Engineering bacteria to fight cancer

TJC 14.2.2017

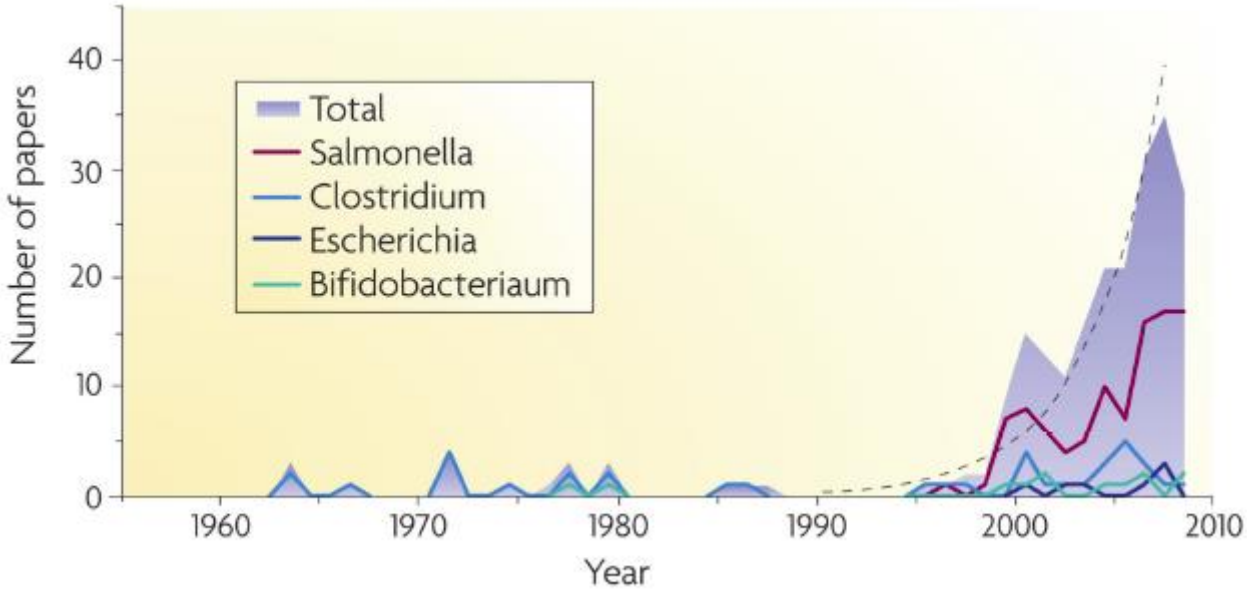
Patrick Schürch (Theocharides/Manz)

Introduction

In the 19th century several physicians observed that erysipelas lead to the shrinkage of tumors

In 1891 William B. Coley injected streptococcal organisms into a cancer patient in order to cause erysipelas and stimulate the immune system of bone sarcoma patients (McCarthy, 2006)
=> Development of Coley's toxin (heat-killed streptococcal organism combined with Serratia marcescens)

William B. Coley (1862-1936)



Published in final edited form as:
Nat Rev Cancer. 2010 November ; 10(11): 785–794. doi:10.1038/nrc2934.

Engineering the perfect (bacterial) cancer therapy

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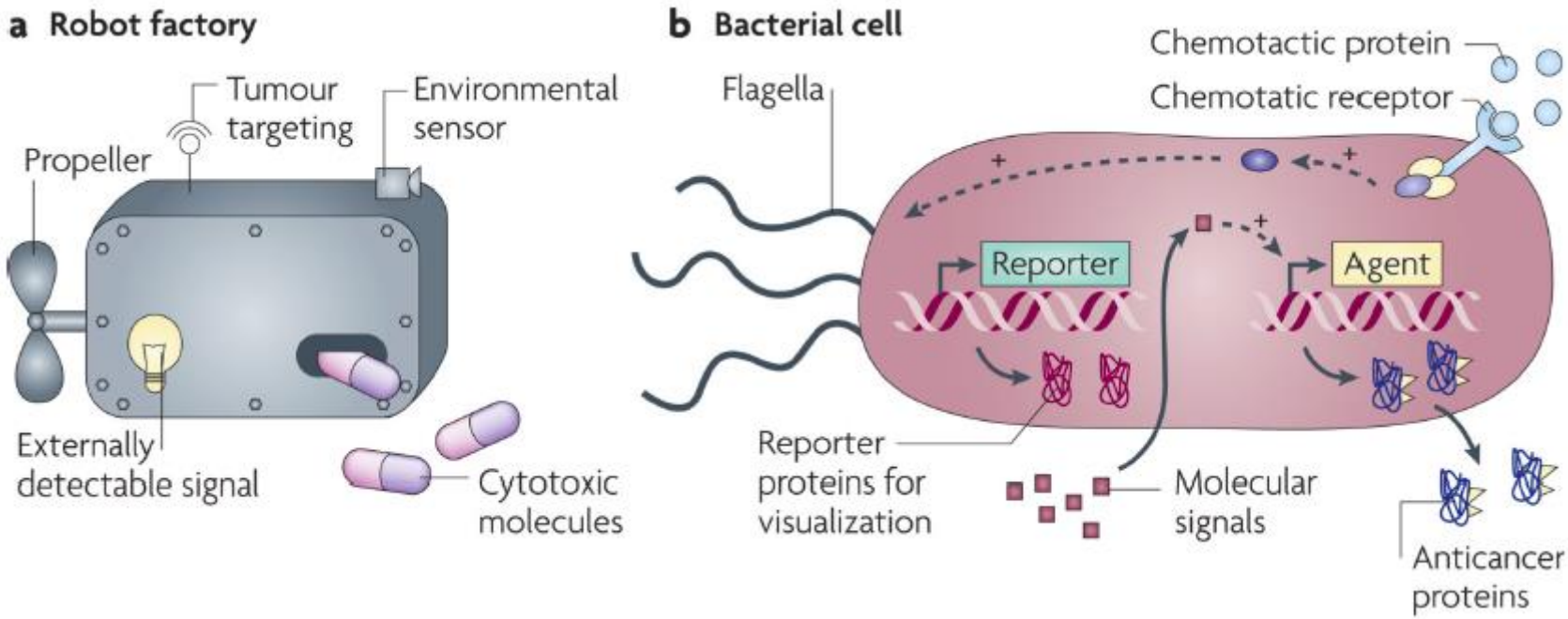
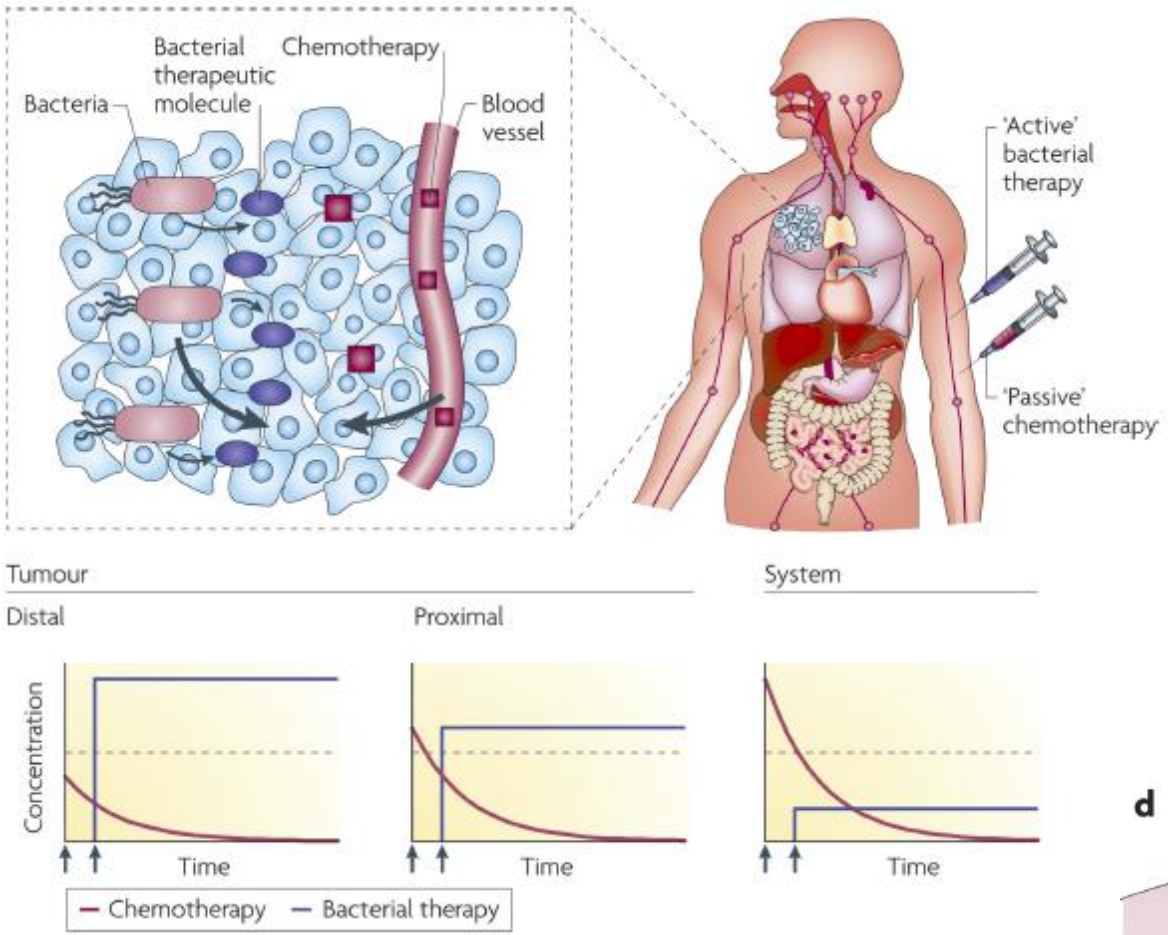
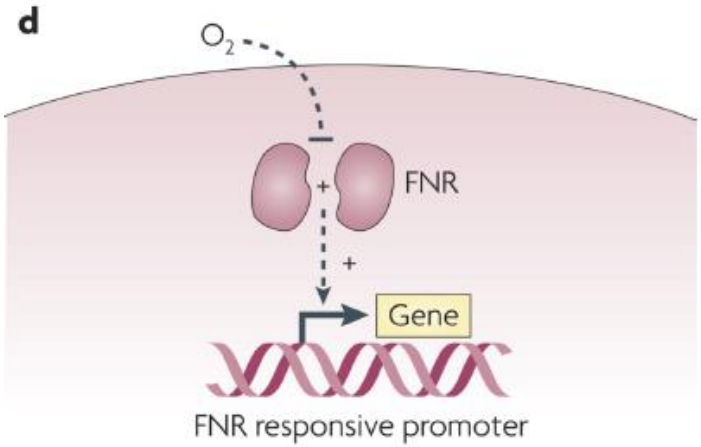

a Robot factory
Tumour targeting
Environmental sensor
Propeller
Externally detectable signal
Cytotoxic molecules
b Bacterial cell
Chemotactic protein
Chemotatic receptor
Flagella
Reporter
Agent
Reporter proteins for visualization
Molecular signals
Anticancer proteins


Bacterial therapeutic molecule
Chemotherapy
Bacteria
Blood vessel
'Active' bacterial therapy
'Passive' chemotherapy
Tumour
System
Distal
Proximal
Concentration
Time
Time
Time
Chemotherapy
Bacterial therapy


d
O_2
FNR
Gene
FNR responsive promoter

Published human trials using bacterial cancer therapies

Strain	Cancer type	*n*	Responses	Ref.
C. butyricum M-55	Squamous cell carcinoma, metastatic, malignant neuroma, leiomyosarcoma, melanoma, sinus carcinoma	5	Oncolysis (3)	63
C. butyricum M-55	Vascular glioblastoma	49	Oncolysis	138
S. typhimurium VNP20009	Metastatic melanoma and renal cell carcinoma	25	Focal tumor colonization (3)	55
S. typhimurium VNP20009	Metastatic melanoma	4	Tumor biopsy culture positive for VNP20009 (1)	54
S. typhimurium VNP20009 TAPET-CD	Squamous cell carcinoma, adenocarcinoma	3	Intratumoral bacterial colonization (2)	56

RESEARCH ARTICLE | CANCER

Programmable probiotics for detection of cancer in urine

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Science Translational Medicine 27 May 2015:
Vol. 7, Issue 289, pp. 289ra84
DOI: 10.1126/scitranslmed.aaa3519

Rationale

Metastatic spread of cancer is responsible for around 90% of all cancer-related deaths

- Liver metastases are clinically challenging due to their small size and multiplicity (Schroeder et al., 2011)

Therefore there is a need for methods to detect small liver metastases that are below the detection limit of existing diagnostic tools

Nonvirulent probiotic *E. Coli* Nissle 1917 (EcN) was engineered to express gene circuits that allow the noninvasive detection of small liver metastases

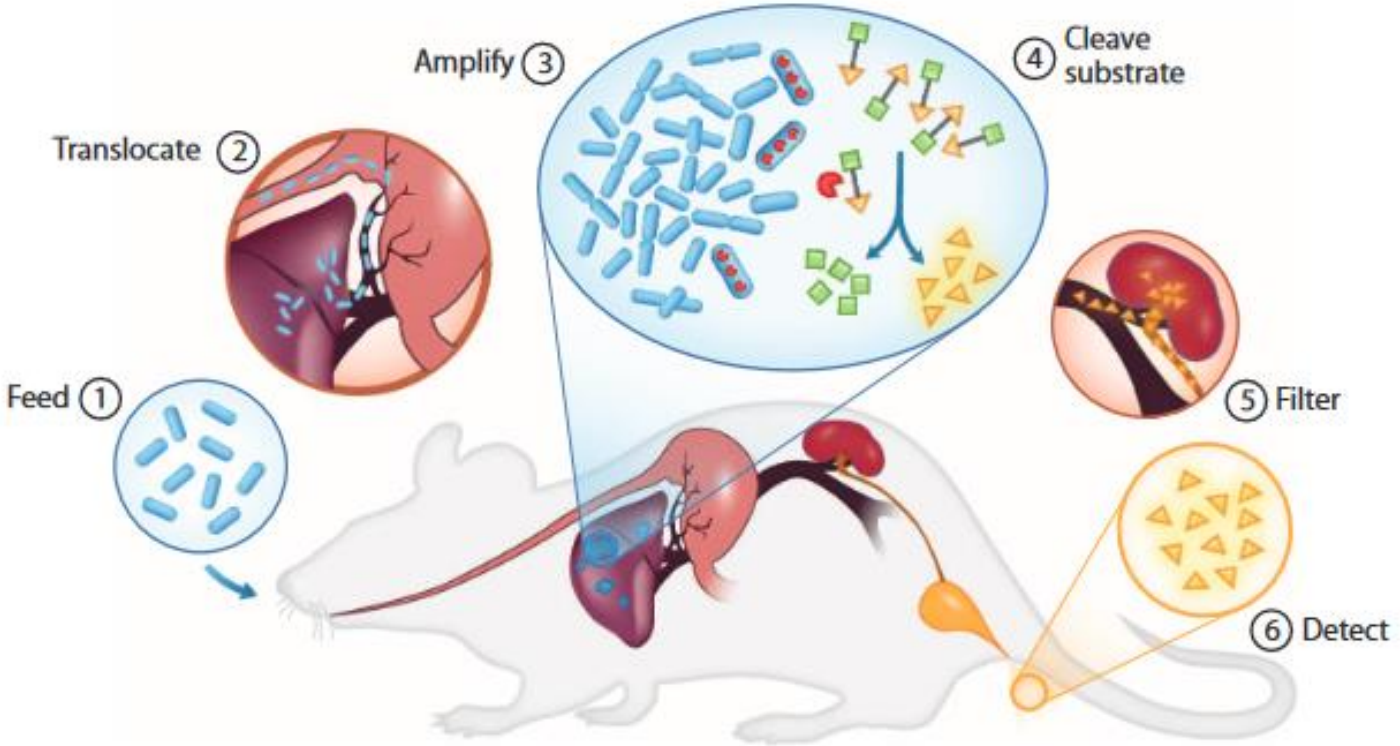


Fig. 1. PROP-Z probiotics for noninvasive cancer detection. The PROP-Z diagnostic platform is made up of probiotic EcN bacteria transformed with a dual-stabilized, high-expression lacZ vector as well as a genomically integrated luxCDABE cassette that allows for luminescent visualization without providing exogenous luciferin (blue). (1) PROP-Z is delivered orally. (2) Probiotics rapidly (within 24 hours) translocate across the gastrointestinal tract and (3) specifically amplify within metastatic tumors present in the liver. (4) PROP-Z expresses high levels of the enzyme lacZ (red), which can cleave systemically injected, cleavable substrates (green and yellow). Cleavage products of the substrates (yellow) filter through the renal system (5) into the urine for detection (6).

Designing diagnostic probiotics

Table 1. Bacterial strains and mammalian cell types used in this study. AHL, *N*-(3-oxohexanoyl)-L-homoserine lactone; GEMM, genetically engineered mouse model.

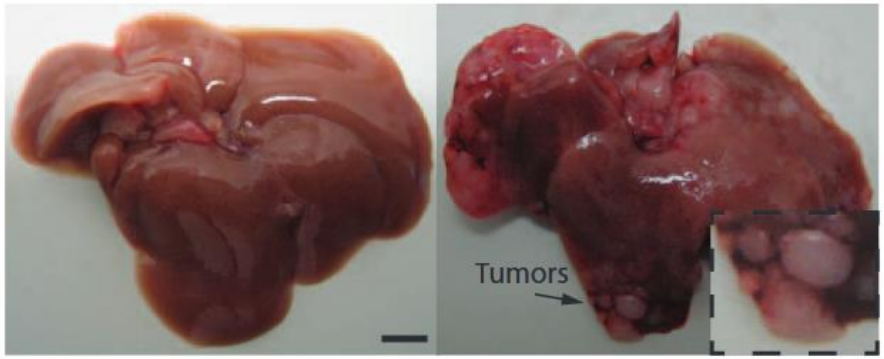
Label	Bacterial strain or mammalian cell	Genomic/plasmids	Use(s)
PROP-Z	EcN	luxCDABE (genomic), IPTG-inducible lacZ (stabilized plasmid)	Bioluminescent imaging of bacteria, urine diagnostic assay
PROP-Luc	EcN	luxCDABE (genomic), luxCDABE (plasmid)	Bioluminescent imaging of bacteria
PROPi-Luc	EcN	AHL-inducible luxCDABE (plasmid)	Bioluminescent imaging of bacteria
Non-lacZ	Mach One	lacZΔM15 (genomic mutant)	Non-lacZ control for urine diagnostic assay
MC26-LucF	Metastatic colorectal mouse cell line	Firefly luciferase (genomic)	Subcutaneous xenografts and liver metastasis models
LS174T-LucF	Human colorectal adenocarcinoma cell line	Firefly luciferase (genomic)	Subcutaneous xenograft models
393M1-LucF	GEMM lung mouse cell line	Firefly luciferase (genomic)	Subcutaneous xenografts and liver metastasis models

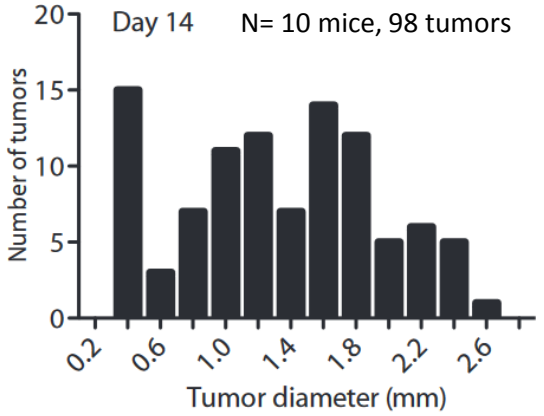
The Prop-Z (**Pro**grammable **p**robiotics with lac**Z**) strain harbours:

1. Genomic luxCDABE cassete (ERYC) => generates endogenous luminescent signal, detectable in vitro and by whole-animal imaging, can be discriminated from luminescence generated by engineered mammalian cells with firefly luciferase
2. pTKW106alp7A plasmid (Kn) => IPTG-inducible lac-Z expression

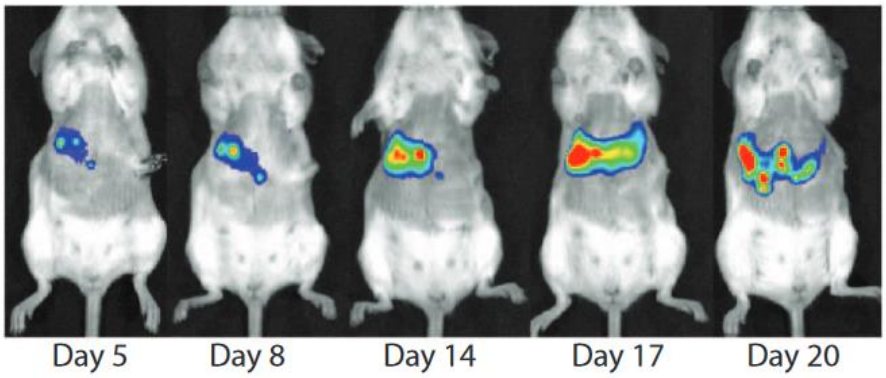
Murine model of colorectal cancer metastases

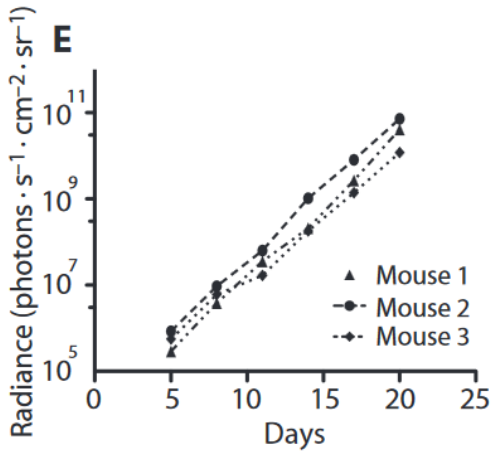
Intrasplenic injection of metastatic murine colorectal cell line (MC26-LucF) into Balb /c mice





IVIS images for luminescence of M26-LucF:Balb/c liver metastasis



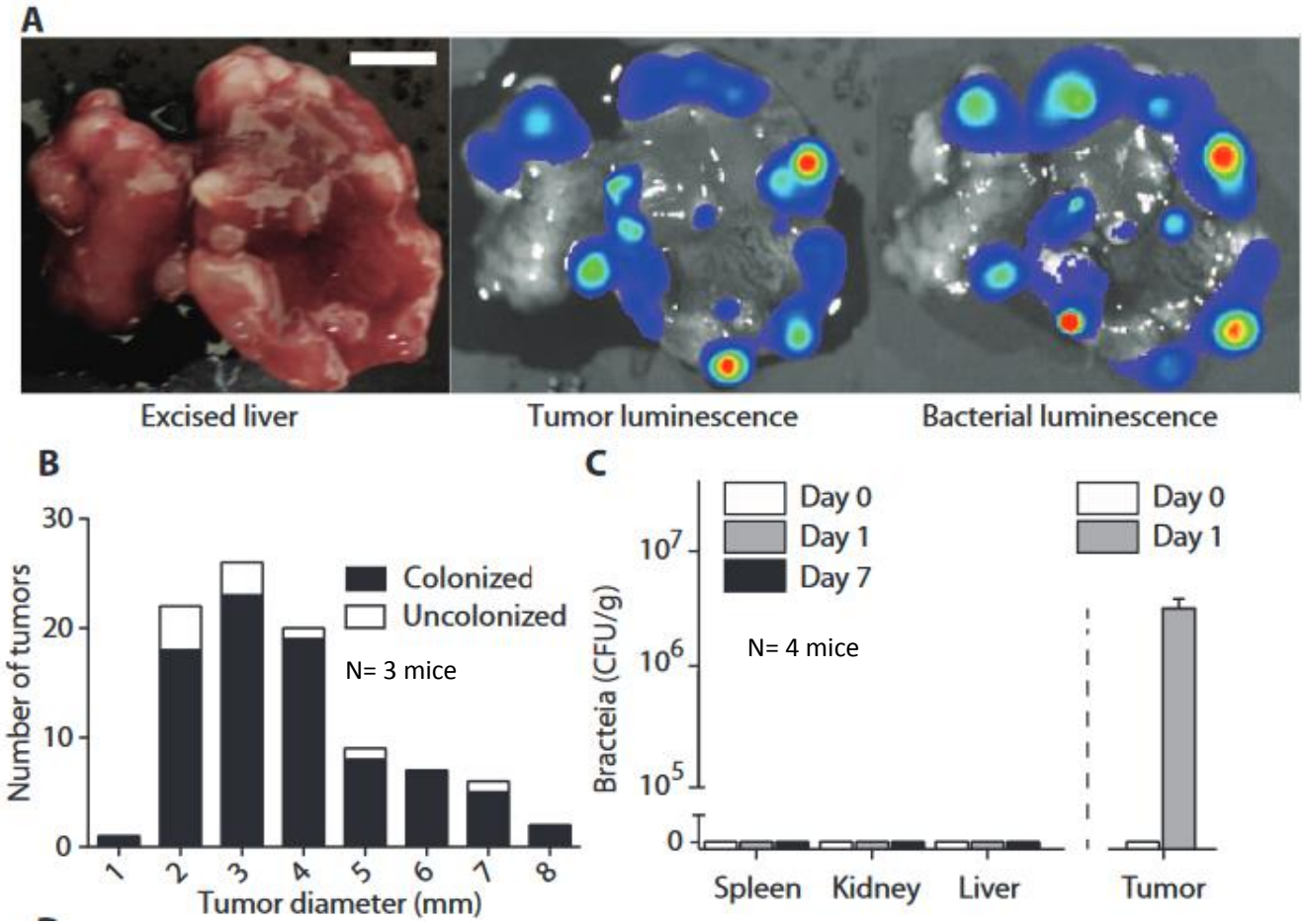


Probiotics can cross the GI tract and colonize large and small hepatic metastases

Oral delivery of 5 x 10E9 CFU to Balb/c mice carrying liver metastases (21 days)

→ Organs were analyzed 24 hours later

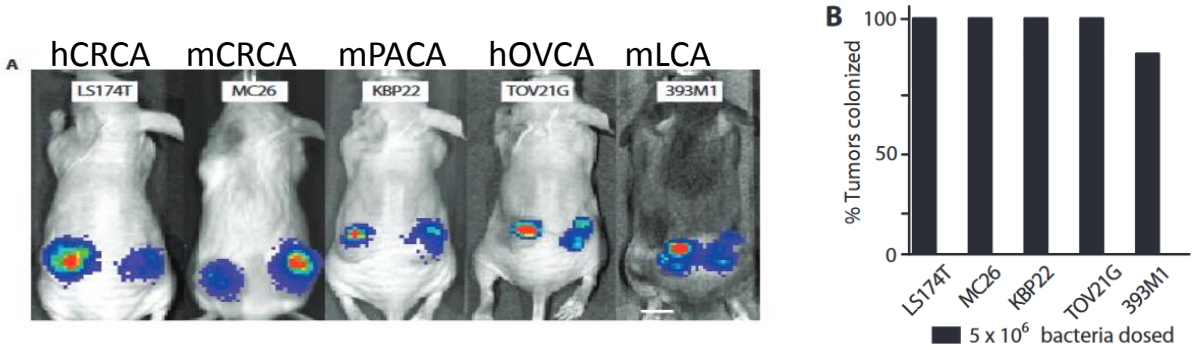
→ In addition, they checked tissue sections (brain, spleen, liver, kidney, heart, lungs) for inflammation, tissue damage or cell death (not shown)



Healthy mice (qPCR), 24 h post oral administration

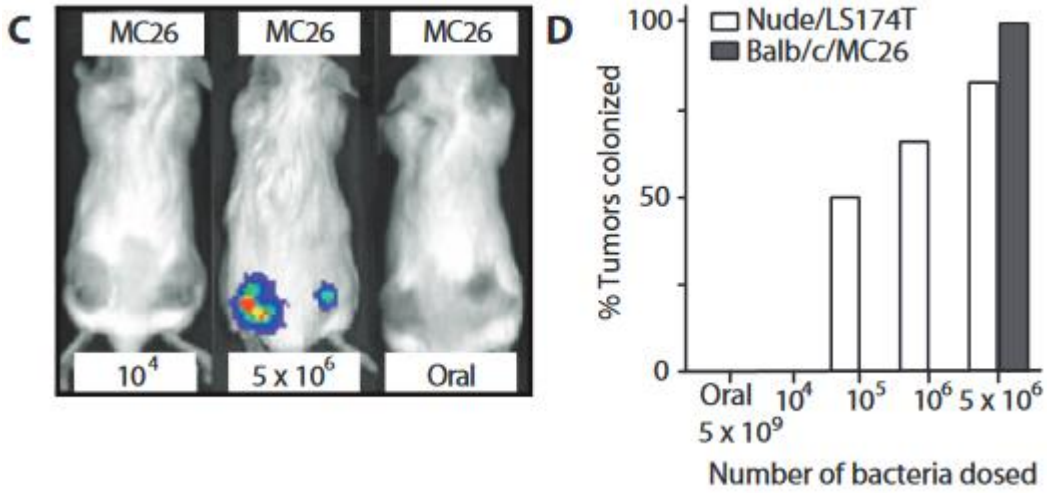
Testing of subcutaneous tumors

Systemic intravenous injection of probiotics (5 x 10E6 CFU) lead to complete colonization in all but one subcutaneous tumor model



IVIS images showing colonization of subcutaneous tumors by PROP-Z in immunocomp. mice (3 d.p. IV)

Compared to the liver metastasis models, the subcutaneous tumors can only be colonized by injected PROP-Z



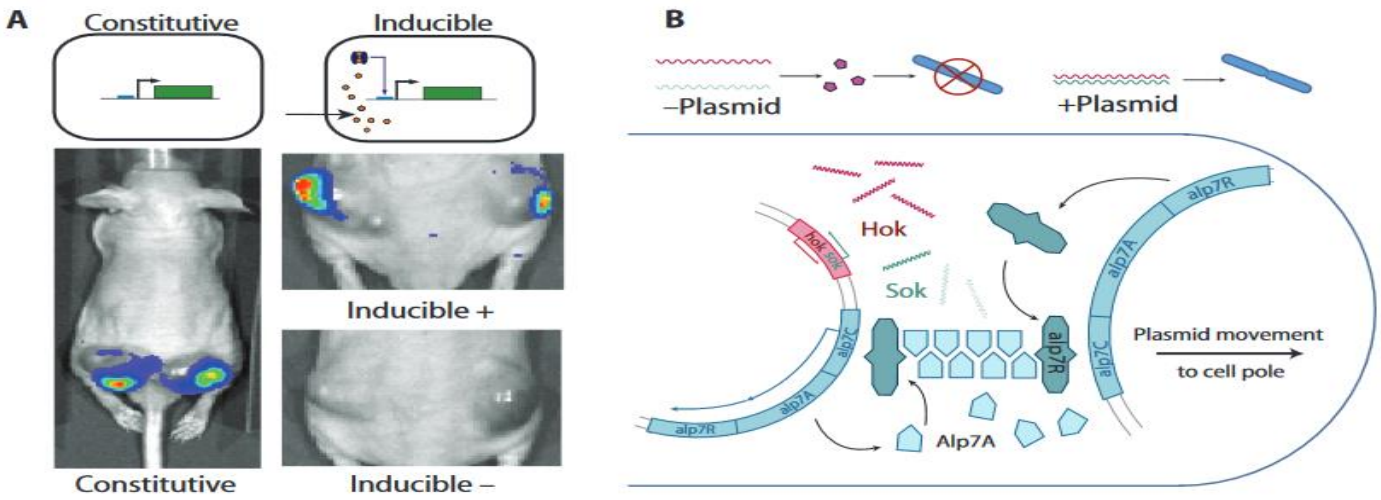
IVIS images of MC26-LucF:Balb/c

- Tumor colonization may depend more on the local concentration of bacteria than on the tumor type
- Dose-dependancy observed

Engineered PROP-Z maintain plasmid expression over time

A dual-maintenance vector was engineered to ensure long-term stability of the PROP platform

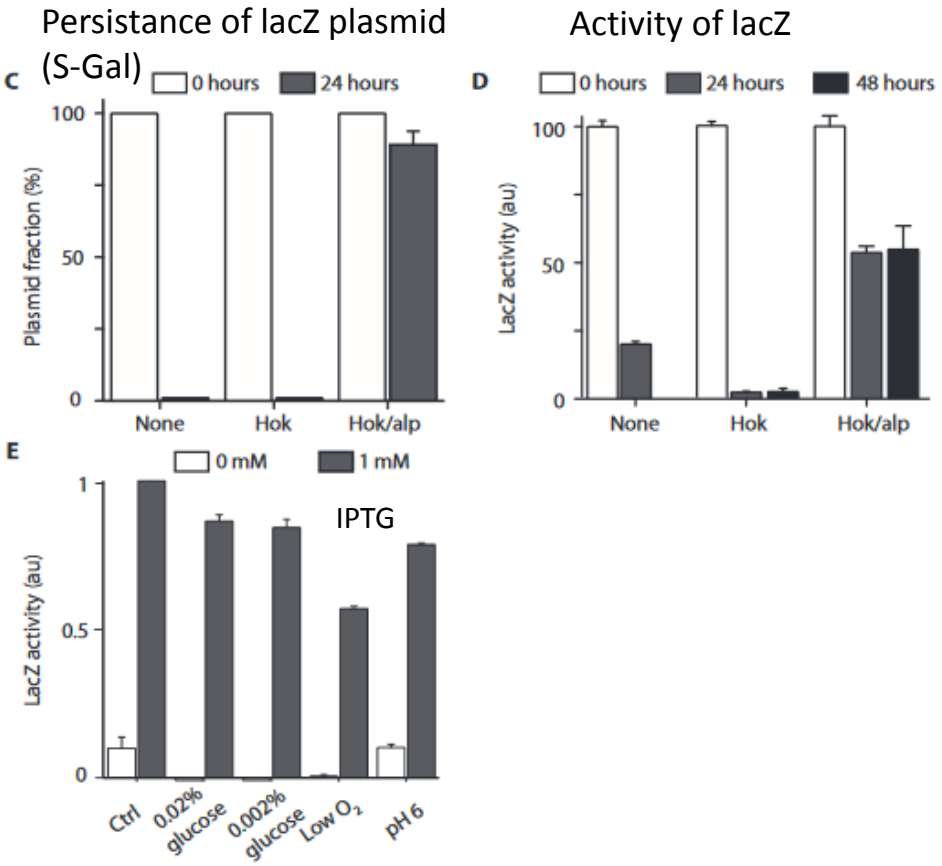
1. toxin-antitoxin system that produces toxin (hok) and short-lived anti-toxin (sok)
2. Alp7 (*B. subtilis*) produces filaments that push plasmid and ensures equal segregation during cell division



Bacterial Luciferase signals 24 h. post IV from constitutive luxCDABE circuit (PROP-Luc) or an AHL-inducible luxCDABE (PROPi-Luc)

Engineered PROP-Z maintain plasmid expression over time

Bacteria were grown overnight with antibiotics and then subcultured without antibiotics (**C+D**).



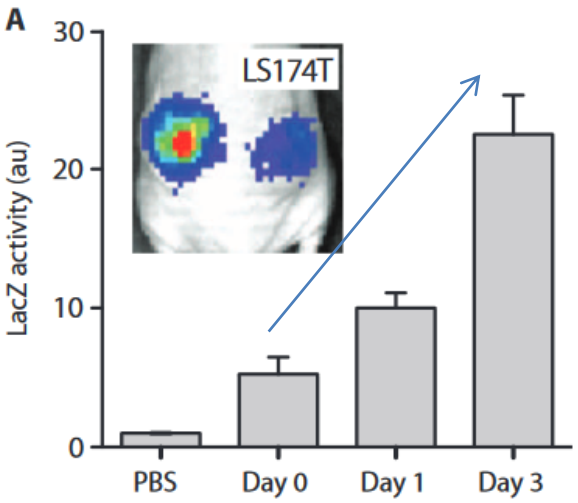
Activity of lacZ under «tumor conditions» *in vitro* (left) / *in vivo*

Bacteria were injected into subcutaneous cancer model (LS174-LucF, nude mice). Tumors were analyed by colony counts on plasmid selective (Kn+ERYC) or non-plasmid selective (ERYC) (**F**).

Detection of metastatic tumors by PROP-Z urine diagnostic

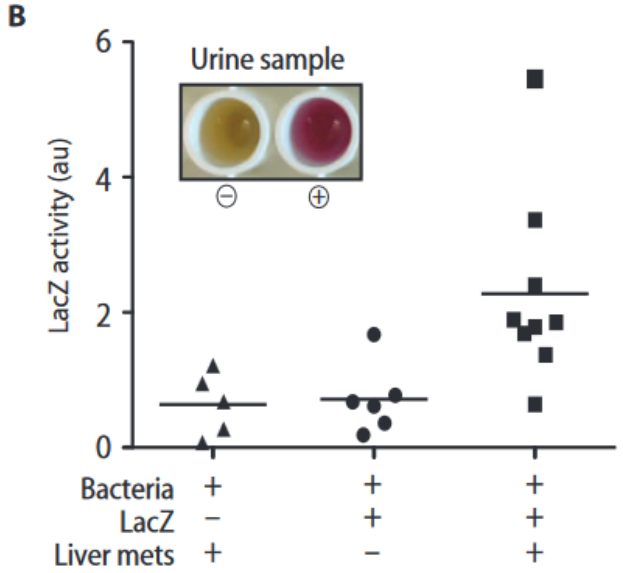
Basis of non-invasive detection of tumor metastases: The capacity of PROP-Z to activate a systemically administered agent. The product will be excreted and detected in the urine.

lacZ activity in PROP-Z-colonized tumors

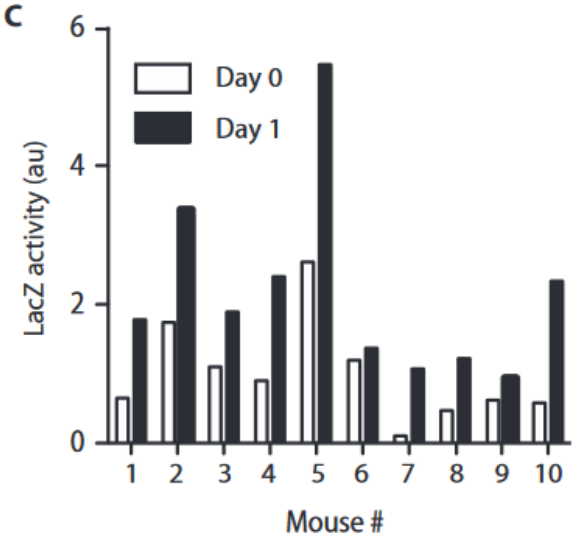


- Proof-of-concept study
- Subcutaneous model (nude mice)
- PROP-Z injected IV
- Colorimetric CPRG assay performed on excized/homogenized tumors

Liver metastasis model (MC26-LucF)



- Oral delivery of PROP-Z or lacZ-deficient bacteria
- Inject (IV) Lu-Gal into mice
- Prop-Z converts Lu-Gal into Luciferin + Galactose (via LacZ)
- Luciferin is detected in urin (1 ul suffices for Luciferase assay) after 24 h
- SNR=3.6



Conclusion

1. A probiotic diagnostic tool was developed, allowing the sensitive, specific and safe detection of hepatic tumors in two murine models of liver metastasis (LM)

2. Nonvirulent probiotic *E. Coli* Nissle 1917 (EcN) homes to tumor
   - The colonization is dose-dependent
   - LM model → oral administration (5 x 10E9 CFU)
   - SC model → IV injection (5 x 10E6 CFU)

3. Early detection of very small liver metastases is possible
   - Even small numbers of PROP bacteria colonize the tumor, expand and thus amplify the signal against the in vivo background signal

4. This technology could help detect primary hepatocellular carcinoma in patients at risk for malignant transformation (e.g. chronic viral Hepatitis) or detect liver metastases in primary liver, colorectal, breast and pancreatic cancer

Clinical Translation

1. The host strain EcN is already being proscribed to patients with GI disorders and its safety record has been confirmed in clinical trials

2. However, the selective trafficking or oral PROP to the LM via the gut wall must also be investigated in humans due to species-specific differences and variability of the gut microbiome

3. Special attention must be paid when combing PROP with therapies that might alter the host's immune system (radiation, cytotoxic chemotherapy, immunotherapy)

4. The regulatory approval of engineered bacteria might profit from the increasing usage of faecal transplantation

LETTER

Synchronized cycles delivery

M. Omar Din[1]*, Tal Danino[2]†*, Arthur Prindle[1], Ma
Lev S. Tsimring[3], Sangeeta N. Bhatia[2,4,5,6,7,8]§ & Jef

'Kamikaze Bacteria' Self-Destructs to Kill Cancer Cells

By **Jamie A.**

Jul 21, 2016 04:20 AM EDT

Scientists have engineered a self-destructing bacteria that could kill cancer cells in the body.
(Photo : nikles5 / Pixabay)

www.natureworldnews.com

Rationale

Several bacteria have been reported to preferentially grow in tumor tissue

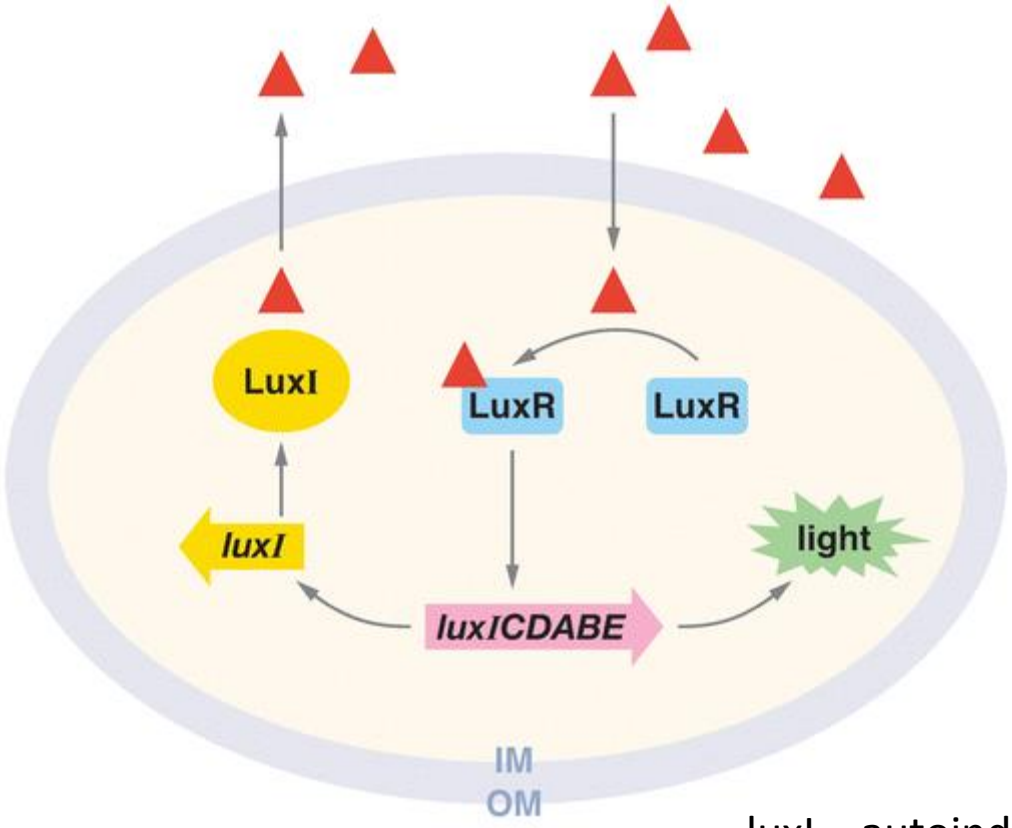
Such strains can be be genetically programmed to deliver therapies into the tumor tissue

- Feature 1: limited bacterial growth within tumor
- Feature 2: Repeatedly release of cytotoxic agent *in situ*
- Feature 3: Attenuated strain

The authors engineered an attenuated *Salmonella enterica* subsp. *Enterica* serovar Typhimurium to lyse synchronously at a defined threshold population density (termed "quorum lysis") in order to release genetically engineered cargo

The surviving bacteria reseed the colony within the tumor and the cycle starts again

Quorum Sensing

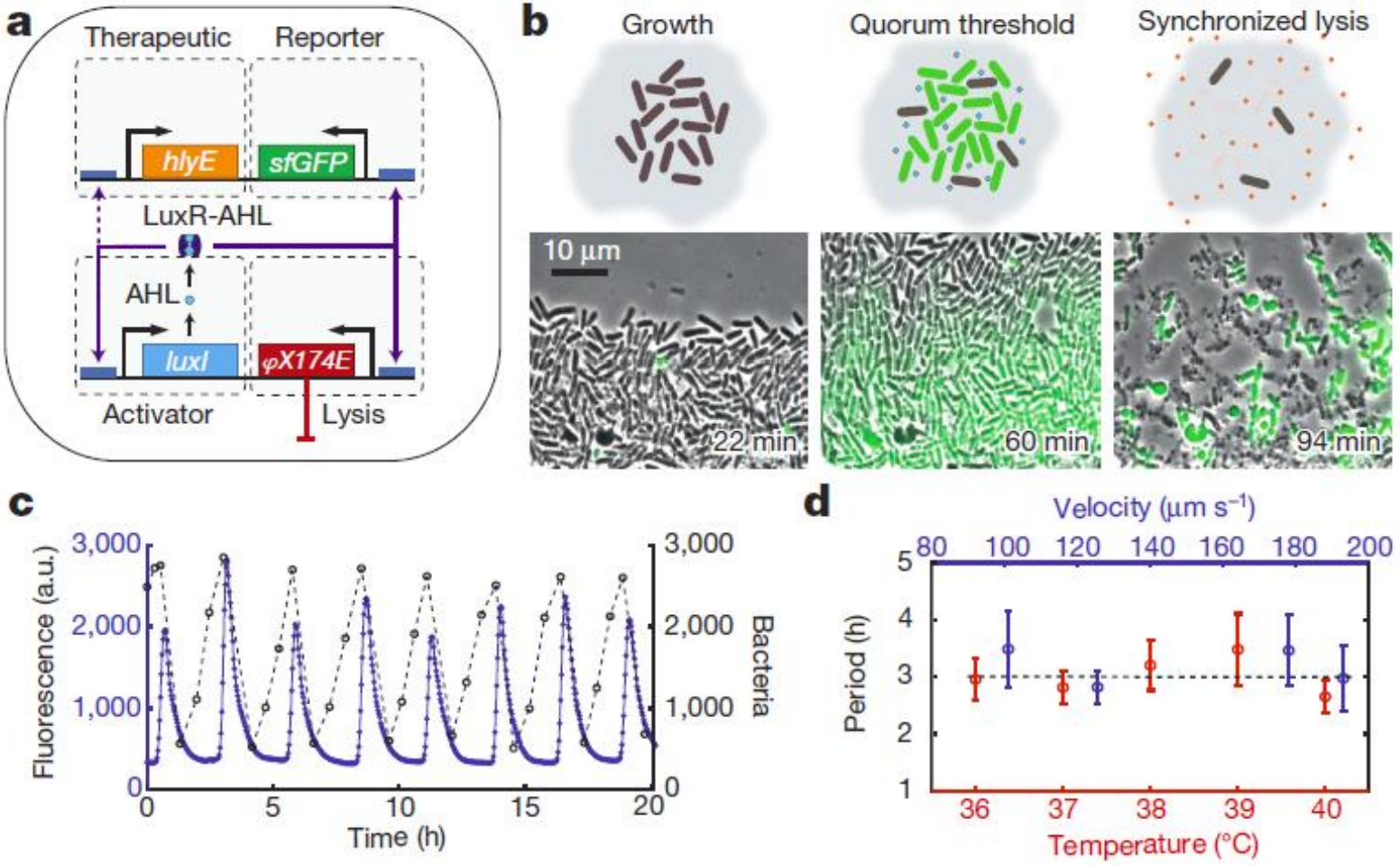


Waters CM, Bassler BL. 2005.
Annu. Rev. Cell Dev. Biol. 21:319–46

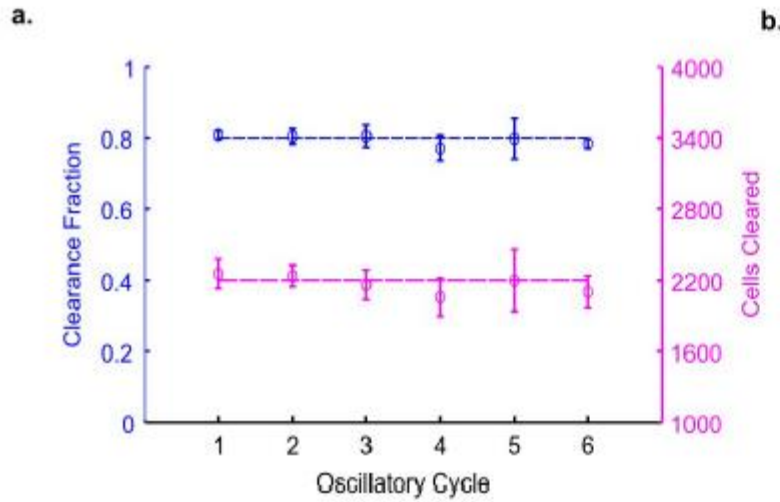
luxI = autoinducer synthase
AHL = autoinducer
luxR = transcriptional activator

Construction of synchronized lysis circuit (SLC)

Circuit consists of a common luxI promoter that drives expression of both ist own activator AHL/luxR (pos feedback) and a lysis gene (neg. feedback)



«Firing»


a.
Clearance Fraction
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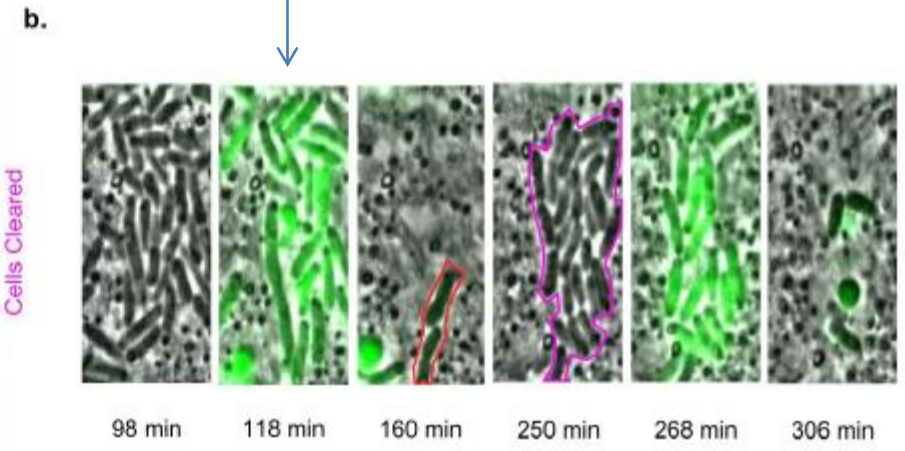
b.
98 min
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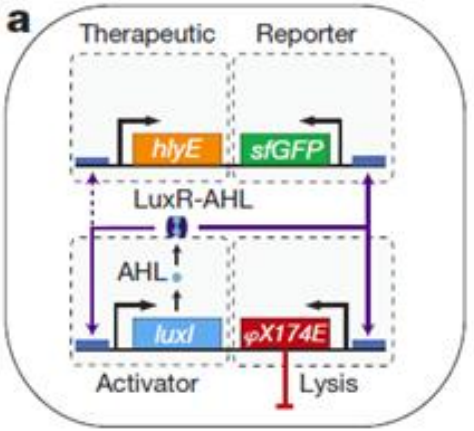
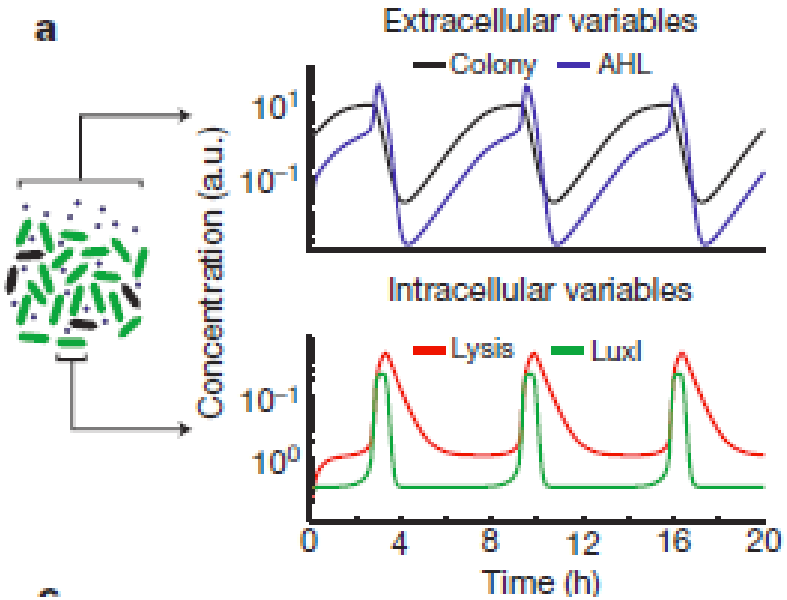
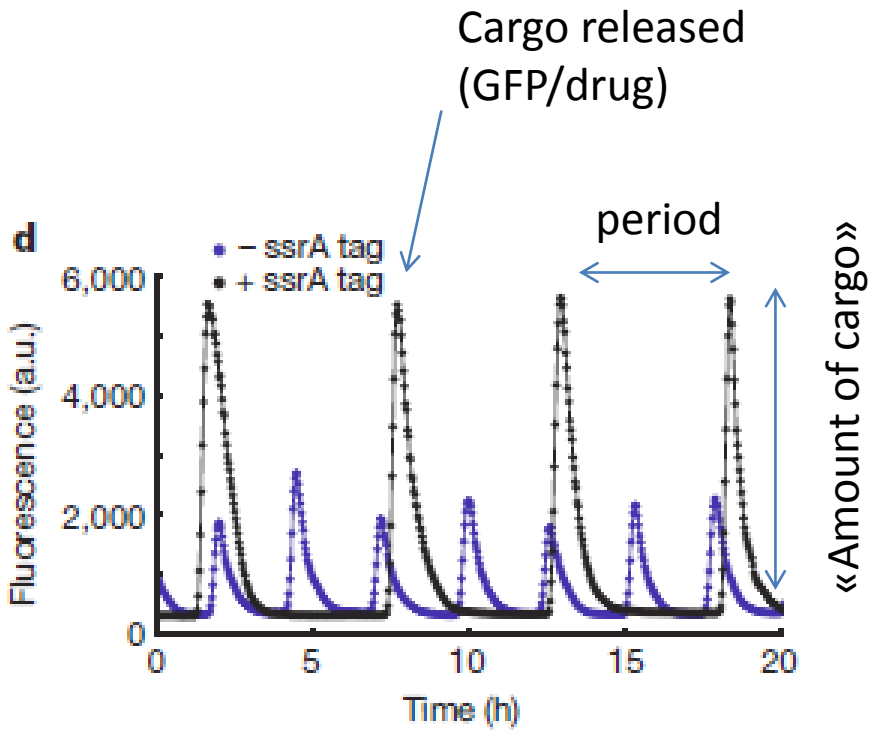
268 min
306 min


a
Therapeutic
Reporter
hlyE
sfGFP
LuxR-AHL
AHL
luxI
φX174E
Activator
Lysis

The SLC enables tuning of period and magnitude of delivery



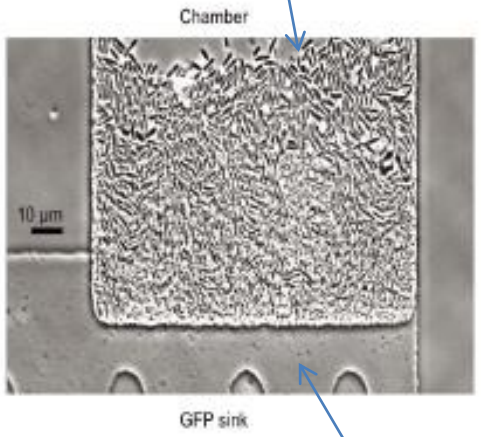


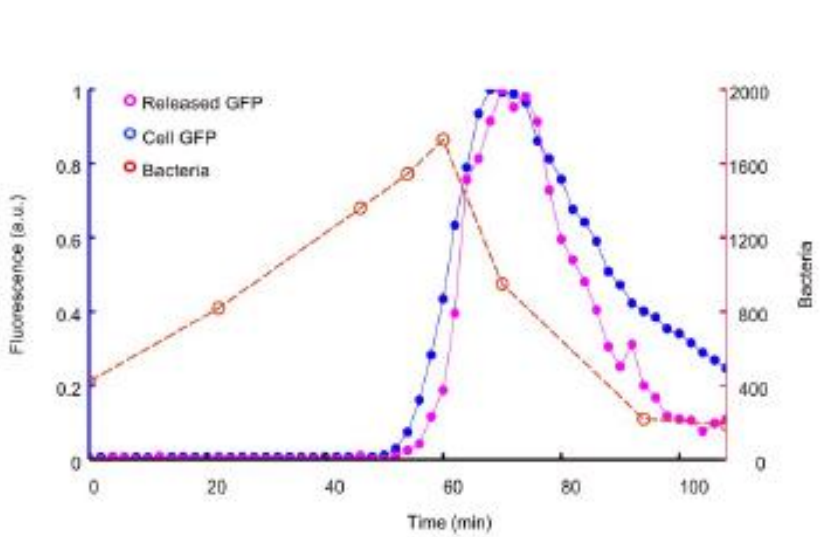
ssrA tag on LuxI
→degradation rate of LuxI increased
→ rate of AHL accumulation lower
→ More cells required to pass threshhold

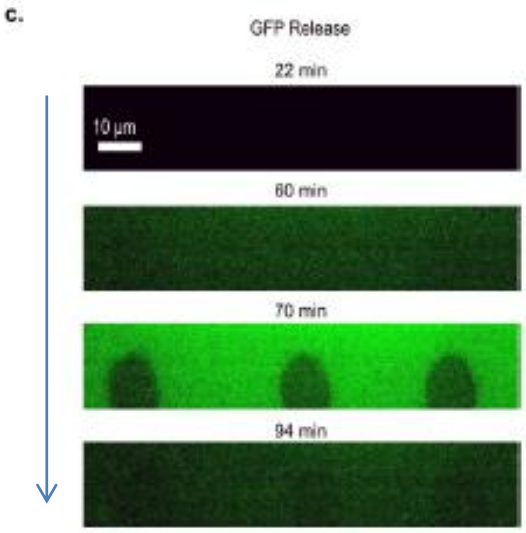
Circuit is able to release intracellular contents

S. Thyphimurium +
SLC circuit

oscillation



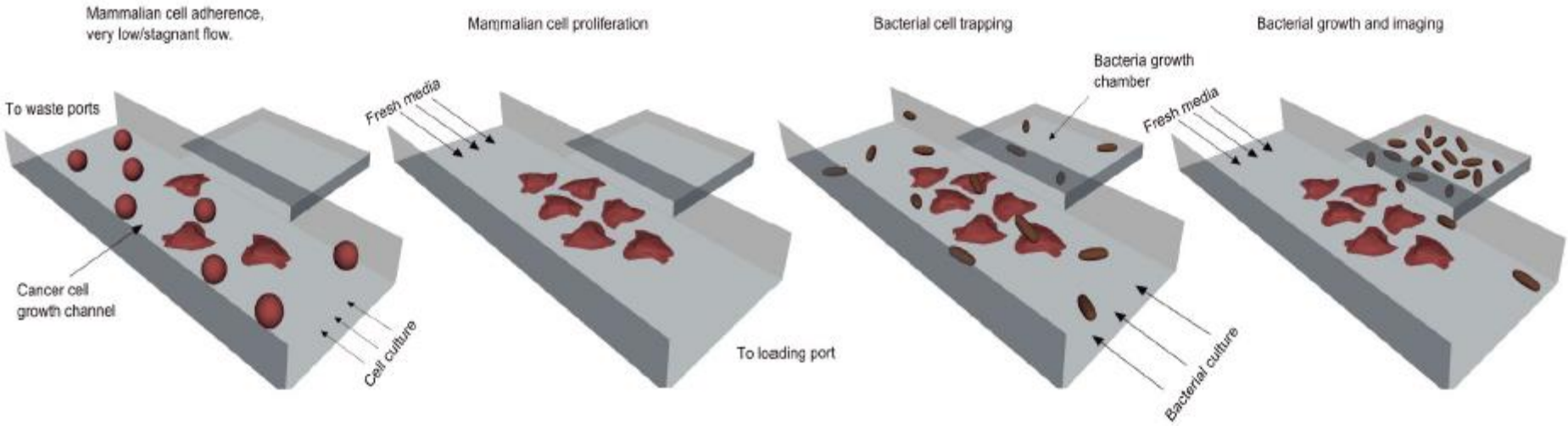




Visualize release of cargo via sfGFP that is collected in a small microfluidic sink located beneath the growth chamber

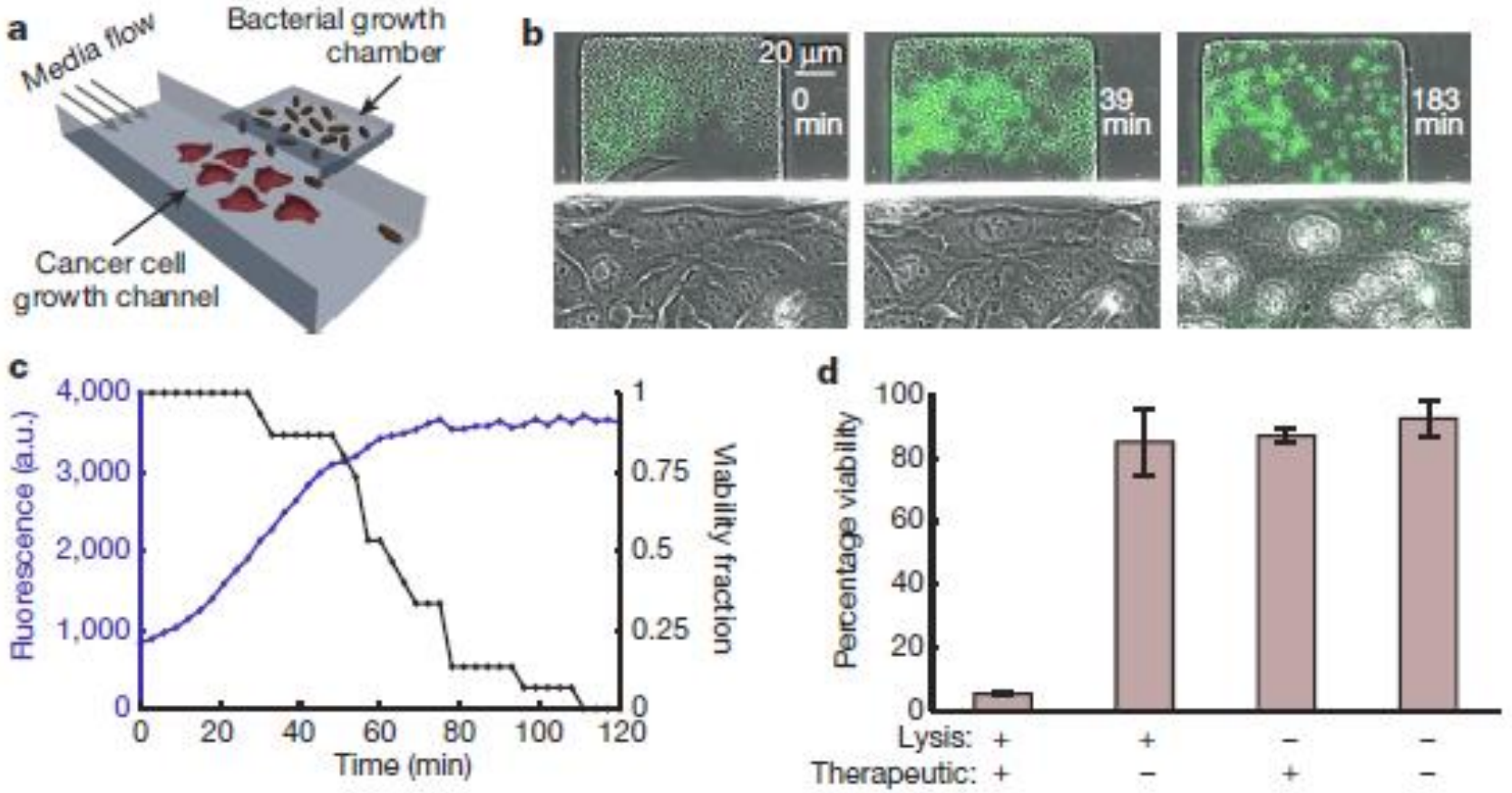
Visualize bacterial lysis and killing of cancer cells *in vitro*

Visualization



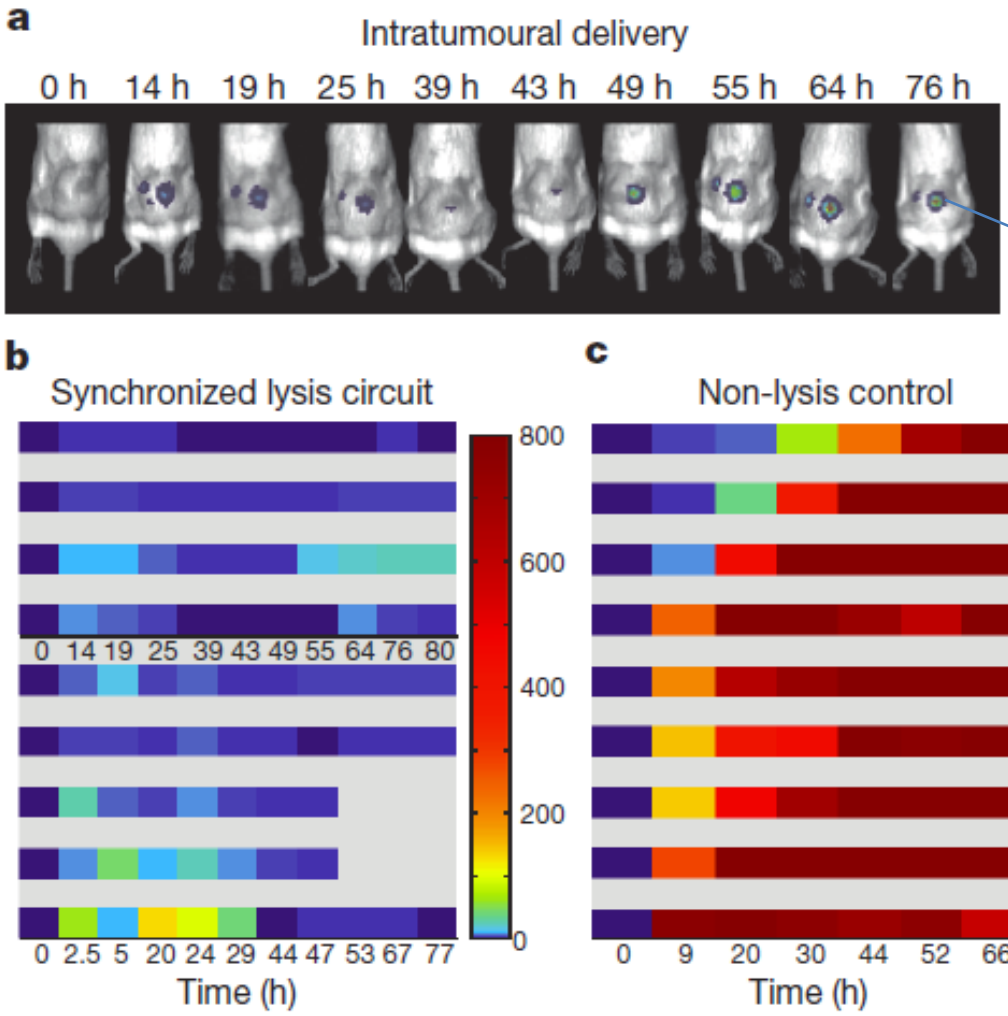
Cargo: Haemolysin E (hylE, *E. coli*) + sfGFP

Visualize bacterial lysis and killing of cancer cells *in vitro*

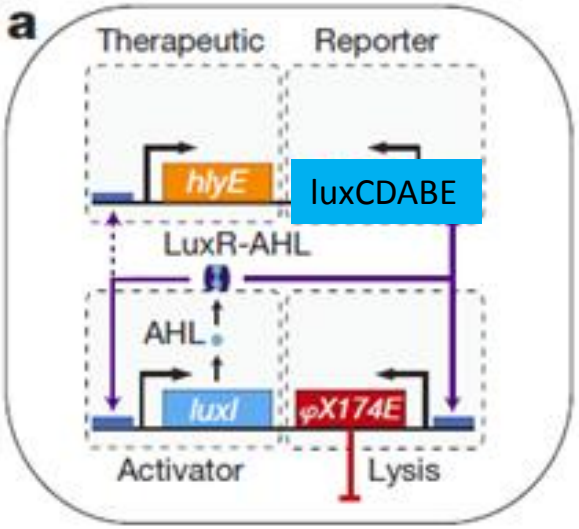


«Initial seeding with a larger volume of bacteria resulted in increased firing rates which correspond to shorter HylE exposure times until death, consistent with greater magnitude of lysis and payload release (…)»

Luc Reporter to monitor bacterial population dynamics in subcutaneous model of colorectal cancer (MC26 cells) in immunocompetent mice

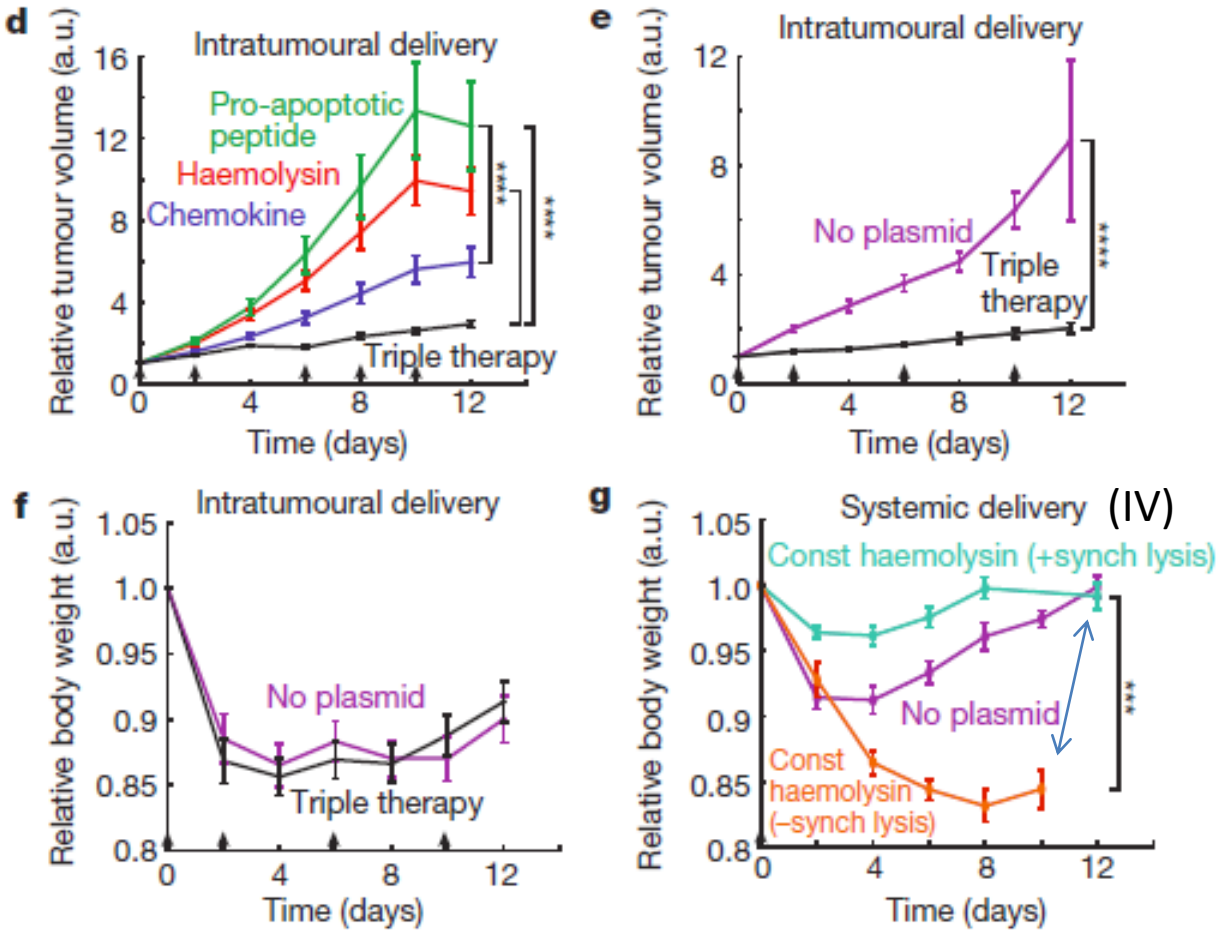


«stabilized» Salmonella typhimurium + SLC



Pulsatile bacterial population dynamics within tumor

3 SLC-strains (1 cargo each) injected intratumorally



SLC-Haemolysin

SLC-CCL21 (recruits T- and DC cells)

SLC containing cell death domain of Bit1 fused to tumor-penetrating peptide iRGD

SLC-3 triple-strain → triple therapy

Combination therapy

Oral delivery of these bacterial strains resulted in an efficient colonization of hepatic colorectal metastases

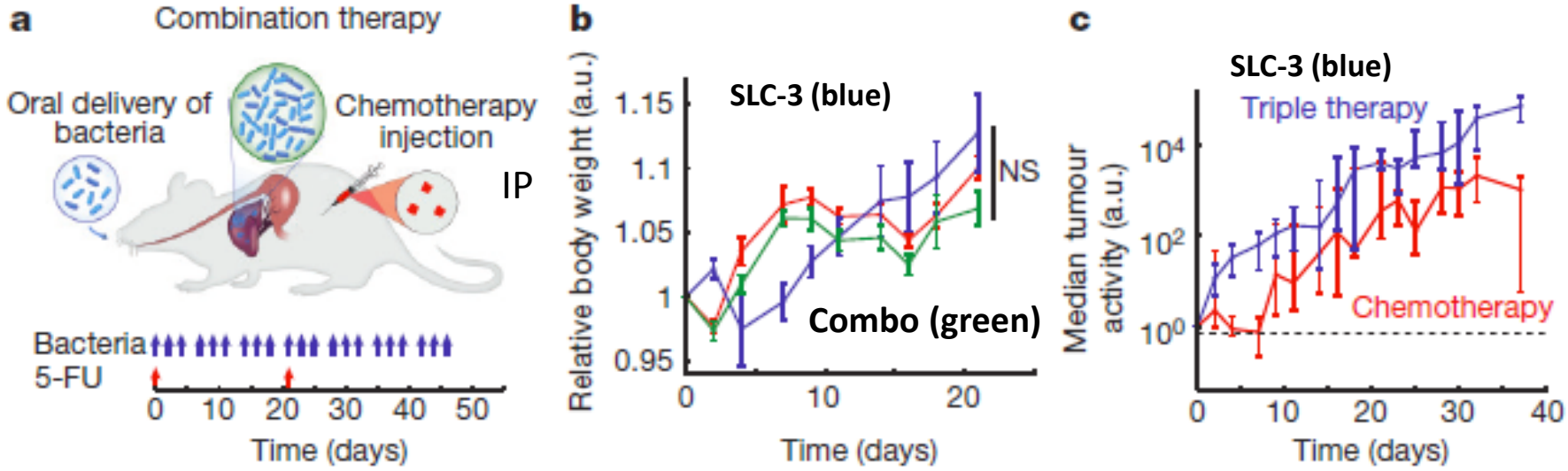
Additionally, mice tolerated repeated dosing without adverse effects (next slide)

Previous studies proved that anaerobic bacteria can occupy avascular tumor compartments where traditional chemotherapy might be ineffective due to poor drug delivery

Therefore, the authors set out to explore the synergistic effect when bacteria deliver drugs to the necrotic core while the standard chemotherapy targets vascularized regions

Combination therapy

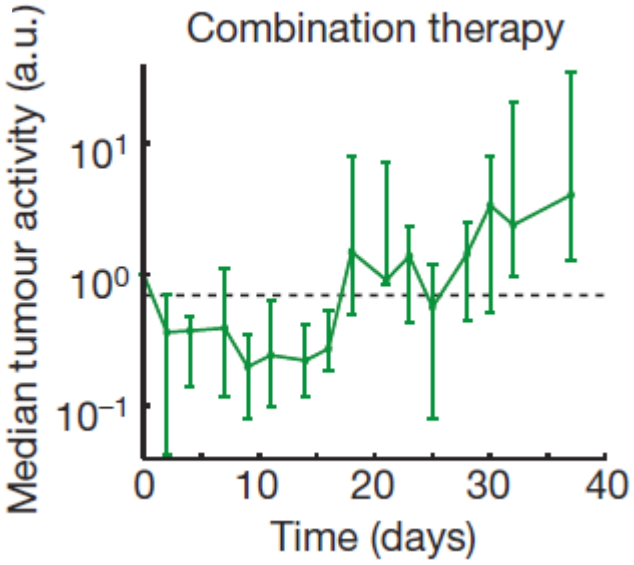
In vivo testing in an experimental model of colorectal metastases in the liver via oral delivery of bacteria (SLC-3)

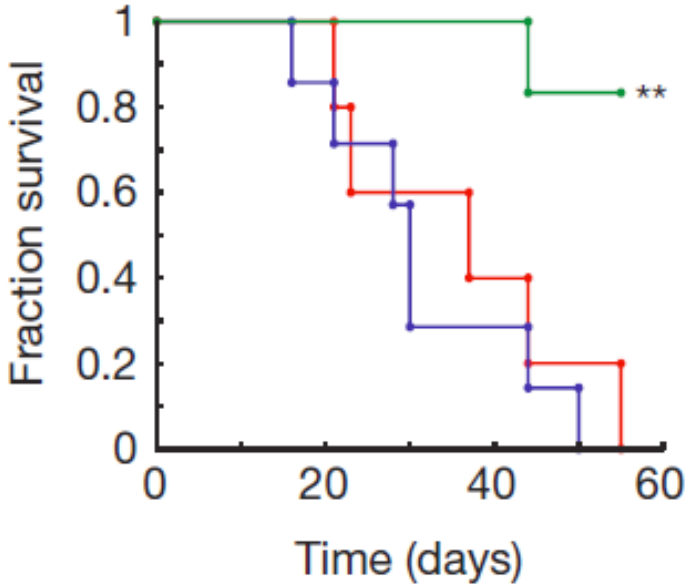


Combination therapy

Triple strain + chemotherapy

- tumor activity shrank by 30% in the first 18 days (not shown)
- Roughly a 50% increase in mean survival time for animal carrying incurable colorectal metastases





Conclusion

1. The synchronized lysis paradigm allows bacteria to release the cargo in a synchronized and repeated fashion, while also controlling the absolute numbers of bacteria in the tissue

2. Compared to other drug-delivery methods, no pre-loading of the drug is necessary and no secretory system has to be engineered

3. The circuit can be modulated and adjusted to *in silico* predictions
   - Amount of cargo (amplitude)
   - Frequency of drug release (period)
   - Seeding numbers
   - Combination of different toxins in one strain
   - Combination with conventional therapies

4. Efficacy has been proven *in vitro* and in murine models

Thank you for your attention!