Nuevos fármacos

Pere Domingo
Malalties Infeccioses
Hospital de la Santa Creu i Sant Pau
Barcelona
pdomingo@santpau.cat

UPDATE. 18th CONFERENCE ON RETROVIRUSES AND OPPORTUNISTIC INFECTIONS
<table>
<thead>
<tr>
<th>Fármaco</th>
<th>Compañía</th>
<th>Familia</th>
<th>Nº abstract</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS-7340</td>
<td>Gilead</td>
<td>ITIAN</td>
<td>152LB</td>
</tr>
<tr>
<td>GSK 2248761</td>
<td>GSK</td>
<td>ITINAN</td>
<td>520, 628, 631</td>
</tr>
<tr>
<td>Cenicriviroc</td>
<td>Takeda Pharm</td>
<td>CCR5</td>
<td>54LB</td>
</tr>
<tr>
<td>VIRIP</td>
<td>MHH</td>
<td>Inh. entrada</td>
<td>58LB</td>
</tr>
<tr>
<td>Ibalizumab</td>
<td>ADCAR</td>
<td>AcAnti-CD4</td>
<td>585</td>
</tr>
<tr>
<td>BMS-663068</td>
<td>BMS</td>
<td>Inh. unión</td>
<td>487</td>
</tr>
<tr>
<td>BMS-626529</td>
<td>BMS</td>
<td>Inh. unión</td>
<td>518</td>
</tr>
<tr>
<td>BMS-488043</td>
<td>BMS</td>
<td>Inh. unión</td>
<td>588</td>
</tr>
</tbody>
</table>

**UPDATE. 18 th CONFERENCE ON RETROVIRUSES AND OPPORTUNISTIC INFECTIONS**
<table>
<thead>
<tr>
<th>Fármaco</th>
<th>Compañía</th>
<th>Familia</th>
<th>Nº abstract</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS-7340</td>
<td>Gilead</td>
<td>ITIAN</td>
<td>152LB</td>
</tr>
<tr>
<td>GSK 2248761</td>
<td>GSK</td>
<td>ITINAN</td>
<td>520, 628, 631</td>
</tr>
<tr>
<td>Cenicriviroc</td>
<td>Takeda Pharm</td>
<td>CCR5</td>
<td>54LB</td>
</tr>
<tr>
<td>VIRIP</td>
<td>MHH</td>
<td>Inh. entrada</td>
<td>58LB</td>
</tr>
<tr>
<td>Ibalizumab</td>
<td>ADCAR</td>
<td>AcAnti-CD4</td>
<td>585</td>
</tr>
<tr>
<td>BMS-663068</td>
<td>BMS</td>
<td>Inh. unión</td>
<td>487</td>
</tr>
<tr>
<td>BMS-626529</td>
<td>BMS</td>
<td>Inh. unión</td>
<td>518</td>
</tr>
<tr>
<td>BMS-488043</td>
<td>BMS</td>
<td>Inh. unión</td>
<td>588</td>
</tr>
</tbody>
</table>

UPDATE. 18th CONFERENCE ON RETROVIRUSES AND OPPORTUNISTIC INFECTIONS
GS-7340 Demonstrates Greater Declines in HIV-1 RNA than Tenofovir Disoproxil Fumarate During 14 Days of Monotherapy in HIV-1 Infected Subjects

M Markowitz, 1 A Zolopa, 2* P Ruane, 3 K Squires, 4 L Zhong, 5 BP Kearney, 5 and W Lee 5

1 Aaron Diamond AIDS Research Center, New York, NY; 2 Stanford University Positive Care Clinic, Palo Alto, CA; 3 Lighthouse Medical, Los Angeles, CA; 4 Thomas Jefferson University, Philadelphia, PA; 5 Gilead Sciences, Foster City, CA

18th Conference on Retroviruses and Opportunistic Infections
March 2, 2011
Paper # 152LB
Introduction

- GS-7340 is a novel amidate prodrug that was designed to deliver high concentrations of tenofovir diphosphate to lymphoid cells.
- The targeted delivery to lymphatic tissue should allow for a low dose and minimal systemic levels of tenofovir.
- Chronic safety studies in dogs and rats demonstrate a greater therapeutic index relative to TDF.
**GS-7340: Targeting Lymphoid Cells**

- GS-7340 is 400-fold more potent than tenofovir in PBMCs\(^1\)
- GS-7340 is 200-fold more stable in plasma than TDF resulting in circulating levels of prodrug\(^1\)
- GS-7340 is rapidly metabolized inside the lysosomes of lymphoid cells by the enzyme cathepsin A\(^2\)

\(^1\) Lee et al. Antimicrob Agents Chemother 2005
Increased Distribution to PBMCs *In Vivo*

Plasma to PBMC ratio following administration of TFV, TDF or GS-7340 to dogs (10 mg-eqv/kg)\(^1\)

M Markowitz, et al., CROI 2011; Paper # 152LB.

\(^1\)Lee et al. Antimicrob Agents Chemother 2005
Objectives

• Primary Objectives
  – To evaluate the antiviral potency of 2 different doses of GS-7340 as compared to TDF
    • Primary endpoint: DAVG at Week 2
  – To determine the safety of GS-7340 over 14 days

• Secondary Objectives
  – To determine the plasma and intracellular PK of GS-7340
  – To determine the viral dynamics of HIV-1 RNA in plasma
• HIV-1-infected adults
  – ART Treatment-naïve
  – HIV-1 RNA ≥ 15,000 c/mL
  – CD4 count ≥ 200 cells/mm³
• Randomized, double-blind 3 arm study
  – TDF 300 mg (active control arm)
  – GS 7340 - 50mg
  – GS 7340 - 150 mg
• Monotherapy for 14-day once-daily dosing
# Baseline Characteristics

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>TDF 300mg (N=10)</th>
<th>GS-7340 50 mg (N=10)</th>
<th>GS-7340 150 mg (N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>6</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Black</td>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Latino</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>34.8 ± 7.6</td>
<td>36.6 ± 9.7</td>
<td>35.4 ± 6.5</td>
</tr>
<tr>
<td>Sex (males)</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Mean HIV-1 RNA (log_{10} copies/mL)</td>
<td>5.03 ± 0.77</td>
<td>4.73 ± 0.58</td>
<td>4.72 ± 0.30</td>
</tr>
<tr>
<td>Mean CD4 cell count</td>
<td>384 ± 153</td>
<td>454 ± 201</td>
<td>432 ± 108</td>
</tr>
</tbody>
</table>

M Markowitz, et al., CROI 2011; Paper # 152LB.
# Primary Efficacy Endpoint

<table>
<thead>
<tr>
<th>Treatment (10 pts/arm)</th>
<th>Mean DAVG$<em>2$ [log$</em>{10}$ c/mL]</th>
<th>p-value vs. TDF 300 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS-7340 50 mg</td>
<td>-0.95 ± 0.32</td>
<td>0.0211</td>
</tr>
<tr>
<td>TDF 300 mg</td>
<td>-0.54 ± 0.32</td>
<td>-</td>
</tr>
<tr>
<td>GS-7340 150 mg</td>
<td>-1.07 ± 0.14</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
## Viral Dynamics

<table>
<thead>
<tr>
<th>Treatment (10 pts/arm)</th>
<th>Mean $\Delta VL$ Day 14 [log$_{10}$ c/mL]</th>
<th>p-value of mean $\Delta VL$ vs. TDF 300 mg</th>
<th>Mean first phase decay slope</th>
<th>p-value of mean decay slope vs. TDF 300 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF 300 mg</td>
<td>- 0.94 ± 0.49</td>
<td>-</td>
<td>- 0.36 ± 0.14</td>
<td>-</td>
</tr>
<tr>
<td>GS-7340 50 mg</td>
<td>- 1.57 ± 0.53</td>
<td>0.0257</td>
<td>- 0.63 ± 0.13</td>
<td>0.0003</td>
</tr>
<tr>
<td>GS-7340 150 mg</td>
<td>- 1.71 ± 0.24</td>
<td>0.0010</td>
<td>- 0.64 ± 0.13</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

M Markowitz, et al., CROI 2011; Paper # 152LB.
Viral Dynamics

\[ \Delta \text{Viral Load from Baseline (log}_{10} \text{c/mL)} \]

Day

TDF 300 mg
GS-7340 50 mg
GS-7340 150 mg

M Markowitz, et al., CROI 2011; Paper # 152LB.
Tenofovir Levels in Plasma: PK Profile on Day 1

- AUC<sub>0-24h</sub>
- TDF 300 mg
- GS-7340 150 mg GS-7340 50 mg

* p-value <0.001

M. Markowitz, et al., CROI 2011; Paper # 152LB.
Tenofovir Diphosphate in PBMCs

Day 3

- TDF 300 mg: 8x
- GS-7340 50 mg: 18x*
- GS-7340 150 mg: 33x

Day 14

- TDF 300 mg: 4x

* p-value <0.05
Safety and Resistance

- No dose interruptions or discontinuations
- No serious adverse events
- No clinically significant laboratory abnormalities
- Most frequent adverse events were mild to moderate headache and nausea
- No resistance mutations to GS-7340 or TDF were detected at day 14 in any subject
Summary

- Monotherapy with GS-7340 at 50 or 150 mg led to significantly greater decreases in HIV-1 RNA and at lower systemic tenofovir exposures than with TDF 300 mg
- GS-7340 is a next generation oral prodrug of tenofovir that has the potential to improve upon the efficacy and safety of TDF for the treatment of HIV
- The lower dose of GS-7340 will permit the development of new single tablet regimens that are not possible today
- GS-7340 has the potential of making tenofovir more widely available in resource limited settings given the relative manufacturing expense compared to TDF
BMS-663068
Inhibidores del acoplamiento
Pharmacodynamics, Safety, and Pharmacokinetics of BMS-663068, a Potentially First-in-Class Oral HIV Attachment Inhibitor

Richard Nettles and Dirk Schurmann, Li Zhu, Michele Stonier, Shu-Pang Huang, Caly Chien, Mark Krystal, Megan Wind-Rotolo, Richard Bertz and Dennis Grasela

HIV ATTACHMENT INHIBITOR

BMS-663068

Conference on Retroviruses and Opportunistic Infections. February 27 - March 2, 2011
Boston, MA
Abstract J-126
HIV-1 Attachment Inhibition to Block HIV-1 Entry

CD4 Binding → Coreceptor Binding → Virus-Cell Fusion

Attachment inhibitor

gp41 → gp120

CD4

Cell membrane

CCR5/CXCR4 (R5/X4)

CCR5 antagonists

CXCR4 antagonists

Fusion inhibitors
Proof of Concept Achieved With Prior HIV-1 Attachment Inhibitor (BMS-448043)

Change in Plasma HIV-1 RNA, (log10 copies/mL)

Treatment period

Bars show 90% Confidence Intervals

BMS-663068: Prodrug of a Next Generation HIV-1 Attachment Inhibitor BMS-626529

- Increased potency (~6-fold) and slower off-rate (~16-fold) compared to prior attachment inhibitor (BMS-488043)
- Prodrug and extended release formulations developed to address dissolution issues and rapid clearance
- Spectrum of activity against HIV-1 with median IC\textsubscript{50} in low nM range
- Synergistic or additive antiviral effects in combination with 19 marketed anti-HIV drugs

BMS-663068 is: (3-(2-(4-benzoylpiperazin-1-yl)-2-oxoacetyl)-4-methoxy-7-(3-methyl-1H-1,2,4-triazol-1-yl)-1H-pyrrolo[2,3-c]pyridin-1-yl)methyl dihydrogen phosphate; BMS-626529 is: 1-(4-benzoylpiperazin-1-yl)-2-(4-methoxy-7-(3-methyl-1H-1,2,4-triazol-1-yl)-1H-pyrrolo[2,3-c]pyridin-3-yl)ethane-1,2-dione

BMS HIV-1 attachment inhibitor non-clinical profile is reported in poster presentation no. 518
BMS-663068: Generally Well Tolerated With a Favorable PK Profile in Early Development

> 200 non-infected and 50 HIV-1-infected subjects in 8 clinical studies across a dose range of 20 to 2400 mg total daily dose

- Double-blind, placebo controlled, sequential, ascending single-dose study of 20-1000 mg
- Double-blind, placebo-controlled, sequential, 14-day ascending dose study of 100 mg Q8H to 1200 mg Q12H
Proof of Concept Study: Design

**HIV-1 clade B-infected males and females**

**Antiretroviral naive or experienced (off-treatment for > 8 weeks)**

**Plasma HIV-1 RNA > 5000 copies/mL**

**CD4+ T-cell Count > 200 cells/μL**

<table>
<thead>
<tr>
<th>Treatment period</th>
<th>Outpatient visit</th>
<th>Day 50 (±3 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMS-663068 600 mg Q12H + RTV 100 mg Q12H</strong></td>
<td><strong>N = 10</strong></td>
<td><strong>Outpatient visit and discharge</strong></td>
</tr>
<tr>
<td><strong>BMS-663068 1200 mg QHS + RTV 100 mg QHS</strong></td>
<td><strong>N = 10</strong></td>
<td></td>
</tr>
<tr>
<td><strong>BMS-663068 1200 mg Q12H + RTV 100 mg Q12H</strong></td>
<td><strong>N = 10</strong></td>
<td></td>
</tr>
<tr>
<td><strong>BMS-663068 1200 mg Q12H + RTV 100 mg QAM</strong></td>
<td><strong>N = 10</strong></td>
<td></td>
</tr>
<tr>
<td><strong>BMS-663068 1200 mg Q12H</strong></td>
<td><strong>N = 10</strong></td>
<td></td>
</tr>
</tbody>
</table>

Inpatient days: day -1 to day 11
50 subjects enrolled (safety population)

48 subjects with steady-state PK

39 subjects completed dosing and had baseline IC$_{50}$ \leq 0.1 \mu M (included in PD subpopulation)

2 ineligible subjects were discontinued from dosing

2 subjects without baseline IC$_{50}$ value

7 subjects with baseline IC$_{50}$ > 0.1 \mu M$^\dagger$

$^\dagger$Phenosense® Entry Assay (Monogram Biosciences)
## Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>600 mg Q12H + RTV 100 mg Q12H (N = 10)</th>
<th>1200 mg QHS + RTV 100 mg QHS (N = 10)</th>
<th>1200 mg Q12H + RTV 100 mg Q12H (N = 10)</th>
<th>1200 mg Q12H + RTV 100 mg QAM (N = 10)</th>
<th>1200 mg Q12H (N = 10)</th>
<th>Overall (N = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>9/1</td>
<td>9/1</td>
<td>10/0</td>
<td>10/0</td>
<td>47/3</td>
<td></td>
</tr>
<tr>
<td>Age, years; median (min, max)</td>
<td>44.5 (20-48)</td>
<td>38.0 (25-70)</td>
<td>43.0 (31-48)</td>
<td>40.0 (26-48)</td>
<td>41.5 (26-48)</td>
<td>42.0 (20-70)</td>
</tr>
<tr>
<td>Antiretroviral treatment: naïve/experienced</td>
<td>7/3</td>
<td>7/3</td>
<td>7/3</td>
<td>6/4</td>
<td>7/3</td>
<td>34/16</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA, log_{10} copies/mL; median (min, max)</td>
<td>4.74 (4.034-5.173)</td>
<td>4.40 (3.771-4.854)</td>
<td>4.41 (3.297-5.317)</td>
<td>4.24 (3.776-6.066)</td>
<td>4.29 (3.540-5.324)</td>
<td>4.40 (3.297-6.066)</td>
</tr>
<tr>
<td>CD4+ T cells, cell/μL; median (min, max)</td>
<td>426.0 (232-878)</td>
<td>422.0 (314-921)</td>
<td>414.0 (206-575)</td>
<td>462.5 (217-877)</td>
<td>456.5 (229-658)</td>
<td>432.0 (206-921)</td>
</tr>
<tr>
<td>Eligible subjects with IC_{50} ≤ 0.1 μM</td>
<td>6</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>6</td>
<td>39</td>
</tr>
</tbody>
</table>
Median Maximum Change in HIV-1 RNA From Baseline With Monotherapy

- 600 mg Q12H + 100 mg RTV Q12H (N = 9)  -1.64
- 1200 mg QHS + 100 mg RTV Q12H (N = 9) -1.59
- 1200 mg Q12H + 100 mg RTV Q12H (N = 10) -1.78
- 1200 mg Q12h + 100 mg RTV QAM (N = 10) -1.63
- 1200 mg Q12H (N = 10) -1.22
- Overall (N = 48) -1.64
Median Maximum Change in HIV-1 RNA From Baseline With Monotherapy*

- 600 mg Q12H + 100 mg RTV Q12H (N = 6) -1.76
- 1200 mg QHS + 100 mg RTV QHS (N = 9) -1.59
- 1200 mg Q12H + 100 mg RTV Q12H (N = 9) -1.77
- 1200 mg Q12h + 100 mg RTV QAM (N = 9) -1.66
- 1200 mg Q12H (N = 6) -1.60
- Overall (N = 39) -1.69

*Excluding ineligible subjects and subjects with missing baseline IC_{50} value or baseline IC_{50} > 0.1 μM.
Median Increase in CD4+ and CD8+ T-Cell Counts at Day 8*

* Excluding ineligible subjects and subjects with missing baseline IC₅₀ value or baseline IC₅₀ > 0.1 μM.
Mean BMS-626529 Plasma Concentrations on Day 8

- 600 mg Q12H + RTV 100 mg Q12H
- 1200 mg QHS + RTV 100 mg QHS
- 1200 mg Q12H + RTV 100 mg Q12H
- 1200 mg Q12H + RTV 100 mg QAM
- 1200 mg Q12H

Median protein binding adjusted IC₅₀ = 9.6 ng/mL** (N = 46)

* Protein binding adjusted IC₅₀ (ng/mL) = sc × mw × IC₅₀ (μM)/fu; where sc is a factor that scales IC₅₀ from IC₅₀ (sc = 5.5); mw is the molecular weight of BMS-626529 free base (mw = 473.48 g/mol); fu is the mean estimated unbound fraction of BMS-626529 (fu = 0.14)
IC$_{50}$: a Potential Predictor of the HIV-1 RNA Response

*Phenosense® Entry Assay (Monogram Biosciences); limit of quantitation = 0.1 µM.
## Adverse Events

<table>
<thead>
<tr>
<th></th>
<th>600 mg Q12H + RTV 100 mg Q12H (N = 10)</th>
<th>1200 mg QHS + RTV 100 mg Q12H (N = 10)</th>
<th>1200 mg Q12H + RTV 100 mg QAM (N = 10)</th>
<th>Overall (N = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1 AE(s), n (%)</td>
<td>8 (80)</td>
<td>5 (50)</td>
<td>9 (90)</td>
<td>39 (78)</td>
</tr>
<tr>
<td>Deaths, n (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>≥1 serious AE(s), n (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Discontinuations due to AE(s), n (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Treatment-related AEs*, n (%)</td>
<td>7 (70)</td>
<td>3 (30)</td>
<td>7 (70)</td>
<td>33 (66)</td>
</tr>
<tr>
<td>Incidence of AEs reported for at least 5 subjects, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>4 (40)</td>
<td>3 (30)</td>
<td>4 (40)</td>
<td>18 (36)</td>
</tr>
<tr>
<td>Rash</td>
<td>2 (20)</td>
<td>1 (10)</td>
<td>2 (20)</td>
<td>8 (16)</td>
</tr>
<tr>
<td>Micturition urgency</td>
<td>1 (10)</td>
<td>1 (10)</td>
<td>1 (10)</td>
<td>7 (14)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>1 (10)</td>
<td>0</td>
<td>3 (30)</td>
<td>6 (12)</td>
</tr>
</tbody>
</table>

* All AEs were Grade 1 or 2; AE: adverse event.
Summary

- BMS-663068, an oral prodrug of the HIV-1 attachment inhibitor BMS-626529, was generally safe and tolerated in Phase I-IIa studies
- Short-term monotherapy dosing in HIV-1-infected subjects resulted in a substantial decline in plasma HIV-1 RNA with increases in CD4+ lymphocytes
- PK/PD profile supports assessing lower doses than currently studied, given once or twice daily, without the requirement for RTV co-administration in patients with susceptible virus
- Overall, the data support initiation of Phase IIb clinical trials of BMS-663068 as part of combination antiretroviral therapy
  - A Phase IIb study in treatment-experienced subjects is planned to start in 2011
Infusión de CD4 autólogos R5/X4-defectivos
A Novel Approach to HIV Therapy: Successful and Persistent Engraftment of ZFN-Modified CCR5-Disrupted Autologous CD4 T-cells (SB-728-T) in Aviremic HIV-infected Subjects on HAART

Jacob Lalezari¹, Ronald Mitsuyasu², Steven Deeks³, Shelley Wang⁴, Gary Lee⁴, Shirley Clift⁴, Katherine Haenfling⁴, Michael Holmes⁴, Philip Gregory⁴, Marty Giedlin⁴, Winson Tang⁴ and Dale Ando⁴

¹Quest Clinical Research, San Francisco, CA; ²UCLA, Los Angeles, CA; ³UCSF, San Francisco, CA and ⁴Sangamo BioSciences Inc, Richmond, CA
Sangamo Phase I Study (SB-728-T): Background and Rationale

- CCR5 is the major co-receptor for HIV entry
- CCR5 delta-32 mutation produces a nonfunctional form of the protein
  - Homozygotes are resistant to HIV infection
  - Heterozygotes have slower disease progression
- The “Berlin Patient” is HIV-free w/o HAART for 3.5 years following hematopoietic stem cell transplant (HSC) from an allogeneic, HLA matched, CCR5 delta-32 donor.
- Zinc Finger Nuclease (ZFN) technology enables precise genetic modification of CCR5 resulting in elimination of receptor expression.
- The therapeutic potential of CCR5 modification as seen in the natural mutation can be extended with ZFN modification of autologous CD4+ T cells in HIV subjects.
Zinc Finger Nucleases (ZFNs) “Designer Restriction Enzymes”

- Comprised of two domains:
  - Nuclease domain of FokI restriction enzyme
  - Engineered zinc finger protein (ZFP) provides DNA binding specificity
  - Targets 24-bps of DNA

- ZFN cleaves as a heterodimer within a 5-6 bp gap between the two binding domains

- Delivered with a non-integrating, replication-deficient adenoviral vector that transiently expresses the ZFNs
Targeting the CCR5 Locus with ZFNs

Δ32 mutation

CCR5 ZFN modification
Site 165

ZFN pairs targeted to region upstream of the Δ32 mutation
Mechanism of ZFN-mediated Targeted CCR5 Gene Disruption

1. Endogenous CCR5 gene targeted for disruption

2. ZFNs dimerize and introduce a double stranded DNA break in the CCR5 gene
   Break repaired by either homologous or non-homologous end-joining (NHEJ) – resulting in permanent CCR5 gene disruption

3. CCR5 gene disrupted

A 5-bp duplication (Pentamer) occurs in 25% of modified cells at target site allowing PCR quantification
Disruption of CCR5 Does Not Affect Human CD4+ T-cell Proliferation

- Efficient CCR5 disruption can be achieved at clinical scale with an approximate 25% modification level.
- ZFN disrupted T cells exhibit similar proliferation rates as non-disrupted T cells.
SB-728-T GMP Manufacturing Process:
Autologous ZFN CCR5-Disrupted CD4+ T-cells

1. Leukapheresis
2. Monocyte and CD8+ T cell depletion → Enriched CD4+
3. Activate with Anti-CD3/28 beads
4. Adenoviral SB-728 transduction and CCR5 modification of CD4 cells
5. Expansion in WAVE
6. Cryopreserve cell product (SB-728-T) ~25% CCR5 modification
7. Infuse
**Sangamo SB-728-0902**

**Phase 1 Study Design**

- Open label, single-dose study
- Study population – HIV+ subjects on HAART
  - Aviremic
  - CD4 T-cells 200 - 500 cells/mm$^3$
- Single infusion of SB-728-T
  - Cohort 1 (N=3): $0.5 - 1.0 \times 10^{10}$ cells
  - Cohort 2 (N=3): $2.0 \times 10^{10}$ cells
  - Cohort 3 (N=3): $3.0 \times 10^{10}$ cells

**Clinical Outcomes**
- Safety and tolerability
- Change in CD4 count, CD4:CD8 ratio
- Engraftment and expansion, persistence and distribution of ZFN CCR5 disrupted T-cells
## SB-728-902 Demographics

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Sex</th>
<th>Ethnicity</th>
<th>Age</th>
<th>CD4 Count</th>
<th>% CD4</th>
<th>CD4:CD8</th>
<th>Viral Load</th>
<th>Years of HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01-102</td>
<td>M</td>
<td>Hispanic</td>
<td>52</td>
<td>376</td>
<td>34.2</td>
<td>1.10</td>
<td>&lt;48</td>
<td>25</td>
</tr>
<tr>
<td>01-103</td>
<td>M</td>
<td>Caucasian</td>
<td>53</td>
<td>269</td>
<td>19.2</td>
<td>0.44</td>
<td>&lt;48</td>
<td>20</td>
</tr>
<tr>
<td>01-104</td>
<td>M</td>
<td>Caucasian</td>
<td>40</td>
<td>272</td>
<td>27.2</td>
<td>0.63</td>
<td>&lt;48</td>
<td>21</td>
</tr>
<tr>
<td>Cohort 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01-201</td>
<td>M</td>
<td>Caucasian</td>
<td>54</td>
<td>450</td>
<td>28.1</td>
<td>0.52</td>
<td>&lt;48</td>
<td>30</td>
</tr>
<tr>
<td>01-203</td>
<td>M</td>
<td>Caucasian</td>
<td>53</td>
<td>281</td>
<td>16.5</td>
<td>0.30</td>
<td>&lt;48</td>
<td>21</td>
</tr>
<tr>
<td>02-302</td>
<td>M</td>
<td>Hispanic</td>
<td>53</td>
<td>384</td>
<td>25.6</td>
<td>0.78</td>
<td>&lt;48</td>
<td>19</td>
</tr>
</tbody>
</table>
SB-728-T Infusion is Safe and Well Tolerated (Cohort 1 & 2)

- **Serious Adverse Events** - None reported to date with a median follow up of 192 days (range: 85-366 days)

- **Adverse Events** - 32 AEs reported by 6 subjects
  - 30 of mild severity and 2 of moderate severity (flatulence, sweats)
  - 24 drug related occurring within 48 hrs of infusion
    - Include: chills, fever, headache, sweats, dizziness, fatigue, and a “garlic” body odor
  - All AEs were reversible and resolved without sequelae
  - No LFT or other lab abnormalities

- **Immunogenicity**
  - Engraftment and expansion of CCR5 disrupted T cells despite a transient increase in anti-adenoviral antibodies post-infusion
  - One subject with higher pre-infusion anti-adenoviral antibodies had lower level engraftment
Increased CD4 T-cell Counts from Baseline after Single SB-728-T Infusion

Sustained increase from baseline observed in 5 of 6 subjects at most time points
Normalization of CD4:CD8 T-cell Ratio after Single SB-728-T Infusion

CD4:CD8 reversal (from <1 to >1) in 3 of 5 subjects
Analysis of Fold Increase of CCR5-Modified Cells in Subject PBMC by Pentamer Assay Shows Robust Engraftment

Median 2.9-fold increase of ZFN-modified cells at D14 post-infusion, suggests in vivo expansion
Median Persistence in Peripheral Blood of ZFN-Modified Cells at D90 by Pentamer Assay is $15,504/10^6$ or 1.6% of CD4 T-cells
ZFN-Modified CD4 T-cells Traffic and Persist in the Rectal Mucosa (Median 16,891/10^6 or 1.7% at 90 Days by Pentamer Assay)
Sangamo SB-728-0902 Summary

- **SB-728-T** can be manufactured at doses of 10-30 billion cells from a single apheresis with a CCR5 disruption frequency of ~25%

- **SB-728-T** treatment is well-tolerated
  - Minor reversible infusion-related symptoms

- Improved and sustained increase in total CD4+ T-cell counts seen in 5/6 subjects

- Normalization of CD4:CD8 ratios seen in 3/5 subjects

- **ZFN-modified T-cells** engraft, expand, and persist in peripheral blood
  - ZFN-modified CD4+ T-cells detected at frequencies up to 7-fold higher (median 2.9) than predicted input on day 14
  - Expansion of ZFN-modified T cells in PBMC may be due to cell proliferation and/or altered distribution

- **ZFN-modified T-cells** engraft and persist in rectal mucosa
  - Engraftment and persistence of ZFN-modified T cells in rectal mucosa demonstrated normal homing to this important tissue
Conclusion and Next Steps

- Preliminary data from this Phase 1 study (SB-728-902) suggest that ZFN-mediated gene-disruption of CCR5 provides a feasible approach to HIV therapy and offers the hope of providing a protected reservoir of CD4+ T-cells that are resistant to HIV-infection.

- These data support the evaluation of SB-728-T in treatment naïve viremic subjects in an ongoing Phase 1/2 trial SB-728-1002.
Creating HIV-resistant CD4+ T cells with CXCR4- zinc finger nucleases

Craig Wilen
Lab of Robert Doms
University of Pennsylvania
Long term-goal: recapitulate the Berlin patient by genome editing the HIV coreceptor genes

- Adoptive therapy phase I trial with CCR5-ZFNs is currently underway.
- ~50% of ART-experienced individuals have R5X4 or X4 HIV.
- CXCR4-ZFNs may protect against X4 HIV and can be combined with CCR5-ZFNs treatment to create completely HIV-resistant CD4+ T cells.
- By disrupting CXCR4 in CD4+ T cells we avoid toxicities associated with systemic disruption.
X4-ZFNs specifically bind, cleave, and disrupt the cxcr4 gene
CXCR4-ZFNs efficiently disrupt *cxcr4* in CD4+ T cells and do not adversely affect cell growth.
The most common CXCR4 mutation is an in-frame deletion that does not traffic to the cell surface

- 80% of mutations are deletions.
- In-frame deletions occur 2.5 fold more frequently than predicted.
- An 18bp deletion, CXCR4Δ18, is the most common mutation and does not traffic to the cell surface.
X4-ZFN treatment of CD4+ T cells preserves cell growth and viability in the presence of HIV
Survival advantage in the presence of HIV is due to CXCR4 disruption

19 days post infection
CXCR4 disruption confers protection from X4 HIV in humanized mice

- X4-ZFNs conferred protection by 14 dpi, but this effect waned over time.
- Evolution or outgrowth of preexisting R5X4 HIV observed in X4-ZFN but not R5-ZFN-treated mice.
Conclusions

- CXCR4-ZFNs specifically and efficiently disrupt CXCR4.
- X4-ZFNs confer robust protection of CD4+ T cells from X4 HIV challenge in vitro.
- CXCR4 disruption confers protection from X4 HIV in humanized mice, resulting in outgrowth of R5X4 HIV.
- Future studies aim to combine CCR5- and CXCR4-ZFNs to eliminate HIV coreceptor expression.
“La crisis se produce cuando lo viejo no acaba de morir y cuando lo nuevo no acaba de nacer”

Bertolt Brecht
(1898-1956)