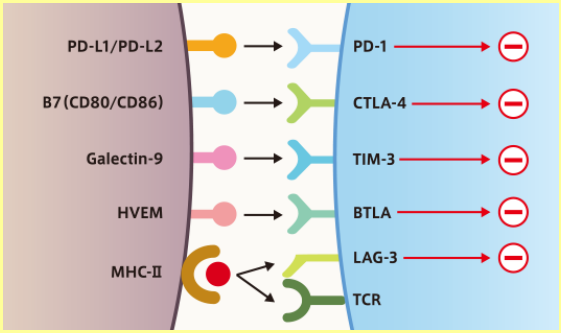


Figure 3 | A coordinated cellular and humoral reaction mediates tumour destruction. Following stimulation, natural killer (NK)

Glenn Dranoff, *Nature Reviews Cancer*,**2004**, *4*, 11

- Immune checkpoints



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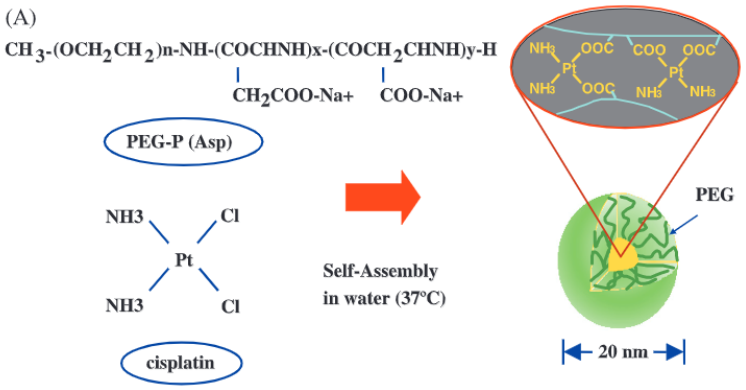
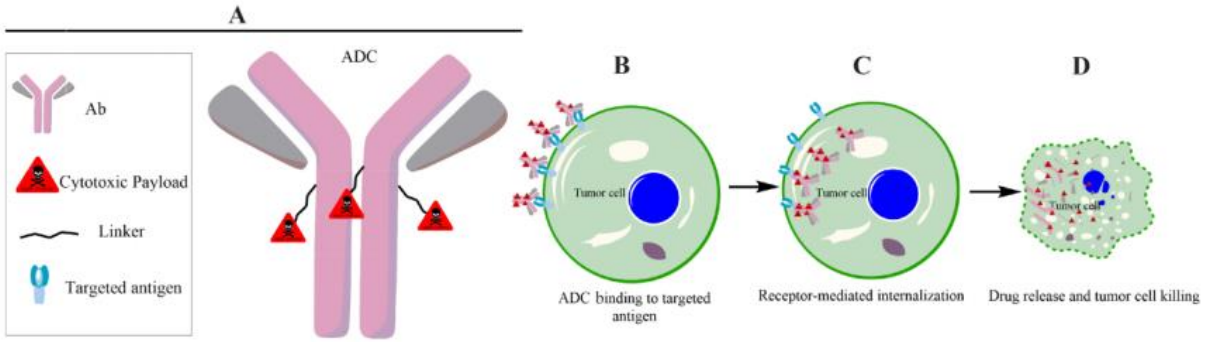
Drug delivery systems to solid tumors

- Nanoparticle (NP)

Matsumura Y, *et al.*, *Cancer Sci.*, **2009**, *100*, 572



- Antibody-Drug Conjugate (ADC)



Nejadmoghaddam MR, *et al.*, *Avicenna J Med Biotechnol,* **2019**, *11*, 3

- These drug delivery systems are not effective for some solid tumors.
- A novel DDS is required to be invented to cope with any solid tumors

Summary of Well-known cancer therapies

- Many types of cancer therapy were developed and are being developed now.
- Some solid tumors are resistant to various therapies.
- A new type of DDS is required to be established to cope with the solid tumors.

Contents

Solid tumor characteristics

- Hypoxia: anaerobic environment
- Oxygen pressure
  - Normal tissues: 3-5% and 20-100 mmHg
  - Solid cancer tissues: <1% and 0-20 mmHg
- Angiogenesis: supplying several nutrients and oxygen and metastasis.
- How is such a low oxygen condition created despite angiogenesis in evident enhancement of malignant tumors?

Solid tumor characteristics

- Hypoxia: anaerobic environment
- Oxygen pressure
  - Normal tissues: 3-5% and 20-100 mmHg
  - Solid cancer tissues: <1% and 0-20 mmHg
- Angiogenesis: supplying several nutrients and oxygen and metastasis.
- The angiogenesis is outpaced by the tumor growth.
- The blood vessels formed by angiogenesis don't have ability to supply enough O_2.

Hypoxia

- HIF-1 (Hypoxia-inducible factors)
  - Stabilized by hypoxic conditions
  - Upregulates glycolysis enzymes and VEGF

glycolysis enzymes → ATP synthesis
vascular endothelial growth factor (VEGF) → angiogenesis.

the 2019 Nobel Prize in Physiology or Medicine
William G. Kaelin Jr., Sir Peter J. Ratcliffe
and Gregg L. Semenza
for their discoveries of how cells sense and adapt to oxygen availability

Announcement of the Nobel Prize in Physiology or Medicine 2019

Contents

Bacterial therapy for cancer

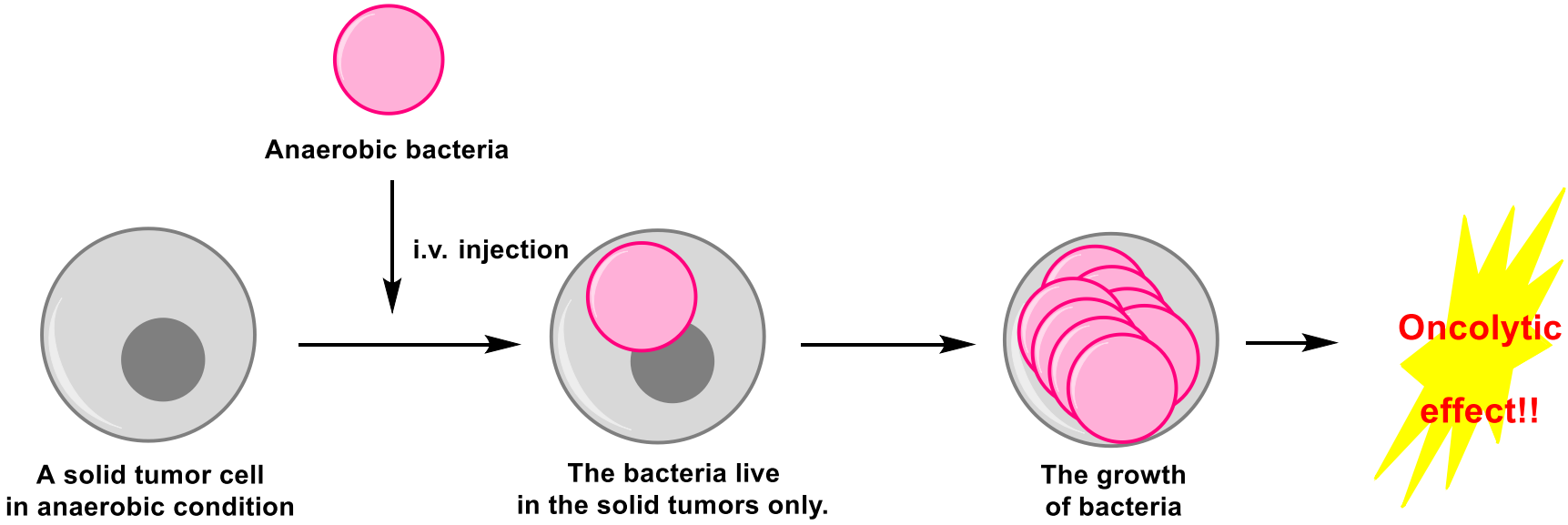
- The original observation of tumor spontaneous recovery and regression of certain cancer patients from concurrent bacterial infections was made over 300 years ago.

- Numerous case reports, review papers and book chapters on the subject have been published, including about 1000 cases by the year 1987 and mean 10 reports per year by 2003.

M.Q Wei *et al.*, *Cancer Letters*, **2008**, *259*, 16

Bacterial therapy for cancer

- Concept



Phase 1 study of *Salmonella* VNP20009

- 25 patients received an intravenous dose of VNP20009.

- the maximum tolerated dose ($3x10^8$ cfu/m^2)
- Shrinking of the tumor was not observed in any case.
- The existence of bacteria in the tumor was detected in only three patients.
- In four patients, bacteriosis was observed but the bacteria were not detected in the tumors.

John F. T, *et al.*, *J. Clin. Oncol.*, **2002**, *20*, 142

This clinical test was failed.

Anaerobic bacteria grows in tumors only.

- *Clostridium tetani* produces toxins.
- The injection of *Cl. tetani* spores resulted in tetanic death in the tumor-bearing host in approximately 48 hours, regardless of the tumor size, the tumor type, or the spore dose.

EFFECT OF THE INTRAVENOUS INJECTION OF *Cl. tetani* SPORES ON TUMOR-BEARING AND NORMAL C3H/He MICE

Tumor	Tumor size (gm.)	Spore dose	No. dead of tetanus*†
C3HBA mammary	2–7	2,400,000	6/6
"	2–5	"	3/3‡
"	2–4	1,200,000	2/2
"	2–4	600,000	2/2
"	3–10	300,000	2/2
"	4	150,000	2/2
"	3–6	75,000	2/2
"	3–9	37,500	2/2
"	3	18,750	2/2
"	2.5	9,375	2/2
Spontaneous mammary	0.3–3	150,000	2/2
98/15 Hepatoma	1–3	"	4/4
HE 8971 fibrosarcoma	2–11	"	2/2
None		2,400,000	0/3
"		150,000	0/6
"		75,000	0/6
"§		"	0/9

* All animals dying of tetanus expired at approximately 48 hours after the spores were injected.

† The numerator represents animals which died. The denominator represents animals injected.

‡ Spores administered intracardially.

§ Strain BALB/c.

Malmgren RA, Flanigan CC, *Cancer Res*, **1955**, *15*, 473

Anaerobic bacteria grows in tumors only.

- *Clostridium tetani* grew a lot in the tumors only.
- Numbers of the bacteria in other organs were not different between in tumor-bearing mice and non- tumor-bearing mice.

Cl. Tetani ORGANISMS/MG OF TISSUE HOMOGENATE DETERMINED BY COLONY PLATE COUNTS

All treated animals received an intravenous injection of 600,000 spores.

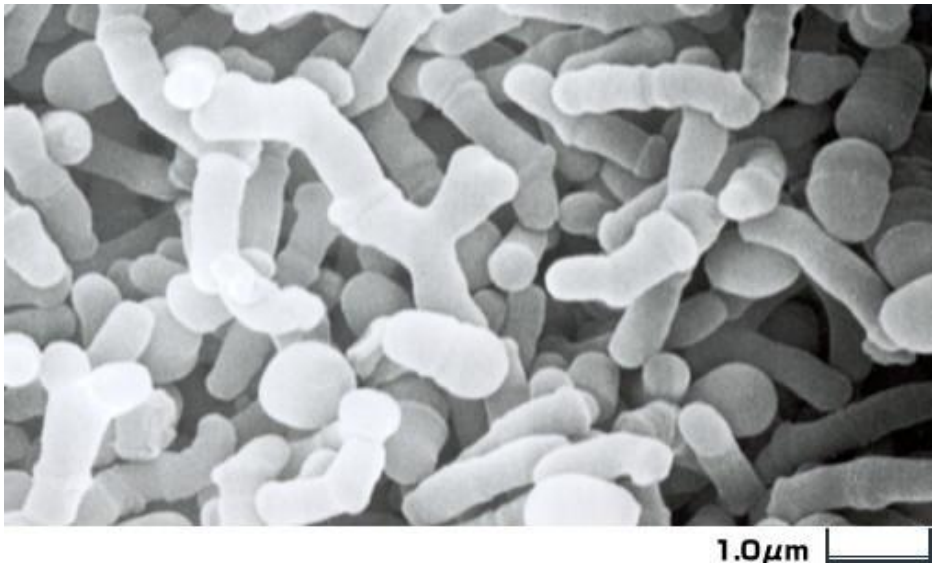
TUMOR	DAYS AFTER SPORE INJECTION	ORGANISMS/MG OF TISSUE Tumor Heated†	Tumor Unheated	Organs* Heated†	Organs* Unheated
C3HBA mammary	4	0	TNC‡	32	48
"	5	2	2,740,000	156	138
"	7	0	2,220,000	114	78
"	7	0	2,760,000	400	546
"	9	0	4,340,000	262	294
"	9	102	2,540,000	252	410
"	9	8	2,000,000	190	210
"	13	6	2,500,000	48	36
HE 8971 fibrosarcoma	3	0	TNC‡	62	48
"	4	22	TNC‡	128	120
"	5	60	1,710,000	20	170
"	7	2	TNC§	160	206
98/15 hepatoma	3	6	TNC‡	122	140
BALB/c spontaneous mammary	5	4	58,000	264	154
C3H/He spontaneous mammary	4	0	120,000	216	304
"	4	0	528,000	230	250
None	30#			582	626
"	30#			358	402
"	30#			708	800
"	40			48	58
"	40			60	72
"	40			40	40

* Organs included liver, spleen, kidneys, and lungs.
† Heated to 73° C. for 20 minutes.
‡ Too numerous to count at a dilution of 1/10,000.
§ Too numerous to count at a dilution of 1/10 million.
# 2,400,000 spores injected instead of 600,000.

Malmgren RA, Flanigan CC, *Cancer Res*, **1955**, *15*, 473

Bifidobacterium

https://www.yakult.co.jp/products/item0228.html



Bifidobacterium bifidum
https://institute.yakult.co.jp/bacteria/4230/

- *Bifidobacterium* has long been prescribed for infant patients in Japan.
- It was considered as safe bacteria injected intravenously.

Bifidobacterium grows only in tumor tissues

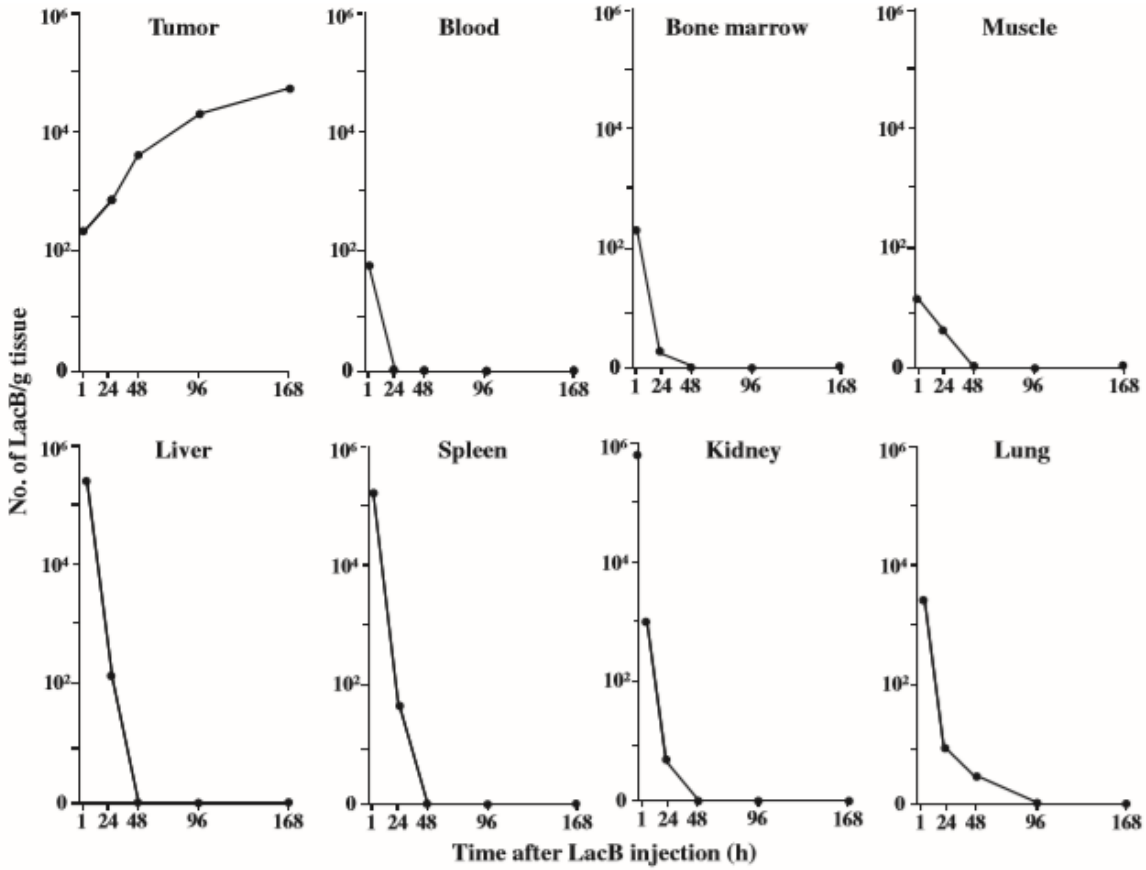
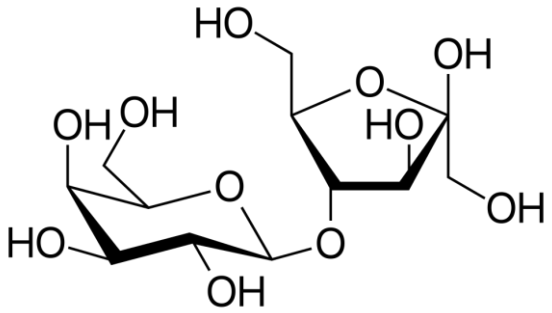


Fig. 1. Specific distribution of *Bifidobacterium bifidum* (LacB) in tumor tissues[54] following a single i.v. injection of 5×10^6 viable bacilli into Ehlich solid tumor-bearing mice. Each point represents the mean of the number of bacilli per gram tissue of eight mice.

Bifidobacterium bifidum grows only in tumor tissues.

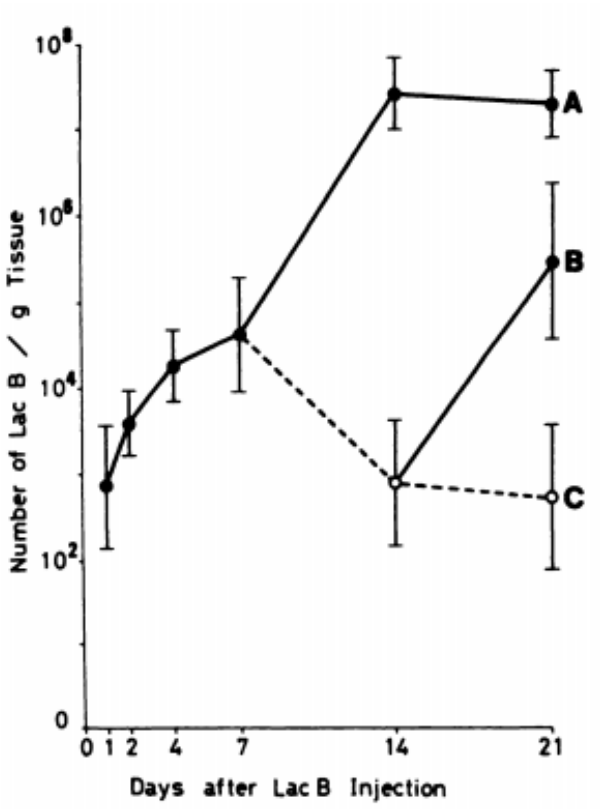
Kimura NT, et al., *Cancer Res*, **1980**, *40*, 2061

B. bifidum (Lac B) & lactulose



Lactulose:
A synthesized disaccharide from lactose.
It is not metabolized by mammalian tissue cells.

Chart 2. Effect of lactulose administration or of its interruption on intratumoral growth of Lac B in Ehrlich solid tumor-bearing mice. ——, period when lactulose was given; - - - -, period when lactulose was not given. Each *point* represents the mean of the number of Lac B per g tumor tissue of 10 mice; *bars*, S.E. The mean value of Group A is significantly different from that of Group B plus C on Day 14 ($p < 0.05$, *t* test). The differences between Groups A and C and between Groups B and C on Day 21 are also significant ($p < 0.05$, *t* test). Difference between the Groups A and B on Day 21 is not significant ($p > 0.05$, *t* test).



Lactulose *in vivo* stimulates the growth of *B. bifidum* in the tumor.

Kimura NT, et al., *Cancer Res*, **1980**, *40*, 2061

The non-pathogenicity of *B. bifidum* (Lac B)

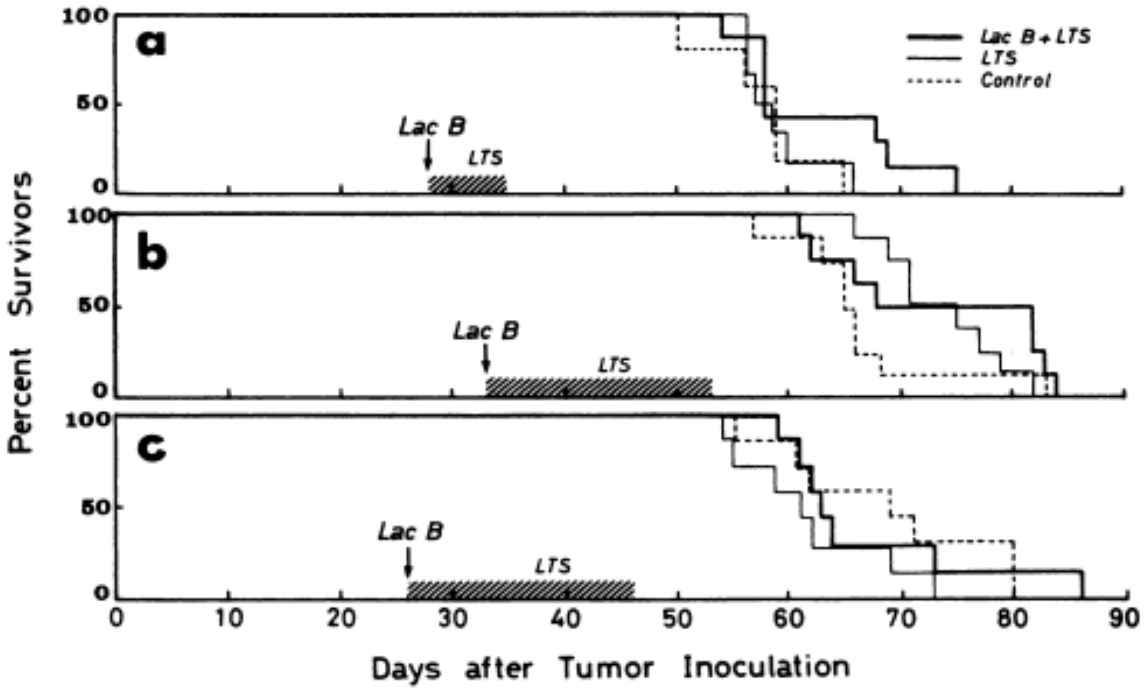


Chart 3. Effect of i.v. administration of Lac B on life span of Ehrlich solid tumor-bearing mice. Number of mice per group was 6, 8, and 7 for Experiments *a*, *b*, and *c*, respectively. Doses of Ehrlich tumor cells were 1.4, 3.6, and 3.7 × 10^6 for Experiments *a*, *b*, and *c*, respectively. Dose of viable Lac B was 2.0 × 10^7 bacilli for Experiment *a* and 2.4 × 10^7 bacilli for Experiments *b* and *c*. *Hatched bars*, period of lactulose (*LTS*) administration. *Curves* are survival curves.

- The animals expressed no visible adverse symptoms.
- It was confirmed that *B. bifidum is* safe bacteria injected intravenously.

Kimura NT, et al., *Cancer Res*, **1980**, *40*, 2061

Anaerobic bacteria characteristics

Three classes of anaerobic and facultative anaerobes have been tested for anticancer therapy

Class	Species	Features	Advantages	Disadvantages
Class I: Bifidobacteria	*B. longum* *B. adolescentis* *B. infantis*	Gram$^+$ non-motile obligate anaerobes	Non-pathogenic present in common intestinal flora, Have been used in human for many years Probiotic bacteria Can be used for intravenous or oral administration Expression of recombinant protein	No obvious oncolytic effect Non-spore former More susceptible to non-permissive conditions More difficult to store and handle
Class II: Facultative intracellular Bacteria	*Salmonella* *S. typhimurium* *S. choleraesuis*	Gram$^-$ facultative anaerobes Agent for intestine infection	Attenuated vaccine strain has been proved safe clinically in human, Biochemistry pathways and genomes are well characterized Auxotrophic isolates for solid tumours have intrinsic antitumour activity	Intracellular bacteria, thus may have difficulty to infect and lyse quiescent cell Have a tumour to normal tissue ratio of 1000:1, therefore a significant number of bacteria colonize normal organs Cell wall components are immunogenic
	Listeria *L. monocytogenes* *E. coli*	Gram$^+$, facultative anaerobes Gram$^-$, facultative anaerobes	Grow under aerobic and anaerobic conditions, thus can target both large and small tumours, enter professional antigen presenting cells and induce strong innate immune response Have the potential as a vaccine vector for tumour therapy Biology is well studied and known	Virulence factors exist, especially LPS in the bacterial cell wall, thus safety is an issue when large amount of bacteria are delivered Virulence factors exist, such as LPS
Class III: Strictly Anaerobic bacteria	*Clostridium* Proteolytic *C. sporogenes* Saccharolytic *C. novyi* *C. butyricum* *C. acetobutylicum* *C. oncolyticum* *C. beijerinckii*	Gram$^+$, strictly anaerobes normal habitat in the soil, aquatic sediments, and intestinal tract of both animals and humans	Spore former Spores are stable, easy to produce and economic to use Clostridial spores can be delivered non-invasively and systemically, i.e. intravenous injection Have shown extensive oncolytic ability Spores are non-immunogenic and can be repeatedly delivered Oncolysis occurs irrespective of tumour cells' heterogeneity or growth status	Some strains are pathogenic Some strains are difficult to manipulate genetically Only colonize in large tumours with area of hypoxia/necrosis Oncolysis interrupted at the rim causing incomplete tumour lysis

M.Q Wei *et al.*, *Cancer Letters*, **2008**, *259*, 16

Summary of Anaerobic bacteria characteristics

- Obligate anaerobic bacteria intravenously injected accumulates only in tumor cells.
  - *Bifidobacterium* is non-pathogenic, but it doesn't show oncolytic effect.
  - *Clostridium* extensive oncolytic effect, but some strains are pathogenic.

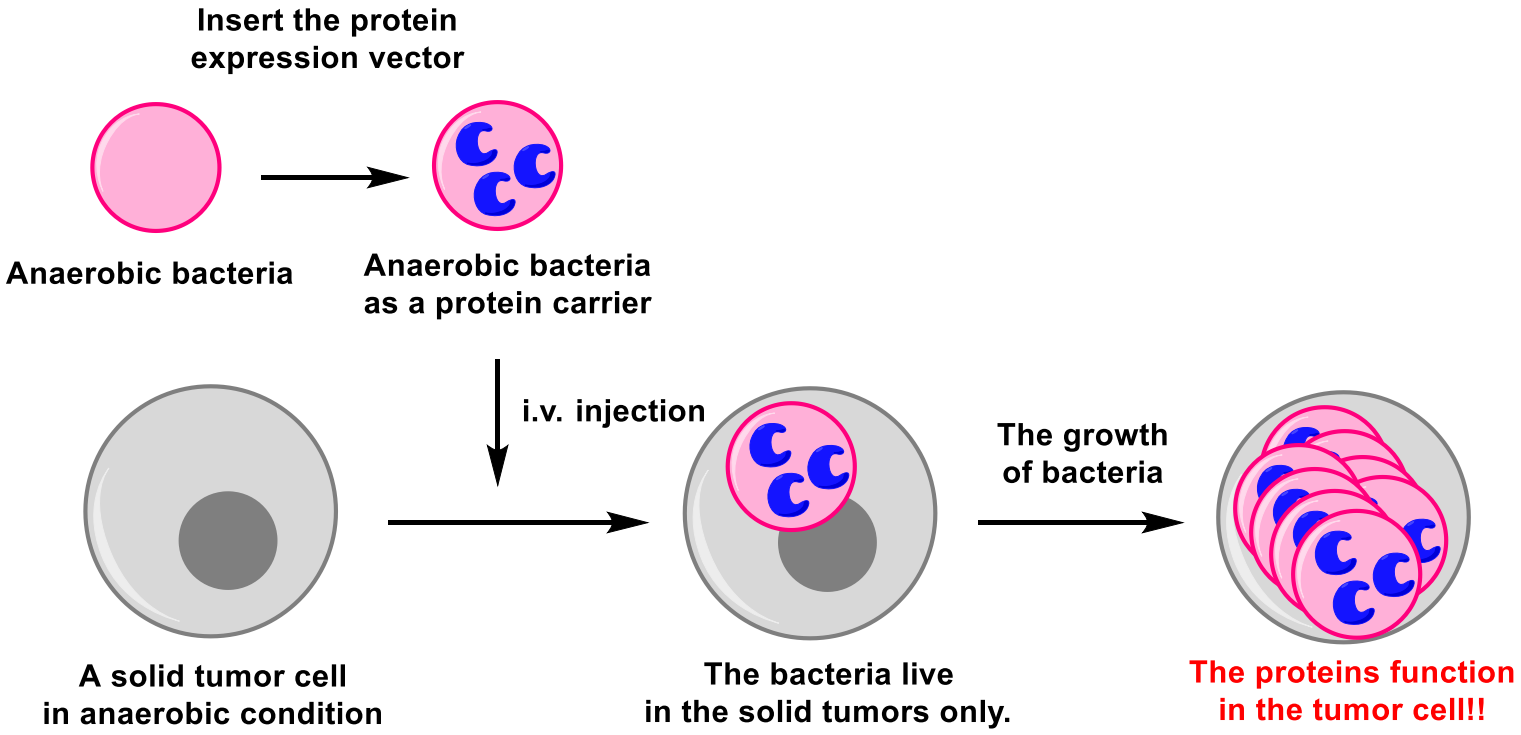
- Facultative anaerobic bacteria shows pathogenic or toxic effect on both tumor cells and normal cells.
  - *Streptococcus*, *Salmonella*, *Listeria*, *E. coli*, etc…

Summary of bacterial therapy for cancer

- The original bacterial therapy for cancer was performed over 300 years ago.
- Obligate anaerobic bacteria intravenously injected accumulates only in tumor cells.
- Obligate anaerobic bacteria (*Clostridium* and *Bifidobacterium*) treatment alone was not enough to control tumor significantly.

Contents

Bifidobacterium used for a novel DDS



- *Bifidobacterium are* non-invasive bacteria.
- Expression vector insertion enables to carry the desired peptides and proteins into the solid tumors.

Examples of *Bifidobacterium* DDSs

- Anti-PD-1 antibody scFv producing *B. longum*

Abstract only

- Trastuzumab scFv producing *B. longum*

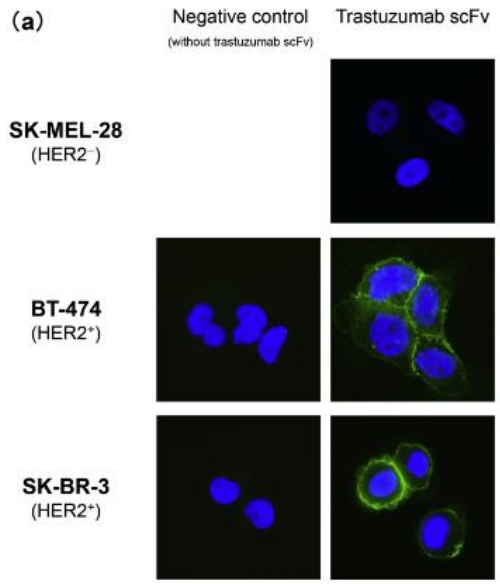


Fig. 2. Immunostaining and FACS analysis of cultured cells by His-tag-purified trastuzumab scFv from *B. longum* H2.
(a) Immunofluorescent staining. Blue: nucleus. Green: stained with trastuzumab scFv from H2. Left panels: negative control (without trastuzumab scFv). Right panels: stained with trastuzumab scFv. Original magnification of all images was ×400. (b) FACS analysis. SK MEL 28 (HER2), BT 474 (HER2 /), and SK BR 3 (HER2) cells were stained with His tag purified trastuzumab scFv. Blue line: control (buffer alone). Red line: stained with trastuzumab scFv from H2.

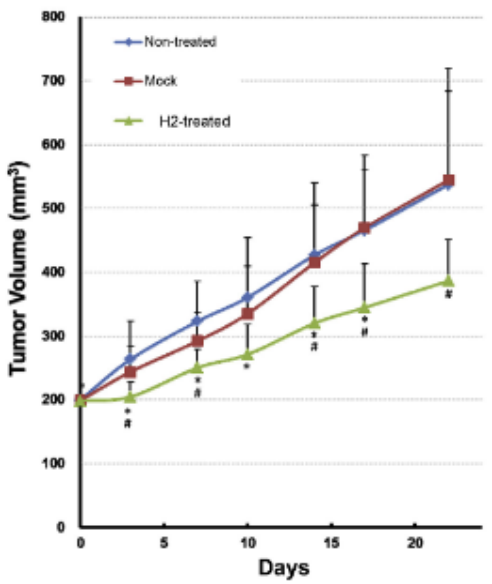
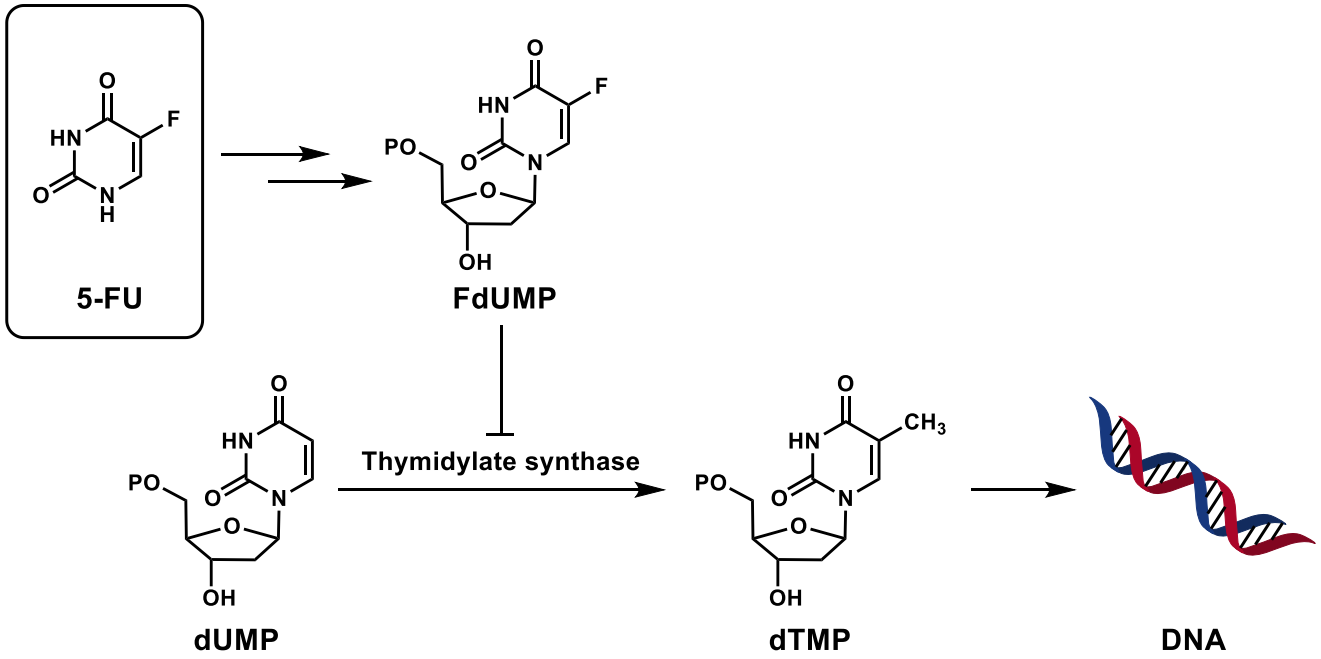


Fig. 3. *In vitro* and *in vivo* anti-cancer effects of trastuzumab scFv from *B. longum* H2.
(a) Antiproliferative activity of trastuzumab scFv against cultured HER2 ( ) cancer cells. (b) Growth suppression of a human HER2 ( ) carcinoma transplanted into nude mice by recombinant *Bifidobacterium* H2.
B. longum mock and H2 were *iv* administered to NCI-N87 human gastric cancer tumor bearing mice twice a week. Mean ± standard deviation values of eight mice. *: $P < 0.05$ versus non treated group, #: $P < 0.05$ versus mock treated group.

T. Kikuchi, *et al.*, *Biochem. Biophys. Res. Commun.*, ***2017***, *493*, 30

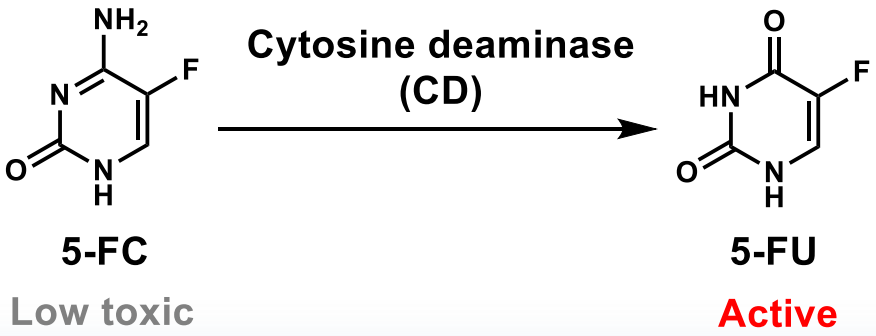
5-Fluorouracil (5-FU)

- 5-FU
  - a medication used to treat cancer.
  - a thymidylate synthase (TS) inhibitor.
- Mechanism
  - 5-FU inhibits Thymidylate synthase resulting in impaired DNA synthesis.



Cytosine deaminase (CD)

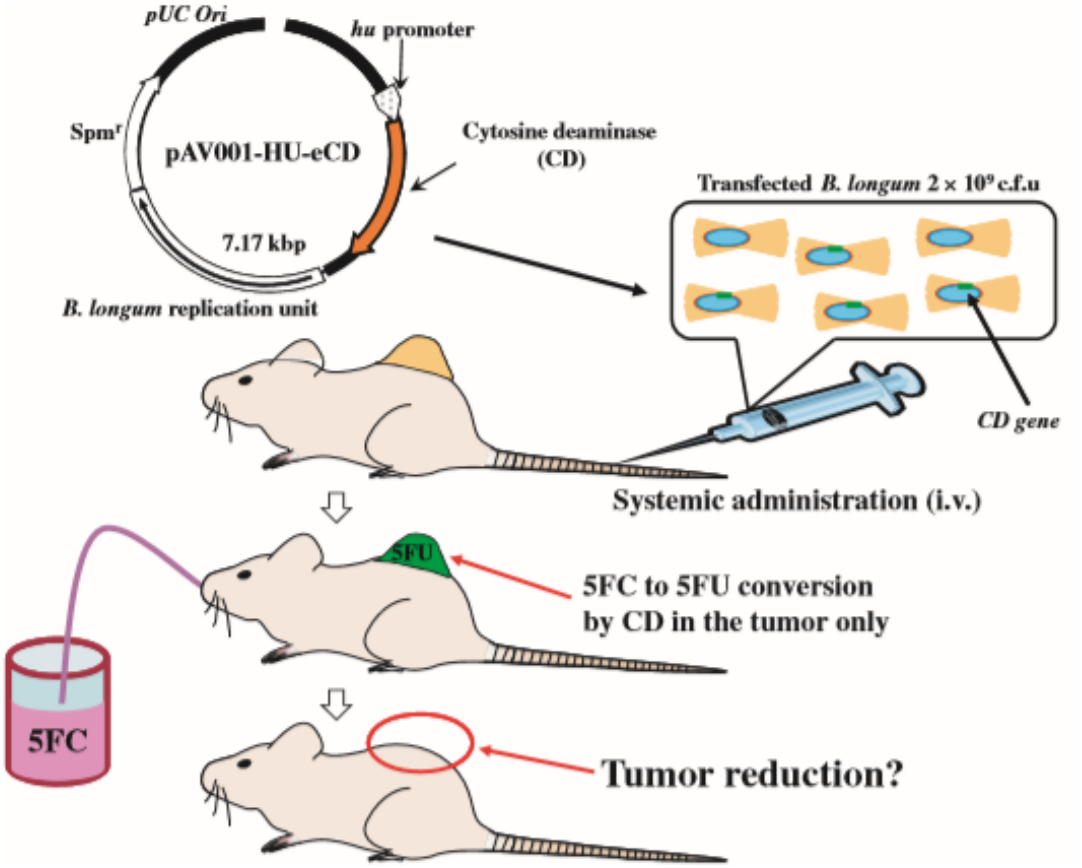
- Cytosine deaminase (CD)
  - CD converts low-toxic 5-fluorocytosine (5FC) to active 5-fluorouracil (5FU)



- The cytosine deaminase of *Escherichia coli* (e-CD) was inserted into the plasmid under the promoter region of the plasmid.

CD producing *B. longum*

- Concept



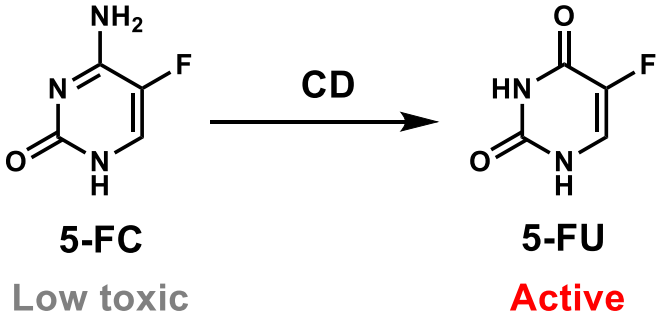


Fig. 2. Concept of cancer treatment by combining cytosine deaminase of *Escherichia coli* (e-CD)-transformed *Bifidobacterium longum* (i.v.) with the prodrug 5-fluorocytosine (5FC) (given orally). 5FU, 5-fluorouracil.

- 5-FC is produced and activated in tumor cells only.

CD producing *B. longum*

Rats

Nude mice

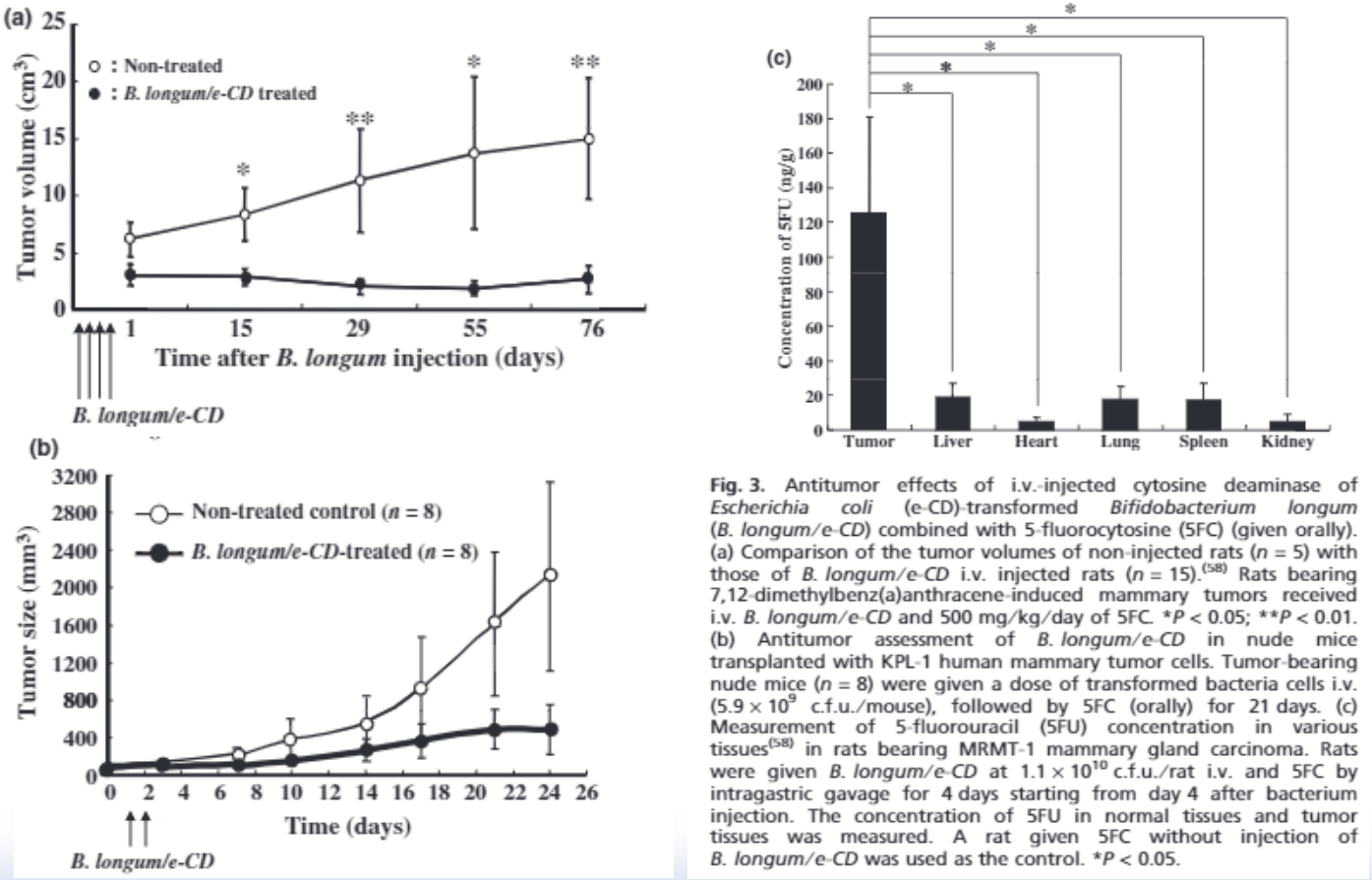


Fig. 3. Antitumor effects of i.v.-injected cytosine deaminase of *Escherichia coli* (e-CD)-transformed *Bifidobacterium longum* (*B. longum*/e-CD) combined with 5-fluorocytosine (5FC) (given orally). (a) Comparison of the tumor volumes of non-injected rats ($n = 5$) with those of *B. longum*/e-CD i.v. injected rats ($n = 15$).[58] Rats bearing 7,12-dimethylbenz(a)anthracene-induced mammary tumors received i.v. *B. longum*/e-CD and 500 mg/kg/day of 5FC. $*P < 0.05$; $**P < 0.01$. (b) Antitumor assessment of *B. longum*/e-CD in nude mice transplanted with KPL-1 human mammary tumor cells. Tumor-bearing nude mice ($n = 8$) were given a dose of transformed bacteria cells i.v. (5.9×10^9 c.f.u./mouse), followed by 5FC (orally) for 21 days. (c) Measurement of 5-fluorouracil (5FU) concentration in various tissues[58] in rats bearing MRMT-1 mammary gland carcinoma. Rats were given *B. longum*/e-CD at 1.1×10^{10} c.f.u./rat i.v. and 5FC by intragastric gavage for 4 days starting from day 4 after bacterium injection. The concentration of 5FU in normal tissues and tumor tissues was measured. A rat given 5FC without injection of *B. longum*/e-CD was used as the control. $*P < 0.05$.

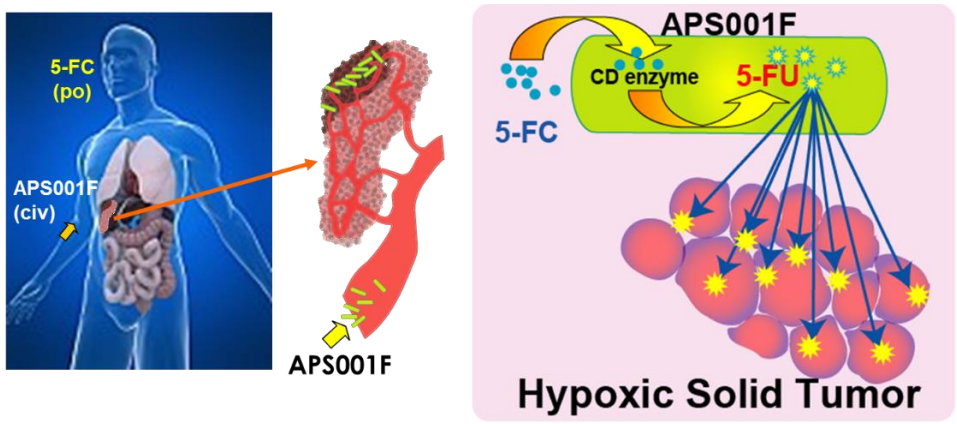
- Suppression of tumor growth was observed in the groups treated with i.v. injection of the bacteria and the prodrug 5-FC given orally.

Sasaki T, *et al.*, *Cancer Sci.*, **2006**, *97*, 649

Anaeropharma Science

- Anaeropharma Science was established in Tokyo, Japan in 2004.

- The Phase I/II trial of APS001F is currently ongoing in US.



- This company works on a joint development project with *Eisai Co., Ltd.* and *Astellas Pharma Inc.* respectively.

Contents

Summary

- Obligate anaerobic bacteria intravenously injected accumulates only in tumor cells because of its anaerobic condition.

- Obligate anaerobic bacteria (*Clostridium* and *Bifidobacterium*) treatment alone was not enough to control tumor significantly.

- *Bifidobacterium* is being used to treat cancer as a novel DDS, or a safety carrier of the therapeutic proteins.

Clinical trial of *Salmonella* VNP20009

Table 1. Characteristics of Patients Receiving VNP20009

	No. of Patients (N = 25)	%
Sex		
Male	16	64
Female	9	36
Performance status		
0	23	92
1	2	8
2	0	
3	0	
Prior treatment		
Surgery	25	100
Chemotherapy	15	60
Radiotherapy	6	24
Immunotherapy	25	100

← Patients' information

Results of counting No. of bacteria in tumors →

FNA: fine needle aspiration cytology
穿刺吸引細胞診

個数 ↓

Table 3. Tumor Biopsy Cultures After the Administration of VNP20009

Dose (cfu/m^2)	Patient No.*	Tumor Biopsy Type*	Tumor Biopsy Day	Tumor Biopsy cfu/g
1×10^6	1	FNA, excised	5, 14	0
	2	FNA	13	0
	3	FNA	2, 15, 35	0
3×10^6	4	FNA	3, 14, 30	0
1×10^7	7(1)	FNA	3	0
	8	FNA	3, 15	0
3×10^7	10	FNA	2, 16	0
	12	FNA	2, 13, 18	0
1×10^8	13(2)	FNA	2, 16	0
	14	FNA, excised	4	0
	15	Excised, FNA	6, 15	0
3×10^8	23	FNA	3	0
	24	FNA (rt lower leg)	4	0
		Excised (rt lower leg)	4	11,000
		FNA (rt upper leg)	4	0
	25	FNA (liver)	5	0
		FNA (liver)	9	0
1×10^9	19	FNA	2	100
		FNA	15, 25	0
	20	FNA	3, 14	0
	21	FNA (ant chest)	4	6.4×10^5
		FNA (ant chest)	6	8.7×10^8
		FNA (ant chest)	8	7.0×10^9
		FNA (lat chest)	4	0
		FNA (lat chest)	6	0
		FNA (lat chest)	8	36
	22	FNA	2, 7	0

Abbreviations: rt, right; ant, anterior; lat, lateral.
*Number in parentheses indicates the patient's treatment cycle.