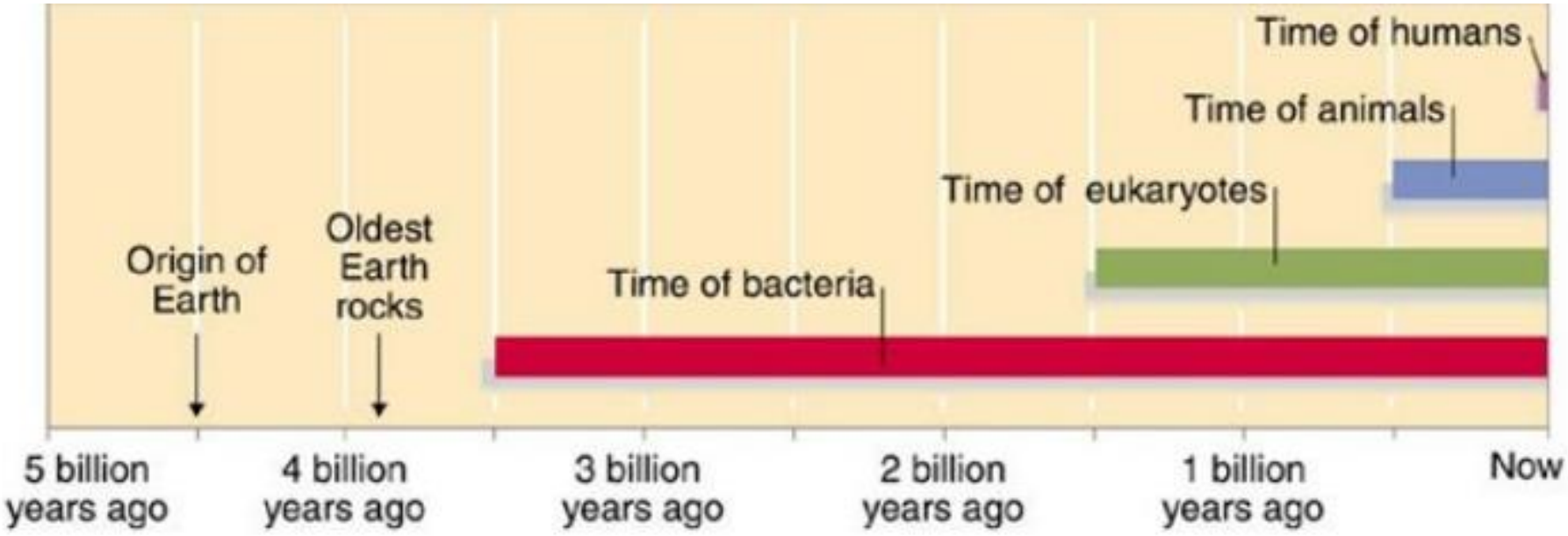
Antibiyotik Dışı Tedaviler

Dr. Özlem Kurt Azap

Başkent Üniversitesi Tıp Fakültesi

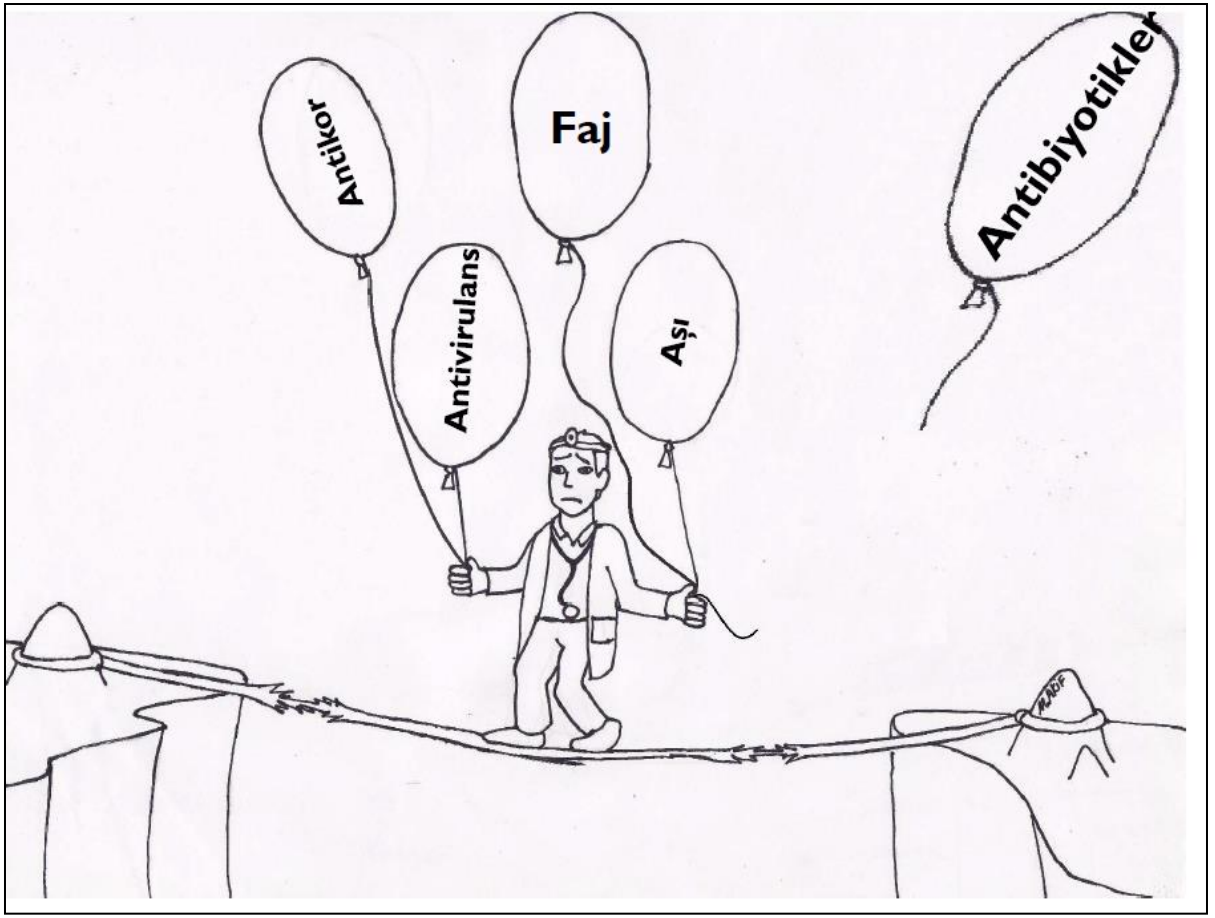
Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji AD

Bakterilerle uğraşmak zor!



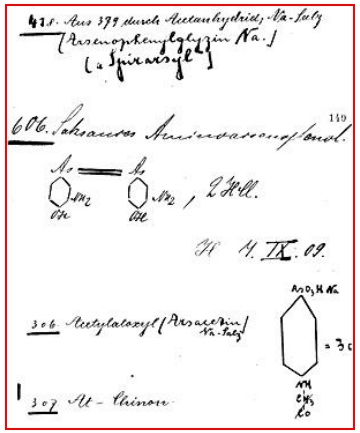
Geldiğimiz nokta:

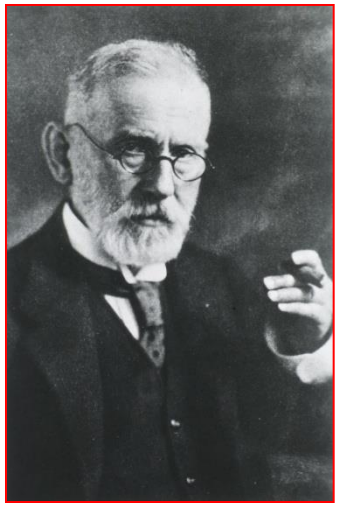
“**Sihirli** bir **mermi**”ye ihtiyacımız var!



Magic Bullet (Sihirli Mermi)

"İnsana zarar vermeyip sadece hedefi yok eden"





Paul Ehrlich (1854–1915)
Nobel Ödülü-1908

Salvarsan 606: Sifiliz tedavisinde,
"hayat kurtaran arsenik"

Alternatives to antibiotics—a pipeline portfolio review

Lloyd Czaplewski, Richard Bax, Martha Clokie, Mike Dawson, Heather Fairhead, Vincent A Fischetti, Simon Foster, Brendan F Gilmore, Robert E W Hancock, David Harper, Ian R Henderson, Kai Hilpert, Brian V Jones, Aras Kadioglu, David Knowles, Sigríður Ólafsdóttir, David Payne, Steve Projan, Sunil Shaunak, Jared Silverman, Christopher M Thomas, Trevor J Trust, Peter Warn, John H Rex

Antibiotics have saved countless lives and enabled the development of modern medicine over the past 70 years. However, it is clear that the success of antibiotics might only have been temporary and we now expect a long-term and perhaps never-ending challenge to find new therapies to combat antibiotic-resistant bacteria. A broader approach to address bacterial infection is needed. In this Review, we discuss alternatives to antibiotics, which we defined as non-compound approaches (products other than classic antibacterial agents) that target bacteria or any approaches that target the host. The most advanced approaches are antibodies, probiotics, and vaccines in phase 2 and phase 3 trials. This first wave of alternatives to antibiotics will probably best serve as adjunctive or preventive therapies, which suggests that conventional antibiotics are still needed. Funding of more than £1·5 billion is needed over 10 years to test and develop these alternatives to antibiotics. Investment needs to be partnered with translational expertise and targeted to support the validation of these approaches in phase 2 trials, which would be a catalyst for active engagement and investment by the pharmaceutical and biotechnology industry. Only a sustained, concerted, and coordinated international effort will provide the solutions needed for the future.

- Akademi ve endüstriden 24 bilim insanı
- Wellcome Trust, İngiltere Sağlık Bakanlığı desteği ile
- 50 sayfalık bir rapor hazırlanmış
- Topikal tedaviler incelemeye alınmamış
- Oral, parenteral ve inhaler tedavi seçenekleri dahil edilmiş

Lancet Infect Dis 2016; 16: 239–51

	Comment	Probable spectrum of activity and initial use	Recommendation over the next 5 years
Tier 1 approaches (translational funding to clinical evaluation at phase 2)			
Antibodies[4,6-13]	Antibodies that bind to and inactivate a pathogen, its virulence factors, or its toxins were widely considered one of the alternative approaches most likely to have major clinical impact. Antibodies were considered a low-risk area with strong science basis, history of safe use, and a high degree of technical feasibility	Prevent Gram-positive and Gram-negative infection; possibly adjunct use	Basic research and development and translational
Probiotics[14-18]	Pro… hos… the… dia… diff… bac…	…eat …ociated …sociated	Translational
Lysins[19-27]	Pha… rep… bac… ber…	…positive	Basic research and development and translational
Wild-type bacteriophages[28-32]	Wi… Bac… an… uni… pro…	…positive and …ive infection	Basic research and development and translational
Engineered bacteriophages[33-36]	The… of … res… adv… a d…	…positive and …ive infection	Basic research and development and translational
Immune stimulation[37-45]	Suc… pot… exp… Ora… gro… enc… TLR… The… translational research in this specialty. Generally, there was insufficient target validation for bacterial infection, a high potential for side-effects, variable responses and polymorphisms in patient populations, and responses specific to bacterial species and strain. The clinical development path for host-targeted therapies will probably use non-human primates during product development	…rovide adjunct …ram-positive …egative	Basic research and development and translational
Vaccines[46-60]	The long established investment in vaccines for new targets should continue given their potential to substantially reduce the incidence of infection and, therefore, the need for antibiotics. In view of the ageing human population, we need better knowledge of the potential for vaccination in the elderly and how best to dose immune compromised individuals	Prevention, Gram-positive more than Gram-negative infection	Basic research and development, especially new adjuvants

Translasyonel aşamadan Faz 2'ye kadar olanlar

- Antikorlar
- Probiyotikler
- Lizinler
- Bakteriyofajlar
- İmmünstimülasyon
- Aşılar

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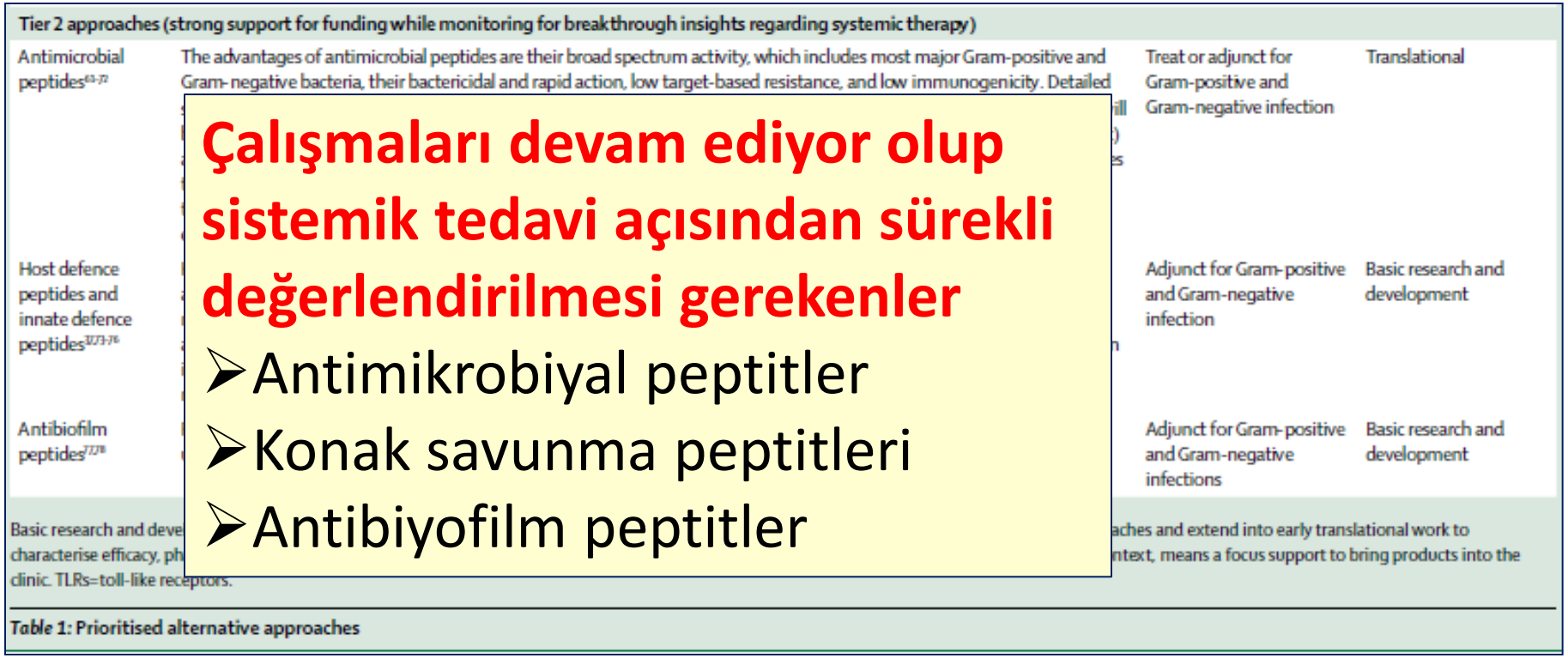

Tier 2 approaches (strong support for funding while monitoring for breakthrough insights regarding systemic therapy)

Antimicrobial peptides[63-72]	The advantages of antimicrobial peptides are their broad spectrum activity, which includes most major Gram-positive and Gram-negative bacteria, their bactericidal and rapid action, low target-based resistance, and low immunogenicity. Detailed	Treat or adjunct for Gram-positive and Gram-negative infection	Translational
Host defence peptides and innate defence peptides[3,73-76]		Adjunct for Gram-positive and Gram-negative infection	Basic research and development
Antibiofilm peptides[77,78]		Adjunct for Gram-positive and Gram-negative infections	Basic research and development

Basic research and deve… …aches and extend into early translational work to characterise efficacy, ph… …ntext, means a focus support to bring products into the clinic. TLRs=toll-like receptors.

Table 1: **Prioritised alternative approaches**

Çalışmaları devam ediyor olup sistemik tedavi açısından sürekli değerlendirilmesi gerekenler

- Antimikrobiyal peptitler
- Konak savunma peptitleri
- Antibiyofilm peptitler

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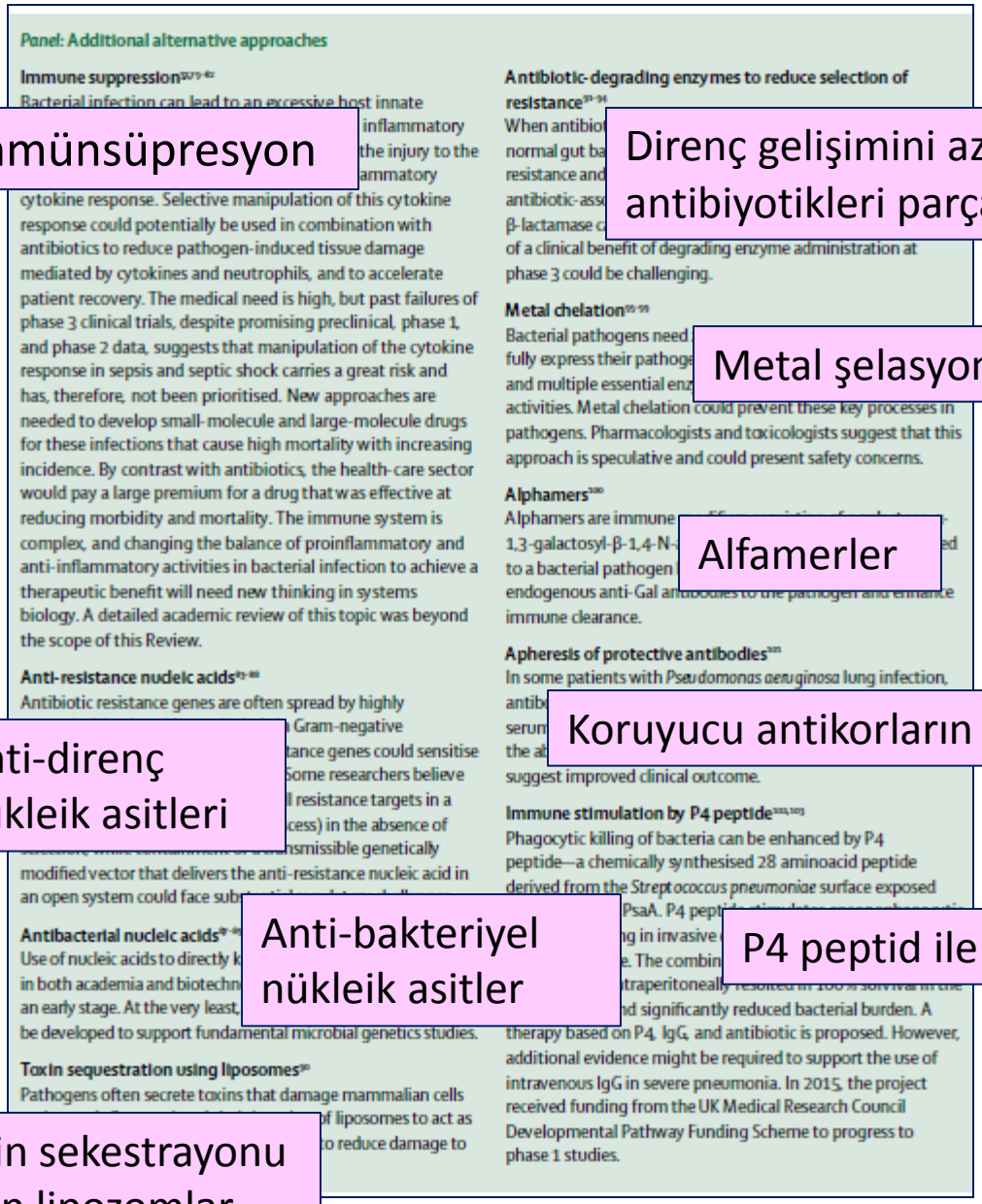
Yeni ufuklar

	Target	Product name, reference	Phase as of January-March, 2015	Earliest anticipated registration	Probability of registration by 2025	Risk-adjusted cost of projects; current phases, subsequent phases (£ million)	Pipeline investment needed for additional phase 2 validation (£ million)
Antibodies							
Merck	Clostridium difficile	Bezlotoxumab[72,73]	Phase 3 ongoing	2017	..	..	..
MedImmune	Staphylococcus aureus	MEDI4893[74]	Phase 2 ongoing	2021	..	..	..
Aridis	Pseudomonas aeruginosa	AR-101[75]	Phase 2a complete	2021	..	..	..
Aridis	S aureus	AR-301[76]	Phase 2a ready	2022			
MedImmune	P aeruginosa	MEDI3902[6]	Phase 1 ongoing	2023			
XBiotech	S aureus	514G3[30]	Phase 1 ongoing	2023			
Aridis	P aeruginosa	Aerucin[30]	IND ready	2025			
Combined	..	..	..	..	183%	60, 120	..
Probiotics							
Seres	C difficile	SER-109[120]	Phase 3 ready	2018	..	..	..
Rebiotix	C difficile	RBX2660[121]	Phase 2 ongoing	2019	..	..	..
Shire (Viropharma)	C difficile	VP20621[122]	Phase 2 ready	2022	..	..	..
Combined	..	..	..	..	124%	52, 53	..
lysins							
Intron Biotechnology	S aureus	SAL200[37]	Phase 1 ongoing	2022	..	..	..
ContraFect	S aureus	CF-301[36]	Phase 1 ongoing	2022	..	..	..
Combined	..	..	..	..	26%	12, 28	135
Bacteriophages							
Wild-type bacteriophages							
AmpliPhi	C difficile	AmpliPhage-004[123]	Pre-phase 1	2023	..	..	..
AmpliPhi	P aeruginosa	AmpliPhage-001[121]	Pre-phase 1	2023			
Engineered bacteriophages							
Phico Therapeutics	P aeruginosa	PT-3.1[54]	Pre-phase 1	2023			
Combined	..	..	..	..			
Immune stimulation							
Akthelia	C difficile	Phenylbutyrate/vitamin D[39,40]	Phase 2 ready	2021			
Various	Various	Bacterial extracts[41]	Phase 1 ready	2022			
Combined	..	..	..	..	43%	0, 55	..
Vaccines							
Sanofi Pasteur	C difficile	C difficile toxoid vaccine[124]	Phase 3	2019			..
Valneva	P aeruginosa	IC43[74,126]	Phase 2 and Phase 3 ongoing	2019			..
Valneva	C difficile	IC84[126]	Phase 2 ongoing	2021			..
Pfizer	S aureus	SA4Ag[127]	Phase 2 ready	2021			..
Combined	..	..	..	..	188%	74, 66	..

Antikorlar

Bakteriyofajlar

Aşılar



Panel: Additional alternative approaches

Immune suppression

Bacterial infection can lead to an excessive host innate inflammatory the injury to the ammatory cytokine response. Selective manipulation of this cytokine response could potentially be used in combination with antibiotics to reduce pathogen-induced tissue damage mediated by cytokines and neutrophils, and to accelerate patient recovery. The medical need is high, but past failures of phase 3 clinical trials, despite promising preclinical, phase 1, and phase 2 data, suggests that manipulation of the cytokine response in sepsis and septic shock carries a great risk and has, therefore, not been prioritised. New approaches are needed to develop small-molecule and large-molecule drugs for these infections that cause high mortality with increasing incidence. By contrast with antibiotics, the health-care sector would pay a large premium for a drug that was effective at reducing morbidity and mortality. The immune system is complex, and changing the balance of proinflammatory and anti-inflammatory activities in bacterial infection to achieve a therapeutic benefit will need new thinking in systems biology. A detailed academic review of this topic was beyond the scope of this Review.

Anti-resistance nucleic acids

Antibiotic resistance genes are often spread by highly Gram-negative tance genes could sensitise Some researchers believe l resistance targets in a cess) in the absence of nsmissible genetically modified vector that delivers the anti-resistance nucleic acid in an open system could face sub

Antibacterial nucleic acids

Use of nucleic acids to directly k in both academia and biotechn an early stage. At the very least, be developed to support fundamental microbial genetics studies.

Toxin sequestration using liposomes

Pathogens often secrete toxins that damage mammalian cells f liposomes to act as o reduce damage to

Antibiotic-degrading enzymes to reduce selection of resistance

When antibio normal gut ba resistance and antibiotic-ass β-lactamase c of a clinical benefit of degrading enzyme administration at phase 3 could be challenging.

Metal chelation

Bacterial pathogens need fully express their pathoge and multiple essential enz activities. Metal chelation could prevent these key processes in pathogens. Pharmacologists and toxicologists suggest that this approach is speculative and could present safety concerns.

Alphamers

Alphamers are immune 1,3-galactosyl-β-1,4-N- ed to a bacterial pathogen endogenous anti-Gal antibodies to the pathogen and enhance immune clearance.

Apheresis of protective antibodies

In some patients with *Pseudomonas aeruginosa* lung infection, antib seru the ab suggest improved clinical outcome.

Immune stimulation by P4 peptide

Phagocytic killing of bacteria can be enhanced by P4 peptide—a chemically synthesised 28 aminoacid peptide derived from the *Streptococcus pneumoniae* surface exposed PsaA. P4 pept g in invasive e. The combin traperitoneally resulted in 100% survival in the nd significantly reduced bacterial burden. A therapy based on P4, IgG, and antibiotic is proposed. However, additional evidence might be required to support the use of intravenous IgG in severe pneumonia. In 2015, the project received funding from the UK Medical Research Council Developmental Pathway Funding Scheme to progress to phase 1 studies.

İmmünsüpresyon

Direnç gelişimini azaltmak için antibiyotikleri parçalayan enzimler

Metal şelasyonu

Alfamerler

Koruyucu antikorların aferezi

Anti-direnç nükleik asitleri

Anti-bakteriyel nükleik asitler

P4 peptid ile immünstimülasyon

Toksin sekestrayonu yapan lipozomlar

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Maliyet

	Preclinical	Phase 1	Phase 2	Phase 3	Registration	Total
Stage probability of success	23%	45%	47%	71%	90%	..
Number of projects	34	8	4	2	1	..
Cost of phase (£ million)	12·5	6	10	45	1·3	..
Portfolio cost (£ million)	425	48	40	90	1·3	604

The calculation used to estimate the costs of funding a relatively new alternative approach to provide sufficient number of preclinical projects to survive standard rates of attrition and to have a reasonable chance of product registration is shown.

Table 3: Estimate of the project pipeline cost for host defence and antibiofilm peptides

Key messages

- Alternatives to antibiotics: non-compound (ie, non-classic antibacterial compounds) approaches that target bacteria or approaches that target the host to treat bacterial infection
- Academics and industry have produced at least 19 approaches that need to be further assessed
- Understanding of the potential of alternatives to antibiotics will need experimental clinical medicine and not just drug discovery
- Enhanced translational expertise should be used to help validation and progression of these alternatives to antibiotics
- Model projects must be advanced to phase 2 clinical trials to enable validation of approaches
- Antimicrobial resistance needs to grow into big science to deliver new innovative therapies
- The Large Hadron Collider project cost roughly £6 billion and the International Space Station £96 billion; antimicrobial research and development to address the problem of antibiotic resistance probably needs an effort that is somewhere between these two projects

- "**İnnovasyon**"
- Akademi, mühendislik, endüstri…işbirliği
- Maliyeti göze alınmalı

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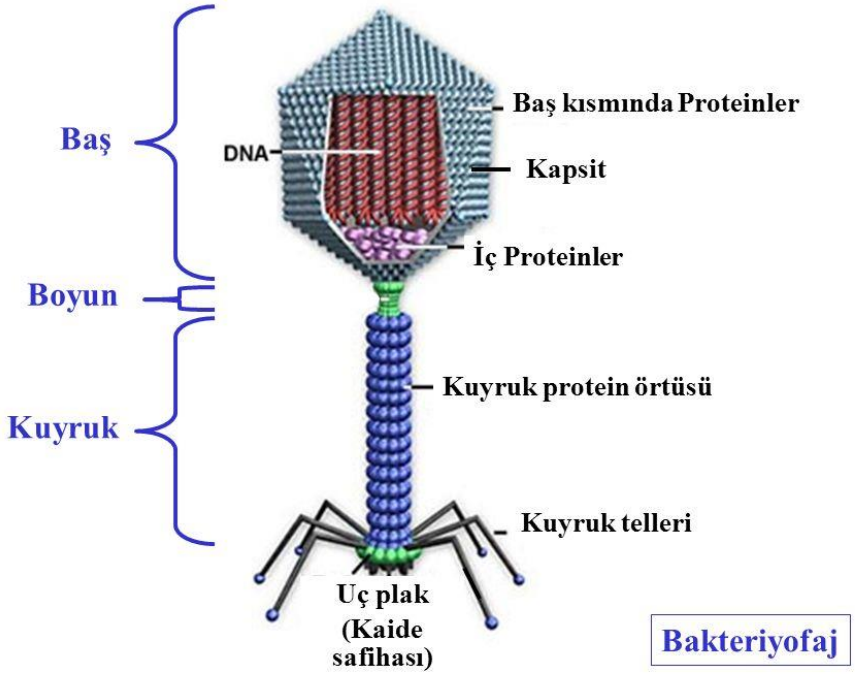
Başka neler üzerinde çalışılıyor?

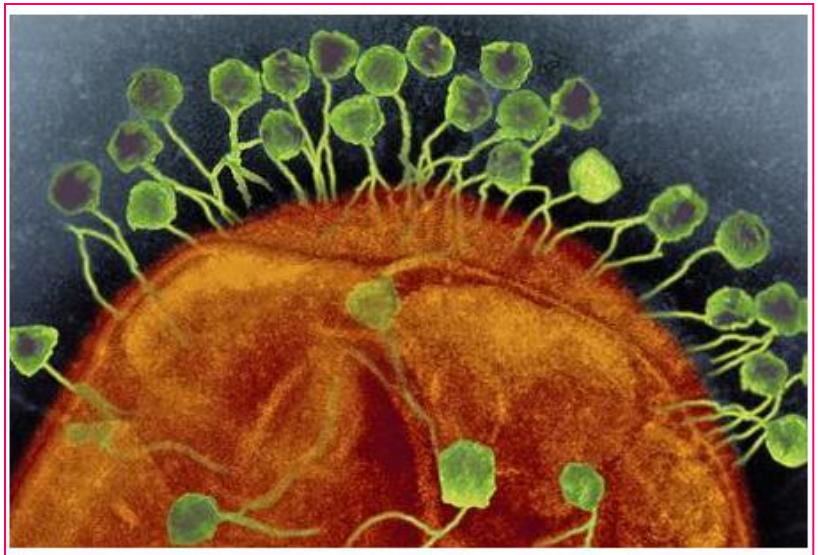
- Hemofiltrasyon cihazları
- Mikrobiyom modülasyonu:

  Prebiyotikler, probiyotikler, fekal transplantasyon
- Quorum sensing inhibitörleri
- “Nanoteknoloji”
- …..

Faj tedavisi

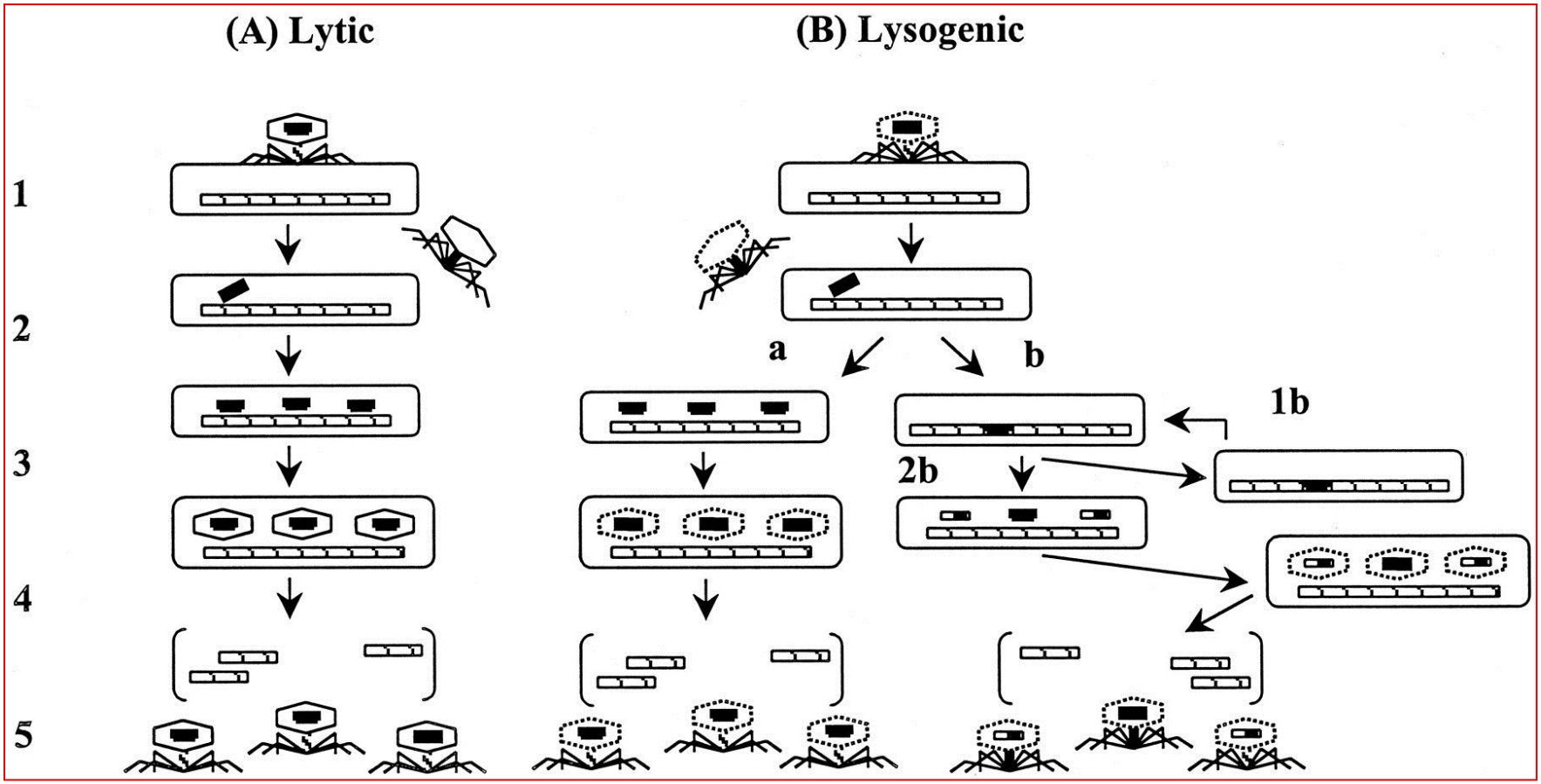
Bakteriyofajlar…





Bacteriophages (green) attacking a bacterium (orange). Image courtesy of Graham Beards.

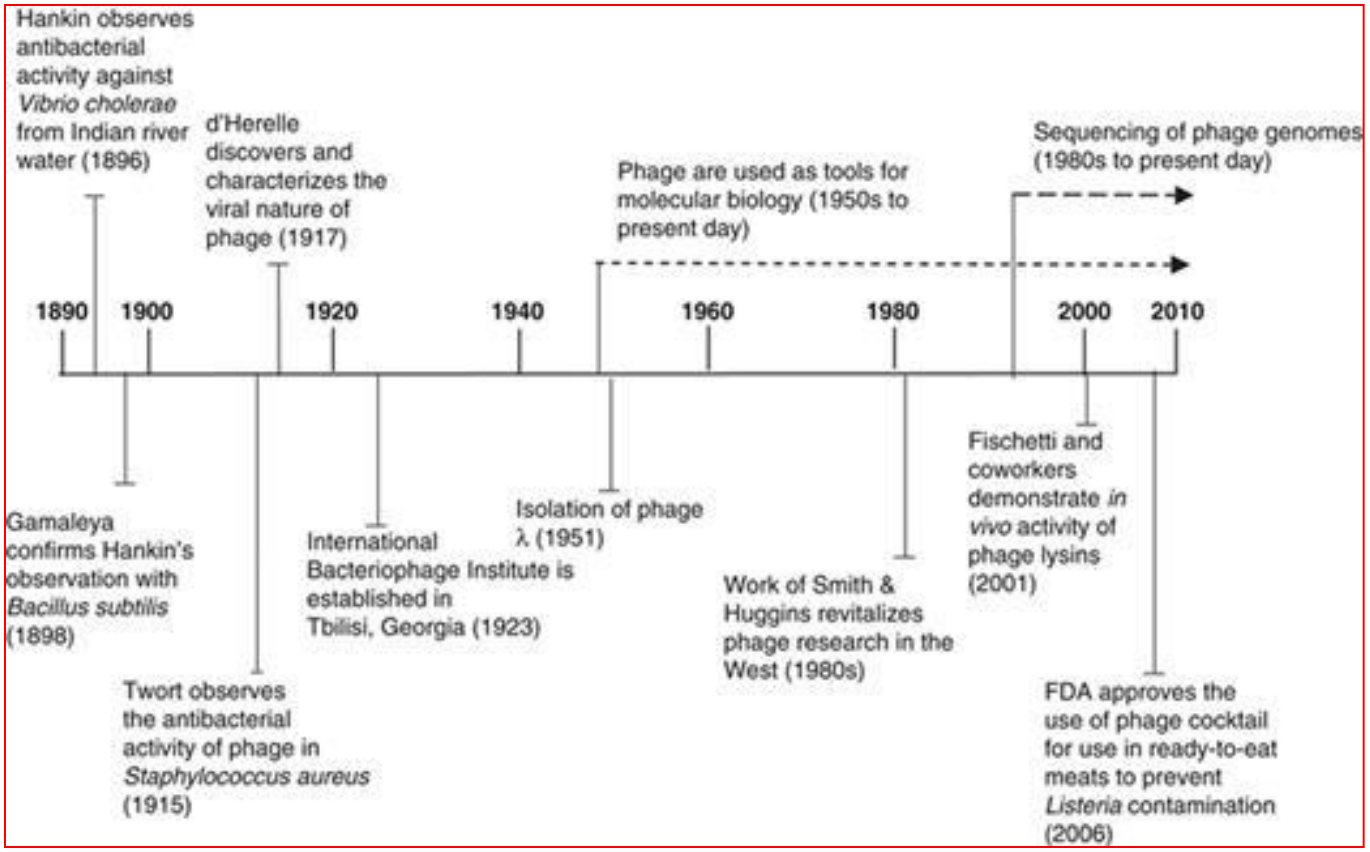
Bakteriyofaj Türleri



Dünyada

- **$8x10^9$** insan
- **5×10^{30}** tane bakteri
- **10^{31}'**den fazla faj var!

Zaman Çizelgesi



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From Science Fiction to Clinical Trial: The Use of Phage to Treat Antibiotic-Resistant Infections

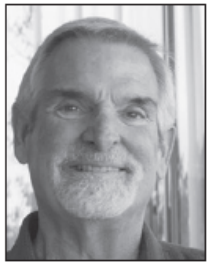
Sunday, October 7, 2018
9:15 - 10:45 a.m.
Moscone Center: West 2005-2024

Turning the Phage on Antibiotics

Steffanie Strathdee, PhD

Steffanie Strathdee, PhD, is an infectious disease epidemiologist who is renowned for her research on the intersection of HIV and drug use, having generated over 500 scholarly publications. She is Associate Dean of Global Health Sciences and Harold Simon Professor of Medicine at the University of California San Diego, where she directs a campus-wide Global Health Institute. She is married to Thomas L. Patterson, Professor of Psychiatry at UC San Diego, where they co-direct a research and training program on the Mexico-U.S. border.

Dr. Strathdee is credited with saving her husband's life from a deadly superbug infection using bacteriophage therapy. The case, which involved cooperation from three universities, the U.S. Navy, and researchers across the globe, shows how phage therapy is a future weapon against multidrug-resistant bacterial infections, which are expected to kill 10 million people per year by 2050.



Tom Patterson, PhD

Tom Patterson, PhD, is distinguished professor of Psychiatry at the University of California San Diego. Dr. Patterson's HIV research has focused primarily on counseling interventions for high-risk individuals to increase condom use and reduce HIV transmission risk. He has published over 500 peer reviewed journal articles and numerous book chapters.

Together, Drs. Strathdee and Patterson are working on a book which chronicles how phages were used to save Dr. Patterson's life and why they have been largely ignored by clinicians. Their presentation will focus on this experience in a talk titled "Turning the Phage on Antibiotics."

Robert "Chip" Schooley, MD, professor of medicine and chief of the Division of Infectious Diseases in the UC San Diego School of Medicine.

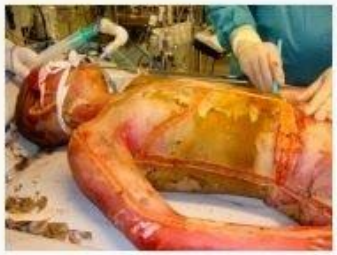

Phage administration
▪ Phage treatment by : Oral, topical,
intraperitoneal, intravenous and intrana
ending on site infection.
travenous treatment was
faster than the intramammary one.
(Chilamban et al., 2004)
Phage concentration (MOI)
varied from 0.01 to 100
e and moment of treatment
ation of phage was most useful when
tment was
▪If treated early, m
than a single dose.
(Huff et al., 2003 and 2004)
Specificity
Phages must be lytic and able to infect the
Use
ncrease
the spectrum of phage activity.
(O'Flaherty et al., 2005a)
Effectiveness of phage treatment
Environment conditions
Example : The proliferation of several phages is limited when pH is lower than 4.5
Resistance to phage
Bacteria may become resistant to phage
Isolate the new phages
(Pirnay et al. 2012)
Accessibility to target bacteria
Pathogens develop in tissue or organ
matrices (Guenther et al., 2009)
Immune factors in raw milk could protect bacteria from phages (O'Flaherty et al., 2005b)
Neutralization
Phages may be neutralized by antibodies or other compounds (Sulakvelidze, et al., 2001)
more resistant or protection of phages by encapsulation
Hangi konsantrasyonda?
Nasıl?
Ne zaman?
Özgüllük?
Hangi koşullarda?
Direnç sorunu
Bakteriye ulaşım?
Nötralizasyon sorunu

Phagoburn Projesi

PhagoBurn

SEVENTH FRAMEWORK PROGRAMME

- Project initiated on June 1st 2013
- Grant of 3.85 M€. Budget: 4.9 M€
- Treatment of infected areas:
  - in the burn wound
  - in the donor skin site
  - on the grafted skin



- Two products
  - PP0121 against *Escherichia coli* infections (inc. ESBL strains): 15 phages
  - PP1131 against *Pseudomonas aeruginosa*: 13 phages
- FP7 partners and project participants

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CENTRE HOSPITALIER UNIVERSITAIRE DE NANTES

France Europe Innovation

Management

5 partners

12 participants

Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial

Patrick Jault, Thomas Leclerc, Serge Jennes, Jean Paul Pirnay, Yok-Ai Que, Gregory Resch, Anne Françoise Rousseau, François Ravat, Hervé Carsin, Ronan Le Floch, Jean Vivien Schaal, Charles Soler, Cindy Fevre, Isabelle Arnaud, Laurent Bretaudeau, Jérôme Gabard

Summary

Background Wound infections are the main cause of sepsis in patients with burns and increase burn-related morbidity and mortality. Bacteriophages, natural bacterial viruses, are being considered as an alternative therapy to treat infections caused by multidrug-resistant bacteria. We aimed to compare the efficacy and tolerability of a cocktail of lytic anti-*Pseudomonas aeruginosa* bacteriophages with standard of care for patients with burns.

Lancet Infect Dis 2018
Published Online
October 3, 2018
http://dx.doi.org/10.1016/S1473-3099(18)30482-1

Findings Between July 22, 2015, and Jan 2, 2017, across two recruitment periods spanning 13 months, 27 patients were recruited and randomly assigned to receive phage therapy (n=13) or standard of care (n=14). One patient in the standard of care group was not exposed to treatment, giving a safety population of 26 patients (PP1131 n=13, standard of care n=13), and one patient in the PP1131 group did not have an infection at day 0, giving an efficacy population of 25 patients (PP1131 n=12, standard of care n=13). The trial was stopped on Jan 2, 2017, because of the insufficient efficacy of PP1131. The primary endpoint was reached in a median of 144 h (95% CI 48–not reached) in the PP1131 group versus a median of 47 h (25–122) in the standard of care group (hazard ratio 0·29, 95% CI 0·10–0·79; p=0·018). In the PP1131 group, six (50%) of 12 analysable participants had a maximal bacterial burden versus two (15%) of 13 in the standard of care group. PP1131 titre decreased after manufacturing and participants were given a lower concentration of phages than expected (1×10^2 PFU/mL per daily dose). In the PP1131 group, three (23%) of 13 analysable participants had adverse events versus seven (54%) of 13 in the standard of care group. One participant in each group died after follow-up and the deaths were determined to not be related to treatment. The ancillary study showed that the bacteria isolated from patients with failed PP1131 treatment were resistant to low phage doses.

Interpretation At very low concentrations, PP1131 decreased bacterial burden in burn wounds at a slower pace than standard of care. Further studies using increased phage concentrations and phagograms in a larger sample of participants are warranted.

Funding European Commission: Framework Programme 7.

Open Forum Infectious Diseases

BRIEF REPORT

Phage Therapy for a Multidrug-Resistant *Acinetobacter baumannii* Craniectomy Site Infection

Stephanie LaVergne,[1] Theron Hamilton,[2] Biswajit Biswas,[2] M. Kumaraswamy,[1] R. T. Schooley,[1] and Darcy Wooten[1]

[1]Department of Medicine, Division of Infectious Diseases, University of California, San Diego, La Jolla; [2]Department of Genomics and Bioinformatics, Naval Medical Research Center-Frederick, Fort Detrick, Maryland

In the era of antibiotic resistance, alternative treatment options for multidrug-resistant bacterial infections are being explored. We present a case of multidrug-resistant *Acinetobacter baumannii* infection treated with bacteriophages. Clinical trials are needed to further investigate bacteriophage therapy as an option to treat multidrug-resistant bacterial infections.

Keywords. bacteriophage; multidrug resistance; phage.

Antibiotics have revolutionized treatment for infectious diseases, prolonged life, and may improve quality of life; however, with the globally increasing prevalence of antibiotic resistance, we continue to observe the limitations of antibiotics. At least two million people become infected with bacteria that are resistant to some antibiotics each year in the United States, and at least 23,000 patients die because of their infection [1]. Multidrug-resistant (MDR) bacteria are a result of overuse of antibiotics in the medical setting, availability of antibiotics without prescriptions in some countries, and mass administration of antibiotics to livestock creating selective pressure [2]. Antibiotic production has not matched the rates of antibiotic resistance, and, therefore, the medical community has turned towards alternative treatment for MDR infections. Lytic bacteriophage therapy may be an opportunity to combat the rapidly growing number of MDR bacteria.

Bacteriophages are viruses that are abundant in the environment, and they have been studied for the treatment of bacterial infections for approximately 100 years. They invade and kill target bacteria by lysis and do not attack mammalian cells. Phages are specific to different bacteria, and they bind to receptors on bacterial cell walls to inject deoxyribonucleic acid into the cell and ultimately lyse the cell in the lytic phase [3]. During the lysogenic cycle, phages integrate into their host genome or exist in the cell as plasmids, evolving to coexist with bacteria.

Bacteriophages were first discovered by Twort and d'Herelle in 1919 and used briefly in the early 1900s, but they fell out of favor in Western Europe and the United States after the development of antibiotics. Bacteriophage research and use continued in Eastern Europe, predominately in Russia, Georgia, and Poland; however, no randomized controlled trials were conducted [4].

The US Army studied phages in the 1940s in animal models, which showed promise for the treatment of Gram-negative infections with *Shigella dysenteriae* [5]. Little phage research ensued until animal models re-emerged in the 1980s. A randomized control trial (RCT) with topical phage treatment of venous leg ulcers in 2009 showed that this therapy was not associated with any adverse events [6]. Wright et al [7] performed an RCT to evaluate the efficacy and safety of bacteriophages in patients who had chronic otitis externa infections with antibiotic-resistant *Pseudomonas aeruginosa*. Patients who received phage therapy had improved symptoms and lower colony counts of *P aeruginosa* from external ear culture. More recently, phages were used to treat a patient suffering from necrotizing pancreatitis and MDR *Acinetobacter baumannii* pancreatic pseudocyst infection. Two 4-phage cocktails were administered intravenously and into 3 intra-abdominal drains, resulting in cure of the infection and complete clinical recovery [8].

In this report, we describe a patient with MDR *A baumannii* infection who was treated with bacteriophages. The man was a previously healthy 77-year-old, who suffered assault, subdural hematoma, and traumatic brain injury. He underwent craniectomy complicated by postoperative infection with cerebritis, subdural and epidural empyema, requiring debridement. A subdural drain was left in place. Intraoperative cultures grew MDR *A baumannii*. His isolate was resistant to all antibiotics; however, some isolates were sensitive to colistin. Susceptibility testing included amikacin (minimum inhibitory concentration [MIC] >32 mcg/mL), ampicillin/sulbactam (MIC >16/8 mcg/mL), cefepime (MIC >16 mcg/mL), ceftazidime (MIC = 16 mcg/mL), ciprofloxacin (MIC > 2 mcg/mL), colistin ([COL] MIC = mcg/mL), doripenem (MIC > 32 mcg/mL), gentamicin (MIC = 8 mcg/mL), imipenem (>32 mcg/mL), levofloxacin (>4 mcg/mL), meropenem (MIC >8 mcg/mL), minocycline (MIC = 16 mcg/mL), tetracycline (>8 mcg/mL), tigecycline (4 mcg/mL), tobramycin (>8 mcg/mL), and trimethoprim/sulfamethoxazole (>2/38 mcg/mL). Broth microdilution antimicrobial susceptibility testing and checkerboard assays were performed in cation-adjusted Mueller-Hinton

Received 10 January 2018; editorial decision 12 March 2018; accepted 16 March 2018.
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Open Forum Infectious Diseases®

DOI: 10.1093/ofid/ofy064

The Journal of Infectious Diseases

MAJOR ARTICLE

Reviving Phage Therapy for the Treatment of Cholera

Sudhakar Bhandare,[1,a] Joan Colom,[1,a] Abiyad Baig,[1] Jenny M. Ritchie,[2] Habib Bukhari,[3] Muhammad A. Shah,[3] Banwarilal L. Sarkar,[4] Jingliang Su,[5] Brendan Wren,[6] Paul Barrow,[1] and Robert J. Atterbury[1]

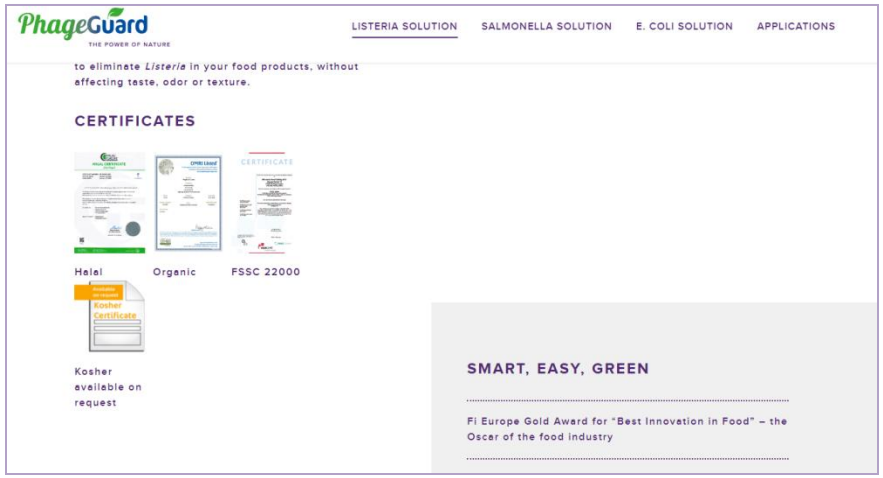
[1]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Leicestershire, [2]Department of Microbial Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, and [6]London School of Hygiene and Tropical Medicine, London, United Kingdom; [3]Department of Biosciences, COMSATS Institute of Information Technology, Chak Shahzad campus, Islamabad, Pakistan; [4]National Institute of Cholera & Enteric Diseases, WHO Collaborating Centre for Diarrhoeal Diseases Research & Training, Kolkata, India; [5]Key Laboratory of Animal Epidemiology and Zoonosis of the Ministry of Agriculture, College of Veterinary Medicine, China Agricultural University, Beijing

Cholera remains a major risk in developing countries, particularly after natural or man-made disasters. *Vibrio cholerae* El Tor is the most important cause of these d ics, so alternative therapies are urgently needed. In this study, a single ba ally and therapeutically in an infant rabbit model. In both cases, phage-t l with 69% of untreated control animals. Bacterial counts in the intestines ny-forming units/g. There was evidence of phage multiplication only in a ant bacterial mutants were isolated from the animals, despite extensive se be effective in the treatment of cholera, without detectable levels of resis d.

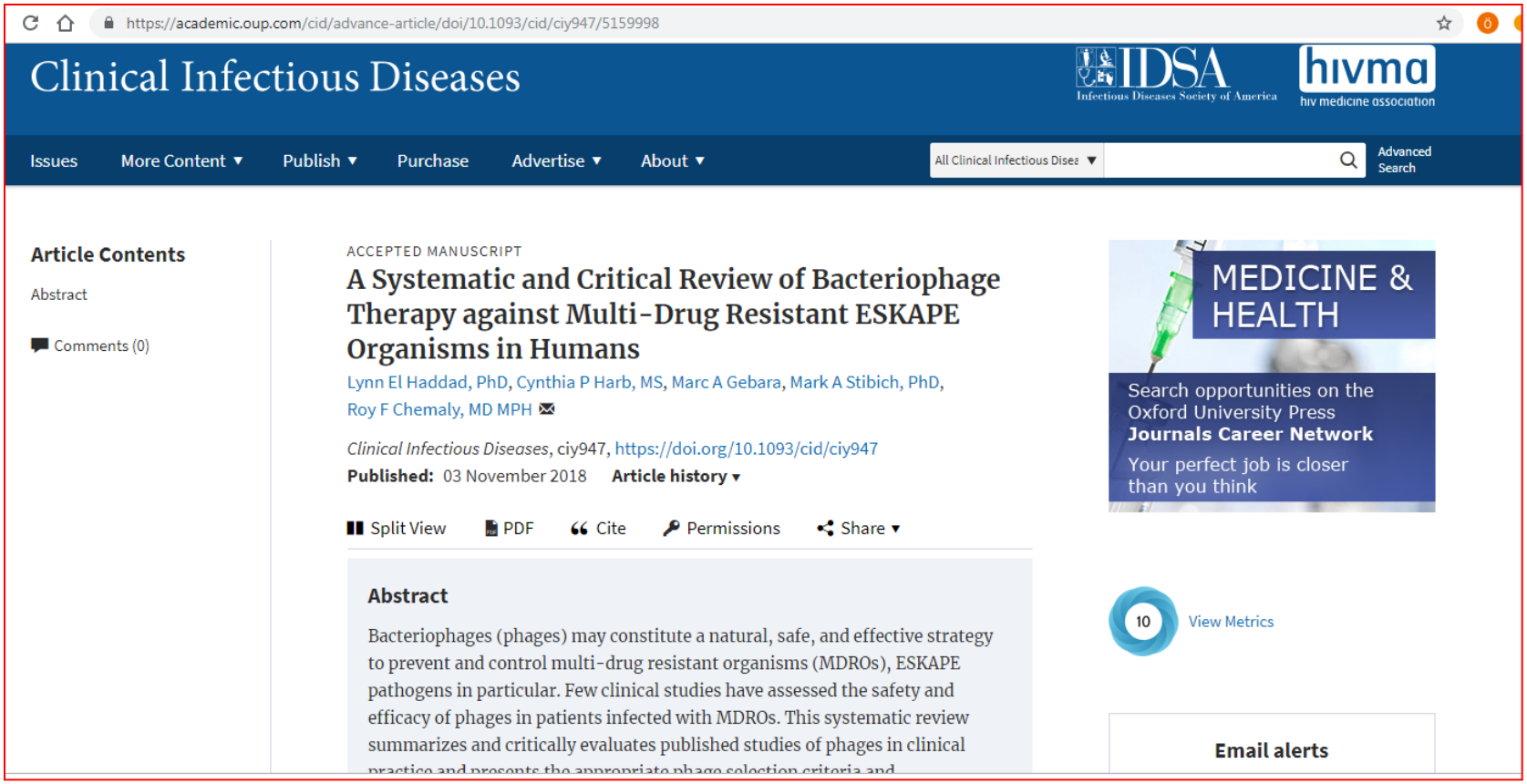
Keywords. bacteriophage t olera.

Characterization of the interaction of Phi_1 and its receptor(s) may provide some clues as to why phage-resistant mutants were not recovered. Prophylactic and therapeutic trials with Phi_1 need to be performed in human volunteers to determine if this treatment is viable. Should this prove successful, bacteriophage therapy could be deployed relatively easily to remote and underserved communities in developing countries owing to the ease and speed with which phage can be prepared, using basic laboratory equipment. Alternatively, preparations of phage can be made using lyophilization, spray drying, emulsification, and microencapsulation, which remain stable for years (recently reviewed in [31]). Phage therapy has significant potential to save hundreds or thousands of lives during outbreaks of cholera that follow natural and man-made disasters, an aim strongly worth pursuing.

ABD’de FDA onaylı Listeria fajı mevcut



ÇİD bakteriler ve fajlar konusunda sistematik derleme



A Systematic and Critical Review of Bacteriophage Therapy against Multi-Drug Resistant ESKAPE Organisms in Humans

Lynn El Haddad, PhD,[1] Cynthia P. Harb, MS,[1] Marc A. Gebara,[1] Mark A. Stibich, PhD,[1,2] and Roy F. Chemaly, MD, MPH[1]

[1]Department of Infectious Diseases, Infection Control, and Employee Health, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

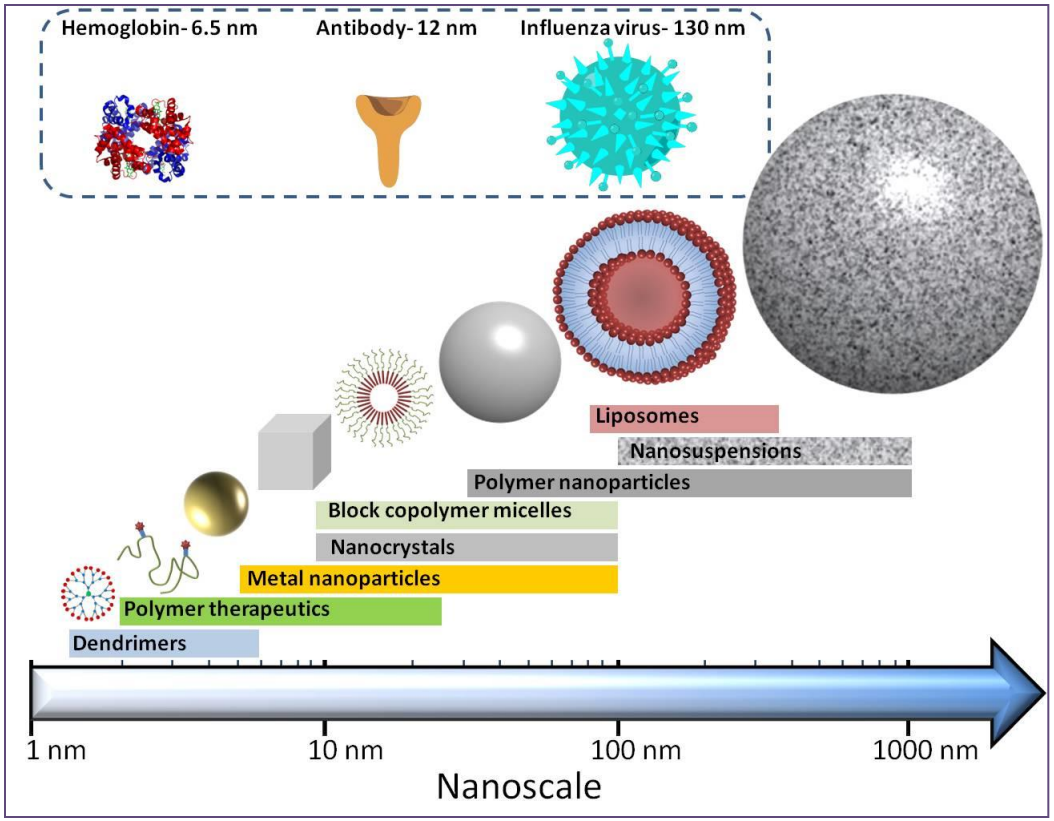
[2]Xenex Disinfection Services, San Antonio, TX, USA

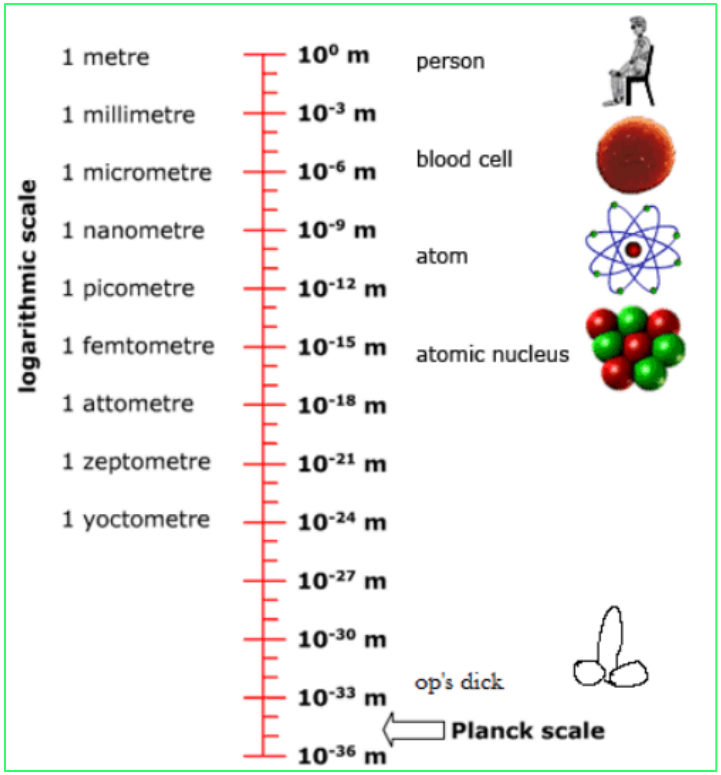
Running title: Phage therapy against MDROs

Summary: Phage therapy in clinical practice seems to be safe and effective for treatment of infections caused by resistant pathogens. Expanding phage libraries and enhancing communication between laboratories and regulatory authorities should be pursued to advance this specific biopharmaceutical strategy.

Nanoteknoloji

Nanoteknoloji…





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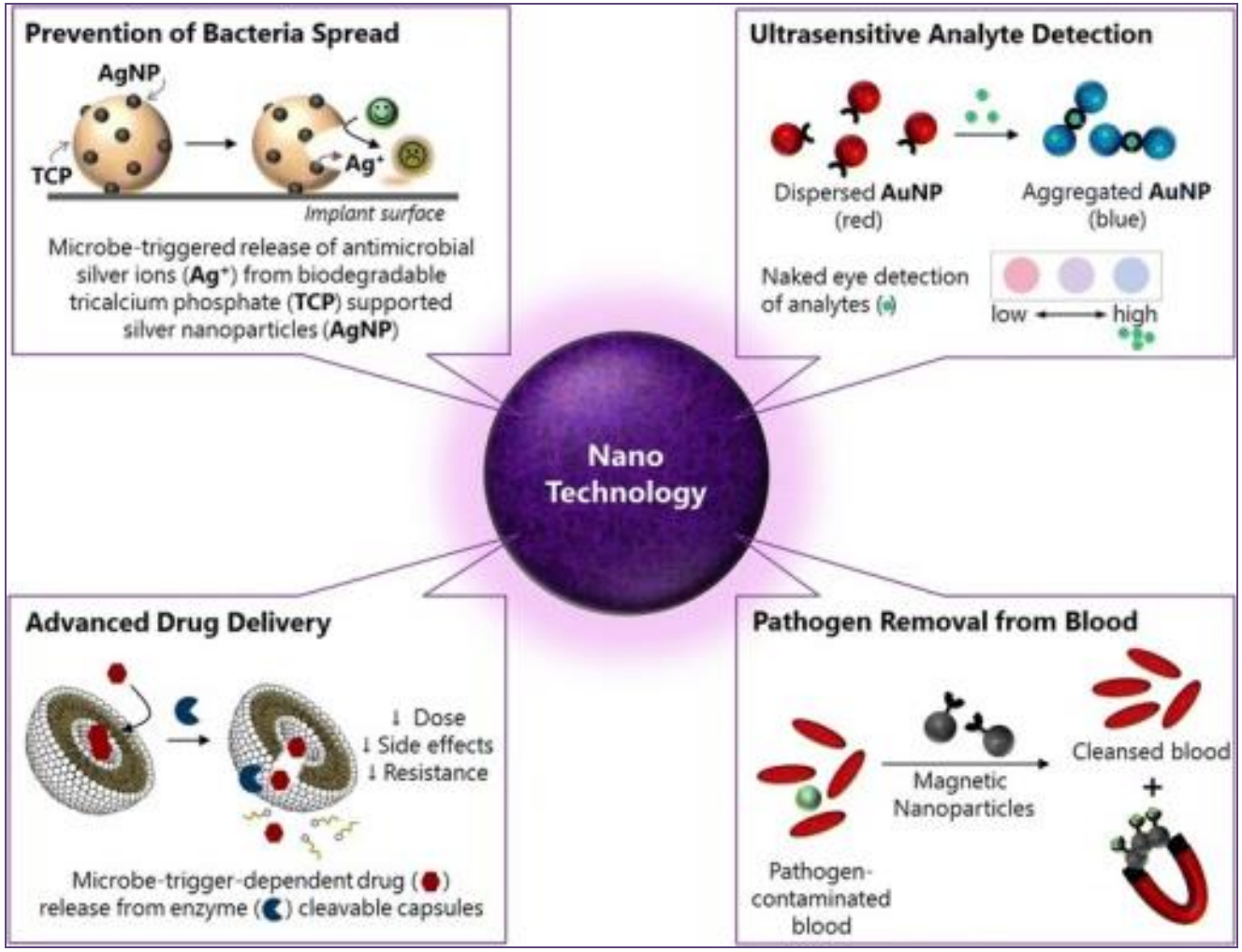


BASIC SCIENCE

Nanomedicine: Nanotechnology, Biology, and Medicine
13 (2017) 2281–2301

Review Article

nanomedjournal.com

Nano-therapeutics: A revolution in infection control in post antibiotic era

Sahar Zaidi, MSc, Lama Misba, MSc, Asad U Khan, PhD*

Medical Microbiology and Molecular Biology Lab., Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, India

Received 22 May 2017; accepted 20 June 2017

Antibakteriyel etki mekanizmaları:

- Oksidatif stres
- Metal iyonlarının serbestleşmesi
- Non-oksidatyif mekanizmalar

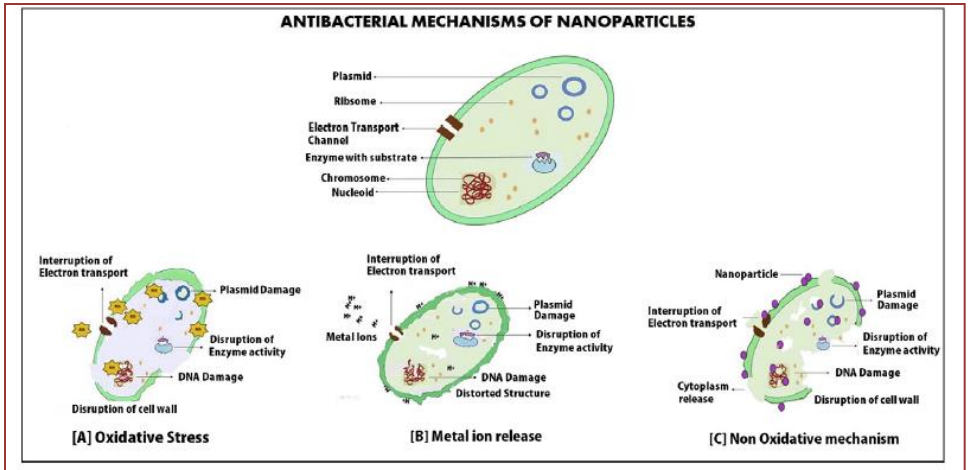
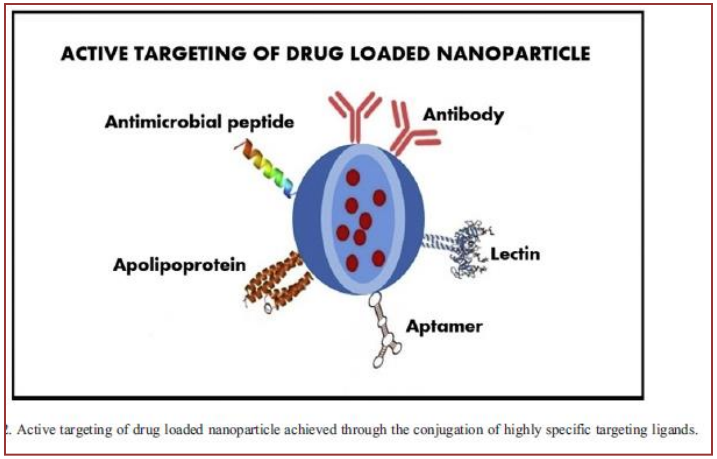


Figure 1. Schematic representation of antibacterial mechanisms of NPs: (**A**) Reactive oxygen species (ROS) mediated antibacterial activity. (**B**) Antibacterial activity due to the release of metal ions. (**C**) Non oxidative antibacterial mechanism.



2. Active targeting of drug loaded nanoparticle achieved through the conjugation of highly specific targeting ligands.

Aşılar ve Nanoteknoloji

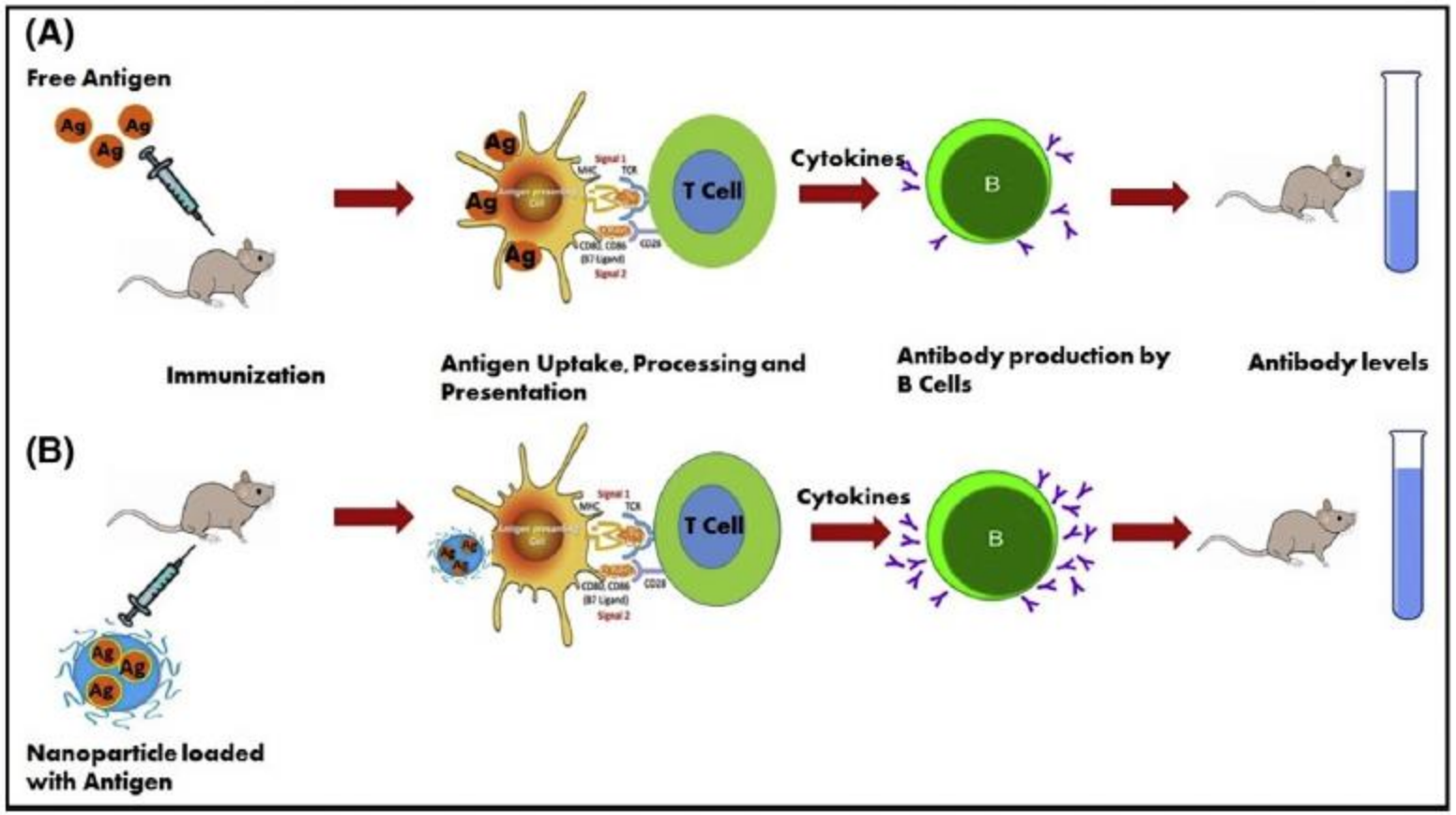


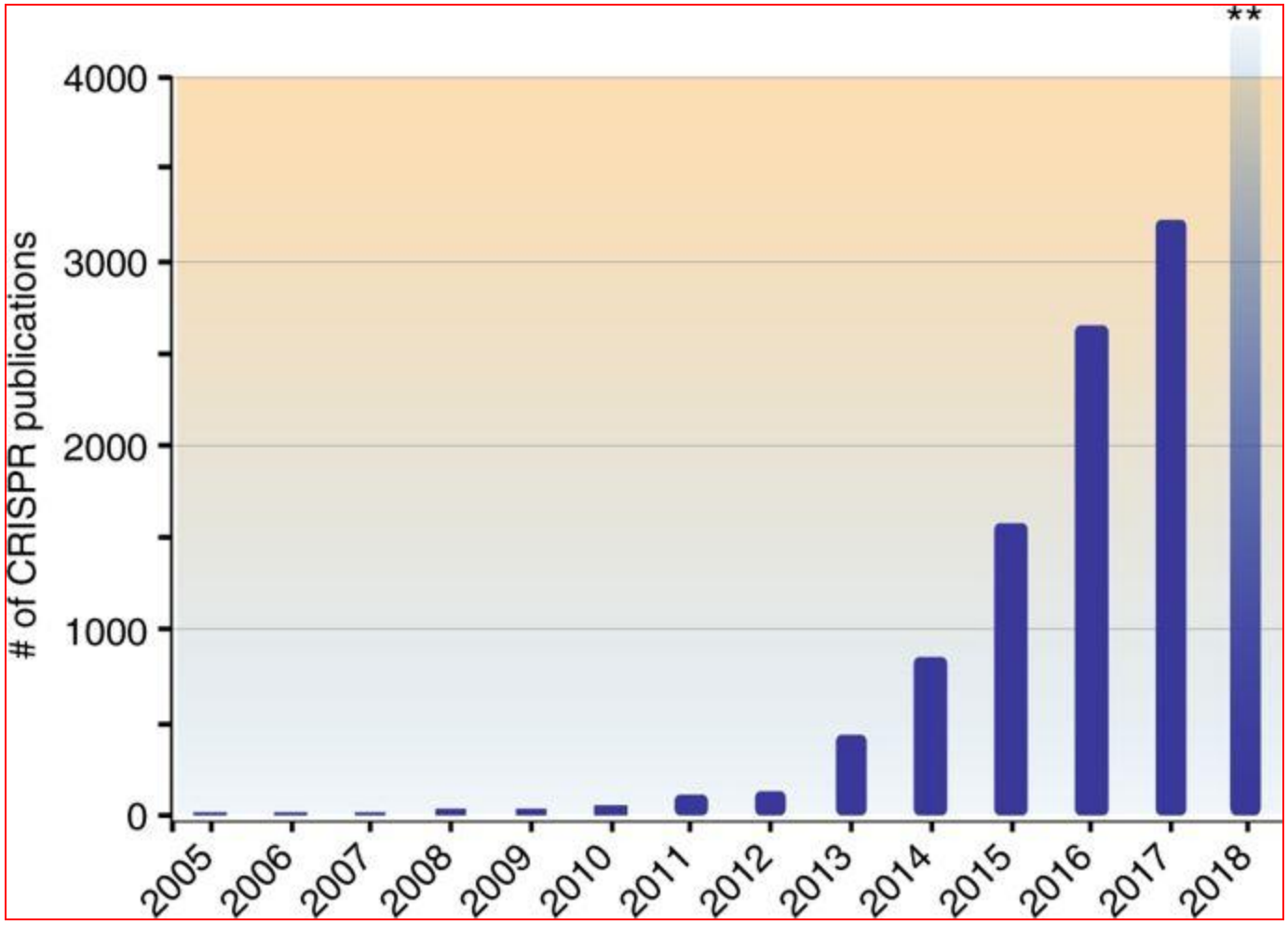
Figure 3. Nanoparticle mediated vaccine delivery: (**A**) Direct antigen (Ag) delivery and (**B**) NP encapsulated Ag, results in better Ag uptake, processing and presentation by antigen presenting cells (APCs) and therefore more antibody production.

Moleküler Cerrahi

Gen (Genom) Düzenleme Teknikleri

Gen (Genome) Editing Techniques

Yayın sayısı



NATURE COMMUNICATIONS | (2018)9:1911

CRISPR-Cas 9 Sistemi

CRISPR

Clustered Regularly Interspaced Short Palindromic Repeat

Düzenli aralıklarla bölünmüş palindromik tekrar kümeleri

Palindromik için örnek:
"ey nihat adana'da tahin ye"

Cas

CRISPR Associate protein

Bir nükleaz veya helikaz enzimi

CRISPR: Bakterilerin adaptif immün sistemi

1. DNA Invasion
Foreign DNA from a virus or plasmid invades the cell.

2. Invading DNA is Incorporated Into CRISPR Array
DNA fragments from the invading DNA are incorporated into the CRISPR locus as spacers. The exact mechanism of incorporation remains unkonwn.

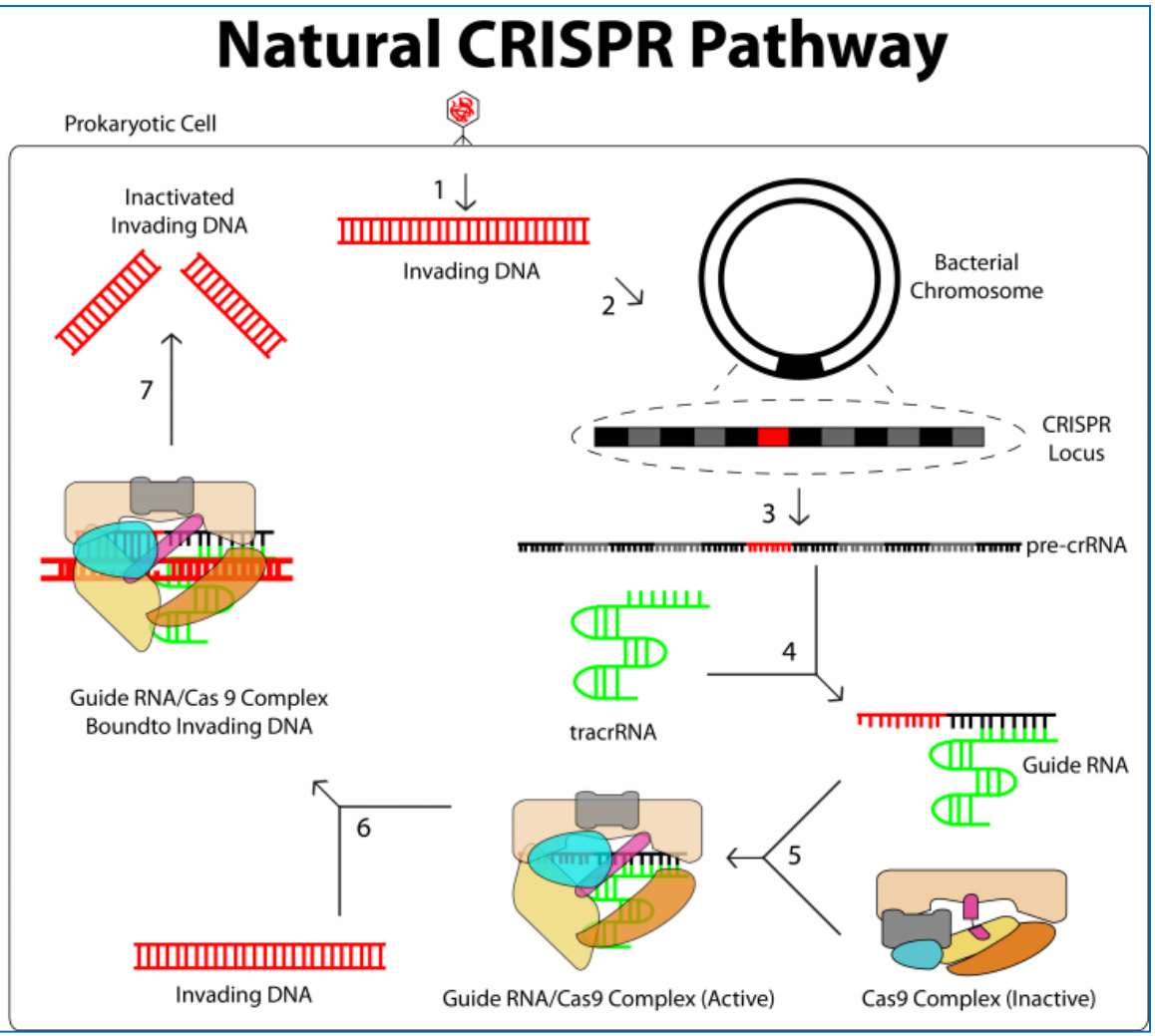
3. Pre-crRNA Transcription
The cell constitutively transcribes a repeat/spacer group into pre-crRNA. Black boxes represent repeats. Grey boxes represent spacers. The red box represents the spacer corresponding to the invading DNA.

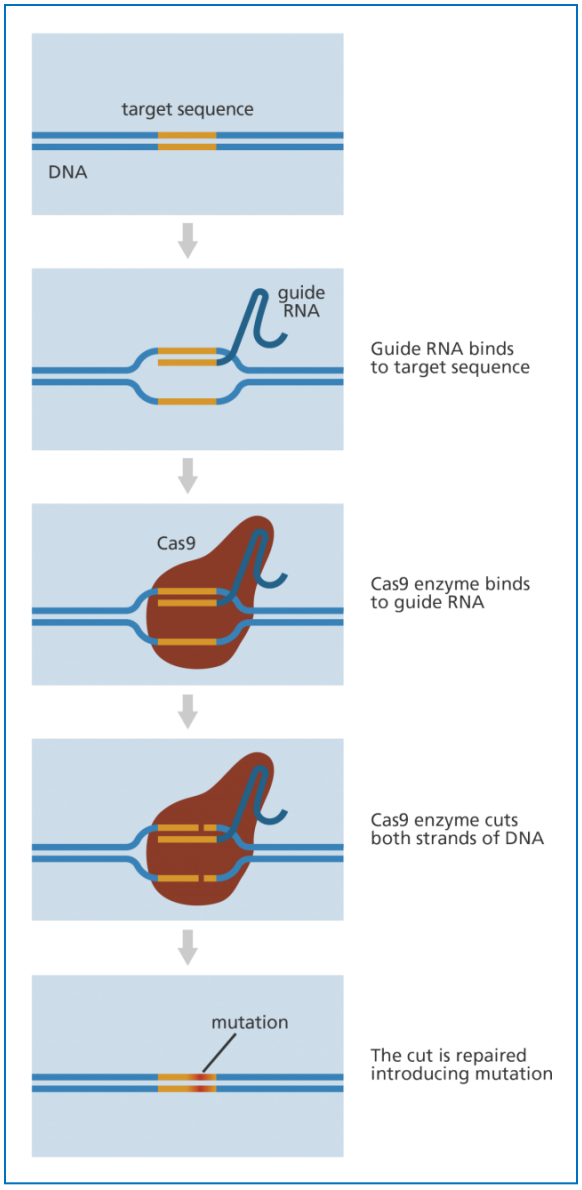
4. Guide RNA Formation
Constitutively expressed transactivating RNA (tracrRNA) base pairs with the CRISPR repeat sequences on the pre-crRNA. RNase III, Csn 1, and other unidentified CRISPR-associated pro-tiens modify the pre-crRNA/tracrRNA duplex to form a guide RNA. (Deltcheva et al. 2011)

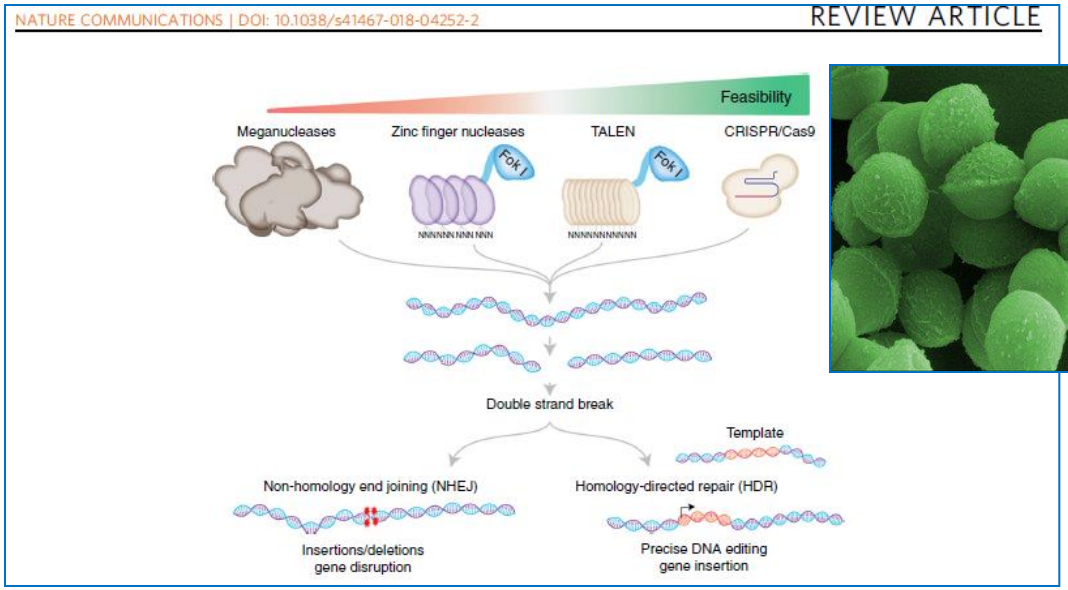
5. Cas9 Activation
Inactive Cas9 protien binds to the guide RNA and becomes activated.

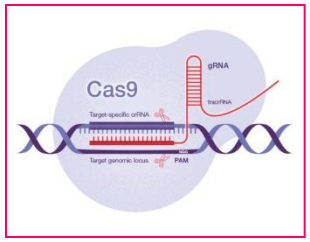
6. Target Binding
The activated guide RNA/Cas9 complex binds with the target DNA. The localization occurs stochastically (Sternberg et al. 2014).

7. Target Cleavage
The Cas9 protein cleaves the invading DNA and inactivates it.



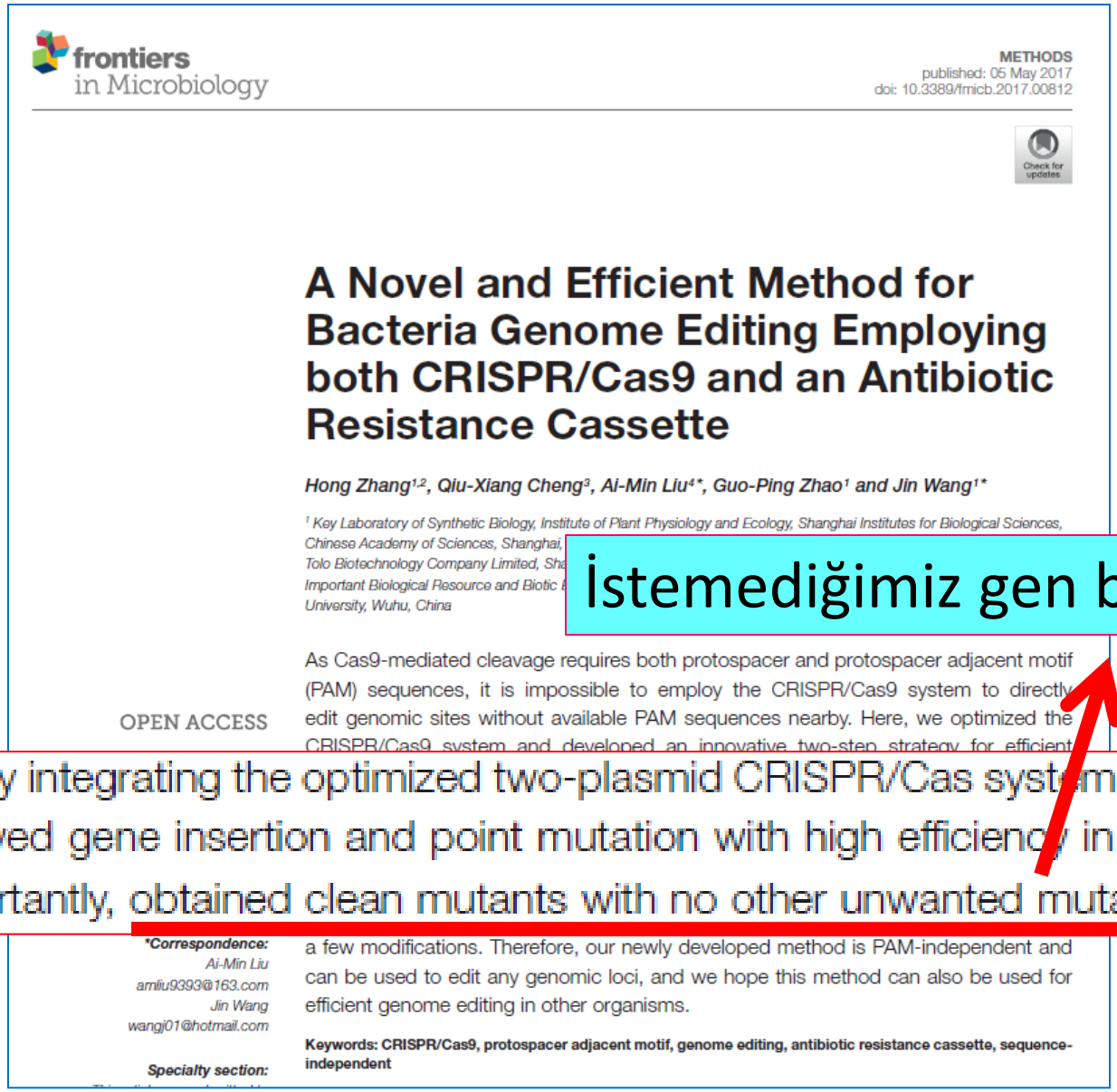






Moleküler bir makas:

Cas 9 enzimi (turuncu), RNA (kırmızı) tarafından seçilen DNA'yı (mavi) kesiyor



frontiers in Microbiology

METHODS
published: 05 May 2017
doi: 10.3389/fmicb.2017.00812

A Novel and Efficient Method for Bacteria Genome Editing Employing both CRISPR/Cas9 and an Antibiotic Resistance Cassette

Hong Zhang[1,2], Qiu-Xiang Cheng[3], Ai-Min Liu[4], Guo-Ping Zhao[1] and Jin Wang[1]**

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Tolo Biotechnology Company Limited, Sha
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University, Wuhu, China

İstemediğimiz gen bölgesi YOK!

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As Cas9-mediated cleavage requires both protospacer and protospacer adjacent motif (PAM) sequences, it is impossible to employ the CRISPR/Cas9 system to directly edit genomic sites without available PAM sequences nearby. Here, we optimized the CRISPR/Cas9 system and developed an innovative two-step strategy for efficient

marker. By integrating the optimized two-plasmid CRISPR/Cas system and donor DNA, we achieved gene insertion and point mutation with high efficiency in *Escherichia coli*, and importantly, obtained clean mutants with no other unwanted mutations. Moreover,

a few modifications. Therefore, our newly developed method is PAM-independent and can be used to edit any genomic loci, and we hope this method can also be used for efficient genome editing in other organisms.

Keywords: CRISPR/Cas9, protospacer adjacent motif, genome editing, antibiotic resistance cassette, sequence-independent

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Dr. **Jennifer Doudna** ve çalışma arkadaşı Emmanuelle Charpentier, “2015 Breakthrough Prize in Life Sciences” ödülünü Cameron Diaz ve Twitter CEO’su Dick Costolo’dan aldı. Araştırmacıların her birine 3 milyon Dolar ödül verildi.

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MARIA OUSPENSKAYA (Franziska Speyer)
MONTAGU LOVE (Prof. Hartmann)
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Written by JOHN HUSTON, HEINZ HERALD, and NORMAN BURNSIDE