Lloyd Klickstein, MD, PhD on behalf of the Novartis/GenVec/KUMC Team

RAC meeting
December 04, 2013
## CGF166 Team Attendees

<table>
<thead>
<tr>
<th>Novartis</th>
<th>Function</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Team Member</strong></td>
<td><strong>Function</strong></td>
<td><strong>Location</strong></td>
</tr>
<tr>
<td>Rhett Behrje</td>
<td>Clinical Trial Leader</td>
<td>East Hanover, NJ</td>
</tr>
<tr>
<td>Constance Clairmont</td>
<td>Regulatory Affairs</td>
<td>Cambridge, MA</td>
</tr>
<tr>
<td>Lois Hinman</td>
<td>Regulatory Affairs</td>
<td>East Hanover, NJ</td>
</tr>
<tr>
<td>Lloyd Klickstein</td>
<td>Translational Medicine Expert</td>
<td>Cambridge, MA</td>
</tr>
<tr>
<td>Andrea Knight</td>
<td>Project Manager</td>
<td>Cambridge, MA</td>
</tr>
<tr>
<td>Tim MacLachlan</td>
<td>Toxicologist</td>
<td>Cambridge, MA</td>
</tr>
<tr>
<td>Peter McArdle</td>
<td>Regulatory Affairs</td>
<td>East Hanover, NJ</td>
</tr>
<tr>
<td>Mark Milton</td>
<td>Biodistribution/Immunogenicity Expert</td>
<td>Cambridge, MA</td>
</tr>
<tr>
<td>Jens Praestgaard</td>
<td>Statistical Scientist</td>
<td>East Hanover, NJ</td>
</tr>
<tr>
<td>Sue Stevenson</td>
<td>Research Lead</td>
<td>Cambridge, MA</td>
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**GenVec**

<table>
<thead>
<tr>
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<th>Function</th>
<th>Location</th>
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<tbody>
<tr>
<td>Doug Brough</td>
<td>Research</td>
<td>Gaithersburg, MD</td>
</tr>
<tr>
<td>Bryan Butman</td>
<td>Development</td>
<td>Gaithersburg, MD</td>
</tr>
</tbody>
</table>

**Kansas University Medical Center (KUMC)**

<table>
<thead>
<tr>
<th>Team Member</th>
<th>Function</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hinrich Staecker</td>
<td>Principal Investigator</td>
<td>Kansas City, KS</td>
</tr>
</tbody>
</table>
Sensorineural Hearing Loss & Vestibular Dysfunction:
29 million Americans (age 20-69) had hearing loss in 2003-2004

- **Disabling conditions with high and increasing prevalence worldwide**
- **90% of hearing loss is sensorineural**
  - Due to damage to hair cells in cochlea
  - Various causes with similar pathology: age, drugs, noise
  - Hair cells are essential for hearing and balance function
  - **Current management is sub-optimal; no pharmacological treatments available**

- **Vestibular dysfunction**
  - Also due to damage to hair cells
  - Vestibular damage may cause dizziness, vertigo and nausea

Sensory Hair Cells are Essential for Hearing and Balance


- Located in the organ of Corti throughout the cochlea and in the vestibular system; sensitive to injury
- Critical for the mechanosensory signal transduction of auditory and positional stimuli
- Humans are born with ~30,000 sensory cells that cannot regenerate if destroyed, unlike avian
- Organ of Corti:
  - **IHC**: responsible for signal transduction
  - **OHC**: enhances the cochlear sensitivity and frequency selectivity
- Vestibular Organ:
  - Type I and Type II HC: responsible for sensation of motion and spatial orientation
ATOH1 is a Master Regulator Responsible for Sensory Hair Cell Development

- Atonal is a bHLH transcription factor involved in cell fate determination; differentiation; cell cycle and apoptosis

- Atoh1 is expressed during embryonic development and epigenetically silenced in adult tissues

- Hair cells are lost in Atoh1 knockout mice

- Atonal is highly conserved across species:
  - Atoh1 = Math1 = Hath1

- Forced expression of Atoh1 leads to the ectopic formation of hair cells that are capable of attracting spiral ganglion innervations

- In animal models, adenoviral-mediated expression of Atoh1 in the mature deaf inner ear induces regeneration of hair cells and improves hearing thresholds

Notch Pathway Inhibition of Atonal Expression

Vector backbone safely administered to over 3000 subjects
- E1, E3, E4-deleted Adenovirus serotype 5 (Ad5) vector
- Replication-incompetent, non-integrating vector
- Engineered to deliver the human atonal transgene expressed under the control of the GFAP (glial fibrillary acidic protein) specific promoter to restrict expression to inner ear supporting cells
Therapeutic Hypothesis:
Hair cell regeneration by transient Atoh1 expression

Image © Novartis
CGF166-Mediated Hair Cell Regeneration

Mouse and Human utricle explants after aminoglycoside injury

A. Untreated

B. Neomycin

C. Neomycin + CGF166

Mouse

Magnification at 20X

2.75 x 10^6vp/µL

Human

Magnification at 60X

1x 10^7vp/µL

A. Untreated

B. Neomycin + Ad5.GFP

C. Neomycin + CGF166

1x 10^7vp/µL
Inner Ear Drug Delivery

*Intra-Labyrinthine (IL) delivery route is species specific*

**Rhesus** Site of IL Delivery: Round Window Membrane OR Stapes Foot Plate

**Rodent** Site of IL Delivery: Posterior Semi-Circular Canal

**Human** Site of IL Delivery: Stapes Foot Plate

**CGF166 In Vivo Delivery and Hair Cell Regeneration**

*Intra-Labyrinthine (IL) delivery in adult mice after vestibular hair cell loss*

**A. CGF166 vector delivery in vivo**

![Graph showing vector DNA levels](image)

<table>
<thead>
<tr>
<th>CGF166 Vector Dose (vp/µL)</th>
<th>Vector DNA (copies/µg genomic DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5x10^8</td>
<td>Mean ± SEM, n=4/group</td>
</tr>
<tr>
<td>1.8x10^8</td>
<td></td>
</tr>
<tr>
<td>5.5x10^7</td>
<td></td>
</tr>
<tr>
<td>1.8x10^7</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion:** The number of tissue-associated viral genomes increases in a dose-dependent manner, assessed 3 days after dosing.

**B. CGF166 utricle hair cell regeneration in vivo**

![Graph showing total number of hair cells](image)

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>Normal HC levels</th>
<th>Hair cells after toxic injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 month</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contralateral ear</th>
<th>CGF166-treated ear</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.75 x 10^8 vp, Mean ± SEM, n=4/group</td>
</tr>
</tbody>
</table>

* ^p<0.05  
* ^p<0.005

**Conclusion:** Hair cells regenerated to ≈60% of normal numbers and are stable over time (at least up to 4 months after treatment).
## Sensory Hair Cell Analysis

**Mouse kanamycin/furosemide ototoxin hearing loss model**

### Evaluation of the presence of sensory hair cells in the cochlea

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (vp/µL)</th>
<th>Apical 1</th>
<th>Apical 2</th>
<th>Medial</th>
<th>Basal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IHC</td>
<td>OHC</td>
<td>IHC</td>
<td>OHC</td>
</tr>
<tr>
<td>Normal</td>
<td>none</td>
<td>100</td>
<td>96</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>Injured</td>
<td>none</td>
<td>64</td>
<td>0</td>
<td>96</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5.5 x 10^8</td>
<td>72</td>
<td>3</td>
<td>87</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>5.5 x 10^7</td>
<td>48</td>
<td>0</td>
<td>68</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5.5 x 10^6</td>
<td>72</td>
<td>0</td>
<td>88</td>
<td>0</td>
</tr>
<tr>
<td>Injured + Ad5.GFAP.Math1</td>
<td>5.5 x 10^8</td>
<td>68</td>
<td>3</td>
<td>76</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5.5 x 10^7</td>
<td>68</td>
<td>0</td>
<td>68</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5.5 x 10^6</td>
<td>52</td>
<td>0</td>
<td>84</td>
<td>0</td>
</tr>
</tbody>
</table>

IHC: Inner hair cells; OHC: Outer hair cells; Percent of hair cells remaining, n=5 mice per group; Kanamycin/furosemide ototoxin-injury hearing loss mouse model used for studies.
Adenoviral *ATOH1* Gene Therapy *In Vivo* Partially Restores Auditory Function

Kanamycin/furosemide ototoxin-injury hearing loss model

*Recovery = -(dB ATOH1 tx groups – dB injured controls)*

**A.** Ad5.GFAP.Math1

**B.** CGF166

Mean ± SEM; *P<0.05*
Guidance on CGF166 Development from FDA

Interactions with CBER Office of Cellular, Tissue and Gene Therapy

- OCTGT has provided guidance to the program since 2010
- Four meetings with OCTGT reviewers to discuss non-clinical program and protocol design for the first-in-man study
- Guidance received in line with public guidance documents, including guidance for preclinical assessment of investigational cellular and gene therapy products and early phase clinical trials
- Agency considered efficacy model showing correlation between hair cell regeneration and functional hearing improvement critical for the program
- Design of GLP toxicology studies in rat and volume distribution studies discussed in detail in order to obtain OCTGT’s buy-in
- Detailed feedback on protocol design including selection of first dose, dose scaling, inclusion/exclusion criteria and duration of patient follow-up
- IND submission planned for Jan, 2014
Preclinical Safety Program Summary

Integration of multiple factors in a volume limited space

**Designed to answer two questions**

1. **what is the safety profile of CGF166 dosed in the inner ear?**
2. **what is a safe volume to administer to the inner ear?**

**Impact of administration volume to rat ear**
- Surgical delivery to semi-circular canal
- Dose escalation of vehicle from 3-10 µL

  *No adverse effects*: 3 µL chosen as top dose volume for subsequent rat studies

**Safety assessment of CGF166 in rat**
- Two (2-month and 6-month) GLP single dose rat toxicology studies
- IL and ICV routes (6 months)

  *No adverse effects observed at any dose level or route, including effects on hearing and balance*

**Distribution of CGF166 in rat**
- GLP biodistribution in rats at maximum feasible dose, multiple time points out to 6-months

  *CGF166 shows limited distribution outside of the dosed ear*

**Impact of administration volume to rhesus ear**
- Three studies conducted in rhesus monkey to assess effects of fluid administration using dosing via the middle ear

  *Top volume of 30 µL well tolerated with no effects on hearing and balance*

**Overall conclusions:**
- CGF166 dosed into the inner ear of rats has limited distribution beyond that site and was without adverse effects
- Administration of 30 µL to the inner ear of rhesus monkey is well tolerated
History of Rhesus Monkey Studies Supporting CGF166

- CBER feedback on preclinical safety program:
  - “We strongly recommend that you conduct a small study in a large animal species in order to identify a potentially safe injection volume and injection rate.”
  - Intention of studies was solely to evaluate volume administration on function of inner ear
  - Agreed with CBER that formal toxicity assessments would be in the rodent safety studies
    - Biodistribution
    - Systemic organ toxicity
    - Worst-case scenario of brain exposure

- Three studies performed:
  1. Pilot study in one rhesus monkey, performed at a CRO
  2. Volume ranging study in six rhesus monkeys, performed at a CRO
    - Challenges to accessing the inner ear via the EAC required an alternative access point
  3. Modified volume ranging study, performed at Johns Hopkins University
Clinical Plan Overview

Open-label, single dose, sequential cohorts design

- Recruit patients with severe acquired hearing loss
- 3 sites distributed across US
  - (Baltimore, Kansas City, San Francisco)
- Surgical delivery of drug under general anesthesia
  - Same surgical approach as used for routine stapedotomy
  - Surgical device designed and tested for Intra-Labyrinthine (IL) delivery
- Patients will be evaluated for 6 months
  - Safety evaluations at 1, 2 and 4 weeks and monthly thereafter
  - Efficacy evaluations monthly
  - Patients then roll over into 5 year follow up study
- Escalate dosing volume, hold CGF166 concentration constant
  - Infusion rate starting at 10 µL/min
- Invert ototoxicity criteria to determine threshold for clinically meaningful improvement
  - ≥10 dB pure tone threshold improvements at 2 or more frequencies or ≥ 20 dB improvement at single frequency
Key Inclusion/Exclusion Criteria

### Inclusion
- Patients 18-70 years old with acquired bilateral hearing loss
- Stable pure tone thresholds for at least 6 months
- Pure tone threshold average > 70 dB (but not deaf)
- Cochlear implantation criteria (score ≤50% on sentence recognition test in candidate ear and ≤60% in non-implanted ear when using hearing aids)
- MRI at screening to confirm anatomy appropriate and establish baseline
- Patients eligible for general anesthesia and surgery

### Exclusion
- Known genetic disease associated with hearing loss (e.g. connexin 26, Alport, etc.)
- Known cause of hearing loss that is associated with structural damage (e.g. penetrating trauma)
- Unilateral vestibular dysfunction in the non-treatment ear
**Rationale for Human Dose Scaling Based on Total Inner Ear Fluid Volume**

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Inner Ear Fluid Volume (µL)</th>
<th>Volume Delivered IL (µL)</th>
<th>Percent Total Inner Ear Fluid Volume</th>
<th>Vector Particles Delivered (vp)</th>
<th>Normalized Dose (vp/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL6 Mouse</td>
<td>2.50</td>
<td>1</td>
<td>40.0 %</td>
<td>$5.50 \times 10^6$</td>
<td>$2.20 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>$5.50 \times 10^7$</td>
<td>$2.20 \times 10^7$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>$5.50 \times 10^8$</td>
<td>$2.20 \times 10^8$</td>
</tr>
<tr>
<td>Wistar Rat</td>
<td>5.50</td>
<td>3 (1:10 dil)</td>
<td>54.5 %</td>
<td>$1.65 \times 10^8$</td>
<td>$3.00 \times 10^7$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>18.2 %</td>
<td>$5.50 \times 10^8$</td>
<td>$1.00 \times 10^8$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>54.5 %</td>
<td>$1.65 \times 10^9$</td>
<td>$3.00 \times 10^8$</td>
</tr>
<tr>
<td>Rhesus Monkey</td>
<td>59.4</td>
<td>10</td>
<td>16.8 %</td>
<td>N/A</td>
<td>*$8.47 \times 10^7$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>50.5%</td>
<td>N/A</td>
<td>*$2.53 \times 10^8$</td>
</tr>
<tr>
<td>Human</td>
<td>191</td>
<td>20</td>
<td>10.5%</td>
<td>$1.00 \times 10^{10}$</td>
<td>$5.24 \times 10^7$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>20.9%</td>
<td>$2.00 \times 10^{10}$</td>
<td>$1.05 \times 10^8$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90**</td>
<td>47.1%</td>
<td>$4.50 \times 10^{10}$</td>
<td>$2.35 \times 10^8$</td>
</tr>
</tbody>
</table>

1 The total inner ear fluid volume was determined at JHU by Micro-CT scanning of temporal bones from each of the listed species.
2 CGF166 drug concentration for nonclinical studies is $5.5 \times 10^8$ vp/µL and for clinical studies is $5.0 \times 10^8$ vp/µL
3 Dose scaling approach based on CBER/FDA feedback
*Theoretical dose prediction in rhesus monkey, as only vehicle was evaluated in volume escalation safety study
**Pre-clinical data will support dosing up to 96.5 µL
CGGF166X2201 Trial Design

NIH OBA protocol #1310-1260

Part A Safety Cohort #1 (N=3) for IL delivery 20 μL

- Dose 1 patient
  - Safety Data review (SDR)*
  - Dose 2nd patient
  - SDR*
  - Dose 3rd patient
  - Cohort #1 Full Safety Review (FDR)**

  ~4 wks

Part B Dose Volume Escalation Cohorts #2-5; N= 3

- Dose 1 patient
  - SDR*
  - Enroll remaining 2 patients
  - Cohort FDR**

  ~4 wks

The proposed dose volumes for cohorts 2-5 in the protocol may be changed based on emerging prior cohort safety data. All dose volumes will be between 20 and 90 μL

Part C Open Label, Efficacy Cohort #6 MTD; N= 20

- Enroll subjects
  - Cohort #6 FDR**

*Safety Data review (SDR) will consist of all data through 4 weeks post dose

**Full Safety Data review (FDR) will consist of all data through 4 weeks post dose
CGF166 Delivery System

Drug Product

Infusion Device

Infusion Pump
Delivery System Validation in Human Cadaveric Temporal Bone

Adaptation of routine stapedotomy procedure

A. Placement of speculum in external auditory canal

B. Tympanic membrane reflected to expose middle ear

C. Stapes footplate exposed

D. Stapedotomy created, cannula in view (arrow indicates stapedotomy)

E. Cannula placed and drug delivered

F. Cannula removed, stapedotomy sealed, tympanic membrane replaced
Primary, Secondary, Exploratory Outcomes

**Primary endpoint:**

- Change from baseline in pure tone audiometry measured at frequencies between 0.5 and 16 kHz
- A stable improvement of 20 dB at any frequency, or 10 dB at two frequencies, is considered clinically meaningful
- With 20 completing subjects study is powered to detect an improvement of 2.5 dB at any frequency
- Sample size will be re-estimated after Cohorts A and B based on observed test-retest variability. May be increased to 30 subjects.

**Secondary endpoints:**

- Speech recognition during audiometry visits
- Caloric nystagmography (when possible), Vestibular evoked myogenic potential (VEMP), subjective visual vertical at baseline and at 2 months after treatment.

**Exploratory endpoints:**

- Jacobson Dizziness Handicap Inventory (DHI)
- Hearing Handicap for Adults (HHIA)
- Tinnitus Reaction Questionnaire (TRQ)
Justification for Primary Endpoint

<table>
<thead>
<tr>
<th>dB Level</th>
<th>Noise Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Normal breathing</td>
</tr>
<tr>
<td>20</td>
<td>Rustling leaves</td>
</tr>
<tr>
<td>30</td>
<td>Quiet conversation</td>
</tr>
<tr>
<td>50</td>
<td>Normal conversation</td>
</tr>
<tr>
<td>60</td>
<td>Loud television</td>
</tr>
<tr>
<td>80</td>
<td>Noisy office</td>
</tr>
<tr>
<td>100</td>
<td>Loud car horn</td>
</tr>
<tr>
<td>120</td>
<td>Jet plane take-off (100 feet)</td>
</tr>
<tr>
<td>130</td>
<td>Threshold of pain</td>
</tr>
</tbody>
</table>

- The minimum criteria for efficacy are an “inversion” of established criteria for ototoxicity
- A 5 dB change is the margin of error for pure tone threshold assessment
- A 10 dB change is considered meaningful

<table>
<thead>
<tr>
<th>Change in dB Level</th>
<th>Noise Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 dB</td>
<td>Generally not perceptible</td>
</tr>
<tr>
<td>3 dB</td>
<td>Just barely perceptible</td>
</tr>
<tr>
<td>5 dB</td>
<td>Clearly noticeable</td>
</tr>
<tr>
<td>10 dB</td>
<td>Twice or ½ as loud</td>
</tr>
<tr>
<td>20 dB</td>
<td>Four times or ¼ as loud</td>
</tr>
</tbody>
</table>
Safety Assessments

- Physical examination & vital signs
- Otoscopy
- Hearing and balance assessment
- Neurological exam
- Pregnancy test
- Clinical laboratory evaluations (hematology, clinical chemistry, urinalysis)
- ECG
- MRI at screening and at 6 mo evaluation
- Sample collection for vector biodistribution/shedding (blood, saliva, nasal and ear swab)
- Immunogenicity assessment: serum anti-Ad5 antibody and anti-Hath1 antibody titers
Study Stopping Rules

- 3 or more patients with drug-related or surgical procedure-related SAEs are reported.
- At least 2 patients in the same cohort experience a similar AE considered severe and related to study drug or surgical procedure.
- Post-operative vestibular dysfunction that persists longer than 4 weeks in 2 patients.
- Post-operative hearing loss that persists longer than 8 weeks in 2 patients.
- 1 patient develops a severe systemic immune reaction to study drug.
- 1 patient develops a severe infection consistent with those caused by adenovirus (if confirmed to be related to study drug).
Summary of CGF166 Gene Therapy
A Novel Therapy for Hearing Loss

- Demonstrated ability to deliver material IL to cochlea and vestibular system (rodent efficacy and toxicology studies)
- Demonstrated efficacy for CGF166 in various models (explants and in vivo)
  - CGF166 treatment regenerated hairs cells that persist over time
  - Ad5.GFAP.Math1 & CGF166 regenerated hair cells that are functional and partially restored auditory function
- Toxicology studies designed with input from FDA
  - GLP rat toxicology showed CGF166 was safe at all tested doses
  - GLP rat biodistribution showed limited distribution of CGF166 beyond injected ear
  - Rhesus monkey volume escalation study showed safe range of volumes
- Clinical study designed to evaluate safety and efficacy of CGF166
  - Surgical procedure minor variation of well known stapedotomy procedure
  - Doses selected to show efficacy and safety in first dose
  - Safety parameters included to minimize patient’s risk