Innovative Biotechnology Companies

and their Academic Origins

Jack Terrett
MacMillan Group Meeting
November 20th, 2013
The Future of Therapeutics

- Highlighting four diverse biotechnology companies

- All four featured companies are based on discoveries and innovations in academic labs

  Tetralogic Pharmaceuticals - Yigong Shi
  Peptidream - Hiroaki Suga
  Scifluor Life Sciences - Tobias Ritter
  Tetraphase Pharmaceuticals - Andy Myers

- Each company is focusing on therapeutic development in totally distinct ways
  - Three small molecule approaches, one peptide-based therapeutic approach

How should drug discovery be accomplished in the 21st century?

How do individual companies stand out and become successful?
Tetralogic Pharmaceuticals

- Pennsylvania-based pharmaceutical company
- Founded in 2003 by Yigong Shi
- Small molecule Smac mimetics for targeting apoptosis of cancer cells

About Yigong Shi

- 1994-1997: Postdoctoral work with Nikola Pavletich (Sloan-Kettering)
- 1998-2001: Assistant Professor, Princeton University (Department of Molecular Biology)
- 2001-2003: Associate Professor, Princeton University
- 2003-2008: Professor, Princeton University
- 2008-present: Professor, Tsinghua University

Featured in NYT article: "Fighting Trend, China is Luring Scientists Home" (Jan. 7, 2010)
Cell Apoptosis

- Insufficient programmed cell death has implications in many diseases, notably cancer
- As such, targeting apoptosis pathways is therapeutically attractive
- Many biological factors are involved in cell death
  - Importantly, caspases are produced in cells as active proteases in cell degradation
  - IAPs (inhibitors of apoptosis proteins) bind caspases, preventing cell death
  - The BIR domain (baculoviral IAP repeat) directly inhibits caspase enzymatic activity
  - Smac (second mitochondria-derived activator of caspases) inhibits BIR, allowing release of caspases
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Cell Apoptosis

- Smac inhibits XIAP and degrades cIAP, releasing caspases (9,3,7)

- In tumour cells, IAPs are over-expressed and Smac levels are low

- Shi’s seminal studies into structure of Smac and BIR binding pocket revealed common motif

  - The N-terminus of Smac/DIABLO homologues are highly conserved (mammals and Drosophila)

  - BIR domain binds substrate predominantly of N-terminal four peptide sequence


Smac Activation of Caspases

- Tetrapeptide motif on Smac binds BIR domain (Ala-Val-Pro-Ile)
- Alanine residue sits in hydrophobic pocket, H-bonds to neighbouring xIAP residues
- Single point mutation of Smac AVPI motif results in loss of binding affinity

Tetralogic Develops Smac Mimetic

- Recognition of tetrapeptide binding unit of Smac lends potential to therapeutics
- Peptidomimetic drugs containing AVPI-type motif should function as effective IAP binder
- Tetralogic developed lead candidate drug, **birinapant**

![Chemical Structure of Birinapant](attachment:image.png)

Birinapant was studied as single agent and combination therapy with TNF-α.

Birinapant degrades cIAP₁ and cIAP₂ allowing TNF-α to signal apoptosis (via caspase-8).

Cotreatment is highly effective against a range of melanoma cell lines.

Tetralogic Develops Smac Mimetic

■ Four cancer cell lines were further analyzed: WM9, WM1366, 451Lu, 1205Lu
■ Absorption at 490nm directly proportional to number of living cells (MTS assay)
■ Increase in sub-G₁ fractions is indicative of apoptosis

Tetralogic Develops Smac Mimetic

- Birinapant shows cIAP1 protein degradation at 100 nmol/L after 1 hour (XIAP unaffected)
- To determine apoptosis is caspase dependent, Z-VAD-FMK was added (caspase inhibitor)
- Necrostatin-1 (RIP1 kinase inhibitor) also reversed effect of birinapant/TNF-α

Tetralogic Develops Smac Mimetic

- Cells grown in 3D spheroid cultures, more similar to *in vivo* environments

- Similar effects as in previous *in vitro* studies for each cell line

**Tetralogic Develops Smac Mimetic**

- *In vivo* studies show birinapant is effective against 451Lu and slows tumor growth in 1205Lu.

- Addition of TNF-α antibodies to WM9 culture shows dependence on endogenous TNF-α.

- Birinapant in combination with cisplatin improves antitumor activity (451Lu, WM1366).

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Tetralogic Moves Birinapant to Clinical Trials

- Birinapant entered Phase I and II clinical trials for a variety of targets:
  - Colorectal cancer (combination with irinotecan)
  - Ovarian cancer (single agent, and combination with conatumumab)
  - AML/ALL (single agent)
  - Myelodysplastic syndrome (MDS) (combination with azacitidine)
  - Hepatitis B (preclinical trials)

- Tetralogic has library of over 3000 Smac mimetics

- Continuing to develop peptidomimetic therapeutics for both oncology and non-oncology indications
  - Both as single agent and combination therapies

- Investors include:
  - Nextech Invest, Clarus Ventures, HealthCare Ventures, Quaker BioVentures, Novitas Capital, Hatteras Venture Partners, Pfizer Ventures, Latterell Venture Partners, The Vertical Group, Amgen Ventures, Kammerer Associates

Having spent $77+ million developing birinapant, Tetralogic plans to raise $90-$100 million in upcoming IPO (rumoured to offer 6.4 million shares at $13-$15)
Peptidream

- Tokyo-based pharmaceutical company
- Founded in July 2006 by Hiroaki Suga
- Novel peptide therapeutics using proprietary Peptide Discovery Platform System (PDPS)

About Hiroaki Suga

- 1994-1997: Postdoctoral work with Jack Szostak (Harvard Medical School)
- 1997-2003: Assistant and Associate Professor at SUNY Buffalo
- 2003-present: Professor at University of Tokyo
Non-standard peptides are appealing therapeutic class

- Very few systematic methods to synthesize and develop as drugs

Non-traditional peptides may include:

- Non-canonical sidechains
- D-amino acids
- N-methyl modification

Macrocyclization and N-methylation improve membrane permeability and bioavailability
Research in the Suga Lab

Artificial Ribozymes

Ribosomal Synthesis of Non-Standard Peptides

Genetic Code Reprogramming

Non-Standard Peptide Probes
A Brief Overview of Translation

Ribsomal Peptide Synthesis (Translation)

Genome British Columbia, www.genomebc.ca/education/articles/translation
Flexizyme Technology

- Aminoacyl-tRNA synthetases (ARSs) catalyze ligation of amino acids to their respective tRNA
- Recombinant ARSs can ligate non-canonical AAs to tRNA, but substrate promiscuity is low

A new approach is necessary!

- **Ribozymes** - an RNA capable of enzymatic processes
- Flexizymes = highly promiscuous aminoacylating ribozyme ARSs


**Flexizyme Technology**

- **Ribozymes** - an RNA capable of enzymatic processes

- Flexizymes = highly promiscuous aminoacylating ribozyme ARSs

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Ito, K.; Passioura, T.; Suga, H. *Molecules* 2013, 18, 3502.
Flexible in vitro Translation (FIT) System

Partial amino acids deficient reconstituted in vitro translation system

Activated non-proteinogenic amino acids

Non-canonical aminocarboxy-IRNAs

Flexible zymo assisted aminoacylation

 Ribosome, Amino acids, IRNAs, ARSs etc.

Natural peptide

Non-standard peptide
Methods for Peptide Macrocyclization

- Replacing N-terminus (initiator codon) with N-2-chloroacetyl amino acid
- Intramolecular thioether bond formation with downstream cysteine
- Spontaneous cyclization (may even occur within ribosome)
Methods for Peptide Macrocyclization

- Bicyclic peptide macrocycles can be synthesized
- Multiple cysteine residues allow thioether and disulphide bond formations

Methods for Peptide Macrocyclization

- Bicyclic peptide macrocycles can be synthesized
- Other functional handles can be manipulated using post-translational reactions

Cu-catalyzed alkyne-azide cyclization

Ito, K.; Passioura, T.; Suga, H. Molecules 2013, 18, 3502.
Methods for Peptide Macrocyclization

- Macrocycles with peptide bond can be synthesized
- FIT system must contain peptide deformylase (PDF) and methionine aminopeptidase (MAP)
- Cys-Pro-glycolic acid motif cyclizes to dkp-thioester
- Enzymatic removal of fMet liberates free NH₂

Ito, K.; Passioura, T.; Suga, H. Molecules 2013, 18, 3502.
RaPID System

- RaPID = Random non-standard Peptides Integrated Discovery system
- Highly efficient system for building up peptide library with high selectivity for a target protein
- The concept involves ligating mRNA strand to its corresponding peptide, observing binding affinity of peptide to protein, then over expressing RNA/peptide that selectively binds
- Overall, combination of FIT system and modified mRNA display
  - selection of bioactive non-standard peptides


RaPID System

How does RaPID work?

Start with a library of diverse mRNA

- Typical sequence: AUG-random sequence (5-15 codons)-UGU-(GGC-AGC)₃-UAG

- AUG = start codon, UGU = cysteine, UAG = stop codon

- G rich section designed to anneal to DNA in puromycin linker

T4 RNA ligase links all mRNA strands to puromycin-DNA oligonucleotide


**RaPID System**

**How does RaPID work?**

FIT system results in translation of mRNA chain by ribosome

- Standard and non-standard amino acids incorporated
- Initiation codon reprogrammed to ClAc-amino acid
- Downstream cysteine cyclizes with N-terminal ClAc group
- At stop codon, ribosome stalls due to lack of Release Factor 1 (RF1)

α-amino group on puromycin linker is ligated to C-terminus of growing peptide chain by ribosome

- Forms the RNA-peptide adduct

![Diagram of puromycin](attachment:puromycin_diagram.png)


Ito, K.; Passioura, T.; Suga, H. *Molecules* 2013, 18, 3502.
RaPID System


Ito, K.; Passioura, T.; Suga, H. Molecules 2013, 18, 3502.
RaPID System

- The RaPID cycle is repeated several times to enrich the mRNA pool
- One round of selection and enrichment is completed in <1 day
- Once complete, the enriched pools are subjected to DNA sequencing
  - Binding is confirmed by resubjecting to target protein
  - Each peptide is then chemically synthesized for further studies of binding affinity and bioactivity

The diversity of non-canonical peptide residues is essentially infinite.

RaPID presents a very fast method for peptide therapeutic development!


Ito, K.; Passioura, T.; Suga, H. *Molecules* 2013, 18, 3502.
RaPID System in Drug Discovery

- Suga and Peptidream have applied the RaPID system towards novel peptide therapeutics

- Proof of concept: discovery of Akt2 inhibitor

- Akt kinase family play critical roles in signal transduction pathways
  - Akt1 and Akt2 indicated as potential oncogenes - over-expression suppresses cell apoptosis
  - Akt3 least understood - activation of growth factors in brain
  - Akt2 involved in insulin receptor signal transduction - possible target for diabetes treatment

- Appealing target for therapeutics, but difficult isoform-selectivity has hampered efforts

**RaPID: Discovery of Akt2 Inhibitor**

- Four classes of Akt2 inhibitors:
  1) Bind to ATP-binding site
  2) Bind to pleckstrin homology (PH) domain
  3) Bind to an allosteric site
  4) Bind to active site (peptide-binding domain)

- Use of RaPID display system with macrocyclic peptides:
  - ClAc\(^1\)Y or ClAc\(^\text{D}\)Y employed as the initiator amino acid
  - Random AA sequence composed of 4-12 units, of standard amino acids
  - End sequence with cysteine (for thioether bond formation) and puromycin linker

- Six rounds of RaPID to generate highly enriched mRNA pool against Akt2

RaPID: Discovery of Akt2 Inhibitor

- From $^{L}$Y- and $^{D}$Y- pools, the best peptide binders were DNA sequenced

- Pakti-L1 and Pakti-D1 were the most abundant sequences

- Inhibitory effects were determined by in vitro kinase assays

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<th>Frequency</th>
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<td>Akt2</td>
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<td>Ac-$^{L}$YILVRNRLRVDCG-NH$_2$</td>
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RaPID: Discovery of Akt2 Inhibitor

- Pakti-L₁, L₂, L₃ showed best inhibitory effect against Akt kinases
- Pakti-L₁ showed tremendous isoform-selectivity for Akt2 (over Akt1 and Akt3)

PKA = Protein kinase A, SGK = serum- and glucocorticoid-regulated protein kinase

Black, dark grey, light grey correspond to 1, 5, and 10 µM peptide concentrations.

RaPID: Discovery of Akt2 Inhibitor

- Remarkably, RaPID system delivered effective inhibitors of Akt2
  - Display system only examines binding affinity, but corresponds well to inhibition
  - Interestingly, LY- and DY-peptides had very difficult sequences indicating different structural orientation

- Pakti-L₁ mode of binding to Akt2 is unknown
  - Possibly interacts with substrate-binding domain or allosteric site

- Pakti-L₁ exhibited unprecedented levels of Akt2-isoform selectivity and potency

Peptidream

Peptidream currently applying Suga technology to novel therapeutic discovery

Peptide Discovery Translation System (PDTS) and Peptide Discovery Display System (PDDS)

Numerous multi-target discovery deals signed:

- Aug. 2010 - Novartis
- Oct. 2010 - Amgen
- Nov. 2010 - BMS
- Dec. 2010 - Pfizer
- Dec. 2010 - Mitsubishi-Tanabe Pharma
- Jul. 2012 - Daiichi Sankyo
- Sep. 2012 - GSK
- Apr. 2013 - Ipsen

Novel non-standard macrocyclic peptides show promise as potent and selective therapeutics!
SciFluor Life Sciences

- Launched in February 2011
- Founded by Tobias Ritter (Harvard)
- Initial $5 million investment by Allied Minds

- Late-stage fluorination of therapeutics (Fluoropeutics)
  
  - Fluorination of already known compounds with established biological targets:
    
    with the goal of improving potency, metabolic stability, binding affinity, bioavailability, and blood-brain barrier penetration
  
  - "De-risked" candidates, due to precedent of parent compound in pre-clinical/clinical trials

- Employing Ritter technology for $^{18}$F PET tracers
SciFluor employs "PhenoFluor" for late-stage fluorination

- Novel deoxyfluorinating reagent discovered by Ritter and coworkers
- Marketed by SciFluor through Sigma-Aldrich and Strem

Other commercially available deoxyfluorinating agents (DAST, DEOXYFLUOR, Xtalflour) gave <1% yield

- Deoxyfluorination of phenols

\[
\text{PhenoFluor} \quad \overset{3 \text{ equiv. CsF}}{\underset{\text{PhCH}_3, \ 110^\circ \text{C}}{\longrightarrow}} \quad \text{PhenoFluor}^{\text{™}}
\]

\[
R - \text{PhOH} \quad \begin{array}{c} \text{PhenoFluor} \quad \overset{3 \text{ equiv. CsF}}{\underset{\text{PhCH}_3, \ 110^\circ \text{C}}{\longrightarrow}} \quad \text{PhenoFluor}^{\text{™}} \\ \text{(1.2 equiv.)} \end{array}
\]

**Proposed mechanistic pathway**

PhenoFluor™

![PhenoFluor](image)

PhenoFluor™

H-bonding interaction facilitates fluorination

a) makes the uronium a better leaving group
b) brings fluoride in closer proximity to *ipso*-carbon

Control experiments: no H-bonding, no reaction

**PhenoFluor™**

- Deoxyfluorination of aliphatic alcohols

![Chemical Structures]

Byproducts observed with conventional fluorination reagents:

![Byproducts]

Deoxyfluorination of aliphatic alcohols

PhenoFluor™

\[
\begin{array}{c}
\text{PhenoFluor} \\
\text{R_1R_2OH} \xrightarrow{\text{PhenoFluor}} \text{R_1R_2F} \\
\text{2 eq. } i\text{-Pr}_2\text{EtNH} \\
\text{2 eq. KF} \\
\end{array}
\]

inversion of stereochemistry

88%
43%
77%
53%

PhenoFluor™

Deoxyfluorination of aliphatic alcohols

oligomycin A

2 eq. \( \text{i-Pr}_2\text{EtNH} \)
2 eq. KF
71% yield

Deoxyfluorination of aliphatic alcohols

PhenoFluor has excellent chemoselectivity

a) 1° alcohols fluorinated selectively over 2° and 3°
b) β,β'-dibranched 2° alcohols react significantly slower (unless allylic)
c) 3° alcohols do not react (unless allylic)
d) hydroxyl groups involved in H-bonding do not react

71% yield

**PhenoFluor™**

PhenoFluor is a versatile tool for SciFluor's late-stage fluorination approach

![PhenoFluor](image)

**Advantages**

a) Air-stable reagent, operationally simple (non-explosive)

b) Excellent selectivity (predictable)

c) Functional group tolerant

d) Avoids byproducts (elimination of H₂O), yields single isomer

**Disadvantage:** stoichiometric waste, not ideal for scale-up


SciFluor's Potassium Channel Opener: SF0034

SciFluor have identified a potent therapeutic for the treatment of partial-onset seizure

Ezogabine (Valeant/GSK)  
Fluoropeutic SF0034

- Ezogabine was the first potassium channel (KCNQ2/3) opener for epilepsy treatment (approved June 2011)
- Binds to voltage-gated K⁺ channel, opening it, and allowing repolarization of the neuron
- Stops the high levels of neuronal action potential burst firing - associated with seizure onset

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SciFluor's Potassium Channel Opener: SF0034

- Selectivity in activating KCNQ2/3 over KCNQ4 is essential
  - KCNQ4 activation results in a urinary retention side effect

SciFluor's Potassium Channel Opener: SF0034

- Selectivity in activating KCNQ2/3 over KCNQ4 is essential
  - KCNQ4 activation results in a urinary retention side effect

Ezogabine

SF0034

SF0034 is 10 times more potent and 5 times more selective than ezogabine

SciFluor's Potassium Channel Opener: SF0034

- SF0034 shows no mutagenicity and reduced hERG inhibition
  - Ames test on SF0034 showed no mutagenicity or cytotoxicity

SF0034 has >10 times higher IC$_{50}$ values for hERG inhibition than ezogabine

SciFluor’s Potassium Channel Opener: SF0034

SF0034 *in vitro* and *in vivo* data

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<td>43 mg/kg (1.6)</td>
<td>11 mg/kg (6.1)</td>
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<td>SF0034</td>
<td>6.6 mg/kg (9.1)</td>
<td>27 mg/kg (2.3)</td>
<td>12 mg/kg (5.0)</td>
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TI = \frac{TD_{50}}{ED_{50}}

SciFluor Life Sciences Pipeline

- SF0034 has demonstrated improved potency and selectivity to ezogabine
  - Overall more favourable phacological profile, including reduced side effect profiles
  - SciFluor seeking industry partner to develop SF0034 as next-generation anti-epileptic drug

- Efforts using fluoropeutics to target cardiovascular disease, infectious disease, CNS, and oncology are currently ongoing

"Precedented drugs" vs. "Me-Too drugs" (Xconomy, Feb. 2013)

"The most fruitful basis for the discovery of a new drug is to start with an old drug."

"A competitor can patent new molecules based on a rival's older drugs. But the competitor must make changes to the original drug that are truly novel, and that would not have been obvious innovation routes for the creators of the original drug."

Arthur Hiller, Former CEO, SciFluor Life Sciences
Tetraphase Pharmaceuticals

- Founded in 2006
- Watertown, MA
- Based on Andrew Myers' tetracycline synthetic efforts (Harvard University)

Tetraphase's mission is to bring novel tetracycline antibiotics to market to target multidrug resistant (MDR) infections.

The Myers/Tetraphase approach to tetracycline synthesis is convergent and allows rapid diversification:
A History of Tetracyclines

- First tetracycline antibiotic isolated in 1948 - Benjamin Duggar, Lederle Laboratories
  - aureomycin (chlorotetracycline)

- In 1950, Pfizer isolated terramycin
  - terramycin (oxytetracycline)

- In 1953, tetracycline was first prepared by Lloyd Conover at Pfizer
  - later determined to be a natural product
**Total Syntheses of Tetracyclines**

- Numerous syntheses of tetracycline and analogues

  - Woodward, Shemyakin, Muxfeldt, Stork, Tatsuta
  - All syntheses apply a "left to right" approach (D ring to A ring)

- Total synthesis of tetracycline analogue accomplished by Woodward in 1968

  ![Chemical Structure](image)

  - 6-deoxy-6-demethyltetracycline
  - 25 steps, 0.002% yield

"the original effort of Woodward has survived as the basic strategy for the total synthesis of this series and at greater than 25 steps is clearly not to be considered as practical....."


History of Tetracycline Antibiotics

- Aureomycin, terramycin, and tetramycin identified as powerful antibiotics

- Three major tetracycline antibiotics over the last 50 years

- Doxycycline (Pfizer 1967)

- Minocycline (Lederle 1972)

- Tigecycline (Wyeth 2005)
History of Tetracycline Antibiotics

Tigecycline

Removal of C6-hydroxyl group improved metabolic stability, retention of antibacterial activity

Derivatization only possible at C7,C9 positions (electrophilic aromatic substitution)

All FDA approved tetracycline antibiotics are made exclusively via fermentation or semi-synthesis

Given the D to A ring synthetic approaches, variation on the D ring is challenging given current methods
History of Tetracycline Antibiotics

- Ribosomal binding

Tetracyclines bind to the 30S subunit of the bacterial ribosome through hydrophilic groups

- Blocks the acceptor site for aminoacylated tRNA

- Prevents binding of amino acid to ribosome
  - inhibits protein synthesis

**History of Tetracycline Antibiotics**

- Ribosomal binding

- Within the 30S subunit, tetracyclines bind to the major groove of 16S RNA
  - predominantly H-bonding with phosphate backbone
  - Mg\(^{2+}\) present in ribosome and helps binding
  - interestingly, the conformation of RNA does not change upon Tc binding

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The Myers Synthesis

A,B ring synthesis

\[
\text{A. eutrophus B9} \quad \xrightarrow{79\%, >95\% \text{ ee}} \quad \text{m-CPBA, EtOAc} \quad \xrightarrow{83\%} \\
\]

1. TMSCHN₂
2. TBSOTf, Et₃N \quad \xrightarrow{70\%} \\

1. LiOTf, PhCH₃, 60°C
2. TFA, CH₂Cl₂ \quad \xleftarrow{62\%} \\

THF, -78°C

(7 steps, 21% yield)

The Myers Synthesis

A,B ring synthesis - deoxy enone 1

1. HCl, MeOH
2. IBX, DMSO
3. TBSOTf, 2,6-lutidine

(11 steps, 10% yield)

The Myers Synthesis

A,B ring synthesis - oxy enone 2

1. CBr₄, PPh₃
2. PhSH, Et₃N

87%

1. P(OMe)₃, MeOH, 70°C

76%

The Myers Synthesis

Key step forms C ring, resulting in ABCD architecture

LDA, TMEDA
THF, −78°C

BnO₂CO
NMe₂

Oxy enone 2
−78°C to 0°C

Michael-Claisen cyclization
excellent diastereoselectivity

The Myers Synthesis

Key step forms C ring, resulting in ABCD architecture

LDA, TMEDA
THF, -78°C

BnO₂CO H NMe₂

Oxy enone 2
-78°C to 0°C

Michael-Claisen cyclization
excellent diastereoselectivity

1. HF, MeCN
2. H₂, Pd, THF, MeOH
90%

(-)-Doxycycline
18 steps, 8.3% yield

The Myers Synthesis

Late-stage C-ring construction allows rapid diversification

1. LDA, TMEDA; 1
   -78°C to 0°C (81%)
2. HF, MeCN
3. H₂, Pd (85%)

1. LDA, DMPU; 1
   -78°C to 0°C (67%)
2. H₂, Pd(OH)₂
3. HCl, MeOH (74%)

1. 1; LDA, HMPA
   -95°C to -50°C (76%)
2. H₂, Pd
3. HF, MeCN (79%)

(-)-6-deoxycyclacetracycline
14 steps, 7.0% yield

Pyridinone derivative
14 steps, 5.0% yield

Pyridine derivative
14 steps, 6.1% yield

The Myers Synthesis

- Late-stage C-ring construction allows rapid diversification

\[ \text{1. } n\text{-BuLi} \quad \text{from -100°C to -70°C (81%)} \]

1. 1; \( n\text{-BuLi} \)
2. HF, MeCN
3. H\(_2\), Pd, (83%)

10-deoxysancycline
14 steps, 6.8% yield

\[ \text{1. } n\text{-BuLi} \quad \text{from -100°C to 0°C (75%)} \]

1. 1; \( n\text{-BuLi} \)
2. HF, MeCN
3. H\(_2\), Pd
4. BBr\(_3\), CH\(_2\)Cl\(_2\)
   -78 to 23°C (74%)

pentacycline derivative
15 steps, 5.6% yield

The Myers Synthesis

The pentacycline derivative showed promising antibacterial activities

<table>
<thead>
<tr>
<th>Bacterial Strains Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-Positive Organisms</td>
</tr>
<tr>
<td>S. aureus ATCC 29213</td>
</tr>
<tr>
<td>S. epidermidis ACH-0016</td>
</tr>
<tr>
<td>S. haemolyticus ACH-0013</td>
</tr>
<tr>
<td>E. faecalis ATCC 700802</td>
</tr>
<tr>
<td>S. aureus ATCC 700699</td>
</tr>
<tr>
<td>Gram-Negative Organisms</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 27853</td>
</tr>
<tr>
<td>K. pneumoniae ATCC 13883</td>
</tr>
<tr>
<td>E. coli ATCC 25922</td>
</tr>
<tr>
<td>E. coli ACH-0095</td>
</tr>
<tr>
<td>E. coli pBR322</td>
</tr>
</tbody>
</table>

(-)-Tetracycline

pentacycline derivative

<table>
<thead>
<tr>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>32</td>
</tr>
</tbody>
</table>

Tetraphase Starts Searching for Leads

- Tetraphase begins investigating various classes of tetracycline analogues
  - Pentacyclines
  - 8-Azatetracyclines
  - Fluorocyclines

- Focus on D ring manipulation to overcome tetracycline-resistance

Two primary tetracycline-resistance mechanisms:
1) active transport via efflux pumps (tetA–tetD, tetK–tetL)
2) ribosomal protection (tetM–tetO)
Tetraphase Starts Searching for Leads

- Pentacyclines deliver potential candidates showing strong in vitro and in vivo data

In vitro MIC data

<table>
<thead>
<tr>
<th></th>
<th>MIC ((\mu g/mL))^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SA101</td>
</tr>
<tr>
<td>29213</td>
<td>MRSA, tetM</td>
</tr>
</tbody>
</table>

| 19a-17              | 3-F-azetidinomethyl | 0.5 | 2 | 0.5 | 0.5 | 2 | 0.125 | 0.25 | 2 | 8 | 1 | >32 | 8 | 8 | 16 |
|                     | minocycline         | 0.0625 | 8 | 0.0313 | 1 | 16 | <0.0156 | 2 | 0.5 | 8 | 0.0625 | 16 | 2 | 1 | 8 |
|                     | tigecycline         | 0.0625 | 0.125 | 0.0625 | 0.0313 | 0.0625 | 0.0156 | 0.0156 | 0.0313 | 0.5 | 0.25 | 8 | 0.25 | 0.125 | 1 |

Tetraphase Starts Searching for Leads

- Pentacyclines deliver potential candidates showing strong in vitro and in vivo data

In vivo pharmacokinetic profile

<table>
<thead>
<tr>
<th>Compound</th>
<th>R^7</th>
<th>R^10</th>
<th>PK (IV)</th>
<th>%F</th>
<th>MIC</th>
<th>PD_{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C_{max}</td>
<td>AUC_{obs}</td>
<td>Cl</td>
<td>V_z</td>
</tr>
<tr>
<td>19a-17</td>
<td>H</td>
<td>3-F-azetidinomethyl</td>
<td>814</td>
<td>3457</td>
<td>4.82</td>
<td>1.4</td>
</tr>
<tr>
<td>tetracycline</td>
<td></td>
<td></td>
<td>583</td>
<td>802</td>
<td>20.5</td>
<td>3.68</td>
</tr>
<tr>
<td>tigecycline</td>
<td></td>
<td></td>
<td>428</td>
<td>1052</td>
<td>15.5</td>
<td>6.12</td>
</tr>
</tbody>
</table>

Tetraphase Starts Searching for Leads

- 8-Azatetracyclines showed promise in overcoming tetracycline-resistance

![Chemical Structures](image)

**In vitro MIC data**

<table>
<thead>
<tr>
<th>compd</th>
<th>R₁</th>
<th>S. aureus</th>
<th></th>
<th>S. pneumoniae</th>
<th></th>
<th>E. coli</th>
<th></th>
<th>K. pneumoniae</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>wild type</td>
<td>tet(M)</td>
<td>wild type</td>
<td>tet(M)</td>
<td>wild type</td>
<td>tet(A)</td>
<td>wild type</td>
<td></td>
</tr>
<tr>
<td>20f</td>
<td>(CH₃)₂N⁻</td>
<td>0.031</td>
<td>16</td>
<td>2</td>
<td>0.063</td>
<td>8</td>
<td>&gt;32</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>24l</td>
<td></td>
<td>0.5</td>
<td>2</td>
<td>0.125</td>
<td>0.016</td>
<td>0.125</td>
<td>0.5</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>tetracycline</td>
<td></td>
<td>1</td>
<td>&gt;32</td>
<td>32</td>
<td>0.25</td>
<td>32</td>
<td>2</td>
<td>&gt;32</td>
<td>4</td>
</tr>
<tr>
<td>minocycline</td>
<td></td>
<td>0.125</td>
<td>16</td>
<td>0.25</td>
<td>&lt;0.016</td>
<td>8</td>
<td>0.5</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

**Tetraphase Starts Searching for Leads**

8-Azatetracyclines showed promise in overcoming tetracycline-resistance

In vivo mouse septicemia model

<table>
<thead>
<tr>
<th>Compd</th>
<th>S. aureus</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (µg/mL)</td>
<td>PD₅₀ (mg/kg)</td>
</tr>
<tr>
<td>20f</td>
<td>0.031</td>
<td>&lt;0.30ᵇ</td>
</tr>
<tr>
<td>24l</td>
<td>0.5</td>
<td>0.36</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.25</td>
<td>0.35</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.063</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Tetraphase Starts Searching for Leads

- 7-Fluoro-9-pyrrolidinoacetamido-6-demethyl-6-deoxytetracycline shows best potency yet

```
\[
\begin{align*}
\text{FP} & \overset{s-\text{BuLi}, \text{TMEDA}}{\longrightarrow} \text{FP} \overset{(\text{COCl})_2, \text{DMF}}{\longrightarrow} \text{FP} \overset{1. \text{BBr}_3, \text{CH}_2\text{Cl}_2}{\longrightarrow} \text{FP} \\
\text{OMe} & \quad \text{Me} \quad \text{OMe} \quad \text{Me} \quad \text{OMe} \quad \text{Me} \quad \text{OMe} \quad \text{Me} \quad \text{OMe}
\end{align*}
\]
```

75% over 2 steps

Tetraphase Starts Searching for Leads

Rapid synthesis of fluorocycline analogue

![Chemical structures and reactions]

**Tetraphase Starts Searching for Leads**

TP-434 shows best efficacy of all Tetraphase compound library

In Vitro Antibacterial Activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>RR'N-</th>
<th>MIC (μg/mL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SA101</td>
</tr>
<tr>
<td>17j</td>
<td></td>
<td>29213</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.125</td>
<td>64</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.0625</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Tetraphase Starts Searching for Leads

TP-434 shows best efficacy of all Tetraphase compound library

In Vivo Activity

<table>
<thead>
<tr>
<th>model</th>
<th>strain</th>
<th>MIC (μg/mL)</th>
<th>PD_{50} (mg/kg)</th>
<th>17j</th>
<th>tigecycline</th>
<th>vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>murine septicemia</td>
<td>EC133 tet (B)</td>
<td>0.125</td>
<td>1.3</td>
<td>0.125</td>
<td>0.125</td>
<td>NT</td>
</tr>
<tr>
<td>neutropenic thigh</td>
<td>SA191 tet (M) (MRSA)</td>
<td>0.25</td>
<td>0.6</td>
<td>0.25</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.75</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>compd</th>
<th>dosage route (mg/kg)</th>
<th>CLs (L/h/kg)</th>
<th>V_{s} (L/kg)</th>
<th>T1/2 (L/kg)</th>
<th>C_{max} (hr)</th>
<th>AUC_{int}</th>
<th>% F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17j</td>
<td>IV (1)</td>
<td>0.564</td>
<td>3.2</td>
<td>4.0</td>
<td>0.812</td>
<td>1.766</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>PO (10)</td>
<td></td>
<td></td>
<td></td>
<td>0.045</td>
<td>0.295</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tetracycline</td>
<td>IV (1)</td>
<td>0.542</td>
<td>1.2</td>
<td>4.6</td>
<td>2.664</td>
<td>3.083</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>PO (10)</td>
<td></td>
<td></td>
<td></td>
<td>0.791</td>
<td>4.536</td>
<td></td>
</tr>
<tr>
<td>tigecycline</td>
<td>IV (1)</td>
<td>0.929</td>
<td>6.12</td>
<td>4.6</td>
<td>0.428</td>
<td>1.052</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>PO (10)</td>
<td></td>
<td></td>
<td></td>
<td>0.0278</td>
<td>0.107</td>
<td></td>
</tr>
</tbody>
</table>

"Eravacycline" Moves Forward

- TP-434 renamed "eravacycline" and is moved onto clinical trials

![Chemical structure of eravacycline]

TP-434 = eravacycline

- Tetraphase timeline:

  Feb. 2012: Biomedical Advanced Research and Development Authority (BARDA) award Tetraphase with $67 million contract for development of eravacycline

  Jul. 2013: Eravacycline designated a Qualified Infectious Disease Product (QIDP) by FDA

  Sept. 2013: Eravacycline entered Phase 3 clinical trials (for cIAI and cUTI)

  TP-834 and TP-271 currently in preclinical development

- Mar. 2013 - Tetraphase initial public offering on NASDAQ (10,714,286 shares at $7.00 each)
  - Currently trading between $10-$12 per share