PRECLINICAL TRIALS REPORT

TUMORIGENICITY TEST IN VIVO:
ADIPOSE-DERIVED MESENCHYMAL STEM CELLS
PROCESSED WITH STEMOPTIMIZER NS 6.X

NeuroSyntek:
Pavel G. Molchanov
Elena N. Golubeva
Pavel M. Bulai
Taras N. Pitlik
Victor V. Boksha

Supported by:
Sylvia Jeewon Kim
Jieying Yang
Hong Zeng

Transgenic, Knockout and Tumor model Center, Stanford Cancer Institute,
School of Medicine, Stanford University, USA

November 2015 - April 2016
## CONTENTS

**FACILITIES**................................................................................................................................. 4

**PROTOCOLS**................................................................................................................................. 4

I  Stem cell cultivation procedure ................................................................................................. 4

II  Electroporation procedure (NeuroSyntek 6.x) ......................................................................... 5

III  Animal model tumorigenicity test ......................................................................................... 6

**RESULTS** ....................................................................................................................................... 9

**CONCLUSION** .............................................................................................................................. 11

**REFERENCES** .............................................................................................................................. 12

**APPENDIX A** ............................................................................................................................... 13

**APPENDIX B** ............................................................................................................................... 19

**APPENDIX C** ............................................................................................................................... 21
FACILITIES

Stem cells culture and expansion was conducted in San Jose BioCube, 5941 Optical Court, San Jose, CA 95138.

All animal tests were conducted by Jeewon Kim, PhD, Jieying Yang, MS in Transgenic, Knockout and Tumor model Center, Stanford Cancer Institute, School of Medicine, Stanford University, Stanford CA 94305, USA.

All animal experimentation protocols were approved by the Stanford University Animal Care and Use Committee.

PROTOCOLS

I Stem cell cultivation procedure

Cell Sample

Adipose-Derived Mesenchymal Stem Cells; Normal, Human (Product Code: PT-5006; Lot Number: 0000421627; Manufacture Date: 23-Jun-2014) were obtained from Lonza Walkersville Inc. (USA) (Appendix A, B).

General terms

Culture medium: DMEM-A (Dulbecco's Modified Eagle Medium with Streptomycin/Penicillin, containing 10% fetal bovine serum (FBS)
Additional reagents: phosphate-buffer solution pH = 7.2 (PBS), trypsin:EDTA (1:4)), saline.
Thaw the cells as described in [1] (if cells were previously cryopreserved). Expand for one week.

Cell culture procedure

1. Remove DMEM-A from Petri dish with cells gently. Wash cells with 4 ml of PBS. Remove the PBS.
2. Add 2 mL of trypsin:EDTA, place the dish in the incubator at 37°C for 3 minutes. Then add 4mL of DMEM-A (containing 20% of FBS) into the Petri. Pipette cells, put into a centrifuge tube.
3. Centrifuge at 1500rpm for 10 minutes at room temperature. Remove the supernatant, add 3mL of DMEM-A.
5. Seed cells on the chip of the NS 6.x system (hAdMSCs-NS) or in Petri dishes (hAdMSCs control) in DMEM-A containing 20% of FBS. Place into the CO2-incubator at 37°C.
6. If cells are not used within 24h, change the medium for DMEM-A (10% of FBS).

**Important:** Avoid high cells confluences (should be less than 30% - 40%), expand to 5x10^6 of cells, use only for 1 - 4 passages. Freeze if needed.
7. For the pre-clinical and clinical studies: trypsinize cells, wash with DMEM-A, centrifuge, replace DMEM-A medium, resuspend in the pharma-grade sterile saline (or saline with Sodium hyaluronate), obtain necessary cells concentration (see below).

**Stem cells cryopreservation**

1. Wash cells with 4mL PBS per 100mm Petri dish. Remove PBS gently.
2. Add 2mL trypsin:EDTA (1:1) to each Petri dish. Place in the CO2 incubator at 37°C for 3 minutes. Add 4mL of DMEM-A (20% FBS) per each P. dish. Resuspend, put the suspension into a centrifuge tube.
3. Centrifuge at 1500rpm, 20C for 10 minutes. Remove supernatant gently, add 6mL DMEM-A (20% FBS), resuspend. Count cells (as described above). Centrifuge.
4. Prepare cells freezing medium: 950μL DMEM-A (20% FBS)+50 μL DMSO per 1x10^6 of cells.
5. Remove supernatant from the tube, add freezing medium (1x10^6 of cells per 1mL of freezing medium). Resuspend the cells. Put 1mL of freezing medium with cells in a vial for freezing.
6. Keep at room temperature for 15 minutes. Keep at + 4°C for 15 minutes. Keep at -20°C for 2 hours. Afterwards, keep cells at -80°C until cells need to be used.

II **Electroporation procedure (NeuroSyntek 6.x)**

**Sample preparation:**

1. Remove medium from each well of the chip with cells.
2. Wash cells with 4mL of HEPES buffered saline.
3. Add 2.5mL of HEPES buffered saline into each well. Work with cells not more than 3h.

Prepare HEPES buffered saline with the following concentrations, filter before use:

<table>
<thead>
<tr>
<th></th>
<th>n [mol/L]</th>
<th>M [g/mol]</th>
<th>V [L]</th>
<th>M [g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.140</td>
<td>58.44</td>
<td>0.05</td>
<td>0.400</td>
</tr>
<tr>
<td>KCl</td>
<td>0.004</td>
<td>74.55</td>
<td>0.05</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Molar Mass</td>
<td>w/v (%)</td>
<td>pKa</td>
<td>Density (g/ml)</td>
</tr>
<tr>
<td>----------------</td>
<td>------------</td>
<td>---------</td>
<td>-----</td>
<td>---------------</td>
</tr>
<tr>
<td>CaCl(_2)×2H(_2)O</td>
<td>147.02</td>
<td>0.002</td>
<td>0.05</td>
<td>0.015</td>
</tr>
<tr>
<td>MgCl(_2)×6H(_2)O</td>
<td>203.31</td>
<td>0.001</td>
<td>0.05</td>
<td>0.010</td>
</tr>
<tr>
<td>HEPES (Na salt)</td>
<td>260.30</td>
<td>0.010</td>
<td>0.05</td>
<td>0.130</td>
</tr>
<tr>
<td>C(_6)H(_12)O(_6)</td>
<td>180.16</td>
<td>0.005</td>
<td>0.05</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Adjust pH to 7.2 - 7.3

For cells staining media use the following buffer solution:
HEPES buffered saline + BSA (BSA-MACS) [9.5 ml + 0.5 ml respectively]

**Light-induced electroporation procedure**

1. Wash cells with HEPES-BSA. Remove buffer.
2. In a separate tube mix 1mL HEPES-BSA and 50μL FcR Blocker, add to a well on the chip.
3. Incubate for 5 minutes at RT.
4. In a separate tube mix 1mL of HEPES-BSA and 50μL of CD105 PE (or any other marker of interest, labeled with PE), add to a well on the chip.
5. Incubate for 15 minutes at RT in the dark. Remove medium.
6. Wash with HEPES-BSA for 3 minutes. Remove medium.
7. Add 2mL - 3mL of HEPES (without BSA). Avoid light exposure. Install the chip in the NS 6.x system. Switch to the luminescence mode. Choose the area of the electroporation. Distinguish the target cells using NS6.x Software. Start the automated light-induced electroporation procedure.
8. After finishing the electroporation procedure, close the program, turn off the device.
9. Remove the module with cells from the slot.
10. Remove the media from each well, wash the cells with HEPES, add 4mL of DMEM (10% of FBS), place the module in the CO\(_2\)-incubator for further cells expansion.

**III Animal model tumorigenicity test**

**Cell sample**

1. Cells provided for all tests were generated in a lab-grade cell processing facility.
2. Cell lines used in the trial included:

**Mesenchymal Stem Cells**
Lipoaspirate derived from white adipose tissue
_Homo sapiens_, human

Product Code: PT-5006; Lot Number: 0000421627; Manufacture Date: 23-Jun-2014, Lonza Walkersville Inc. (USA) (Appendix A, B).

Quality control records of Mesenchymal Stem Cells included:
Input control:
Sterility Test
   Bacteria and Yeast: Negative
   Mycoplasma: Negative

Viral Test
   Hepatitis B: Negative
   Hepatitis C: Negative
   HIV: Negative

Specific Staining
   Positive expression for CD13, CD29, CD44, CD73, CD90, CD105, and CD166.
   Negative expression for CD14, CD19, and CD45.

Output control:
   IDEXX IMPACT II: All negative

HeLa cells
Cervix epithelial adenocarcinoma
   Homo sapiens, human negroid
Lot Number 14G005, ECACC, purchased from Sigma, USA (Appendix A,B)
Quality control records of HeLa cells included:

Input control:
   Sterility Test
      Bacteria and Yeast: Negative
      Mycoplasma: Negative
   Viral Test
      Hepatitis B: Negative
      Hepatitis C: Negative
      HIV: Negative

Output control:
   IDEXX IMPACT II: All negative

General Terms
1. Six- to eight-week-old female BALB/c nude mice should be used as the animal model (obtained from Charles River Laboratories Inc.) All mice should be kept under standard temperature, humidity, and timed lighting conditions and were provided with mouse chow and water ad libitum.
Note: All animal experimentation protocols should be approved by the animal ethics committee (Stanford University Animal Care and Use Committee).

2. Mice should be divided into the following groups:
   I. 6 mice - control-dose group (5x10^6 hAdMSCs-Control/kg B.W) (Approx. 1x10^5 cells per animal)
   II. 5 mice - study-dose group (5x10^6 hAdMSCs-NS/kg B.W) (Approx. 10^5 cells per animal)
   III. 4 mice - positive control group (HeLa cells; human cervix adenocarcinoma; 5x10^6 cells/kg B.W.), (Approx. 10^5 cells per animal)

   **Cells injection procedure**
   1. Adjust the concentration of the cell suspension to 5x10^6/mL viable cells in PBS.
   2. Set cell injection volume to 0.2mL in a low dead-volume syringe with 26G needle.
   3. Deliver cells to 36.6 C prior to the injection.
   4. Inject 0.2mL of the cell suspension subcutaneously unilaterally once with a slow rate 10uL/s for the injection volume.

   **Animals observation**
   1. Animals to be observed once a week for their clinical symptoms (vital signs, appearance, presence and extent of any abnormal response, etc.) for up to 4 first weeks.
   2. After 4 weeks the observation rate should be set to once per two weeks.
   3. Assess the presence of tumors, if any, once in a two weeks interval between the injection and study completion. Measure the tumor dimensions (if there are any tumors) using a caliper in two perpendicular dimensions, and calculate the tumor size using the equation 0.52 x Width x Width x Height (mm).
   4. After 12 weeks of observation sacrifice animals.

   **Reporting results and findings**
   1. Reports of macroscopic findings should be given for stem cells. Macroscopic findings include the conditions of mice during the test period and macroscopic tumor growth, if any. Visible metastases, if any, their location, and frequency should be reported.
RESULTS

Adipose-derived mesenchymal stem cells were purchased from Lonza and seeded on the 18th Jan 2016, HeLa cells (for positive control group of mice) were obtained from Sigma and seeded on the 21st Jan 2016 (Appendix A). Cells were cultured according to protocols provided by suppliers, in order to minimize adverse effects of unexpected cells transformation, proliferation activity modification, etc.

Fig. 1 Reflected light microscopy of cells for the injections. A – ADSCs, B – HeLa.

Fig. 2 Cells preparation with NS StemOptimizer. A – Control cells (Petri dishes) and processed stem cells (in NS-chips); B – on-chip cells sorting procedure; C – NS StemOptimizer setup
On the 24th of Jan cells (both cell lines) were partially frozen for further rodent pathogen, bacteria and mycoplasma testing and shipped to IDEXX BioResearch on the next day for the independent evaluation.

IDEXX report was received in 5 days with all positions tested being negative (Appendix C). One half of ADMSCs cells were processed with NS StemOptimizier in accordance with the previously developed protocol. Another half was expanded in polystyrene cell-culture treated Petri dishes (negative control). Cells viability was determined by trypan blue test. Both cell groups and HeLa cells were used for the injection in immunocompromised mice.

Cells injections were provided in two batches:

<table>
<thead>
<tr>
<th>03 Feb 2016 (1st Part)</th>
<th>Number of animals</th>
<th>Cells number injected per an. (viability)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSCs control</td>
<td>2 mice</td>
<td>4.0×10^5 (96 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.6×10^5 (98 %)</td>
</tr>
<tr>
<td>MSCs-operated with NeuroSyntek study</td>
<td>1 mouse</td>
<td>6.0×10^5 (93 %)</td>
</tr>
<tr>
<td>HeLa w/FBS (10%) positive control</td>
<td>2 mice</td>
<td>1.0×10^6 (80 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0×10^6 (80 %)</td>
</tr>
<tr>
<td>HeLa w/o FBS positive control</td>
<td>2 mice</td>
<td>1.8×10^6 (82 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.8×10^6 (80 %)</td>
</tr>
<tr>
<td>10 Feb 2016 (2nd Part)</td>
<td>Number of animals</td>
<td>Cells number injected per an. (viability)</td>
</tr>
<tr>
<td>MSCs control</td>
<td>4 mice</td>
<td>8.0×10^5 (95 %)</td>
</tr>
<tr>
<td>MSCs-operated with NeuroSyntek study</td>
<td>4 mice</td>
<td>4.8×10^5 (98 %)</td>
</tr>
</tbody>
</table>

Three groups of mice were controlled as described above.
CONCLUSION

No tumors in the control group of mice and mice, injected with ADSCs, processed with NS StemOptimizer, were detected. ADSCs, processed with NS StemOptimizer, showed no the tumor-forming potential after transplantation, as they did not form tumors at the site of transplantation or at distal sites.
REFERENCES


9. U.S. Food and Drug Administration http://www.fda.gov/ohrms/dockets/ac/05/slides/5-4188s1_2.ppt
# APPENDIX A

Lonza Walkersville Inc.
8530 Biggs Ford Road
Walkersville, MD 21793 5415
Tel (301) 898 7025
Fax (301) 845 4024

---

**CERTIFICATE OF ANALYSIS**

<table>
<thead>
<tr>
<th>Product Code:</th>
<th>PT-5006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product:</td>
<td>Adipose-Derived Stem Cells (Amp)</td>
</tr>
<tr>
<td>Lot Number:</td>
<td>0000421027</td>
</tr>
<tr>
<td>Manufacture Date:</td>
<td>23-Jun-2014</td>
</tr>
</tbody>
</table>

## TEST (Method)  | SPECIFICATIONS  | Min.  | Max.  | Results |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Acquisition Number</td>
<td>***</td>
<td>***</td>
<td>27739</td>
<td></td>
</tr>
<tr>
<td><strong>DONOR CHARACTERISTICS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>***</td>
<td>***</td>
<td>34 Y</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>***</td>
<td>***</td>
<td>FEMALE</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td>***</td>
<td>***</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>***</td>
<td>***</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td><strong>VIRUS TESTING</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV Test</td>
<td>***</td>
<td>***</td>
<td>Not Detected</td>
<td></td>
</tr>
<tr>
<td>HBV Test</td>
<td>***</td>
<td>***</td>
<td>Not Detected</td>
<td></td>
</tr>
<tr>
<td>HCV Test</td>
<td>***</td>
<td>***</td>
<td>Not Detected</td>
<td></td>
</tr>
<tr>
<td><strong>MICROBIAL TESTING</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterility Test</td>
<td>***</td>
<td>***</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>***</td>
<td>***</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td><strong>CELL PERFORMANCE TESTING</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell Passage Frozen</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Viability</td>
<td>&gt;=70%</td>
<td>*****</td>
<td>95 %</td>
<td></td>
</tr>
<tr>
<td>Cell Count (Cells/ml)</td>
<td>&gt;=1,000,000</td>
<td>*****</td>
<td>2200000</td>
<td></td>
</tr>
<tr>
<td>Seeding Efficiency</td>
<td>&gt;=25%</td>
<td>*****</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>Doubling Time (hours)</td>
<td>15</td>
<td>70</td>
<td>15 hrs</td>
<td></td>
</tr>
<tr>
<td>CD13, CD29, CD44, CD73, CD90, CD105, CD166</td>
<td>&gt;=90% Pos.</td>
<td>*****</td>
<td>Pass</td>
<td></td>
</tr>
<tr>
<td>CD14, CD31, CD45</td>
<td>&lt;=5% Pos.</td>
<td>*****</td>
<td>Pass</td>
<td></td>
</tr>
<tr>
<td>CD34</td>
<td>% Pos.</td>
<td>FI0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These cells were isolated from donated human tissue after obtaining permission for their use in research applications by informed consent or legal authorization. This product is for research use only. Details concerning the use of our cell and media products can be downloaded from our website at www.lonza.com/cell-protocols.
Product Description: HeLa
Lot Number: 14G005

Test Description:
Sterility Testing of Cell Banks: SOP ECC02

Acceptance Criterion/Specification:
All positive controls (Bacillus subtilis and Candida albicans) show evidence of microbial growth (turbidity) and the negative controls show no evidence of microbial growth (clear). The criterion for a positive test is turbidity in any of the test broths. All broths should be clear for a negative test result.

Test Number: 52053
Test Date: 05/03/2014
Result: Pass

Authorised by [Signature] ECACC, Head of Quality Date 09/01/15

Culture Collections, Public Health England, Porton Down, Salisbury, SP4 0JG, UK
Tel: +44 (0)1980 612512 Email: culturecollections@phs.gov.uk
Product Description: HeLa
Lot Number: 14G005

Test Description:
Cell count, viability and confluency of cells on resuscitation from frozen. SOP ECC103

Acceptance Criterion/Specification:
were judged acceptable if they meet two of the following criteria:
* >70% viable cells
* >2 x 10^6 total cells/amp
* Cell growth as expected within 2 days.

Test Number: 52087
Test Date: 01/08/2014
Result: 2.4 x 10^6 95.6%

Test Description:
Detection of Mycoplasma by PCR using Mycoplasma-specific PCR Primers Validated by ECACC. SOP ECC73

Acceptance Criterion/Specification:
Positive controls yield a single 280 bp amplification product. Negative Control yields no amplified product. The criteria for a positive test result is the yield of a single 280bp PCR product.

Test Number: 52087
Test Date: 01/08/2014
Result: Pass

Authorised by ECACC, Head of Quality Date 09 APR 15

Culture Collections, Public Health England, Porton Down, Salisbury, SP4 0JG, UK
Tel: +44 (0)1980 612512 Email: culturecollections@phe.gov.uk
Product Description: HeLa
Lot Number: 14G005

Test Description:
Detection of Mycoplasma using a Vero indicator cell line and Hoechst 33258 fluorescent detection system. SOP ECC123

Acceptance Criterion/Specification:
The Vero cells in the negative control are clearly seen as fluorescing nuclei with no cytoplasmic fluorescence. Positive control (M. hyorhinis) must show evidence of mycoplasma as fluorescing nuclei plus extra nuclear fluorescence of mycoplasma DNA. Positive test results appear as extra nuclear fluorescence of mycoplasma DNA. Negative results show no cytoplasmic fluorescence.

Test Number: 52087
Test Date: 01/08/2014
Result: Pass

Authorised by [Signature] ECACC, Head of Quality Date 09 APR 15.

Page 2 of 3
Culture Collections, Public Health England, Porton Down, Salisbury, SP4 0JG, UK
Tel: +44 (0)1980 612512 Email: culturescollections@phe.gov.uk
Product Description: HeLa
Lot Number: 14G005

Test Description:
The authentication of human cell lines by STR Profiling, SOP ECC86.
Human cell lines are authenticated using the PowerPlex® 16 HS PCR amplification kit. The
Powerplex kit amplifies 15 STR loci plus the gender marker

Acceptance Criterion/Specification:
Three controls are used for each STR Profiling, two positives: PowerPlex® 16 HS control and
K562, and a negative.
For the test to be deemed valid the control samples must give the correct algorithms.
For a result to be accepted as a pass, the algorithm for the test sample must exhibit a minimum
of 60% homology of the nine core alleles with an agreed source.
This criterion does not apply where the sample itself constitutes the source.

Test Number: 52067
Test Date: 26/03/2014
Result: Pass

Authorised by [Signature] ECACC, Head of Quality Date 09 APR 15.

Page 3 of 3
Culture Collections, Public Health England, Porton Down, Salisbury, SP4 0JG, UK
Tel: +44 (0)1980 612512 Email: culturescollections@phe.gov.uk
Human Pathogenic Virus Testing

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Assay Subtype</th>
<th>Referral Lab ID</th>
<th>Referral Lab Ref No</th>
<th>Report Date</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 (PCR)</td>
<td>HIV-1 env</td>
<td>TDL(920)</td>
<td>14T644147</td>
<td>17/12/2014</td>
<td>NOT Detected</td>
</tr>
<tr>
<td>HIV-1 LTR</td>
<td>TDL(920)</td>
<td>14T644147</td>
<td>17/12/2014</td>
<td>NOT Detected</td>
<td></td>
</tr>
<tr>
<td>HIV-1 gag</td>
<td>TDL(920)</td>
<td>14T644147</td>
<td>17/12/2014</td>
<td>NOT Detected</td>
<td></td>
</tr>
<tr>
<td>HIV-1 pol</td>
<td>TDL(920)</td>
<td>14T644147</td>
<td>17/12/2014</td>
<td>NOT Detected</td>
<td></td>
</tr>
<tr>
<td>Hep C (PCR)</td>
<td>n/a</td>
<td>TDL(920)</td>
<td>15T452354</td>
<td>24/03/2015</td>
<td>NOT Detected</td>
</tr>
<tr>
<td>Hep B (PCR)</td>
<td>n/a</td>
<td>TDL(920)</td>
<td>14T644147</td>
<td>17/12/2014</td>
<td>NOT Detected</td>
</tr>
</tbody>
</table>

Comments:

This virus testing report is supplementary to the original certificate of analysis. Virus testing was carried out by an external referral laboratory.
## APPENDIX B

### Packing List

<table>
<thead>
<tr>
<th>Shipping Point:</th>
<th>Walkersville 21793-0127</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shipping Date:</td>
<td>21793-0127/Walkersville</td>
</tr>
<tr>
<td>Delivery Date:</td>
<td>15 Jan 2016</td>
</tr>
<tr>
<td>Freight Carrier:</td>
<td>FED EX</td>
</tr>
<tr>
<td>Freight Mode:</td>
<td>LBS Overnight Air</td>
</tr>
<tr>
<td>Route:</td>
<td>ZLALI-LBS Overnight Air</td>
</tr>
</tbody>
</table>

**Shipping Terms:** FCA - Free carrier Incoterm2010

**Customer No.: 6438533**

**Ship to:**
- Company: Neurosynetek
- Neurosynetek
- 5941 optical Court
- San Jose CA 95138
- USA

**Sold to:**
- Neurosynetek Ophthalmic LLC
- Elizabeth Garibay
- 4704 Montrose Boulevard
- Houston TX 77006
- USA

**Bill to:**
- Neurosynetek Ophthalmic LLC
- Elizabeth Garibay
- 4704 Montrose Boulevard
- Houston TX 77006
- USA

<table>
<thead>
<tr>
<th>Order No.</th>
<th>Order Date</th>
<th>Customer Order No.</th>
<th>Customer Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>40067677</td>
<td>12-Jan-2016</td>
<td>LZ160112-1</td>
<td>Pavel Molchanov - 832 643 7648</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Line Item</th>
<th>Product Code/ Description</th>
<th>Order Qty</th>
<th>Ship Qty</th>
<th>UOM</th>
<th>Lot No./Ser.Nr.</th>
<th>Expiration Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>010 PT-5006</td>
<td>Adipose Derived Stem Cells (Amp)</td>
<td>1</td>
<td>1</td>
<td>AMP</td>
<td>0000421627</td>
<td></td>
</tr>
</tbody>
</table>

**Delivery Instructions:**

- In the event of an emergency with the products contained in this package, please contact 800-424-9300 or 703-527-3687 (collect calls).
- Returns: Products may not be returned for credit without first obtaining authorization from Lonza. Proof of proper storage may be required. A restocking fee (15% or 25% of product price) may be applied without return authorization or when purchaser is at fault. Damaged, discrepancies or missing product must be reported to Customer Service within 5 business days of receipt.
- Federal ID: 95-3917176

---

19
**SHIP TO:**

PAVEL MOLCHANOV
5541 OPTICAL CT
SAN JOSE CA 95138

**Customer Service 800-325-3010 • sigma-aldrich.com/custserv**

<table>
<thead>
<tr>
<th>DATE</th>
<th>PURCHASE ORDER NUMBER</th>
<th>SHIPPING POINT</th>
</tr>
</thead>
<tbody>
<tr>
<td>07/13/2016</td>
<td>SA10118-1</td>
<td>PAVEL MOLCHANOV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ref: 3015608683</td>
</tr>
</tbody>
</table>

**STOCK NO.**

<table>
<thead>
<tr>
<th>STOCK NO.</th>
<th>LOT NO.</th>
<th>ORDERED</th>
<th>SHIPPED</th>
<th>BRICK (BOX)</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>93021013-1VL</td>
<td>140005</td>
<td>1</td>
<td>1</td>
<td></td>
<td>CINRY OF OR: GB</td>
</tr>
<tr>
<td>*** Shipped in Dry Ice ***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TRMS: OVERNIGHT**

**RECEIVE**

<table>
<thead>
<tr>
<th>ORDERED</th>
<th>SHIPPED</th>
<th>BRICK (BOX)</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td></td>
<td>CINRY OF OR: GB</td>
</tr>
</tbody>
</table>

**SUB TOTAL**

526.00

**TOTAL**

526.00

**TRAN SIMULATION**

42.91

**TOTAL TAX**

46.03

**TOTAL**

514.94
APPENDIX C

IDEXX BioResearch Case # 3849-2016

Submitted By:
Pavel Molchanov
Neurosyntek
932 Lundy Ln
Los Altos, CA 94024

Phone: 650-229-2683
Email: pgnr@neurosyntek.com

Specimen Description
Species: mouse
Description: Cell culture lines
Number of Specimens/Animals: 2

<table>
<thead>
<tr>
<th>Client ID</th>
<th>Add ID</th>
<th>Investigator</th>
<th>Date Placed</th>
<th>Code Placed</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEBAS3</td>
<td>143105</td>
<td>MOLOHANOV</td>
<td>12/4/2016</td>
<td>93021013-1VL</td>
<td>MFG 3/18/2014</td>
</tr>
</tbody>
</table>

Purchase Order #: IDX150122-1

Services/Tests Performed: IMPACT II
- PCR evaluation for: Ectromelia, EDIM, LCMV, LDEV, MABV, MABV1, MABV2, mCMV, mNY, MPV, MVM, Mycoplasma pulmonis, Mycoplasma sp., Polyoma, PVM, REO3, Sendai, TMEV

General Comments: Cell culture lines for rodent pathogen test, frozen, freeze media, inln 1M per vial

Summary: All test results were negative.
### PCR Evaluation

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>AD3CL29</th>
<th>HELASA3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectromelia</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EDIM</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LCMV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LDEV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MA/1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MAV2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>nCMV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MHV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MNV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mycoplasma pulmonis</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mycoplasma sp.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MVM</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MPV</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polyoma</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PVM</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>REd3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sendai</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TMEM</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Legend:** + = positive  - = negative  d/d = pooled sample range  d+Ht = non-range pooled sample  Nt or blank = no test performed  wps = weak positive