Novel Monoclonal Antibodies for the Prevention and Treatment of Bacterial Infections

Steven J. Projan, Ph.D., F.A.A.M.  
Sr. Vice President R&D and Innovative Medicines Head Infectious Disease & Vaccines  
MedImmune/AstraZeneca  
January 21, 2016
Thanks to....

JPIAMR
Swedish Research Council
NIH, especially NIAID
Also I would like to acknowledge the contributions of…..

Otto Cars & John Rex
The Pioneers of Serum Therapy
Emil von Behring, Paul Ehrlich and…..who?

Nobel Prize in 1901
The 1st Prize for Physiology or Medicine

Nobel Prize in 1908
shared with I. Metchnikoff

Posthumous Academy Award in 1973
The Great Race of Mercy - 1925
20 mushers and 150 dogs covered 674 miles

Gunnar Kaasen & Balto

Leonhard Seppala & Togo

Corynebacterium diphtheriae
Antibodies have potential to be a powerful new approach, particularly in preventing infections before they happen

**Specificity**
- No perturbation of the beneficial microbiome
- Would not cause cross resistance in other bacteria

**Safety**
- Potential to be safer due to pathogen specificity
- No drug-drug interactions with small molecules

**Long half-life**
- Potential single dose protection for 1-3 months

**Antibiotic preservation**
- mAb MOA will not select for resistance to small-molecule antibiotics
- Adjunctive use could reduce the potential for resistance development
- Antibody prophylaxis could decrease prophylactic antibiotic usage

**Sometimes antibiotics aren’t enough**
- Complementary mAb MOAs with antibiotics
- Enhance host bacterial clearance
- Inhibition of colonization, cytotoxicity and virulence
- Reduce hyperinflammatory sequelae (tissue damage & sepsis)

Antimicrobial antibody discovery informs the discovery and development of effective vaccines
Serious Bacterial Infections

- **Staphylococcus aureus**
  - Anti-alpha toxin mAb for ICU pneumonia in Ph1
  - Staph combo projects in lead optimization

- **Pseudomonas aeruginosa**
  - Anti-Psl/ Anti-PcrV: bispecific 2014 IND

- **Clostridium difficile**
  - Caused by antibiotic therapy perturbation of microbiome
  - Surpassed *S. aureus* as leading cause of HAIs
  - In-licensed antitoxin A+B mAbs: CD 2014

- **And the usual suspects!**
**S. aureus** and **P. aeruginosa** Pneumonias are costly to patients and payers – **Prevention makes sense**

- **Hospital Days**
  - Control: 7.2 days
  - Staph: 37.9 days
  - Pseudomonas: 55.4 days

- **ICU Stay**
  - Control: 1.1 days
  - Staph: 6.9 days
  - Pseudomonas: 14.8 days

- **All Cause Mortality**
  - Control: 3%
  - Staph: 16%
  - Pseudomonas: 20%

- **In Patient Cost**
  - Control: $33,851
  - Staph: $146,978
  - Pseudomonas: $213,104

- **US claims database study of ~252,000 ICU patients**
- **Control patients without pneumonia: N=201,394**
There is an increasing focus in biotech and big pharma on antibodies for antibacterial therapy

**Staphylococcus aureus**
*Kenta/Aridis – *S. aureus* alpha toxin
*Alopexx/Sanofi – PNAG
*MedImmune/AstraZeneca - *S. aureus* alpha toxin
*Arsanis – multiples Staph toxins

**Pseudomonas aeruginosa**
*Kalobios/Sanofi – PcrV mAbs (Sanofi returned it though – and Kalobios ihas filed for bankruptcy)
Symphogen/Meiji – 5-10 mixed mAbs
*MedImmune/AstraZeneca – PcrV/Psl bispecific mAbs
Aridis – Alginate mAb

**Clostridium difficile**
*MassBio/Medarex/Merck – Toxin A&B mAbs

*Currently in clinical trials
New Antibacterial mAb Lead Generation Strategy

♦ Hybridoma technology
  – Murine and humanized

♦ Human derived mAb phage libraries
  – “Normal human”
  – Patient derived

♦ In licensing and collaboration
  – e.g. RSV C. difficile

♦ Human B-cell technology
  – Healthy immunized and convalescent

• Both validated target-driven and target-agnostic discovery
• Matrixed activity driven screening for protective mechanisms early in lead generation
• Multiple animal models to emphasize different aspects of pathogen infection and disease
• Fc engineering to further enhance activities and half lives
• Multi-specific, multi-functional emphasis for mAb candidates
Tonsils are a primary lymphoid tissue designed to trap antigens and are constantly stimulated by the oropharynx microbiome.

- B cells producing IgG antibodies to Gram positive and Gram negative bacteria are found at high frequencies among tonsil or adenoid B cells.
mAbs for *C. difficile* prophylaxis: a sure bet, or just a crap shoot?

Norah J. Shire, PhD, MPH
Translational Medicine
MedImmune Infectious Diseases
The Human Metagenome

- When we are born we are 100% human, when we die we are 90% microbial
- *Nature* 464, 59-65 (4 March 2010). “To understand the impact of gut microbes on human health and well-being it is crucial to assess their genetic potential. Here we describe the Illumina-based metagenomic sequencing, assembly and characterization of 3.3 million non-redundant microbial genes, derived from 576.7 gigabases of sequence, from faecal samples of 124 European individuals. The gene set, ~150 times larger than the human gene complement, contains an overwhelming majority of the prevalent (more frequent) microbial genes of the cohort and probably includes a large proportion of the prevalent human intestinal microbial genes. The genes are largely shared among individuals of the cohort. Over 99% of the genes are bacterial, indicating that the entire cohort harbours between 1,000 and 1,150 prevalent bacterial species and each individual at least 160 such species, which are also largely shared. We define and describe the minimal gut metagenome and the minimal gut bacterial genome in terms of functions present in all individuals and most bacteria, respectively.”
Clostridium difficile (C. difficile) causes life-threatening diarrhea. These infections mostly occur in people who have had both recent medical care and antibiotics. Often, C. difficile infections occur in hospitalized or recently hospitalized patients.

RESISTANCE OF CONCERN
- Although resistance to the antibiotics used to treat C. difficile infections is not yet a problem, the bacteria spreads rapidly because it is naturally resistant to many drugs used to treat other infections.
- In 2000, a stronger strain of the bacteria emerged. This strain is resistant to fluoroquinolone antibiotics, which are commonly used to treat other infections.
- This strain has spread throughout North America and Europe, infecting and killing more people wherever it spreads.

PUBLIC HEALTH THREAT
- 250,000 infections per year requiring hospitalization or affecting already hospitalized patients.
- 14,000 deaths per year.
- At least $1 billion in excess medical costs per year.
- Deaths related to C. difficile increased 400% between 2000 and 2007, in part because of a stronger bacteria strain that emerged.
- Almost half of infections occur in people younger than 65, but more than 90% of deaths occur in people 65 and older.
- About half of C. difficile infections first show symptoms in hospitalized or recently hospitalized patients, and half first show symptoms in nursing home patients or in people recently cared for in doctors’ offices and clinics.

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention
Biology and Pathogenesis

- Disruption of normal gut flora (antibiotic treatment)
- *C. difficile* dormant spore contact (hardy survivors)
- *C. difficile* colonization

- **C. difficile** bacteria secrete Toxins A and B
- Toxins inactivate Rho GTPases (glycosylation) and disrupt cell junction

- Toxin mediated damage
- Inability to re-establish normal flora
- Relapse
C. difficile hamster model: Weiss* protocol

mAb mixture (IP)
- 20 mg/kg each

oral spore challenge
clindamycin
- 10 mg/kg

day -1 0 1 3 5 8

monitor survival

*University of North Texas Health Science Center
Treatment with monoclonal antibodies against *C. difficile* toxins A and B prevents recurrence

- **Phase 2:** Treatment with anti Toxin A and B mAb (10 mg/kg each) combination reduced the rate of recurrence from 25% to 7%

- **Two phase III clinical trials ongoing**
Progenics anti-toxin A and B mAbs

Protection Against Clostridium difficile Infection With Broadly Neutralizing Antitoxin Monoclonal Antibodies

Andre J. Marozsan, a Dangshe Ma, a,b Kirsten A. Nagashima, Brian J. Kennedy, b Yun (Kenneth) Kang, c Robert R. Arrigale, Gerald P. Donovan, Wells W. Magargal, Paul J. Maddon, and William C. Olson

Progenics Pharmaceuticals, Inc, Tarrytown, New York

The spore-forming bacterium Clostridium difficile represents the principal cause of hospital-acquired diarrhea and pseudomembranous colitis worldwide. C. difficile infection (CDI) is mediated by 2 bacterial toxins, A and B; neutralizing these toxins with monoclonal antibodies (mAbs) provides a potential nonantibiotic strategy for combating the rising prevalence, severity, and recurrence of CDI. Novel antitoxin mAbs were generated in mice and were humanized. The humanized antitoxin A mAb PA-50 and antitoxin B mAb PA-41 have picomolar potencies in vitro and bind to novel regions of the respective toxins. In a hamster model for CDI, 95% of animals treated with a combination of humanized PA-50 and PA-41 showed long-term survival relative to 0% survival of animals treated with standard antibiotics or comparator mAbs. These humanized mAbs provide insight into C. difficile intoxication and hold promise as potential nonantibiotic agents for improving clinical management of CDI.

J Infect Dis. 2012 Sep 1;206(5):706-13
In Vitro Neutralization

- Significantly improved *in vitro* potency against majority of clinical isolates
- Markedly improved *in vitro* potency against hypervirulent 027 hypervirulent and emerging 078 *C. difficile* strains.

J Infect Dis. 2012 Sep 1;206(5):706-13
**C. diff** anti-toxin mAbs are superior to competitor mAbs in *vitro* and *in vivo*

**In vitro**: MedI mAbs have increased potency against clinically relevant strains

**In vivo**: Both anti-Toxin A and anti-Toxin B mAbs are required for protection

**In vivo**: Medi mAbs provide superior *in vivo* protection
MAb Prophylaxis of *C. Difficile*: The Debate

**SURE BET**
- mAbs have demonstrated pre-clinical and clinical activity (Merck)
- Long half-life could ensure coverage for duration of hospital stay
- Exogenous mAb does not rely on robust immune response, unlike vaccines

**CRAP SHOOT**
- Highly enriched patient population required for clinical trials (incidence)
- Requires clinician paradigm shift from treatment to prevention
- Requires payer buy-in; high-risk patients must be identified for acceptable NNT
Pseudomonas aeruginosa

- Versatile environmental bacteria
  - “The bacterial cockroach”

- An opportunistic bacterial pathogen
  - A major threat to critically ill patients

- Resistant to most front line antibiotics
  - Intrinsic and acquired resistance

Given *P. aeruginosa*’s lifestyle versatility, we are taking a multi-mechanistic approach.
Targeting *P. aeruginosa’s* Type 3 Secretion System (T3SS)

- The **T3SS** is responsible for the injection of multiple virulence factors into host cells. **PcrV** is the tip of the needle.
- PcrV knockout strains are greatly reduced in virulence.
- PcrV expression correlates with **acute disease** severity.
- >95% of strains express PcrV under inducing conditions.
Acknowledgements – MEDI3902

ID & ADPE
Antonio DiGiandomenico
Ashley Keller
Margarita Camara
Cuihua Gao
Jonah Rainey
Paul Warrener
Jessica Bonnell
Jameese Hilliard
Ruoyan Chen
Jingying Zha
Randall MacGill
Ryan Fleming
Binyam Bezabeh
Nazzareno Dimasi
Bret Sellman
JoAnn Suzich
Michael McCarthy
Ken Stover

Changshou Gao
Reena Varkey
Lena Shirinian
Kim Rosenthal
Jia Lin
Vineela Aleti
Ralph Minter
Steve Rust
Sandrine Guillard
Lutz Jermutus

Lab animal resources
Donna Goldsteen
Erin Straley
Matt “Doug” Jones
Stephanie Oldham

CMC Development
Marcia Carlson

MedImmune Clinical
Judy Falloon
Hasan Jafri
Genny Losonsky
Pam Griffin

AstraZeneca Clinical
David Melnick
John Rex

Project Mgmt
David Vallo
Phyllis Link

Translational Sciences
Annand Datta
Gabe Robbie
Xiang-Qing Yu
Mark Esser
Psl Exopolysaccharide

- Psl – Abundant surface-expressed, mannose-rich **serotype-independent** exopolysaccharide (slime)
- Promotes initial adherence to epithelial cells (acute)
- Key component in chronic biofilm establishment & maintenance
- Implicated in immune evasion
- Present on ~90% of clinical isolates tested (124/145)

\[
[-\rightarrow 3]-\beta-D-Manp- (1 \rightarrow 3)-\beta-D-Manp- (1 \rightarrow 3) \cdot \alpha-L-Rhap- (1 \rightarrow 3)-\beta-D-Glc\rho- (1 \rightarrow)_n- 
\]

**P. aeruginosa anti-PcrV + anti-Psl mAb Combination: Multi-Functional Bispecific Approach**

**Target 1: PcrV - Virulence**
- MOA: Prevents toxin injection into host cells
- High affinity mAb to low density target

**Target 2: Psl: Colonization-Persistence**
- Dual MOA: Clearance and blocks cell adherence
- Lower affinity mAb to high density target
BS4 is superior to monotherapy in providing protection in prophylactic pneumonia model

Challenge ~5x LD100 mAbs delivered 24 hrs prior to infection

Black circle – cont IgG
Red Sq – anti-PSL
Blue Diamond – anti-PcrV
Purple Circle – BS4
BS4αPa Antibiotic Adjunctive Therapy: Pneumonia

Even at a sub-protective dose, BS4αPa on board facilitates antibiotic treatment of mice in pneumonia model even when antibiotic exposure is subtherapeutic.

Strain 6206 1e6 cfu (5x LD100)
Drugs administered +4 hrs post infection
N=9 mice/group in vivo synergy confirmed by isobologram analysis

*P<0.005

*P<0.02
MEDI3902 for *P. aeruginosa*

- Novel bispecific, multi-functional mAb
- Three synergistic MOAs
- Targets multiple facets of the *P. aeruginosa* infection
- Broad strain coverage including MDR strains
- Promising activity in five different infection models
- Synergistic activity with antibiotics
- Partnership support with
**S. aureus: New paradigm for prevention**

- **S. aureus**: Leading cause of hospitalizations and in-hospital infections
  - 2M *S. aureus* infected cases annually in G7 (top 7 markets)
  - 2.7M excess hospital days annually in the US
  - On a patient basis, *S. aureus* infections add 10-25 days of hospital stay and $34K-$190K in incremental costs in the US

- **Current standard of care is peri-operative antibiotics or antibiotic treatment**
  - Morbidity, mortality and costs remain high despite use of latest antibiotics
  - Increased antibiotic resistance – key commercial driver

- **Immunoprophylaxis is a promising alternative strategy**
  - Targeting alpha toxin is valid regardless of *S. aureus* strain susceptibility to antibiotics, and ~99% of clinical isolates are capable of expressing alpha toxin
  - Preventing disease will obviate the use of antibiotics forestalling resistance
  - Unlike active vaccination, protection is immediate

---

The medical need and opportunity for *S. aureus* is large enough to warrant a multi-pronged strategy.
Virulence Factors of *S. aureus*

Alpha Toxin (AT): Key Virulence Factor in *S. aureus* Disease

- Cytolytic pore-forming toxin
- At sub-lytic concentrations AT binds ADAM10
  - Receptor mediated cell death
  - Cleavage of cell junction
  - Proinflammatory cytokine expression
  - Promotes immune evasion, tissue necrosis and invasion
- AT mutants attenuated in multiple animal models


*ID = Intradermal

Acknowledgements – MEDI4893

- **Infectious Disease**
  - Christine Tkaczyk
  - Melissa Hamilton
  - Yueyue Shi
  - Agnieszka Sadowska
  - Jamese Hilliard
  - Omari Jones
  - Lei Hua
  - Randall MacGill
  - Bret Sellman
  - Ken Stover
  - JoAnn Suzich

- **Molecular Biology**
  - Arnita Barnes
  - Hui Feng
  - Kim Rosenthal
  - Kannaki Senthil
  - Susan Wilson

- **ADPE**
  - Reena Varkey
  - Li Cheng
  - Qun Du
  - Lena Shirinian
  - Mario Cepeda
  - Yan Chen
  - Peter Pavlik
  - Vaheh Oganesyan
  - Rob Woods
  - Melissa Damschroder
  - Changshou Gao
  - Partha Chowdhury
  - William Dall’Acqua

- **CMC**
  - Jennifer Gribskov Panackal
  - Ken Miller
  - Gaurav Chauhan
  - Irina Ramos
  - Roberto Depaz
  - Ian Hart
  - Jean Mudrick
  - Patrick McGeehan
  - Matthew Dickson

- **LAR**
  - Julie Bakken
  - Jose Martinez

- **Translational Sciences**
  - Xiang-Qing Yu
  - Gabriel Robbie
  - Yuling Wu
  - Mark Esser
  - Anand Datta
  - Lily Cheng
Anti-alpha toxin mAb: Protects against acute toxin mediated diseases of *S. aureus*

- Alpha toxin mediates cellular/tissue damage and immune dysfunction
- The vast majority of *S. aureus* clinical isolates express alpha toxin during infection (*in vivo*)
- Anti-toxin serology correlates with protective immunity against community-onset *S. aureus* infection
  - Fritz et al. 2013. CID. Vol. 56. pg. 1554
  - Adhikari et al. 2012. JID. Vol. 206 pg. 915

**Anti-AT Skin & Soft Tissue Protection**

- mAb administered 24hr prior to infection

**Anti-AT Pneumonia Protection**

- Control IgG
- Anti-AT

![Graph showing percent survival over days post infection for different mAb dosages](image)
Anti-AT mAbs Reduce Dermonecrotic Lesion

10A7.5

- 5mg/kg
- 1mg/kg
- 0.2mg/kg

28F6.1

- 5mg/kg
- 1mg/kg
- 0.2mg/kg

R347 5mg/kg
Anti-AT mAbs Prevent Oligomerization

 SDS Sensitive/resistant
 SDS resistant
Anti-AT mAb Significantly Increases Survival In a Murine Model In A Dose Dependent Manner

*** p<0.0001
USA 300 2.7x10^8
Anti-AT prophylaxis prevents death and bacterial dissemination in murine pneumonia model

- Improved survival vs. most prevalent SA isolates**
- Reduction in bacterial load in lungs
- Reduced bacterial dissemination to kidneys
- Preserved lung integrity

**MRSA/MSSA isolates = USA300, USA200, USA100

mAb administered 24h prior to IN* challenge**
Anti-AT Treatment: Wider therapeutic window compared to antibiotics in skin and soft tissue model

ID Challenge: 5 x 10^7 CFU
Vancomycin (VAN)
Linezolid (LZD)
R347=Neg cont
Anti-AT Prophylaxis Promotes Survival in Immunocompromised Mouse Pneumonia Model

Neutrophil Count

Survival Post Infection

- Cyclophosphamide (CPM) administered 4 days prior to infection (150 mg/kg) and day -1 of infection (100 mg/kg).

- Mice infected day 0 with SF8300 (1 x 10^8 CFU)

mAb administered 24hr prior to infection
Anti-AT Antibiotic Combinations Improve Outcome Over Monotherapy in an Immunocompromised Model

Prophylaxis: LZD combo

Prophylaxis: Van combo

• Cyclophosphamide (CPM) administered 4 days prior to infection (150 mpk) and day -1 of infection (100 mpk).

• Mice infected day 0 with SF8300 (1 x 10^8 CFU)

• Note: LC10 is mouse version of MEDI4893
Adjunctive Therapy: Staph Murine Pneumonia Model

**VAN q12 for 3 days**

- Cont. IgG 15 mpk
- VAN 40 mpk/day
- anti-AT 15 mpk
- VAN-40/anti-AT 15

**LZD q12 for 2 days**

- Cont. IgG 15 mpk
- LZD 5 mpk/day
- anti-AT 15 mpk
- LZD-5 + anti-AT 15
Multiple Approaches to *S. aureus*

**Anti-AT mAb Ph1 trial underway.**
- Prevention of *S. aureus* hospital acquired ICU pneumonia
- Phase II scheduled to begin in 2014

**S. aureus combo mAb program**
- AT alone likely not sufficient for all *S. aureus* diseases (e.g., indwelling devices, hemodialysis patients)
- MAb combo product targeting different *S. aureus* virulence mechanisms Prevention or adjunctive treatment of serious *S. aureus* infections including device related infections and infections in hemodialysis patients
- **Primary goal:** Expand disease and isolate coverage
- **Bonus:** Complementary mAb mechanisms enhance efficacy

The medical need and opportunity for *S. aureus* is large enough to warrant a multi-pronged strategy
Murine Osteomyelitis (OM) Model

- Steel pin coated with *S. aureus* (~5e4 CFU) inserted across tibia
- Results in localized infection and significant bone damage
- Micro-CT to evaluate bone damage
- Closely resembles clinical disease
- AT does not play a role in this model
- Conducted by Eddie Schwarz, PhD U. of Rochester, Orthopedics Dept
Alpha Toxin Does Not Play a Role in This Model

WT

Δhla

Is it the model or is it AT?
mAb-2 reduces murine osteomyelitis disease severity
Staphylococcus aureus alpha toxin potentiates opportunistic bacterial lung infections

Taylor S. Cohen
Acknowledgements

◆ Infectious Disease
  – Christine Tkaczyk
  – Melissa Hamilton
  – Agnieszka Sadowska
  – Lei Hua
  – Yueyue Shi
  – Antonio DiGiandomenico
  – Omari Jones
  – Meghan Pennini
  – Ashley Keller
  – Randall MacGill
  – Jامesه Hilliard
  – Ken Stover
  – JoAnn Suzich

◆ Antibody Engineering
  – Reena Varkey
  – Li Cheng
  – Qun Du
  – Melissa Damschroder
  – Changshou Gao
  – Partha Chowdhury

◆ Molecular Biology
  – Arnita Barnes
  – Kim Rosenthal
  – Kannaki Senthil
  – Susan Wilson

◆ LAR
The presence of *S. aureus* is highly correlative with other infections

Fukutani et. al. J Clinical Virology 2015
S. aureus often precedes infection with other bacteria
Major Question

- Will patients just get a different infection?
- Bacterial infection “whack-a-mole”?
Questions:

- Does *S. aureus* promote Gram-negative bacterial infection?
- Would targeting one of multiple infecting pathogens enable clearance of all, or allow for expansion of the non-targeted bacteria?
- How does *S. aureus* influence the immune system such that clearance of co-infecting organisms is impaired?
**S. aureus:** *P. aeruginosa* co-infection potentiates disease

![Graph showing survival rates](image)

*S. aureus SF8300*

*P. aeruginosa* 6077
**S. aureus:** *P. aeruginosa* co-infection potentiates disease
S. aureus potentiates infection with multiple Gram-negatives

**Klebsiella pneumoniae**  
**Acinetobacter baumannii**

![Graphs showing percent survival over days post-challenge for S. aureus (Sa), Klebsiella pneumoniae (Kp), and Acinetobacter baumannii (Ab) individually and in combination.](image-url)
Precision mAbs prevent mortality

MEDI4893*: anti-Sa

Percent survival vs. Day Post-Challenge
Alpha toxin is sufficient for disease progression
Summary

Mono-infection

Mixed-infection

Epithelial Cell  Alveolar Macrophage  Neutrophil  Alpha-toxin Heptamer  Alpha-toxin Monomer  ADAM10  Gram-negative bacterium  S. aureus
What is THE Key Challenge?

• Picking the right patient population(s) to demonstrate efficacy
  - By efficacy we mean superiority to standard of care
• And not breaking the bank in the process
The Long and Winding Road to an Approval…

- Biologics, are in general going to be much narrower in spectrum
  - But this will limit the number of available patients
  - And do we have Guidance for developing such narrow spectrum agents?
  - What about mixtures?
  - How about “adjunctive therapy?”
- Those drugs that target the infecting organism are likely to have good safety profiles
  - So do we need expansive patient safety databases?
  - Rapid diagnostics? Has the time come
- What about immunomodulatory drugs
  - Can we beat “T cell exhaustion”
  - Anti-CTLA4, anti-PD-L1…these are coming and pretty soon
Many thanks for funding support from…

• IMI: COMBACTE – ND4BB
  • MEDI4893 & MEDI3902
• DARPA
  • Novel mAb delivery
Thanks for your attention!

Beware of false dichotomies:

“When you come to fork in the road, take it.”

Lawrence Peter (Yogi) Berra