Combination Therapy for Serious Infections: 
*Staphylococcus aureus*

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Limited

**CLINICAL TRIALS**
Once a disease target is identified, drugs are designed and tested. Both public and privately funded research are involved.

**REGULATORY APPROVAL**
Human trials are completed. FDA approval. Industry is responsible for bringing a drug to market. Safety and evaluation continue after approvals.

**BASIC RESEARCH**
The majority of the research at this stage is publicly funded at universities, colleges and independent research institutions in every state.

17 years Process
Questions to Consider in Combination Therapy…

• Why Talk About Combination Antibiotic Therapy to Treat Bacterial Infections?
• Do we use combination therapy to treat some infections currently?
• If so, why is it such a big deal when the concept is introduced to clinicians?
• Why are serious S. aureus infections a good example where combination antibiotic therapy should be considered?
The Beginning of the Modern (Human) Antibiotic Era

1943 - 4 year old.
There was never a comparator—does that matter?
Why 14 days?
How different were patient hosts in 1943 vs the 21st century?
How many beta-lactam resistant bacterial pathogens were clinically relevant in the 1940’s?

Herrell '43 Proc Staff Meetings Mayo Clinic 18:65-76
March 1942

Life Expectancy at Birth

1st Clinical Use of Penicillin
March 1942
Age Distribution: 1940 and 2010

<table>
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<tr>
<th>Year</th>
<th>Under 18</th>
<th>18-44</th>
<th>45-64</th>
<th>55+</th>
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<td>1940</td>
<td>30.6</td>
<td>42.8</td>
<td>19.8</td>
<td>6.8</td>
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<tr>
<td>2010</td>
<td>24.0</td>
<td>36.5</td>
<td>26.4</td>
<td>13.0</td>
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</table>

Sources: U.S. Census Bureau, 1940 Census and 2010 Census.
Consequence of Medical Progress: Age Senescence of Innate Immunity

Incidence of IE

Does the Human Innate Immune System Have an Approximate 6 Decade Shelf Life??

Sepsis  CAP  UTI
COVID-19 Mortality by Age

- Number of patients
- Fatality (%)
Combating Antimicrobial Resistance Means We Have Set Our Targets and Hit the Mark

PATIENT
- Age
- Comorbid Conditions
- Immune Status
- Site of Infection

DRUG
- Pharmacokinetics
- Pharmacodynamics

BUG
- Susceptibility by Minimum Inhibitory Concentration (MIC)

‘Dead Bugs Don’t Mutate’
Rationale for Combination Antibiotic Therapy

- Synergy: More potent and efficient killing of bacteria (1+1>2)
  - Immunocompromised hosts
  - High inoculum
  - Attack bacteria in different ‘compartments’ (eg. intracellular vs extracellular; biofilms)
  - Target different aspects of pathogenesis (eg. killing; virulence attenuation)

- Broaden spectrum of activity
  - Empiric regimen
  - Beta-lactamase inhibitors

- Reduce the likelihood of antimicrobial resistance
  - High inoculum
  - Organism-specific concerns

- Reduce the incidence of side-effects
  - Reduce dose and boost pharmacodynamics

- Reduce costs
  - Pharmacodynamic boosting of a costly single agent by a second cheaper agent
  - Shorten duration of hospitalization
Factors Determining A Successful Outcome in the Treatment of Infection

Burden of Infection (inoculum)

Host Immunity
- Age
- Immunosuppression

Pathogen
- Virulence (invasion/evasion)
- Antimicrobial Resistance
- Infecting Site

Surgical Source Control

Antibiotic Properties: MIC (S vs R)
- Bactericidal vs Bacteriostatic
- PK/PD
- Penetration into biofilms
- Activity against ‘inert’ forms
- Synergy with Innate Immunity

Duration of Therapy

(distance, speed, time)
REFRACTORY MRSA BACTEREMIA
Rapid MRSA Bacteremia Clearance with High-Dose Daptomycin plus a β-lactam


*Relapsed – 12 wks & 8 wks post-therapy – 1 cleared w/another course; 1 died w/VISA PV IE VAN MIC 3; DAP MIC 1.5
Red VISA; DAP MIC 2-4 - Additional studies performed on the isolates from this case

Daptomycin binds Ca$^{2+}$ in vivo as an integral part of its mechanism of action – i.e. it becomes a de facto cationic peptide.

Cationic antimicrobial peptides are a critical component of mammalian innate immunity.

Nizet V. Curr Issues Mol Biol 8:11-26
Potential beneficial effects of β-Lactam antibiotics not reflected in MIC

- Reduced peptidoglycan O-acetylation
- Reduced peptidoglycan crosslinking
- Decreased shedding of membrane phospholipids
- Decreased expression of MSCRAMMs (e.g., CIA, FnBPA)
- Increased α-toxin release
- Increased lipoteichoic acid shedding

- Increased bacterial susceptibility to lysozyme killing
- Increased cell membrane access
- Reduced invasion of epithelial and endothelial cells
- Activation of the NLRP3 inflammasome
- Increased host macrophage cytokine release
- Increased IL-1β response
- Improved neutrophil recruitment
- Reduced neutrophil apoptosis

Potentiation of cationic host defense peptides
Potentiation of peptide antibiotics (e.g., daptomycin)
Increased TH17 response
Increased neutrophil antibacterial functions
Enhanced clearance of S. aureus bacteremia

CAMERA-2 Study: A Rigorous Look At Combination Therapy for MRSA

- MRSA Bacteremia
- August 2015 - July 2018, 352 adults, 27 sites, 4 countries (Australia, New Zealand, Singapore, and Israel)
- Monotherapy Vancomycin or Daptomycin VS plus an Combination with anti-staphylococcal β-lactam (flucloxacillin, cloxacillin, or cefazolin)
- Mention of daptomycin is highly deceiving
- Vast Majority of combination received vancomycin + flucloxacillin
  - 171/174 (98%) combination group
  - 178/178 (100%) monotherapy group
- Stopped Early Due to Safety Concerns

Tong et al. JAMA Feb 2020
CAMERA-2 Study

- 30% developed acute kidney injury vs 9% in the standard therapy arm
- Increased 90-day mortality in the combination arm vs the standard therapy arm (21% vs 16%)
- Combination arm had less bacteremia persistence, consistent with CAMERA-1
- NOT REALLY THE END OF THE COMBINATION THERAPY DISCUSSION

Tong et al. JAMA Feb 2020
Putting the CAMERA Under the Microscope
Mortality in MRSA Bacteremia: Where Did CAMERA-2 Fall?

Tong et al. 2020 Feb 11;323(6):527-537
CAMERA 2 Did Not Have the Study Population to Answer the Combination Therapy Question

Tong et al. 2020 Feb 11;323(6):527-537
The Real Take Home Message of CAMERA-2
AKI With Vancomycin and Beta-Lactams

![Bar chart comparing Flucloxacillin, Cloxacillin, and Cefazolin](chart.png)

- **Flucloxacillin:** 25/90
- **Cloxacillin:** 5/21
- **Cefazolin:** 1/27

**Statistical Analysis:**

- **P=0.008** Cefazolin vs Flucloxacillin + Cloxacin
- **P=0.007** Cefazolin vs Flucloxacillin
- **P= 0.07** Cefazolin vs Cloxacillin

Tong et al. 2020 Feb 11;323(6):527-537
153 Monotherapy, 444 Combination Therapy (Mostly with cephalosporins)
14% Nephrotoxicity In Both Groups (15% Pip/tazo, 3% Amp/Sulbactam)
Multivariate logistic regression model predictive of clinical failure
- Endocarditis 3.294 (2.115-5.132)
- APACHE II Score 1.045 (1.029-1.072)
- Combination Therapy 0.545 (0.364-0.817)
Multivariate logistic regression model predictive of persistent bacteremia
- Endocarditis 3.331 (2.132-5.205)
- Source Control 1.236 (0.828-1.844)
- Combination Therapy 0.597 (0.393-0.907)
Comparison of Clinical Outcomes Between Patients in Pre-pathway or Post-pathway

Proximal Tubular Cells

BLOOD

TUBULAR LUMEN

Nephrotoxic

Cephaloridine

BLOOD

TUBULAR LUMEN

Cephalothin, Cefaclor

BLOOD

TUBULAR LUMEN

Cephaloglycin, Cephalexin

BLOOD

TUBULAR LUMEN

Ceftazidime (minimal)

BLOOD

• OAT-3 is important in renal and CSF beta-lactam transport

• Used cells expressing mouse and human OAT-3 to assess interactions with 26 beta-lactams
  • 12 inhibitors
  • 14 poorly interactive

• The beta—lactam that were inhibitors were also substrates

• OAT-3 interactive antibiotics were lipophilic (log partition coefficient +1.41 inhibitors vs -1.54 for non-inhibitors)

• Beta-lactams that interact with OAT-3 correlate with nephrotoxicity
  • Piperacillin 0.737
  • Ampicillin -0.873
In 1900 Ernst Overton found entry of dyes through cell membranes of living cells predicted lipophilicity. This predicts beta-lactam CSF penetration (and likely proximal tubule accumulation and vanco-associated nephrotoxicity).
Vancomycin induces reactive oxygen species-dependent apoptosis via mitochondrial cardiolipin peroxidation in renal tubular epithelial cells

Yuya Sakamoto\textsuperscript{a,b,1}, Takahisa Yano\textsuperscript{c, 1}, Yuki Hanada\textsuperscript{a}, Aki Takeshita\textsuperscript{a}, Fumika Inagaki\textsuperscript{a}, Satohiro Masuda\textsuperscript{b,c}, Naoya Matsunaga\textsuperscript{d}, Satoru Koyanagi\textsuperscript{d}, Shigehiro Ohdo\textsuperscript{a}

**Concentration and Time-Dependent Vancomycin Cytotoxicity**

![Graph showing cell viability over different concentrations of vancomycin](image1)

**Vancomycin Mitochondrial Cardiolipin Peroxidation**

![Graph showing time-dependent peroxidation](image2)

Porcine Proximal Tubular Epithelial Cell line LLC-PK

http://dx.doi.org/10.1016/j.ejphar.2017.02.025
Using Ceftaroline as the Beta-Lactam in Daptomycin Combination Therapy in MRSA Bacteremia

Case Series Salvage Regimen
Antimicrobial Stewardship
Pharmacist Emoji
Prospective Randomized Study of Vancomycin vs DAP+CPT in MRSA Bacteremia

Expensive
  Limitations on Disposition
  Third Party Payors
  Subacute Nursing Facilities

Cumbersome
  Many Doses

More drugs = more risk of medical errors and AE’s

CAN DAP+CPT BE TARGETTED TO HIGH-RISK PATIENTS?

HOW TO IDENTIFY HIGH RISK PATIENTS UP FRONT?
Cytokines Predicting Mortality in SaB

• Multi-omic analysis of *S. aureus* bacteremia captured strong mortality biomarkers

• Clustering of proteins and metabolites highlights host metabolic dysfunction

• Therapeutic interventions of host metabolism can influence survival

Induction->Consolidation Paradigm

• MRSA Bacteremia is a dynamic condition, so therapy must be dynamic
• Combination therapy at the beginning to de-escalation to monotherapy
• One small study of 30 patients rx with combo: 15 de-escalated to monotherapy, 15 remained on combination
  • Median bacteremia duration preceding CPT 6 days
  • 1 recurrence in monotherapy; 2 30-day readmission monotherapy; 1 death monotherapy, 3 deaths combo
  • Ahmad O et al. Infect Dis Ther. 2020 Mar;9(1):77-87
• Factors to consider:
  • Bridge to definitive source control (eg. left-sided IE with CNS emboli)
  • Bacteremia cleared 5-7 days
  • Time to discharge and disposition (home vs SNF vs LTAC)
  • CRP reduced >50%
Beta-Lactams in MRSA

• Important adjuncts to daptomycin backbone
• Some are using vancomycin backbone with improved outcomes
• Vancomycin + cephalosporin > Vancomycin + Penicillin due to nephrotoxicity
• How do anti-staphylococcal beta-lactams compare to ceftaroline when added to daptomycin?

• COMBINATION THERAPY IS NOT REQUIRED FOR THE WHOLE DURATION
  • De-escalate to vancomycin (eg LTAC/SNF)
  • Single therapy
  • CRP reduction >50%
Daptomycin+Fosfomycin

• Randomized 1:1 open label 18 medical centers in Spain
• Daptomycin 10 mg/kg IV QD+ Fosfomycin 2g IV Qhr vs Daptomycin 10 mg/kg alone
• Treatment success day 7, day 42
• 6 weeks: Combo 40/74 (54%) vs Mono 34/81 (42%)
• 7 days: Combo 69/74 (93%) vs Mono 62/81 (77%)
• Combo lower rate of microbiological failure at 6 weeks: 0 vs 9 patients (p=0.009)
• 578 patients with high-risk SaB, 313 (54%) received combination therapy
• Evaluated composite outcome of 90-day all-cause mortality plus rates of late complications at 180 days
• 242 received combination with rifamycin; 58 with IV fosfomycin.
• Combination therapy better composite outcome (hazard ratio, 0.65; 95% confidence interval, 0.46–0.92).
• Primary benefit was seen in patients with biomedical devices (HR, 0.53; 95% CI, 0.35–0.79).
• Fosfomycin and rifampicin had similar outcome benefits
REFRACTORY MSSA BACTEREMIA
• 32 yo IVDA but otherwise healthy admitted with worsening fevers x 1 week
• Accompanied by SOB, lethargy
• Found to have leukocytosis, tachycardia, hypotension, admitted to ICU
• Vancomycin+ceftriaxone for 1st 24 hrs
• Blood Cx GPC->ID called->Ceftaroline 600 mg iv q8 hr+ Dapto 8 mg/kg/24 hr x 24 hr
• Verigene Show MSSA in 24 hrs->Nafcillin 2g iv 4 hr
• Imaging Chest, Abdomen, Pelvis: Multifocal pneumonia suggestive of septic pulmonary emboli, some early cavitation
• TEE shows 3.2 cm tricuspid valve vegetation
• Blood Cultures Remain + Despite 5 days nafcillin
• Ertapenem 1g iv q24 hr + cefazolin 2g iv q8hr →Blood cultures clear in 24 hrs!!
• Angiovac was performed→Partial success in debulking the Tricuspid Valve
• Signed out AMA after 4 weeks in the hospital
Consider DAP + Beta-lactam But May Be Antagonistic in MSSA

Carbapenem

Cefazolin
MSSA RAT ENDOCARDITIS MODEL

FUTURE THERAPY COMBINATIONS

- Not necessarily with two antibiotics
- Host response therapies
- Virulence Neutralization
- Repurposing Drugs Used by Other Specialists
Exebacase for patients with Staphylococcus aureus bloodstream infection and endocarditis

Vance G. Fowler Jr., ... , Pamela S. Douglas, Cara Cassino


Graphical abstract
Repurposed Drugs Block Toxin-Driven Platelet Clearance by the Hepatic Ashwell-Morell Receptor to Clear *Staphylococcus aureus* Bacteremia

Josh Sun$^{1,2,3*}$, Satoshi Uchiyama$^4$, Joshua Olson$^1$, Yosuke Morodomi$^6$, Ingrid Cornax$^1$, Nao Ando$^1$, Yohei Kohno$^1$, May M. T. Kyaw$^1$, Bernice Aguilar$^1$, Nina M. Haste$^{1,2,3}$, Sachiko Kanaji$^4$, Taisuke Kanaji$^4$, Warren E. Rose$^3$, George Sakoulas$^2$, Jamey D. Marth$^{6,7}$, Victor Nizet$^{1,2,3*}$

SA α-toxin-mediated platelet clearance compromises host defense

Ticagrelor and oseltamivir preserve platelet counts and antibacterial function
A CASE OF MSSA BACTEREMIA AND PLATELETS

Platelets (×1000/mm³)

Day

Ticagrelor Therapy
MSSA Bacteremia

Platelet Killing of *S. aureus*

**Figure 1.** Ticagrelor (TICA) boosts the bactericidal activity of platelets, frontline components of innate immunity, to kill methicillin-susceptible *S. aureus in vitro* at physiological attainable concentrations (1 μM).
Summary and Conclusions

- Clearly in some more difficult cases of S. aureus bacteremia, combination therapy is better than monotherapy (membrane+cell wall agent; complementary beta-lactams)
- As in most cases of optimal therapy, benefit declines with delays in use
- Unfortunately, days go by until difficult cases define themselves during which the patient’s clinical status and prognosis may decline
- An important goal of future studies will be the a priori identification of challenging cases before they become challenging
- Concept of ‘Induction’ and ‘Consolidation’ Phases of Treatment for SaB
  - Beginning/Middle/'Mop up’ Regimens
- Future therapies will need to consider host-virulence factor attenuation
- Lysin Therapy
- Phage Therapy
Questions to Consider…Answers

• Why Talk About Combination Antibiotic Therapy to Treat Bacterial Infections?
  • Some infections are treated more successfully when using multiple antibiotics (high inoculum size, host level of immunosuppression)
  • Monotherapy Infection Treatment Paradigms Were Established At A Time With More Immunologically Robust Hosts

• Do we use combination therapy to treat some infections currently?
  • Routine in malaria, HIV, Tuberculosis, Early Fungal Meningitis

• If so, why is it such a big deal when the concept is introduced to clinicians?
  • Treatment and diagnostic paradigms that are decades old are very difficult to change
  • Identifying the subset of infections that benefit with 2 vs 1 antibiotics are difficult in broad clinical trials (granularity issue)
  • Antibiotic drug acquisition costs
  • Concern for resistance when using antibiotics

• Why are serious S. aureus infections a good example where combination antibiotic therapy should be considered?
  • High inoculum bloodstream infections do not respond well to single therapy
  • Adjunctive antibiotics may potentiate immune clearance
  • Small low-powered study was able to show a mortality benefit