Advanced dendritic cells for adaptive post-transplant therapy

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Regenerative Immune Therapies Applied

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Hannover Medical School
1. Unmet clinical need: Delayed immune reconstitution after allogeneic stem cell transplantation

**INDICATIONS:**
- Hematologic malignancies
- Primary immune deficiencies
- Hemato-metabolic diseases

**TOP 3 RISKS:**
- Recurrence of malignancy
- Graft versus host disease
- Viral infections:
  1. CMV disease
  2. EBV lymphoma
  3. ADV disease
2. The solution: Advanced “induced dendritic cells” (iDCs)

A) Multicistronic Design in Lentiviral Vector

- CMVp
- GM-CSF
- 2A
- IFN-α
- 2A
- Antigens

Integrate-Defective Lentiviral Vector

Hypothesis: *In vivo* DC Differentiation
Improve Immunity

B) PBMC Harvest from Donor

C) Thaw and administration into patient after HSCT

Induced DCs (iDCs):
- High Viability
- Self-differentiation
- Migration
- Immune-activations

New: Single 24 h *ex vivo* manipulation and storage
3. The invention: Studies in “humanized mice” confirmed iDC high viability and migration

G-CSF Mobilized Blood

Hematopoietic stem cells

Cord blood

CD14+ Monocytes plus LV: iDCpp65

Stem cell Transplantation into Nod.Rag-/-IL2γcR-/-Irradiated and immune deficient mice

iDCpp65 Applied s.c. after HSCT differentiate And migrate

Whole Body Imaging

CMVp GM-CSF 2A IFN-α 2A HCMVpp65

Integrase-Defective Lentiviral Vector
3. The invention: Mode of Actions

1. Highly viable iDCs promote immune regeneration

2. Immunomodulation: cytokines, MHCs, antigens activate T&B

http://webinar.sciencemag.org/webinar/archive/applying-humanizedmouse-models-immune-therapy-research

(Salgueiro et al., J Immunology 2014)
4. Commercial Potential: Donor-derived personalized anti-HCMV cellular vaccines for post-HSCT patients

No approved vaccine against human cytomegalovirus

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>pre-clinical</th>
<th>Clinical (PhI/II)</th>
<th>Efficacy trial</th>
<th>Outcome</th>
<th>Status / Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live attenuated virus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Towne, Ad169</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>protective against disease, no prevention of infection</td>
<td>FAILED</td>
</tr>
<tr>
<td>recombinant Towne/Toledo</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>well tolerated in healthy volunteers</td>
<td>FAILED</td>
</tr>
<tr>
<td>replic. defective</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td></td>
<td>Ongoing</td>
</tr>
<tr>
<td>Individual antigen vaccine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canarypox</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>CTL- and Th-responses in seronegative volunteers, poor NT-Ab response</td>
<td></td>
</tr>
<tr>
<td>MVA, Adeno</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>induction of NT-Abs, CTL and Th in animal models</td>
<td></td>
</tr>
<tr>
<td>Alphavirus replic.</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>induction of NT-Abs, CTL and Th</td>
<td>On hold since 2008</td>
</tr>
<tr>
<td>DNA vaccines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gB+pp65</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>- efficacious in reducing viremia in HSCT recipients</td>
<td>Ongoing</td>
</tr>
</tbody>
</table>

Data from 16,628 patients (Europe, 2013)
…“The treatment is set to begin a phase III trial among haematopoietic stem cell transplant patients in the first half of 2012…

In return for rights to TransVax, manufacturer Vical will receive a total payment of up to $130 million (80.7 million pounds) in upfront and milestone-based fees.”

- See more at: http://www.zenopa.com/news/800625894/Astellas_agrees_licensing_deal_for_cytomegalovirus_vaccine_TransVax#sthash.uVZAqGlk.dpuf
- https://www.nationalcmv.org/default.aspx
5. Comparison with other solutions: Combating HCMV after Hematopoietic Stem Cell Transplantation

<table>
<thead>
<tr>
<th>Approach</th>
<th>Pros</th>
<th>Cons</th>
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</thead>
</table>
| Antiviral Drugs                               | Standard treatment in HSCT Transplantation| • Side effects  
• Myelosuppresion  
• Resistances                                                                 |
| Passive polyclonal antibody infusion CMV-IGIV | Licensed, Prevention                      | • No protection against late reactivation                          |
| Viral vaccines, DNA vaccines                  | In clinical trials                        | • Immune compromised patients after HSCT may not respond            |
| Adoptive T cells Therapy                     | Clinical trials or compassionate use      | • Expensive,  
• Not feasible when donor is HCMV seronegative (high risk group in HSCT) |
| Conventional dendritic cells                 | Evaluated in a phase I trial, no safety concerns| • Production complex  
• Poor viability and bio-distribution in humanized mouse models  
• No B and antibody responses                  |
| Lentivirus-induced dendritic cells           | Adjuvant effects leading to significantly improved immune reconstitution in humanized mouse model | • Gene therapy product  
• First-in-man  
• Clinical safety unknown  
• Clinical potency unknown                  |
6. Differentiation from other solutions: Modular approach validated for different infections and cancer types

Selection of cytokines by co-transduction

"SmartDC"

Bi-cistronic vectors

Antigens added to tricistronic vectors

Against Cancer: Leukemia, Melanoma

Against Chronic Infections: HCMV, HCV

(Koya et al, 2004)

(Daenthanasanmak et al, 2012)

(Koya et al, 2007)

(Daenthanasanmak et al, 2012)

(Pincha et al, 2009, 2012; Salguero et al, 2011)

(Salguero et al, 2014)

(Pincha et al, 2012, Sundarasetty et al, 2016)

(Sundarasetty et al, 2013)

# 7. Development status: iDC platform for clinical use against HCMV, melanoma and leukemia

<table>
<thead>
<tr>
<th>PoC</th>
<th>PoC</th>
<th>GMP Development</th>
<th>GMP Validation</th>
<th>Scientific Advice PEI</th>
<th>Non-clinical pharm-tox</th>
<th>Multi-center Network for Phase I/IIa</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>In vivo</td>
<td></td>
<td></td>
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</table>

- **iDC/ pp65 HCMV after HSCT**
  - MHH (PI Ganser) Heidelberg Würzburg

- **iDC/ TRP2 Metastatic Melanoma**
  - MHH (PI Gutzmer) Heidelberg Göttingen

- **iDC/ tWT1 High-risk Leukemia**
  - MHH (PI Ganser) Heidelberg Dresden
8. IP Status: International Patent Application in Europe, US, China, Japan, Canada

Stripecke R., G. Salguero, A. Daenthasanmak, A. Ganser.

“Induced dendritic cells and uses thereof”

Owner: MEDIZINISCHE HOCHSCHULE HANNOVER

Priority date 07, February 2013

PCT Filing Date Jan 24 2014 PCT/EP2014/051422

PCT Publication Date 14 Aug 2014 WO2014/122035

National Entry: EU, USA, China, Japan, Canada

“The present invention relates to cells engineered to express at least one cytokine and at least one antigen which induces the self differentiation of dendritic cell (DC) progenitor cells into functional antigen-presenting induced DC (iDC). Moreover, therapeutic uses of said iDC for regenerating the immune system after transplantation of hematopoietic stem cells are disclosed. Said iDC are also useful for generating mice with a functional endogenously regenerated humanized immune system producing antigen-specific T and B cell responses which can be used as animal models for the study of the human adaptive immune responses.”
9. Freedom to operate: Vectors, GMP-SOPs, reagents for production and QC validated and available for clinical trials

A) ID-LV “Starting Material”

- Plasmid production (in ccc supercoiled form)
  1. LV-G2a2pp65 (backbone vector)
  2. pcDNA3/pG46V/4xTCE (D4V mutation)
  3. Packaging plasmid pRSV-Rev expressing Rev
  4. Pseudotyping plasmid pMD2.G
- Sequence confirmation of all plasmids
- Thawing 293T master cell bank
- Thawing and expansion 293T master cell bank
- qPEI transfection in 40 stack cell factories
  2.5μl qPEIμg DNA, Transfer vector 2.7 mg, Gag/poli vector 1.8 mg
  VSV-G vector 1.1 mg and Syn rev vector 0.6 mg
- Medium change with benzonase (24 hrs) and harvest (48 hrs)
- Filtration through 0.8 μm and 0.45 μm
- Chromatographic purification (CEX)
- Tangential flow filtration and dialysis
- Sterile filtration 0.45 μm and 0.2 μm
- Filling and storage at -80°C (aliquots separated for research)

B) iDCpp65 “Drug Product”

- LV-G2a2pp65 Transduction (18h)
- In process Q/C: Viability 2×10^6 cells
- MOI = 5
- ID-LV-G2a2pp65 Transduction (18h)
- Cryopreservation (1h)
- Thaw for Q/C

C) After thaw: batch release

- FACS and RT-q-PCR

D) After culture: Product characterization by FACS, RT-q-PCR, luminex

Sundarasetty et al, J Translational Medicine 2015
10. Needed:
- Seed funds for a phase I/IIa trial,
- industrial partner

MH

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Guzman, Carlos, Prof. Preclinical Potency

Fraunhofer

(Wanted: Industrial Partner)
### 11. Risks an bottlenecks: Effects in patients?

<table>
<thead>
<tr>
<th></th>
<th>Low Risk</th>
<th>Moderate Risk</th>
<th>Endpoint Phase I/IIa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Trial:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 patients</td>
<td>• Replication competent lentivirus</td>
<td>• Acute graft-versus-host disease</td>
<td>• Safety</td>
</tr>
<tr>
<td>Open label</td>
<td>• Insertional mutagenesis</td>
<td></td>
<td>• T and B cell reconstitution</td>
</tr>
<tr>
<td>Partially randomized</td>
<td></td>
<td></td>
<td>• Protection against HCMV</td>
</tr>
<tr>
<td>Dose escalation</td>
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</tbody>
</table>

None of these risks were seen in long-term (26 weeks) Pharm-tox pilot study in humanized mice (*Sundarasetty et al, American Journal of Pathology 2017*)


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