The role of Cell Therapy in Crohn´s Disease

Disclosure: Validated evidence based content of the presentation overcomes any eventual Conflict of Interest
The role of Stem-Cells
Alternative Treatments in Perianal Crohn’s Disease

Disclosure: Validated evidence based content of the presentation overcomes any eventual Conflict of Interest
Why using Stem-Cells to treat fistulas?

Potential applications of Stem Cells

- Bone regeneration
- Cartilage regeneration
- Muscle regeneration
- Myocardial regeneration
- Neural regeneration
- Soft tissue repair
  - Fistula as a testing bench

Our choice: Wound Healing
Fistula in Crohn’s Disease: a real problem of wound healing due to auto-immune disease

- Perianal discharge
- Pain
- Swelling
- Bleeding
- Diarrhea
- Skin excoriation
- External opening
Complex perianal fistula
An unresolved problem

A complex perianal fistula is characterized by:
- Anal sphincters involvement
- Multiple tracts
- Associated with perianal abscess and/or connects an adjacent structure
- Recurrence

A complex perianal fistula is characterized by:
- Anal sphincters involvement
- Multiple tracts
- Associated with perianal abscess and/or connects an adjacent structure
- Recurrence

Advanced Flaps

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<tr>
<th>Author</th>
<th>Recurrence (%)</th>
<th>Incontinence (%)</th>
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<td>Schouten 1999</td>
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<td>Sonoda 2002</td>
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<td>Van Koperen 2008</td>
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Cryptoglandular Disease

Crohns´s Disease

Infliximab

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Infliximab
Who is working in this line?

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<thead>
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<td>Recruiting</td>
<td>Allogenic Stem Cells Derived From Lipoaspirates for the Treatment of Recto-vaginal Fistulas Associated to Crohn’s Disease (ALOREVA)</td>
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<td>Conditions: Rectovaginal Fistula; Crohn Disease</td>
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<td>Intervention: Drug: Expanded allogenic adipose-derived adult stem cells</td>
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<td>Interventions: Drug: ASCs (Cx401, company code); Drug: Fibrin adhesive</td>
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<td>Autologous Stem Cells Derived From Lipoaspirates for the Non-Surgical Treatment of Complex Perianal Fistula</td>
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<td>Intervention: Drug: ADIPOPLUS</td>
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<td>Long-term Safety and Efficacy of Adipose-derived Stem Cells to Treat Complex Perianal Fistulas in Patients Participating in the FATT-1 Randomized Controlled Trial</td>
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<td>Evaluation of PROCHYMAltm Adult Human Stem Cells for Treatment-Resistant Moderate-to-Severe Crohn's Disease</td>
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<tr>
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<td></td>
<td>Interventions: Drug: adult human mesenchymal stem cells; Drug: Placebo</td>
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</table>
Stem Cells from Adipose Tissue
Advantages

Adipose derived mesenchymal stem cells:

- **Higher yield** (between 100 and 1000 times higher yield than bone marrow)
- **BM stimulation is not required** (G-CSF)
- Expandable and accessible: **Simple liposuction**
- **Biosafety**: No chromosomal alterations/ tumorigenic behavior after long term *ex vivo* cultures
- **Wide range of potential applications**
Cells extraction by liposuction

- Local anesthesia
- Little incision (0.5 cm.)
- Cosmetic value
- ACS lab manipulation
Technologies involved in Adipose Derived Stem Cells (ASC) Therapy

ASC Harvest
- Liposuction
- Isolation

ASC Implant

ASC manipulation
- In vitro culture
- Master Cell Bank

Cryopreservation
- Cell expansion

Master Cell Bank

Cell expansion

In vitro culture

ASC Harves
ASC Clinical Development in fistula

- **Preclinical**: 1 case, 1 center
- **Proof of Concept**: 8 cases, 1 center
- **Phase I**: 50 cases, 3 centers
- **Phase II**: 207 cases, 10 centers
- **Phase III**: 210 cases, 22 centers

- **Completed**
- **Ongoing**
- **Autologous**
- **Allogenic**

- **CX601**: 24 cases, 3 centers
- **FIS NC**: 10 cases, 1 center
- **FATT1**: 207 cases, 10 centers
- **FATT2**: 210 cases, 22 centers
ASC Clinical Development in fistula

Preclinical
- 1 case
- 1 center

Proof of Concept
- 8 cases
- 1 center

Phase I
- CX601: 24 cases, 3 centers
- FIS NC: 10 cases, 1 center

Phase II
- 50 cases
- 10 centers

Phase III
- FATT1: 207 cases, 10 centers
- FATT2: 210 cases, 22 centers

Completed
- Autologous
- Allogenic

Ongoing
Successful cell treatment of a young woman with a recurrent rectovaginal Crohn’s fistula unresponsive to medical treatment

Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn’s disease: a new cell-based therapy

DOI 10.1007/s00384-003-0490-3
ASC Clinical Development in fistula

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<td>1 center</td>
<td>8 cases</td>
<td>1 center</td>
<td>50 cases</td>
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<td>207 cases</td>
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</table>

CX601: 24 cases, 3 centers
FIS NC: 10 cases, 1 center
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Autologous, Allogenic
### Phase I clinical trial

#### TRIAL SUMMARY

<table>
<thead>
<tr>
<th>Category</th>
<th>Details</th>
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<tr>
<td><strong>Start</strong></td>
<td>April 2002</td>
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<td><strong>Enrollment</strong></td>
<td>5 patients (total of 8 fistulas)</td>
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<td><strong>Design</strong></td>
<td>Open Label; Feasibility / Safety Study</td>
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<td><strong>Administration</strong></td>
<td>Intralesional use</td>
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<tr>
<td><strong>Duration</strong></td>
<td>First evaluation of endpoint: 8 weeks</td>
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<td><strong>Controlled</strong></td>
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<td><strong>Endpoint</strong></td>
<td>Complete closure/healing of the fistula clinically assessed</td>
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<tr>
<td><strong>Results</strong></td>
<td>75% success</td>
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### Fistula closure

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<tr>
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<th>Patients</th>
<th>Treatments</th>
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<th>Partial</th>
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<td>5</td>
<td>8</td>
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</tbody>
</table>

**Diseases of the Colon & Rectum**

A Phase I Clinical Trial of the Treatment of Crohn’s Fistula by Adipose Mesenchymal Stem Cell Transplantation

Damián García-Olmo, M.D., Mariano García-Arranz, Ph.D.,
Dolores Herreros, M.D., Isabel Pascual, M.D., Concepción Peiro, Ph.D.,
José Antonio Rodríguez-Montes, M.D.

---

**NO SEVERE ADVERSE EVENTS**

**NO TUMOROGENIC EVENTS**
Operative technique

- **Enterocutaneous fistula**
  - Tracts cureted
  - External opening closed
- **Rectovaginal fistula**
  - Rectal opening closed
  - Vaginal flap
- **Perianal fistula**
  - Main track cored out
  - Rectal opening closed
ASC Clinical Development in fistula

2002 2003 2004 2005 2006 2007 2008 2009 2010

Preclinical

Proof of Concept

Phase I

Phase II

Phase III

Completed

Ongoing

Autologous

Allogenic

1 case 1 center

8 cases 1 center

50 cases 3 centers

207 cases 10 centers

210 cases 22 centers

FATT1

FATT2

CX601

FIS NC

200 cases

3 centers

24 cases

3 centers

10 cases

1 center

207 cases

10 centers

Completed

Ongoing

Autologous

Allogenic
Injection of ADSC cell suspension

Cell suspension is injected in the internal opening (the syringe enters through the anus) and through the fistula tract walls (the syringe enters through the external opening of the fistula).

(*) 50% of total cell dose placed in the intersphincteric tracts and adjacent to the internal opening; 50% in the tract walls in the direction of the external opening. Superficial injection (< 2mm)
Phase II clinical trial
General considerations

- **Multi-center:**
  - Three major hospitals in Madrid, Spain

- **Randomized**
  - Randomization performed by an independent organization

- **Controlled**
  - Control arm: fibrin glue as fistula tract sealant (one of the elective procedures to avoid conventional surgery)

- **Add-on trial**
  - Treatment arm: Cx401 administered intralesionally and fibrin glue as tract sealant

- **Open-label, primary endpoint evaluated by a blinded committee**
  - Committee formed by three surgeons experts in coloproctology not recruiting patients for the study
  - Analyzed clinical and photographic data

---

**Patient Selection:**
- Older than 18 years
- Both sexes

**Complex perianal fistula pathology fulfilling some of the following conditions:**
- Associated faecal incontinence
- Risk factors of anal incontinence
- At least 1 previous operation for a fistulous disorder
- Rectovaginal fistula
- Crohn's disease

---

**Route of Administration:**
- **Intralesional use:**
  - ½ in the fistula wall (*)
  - ½ mixed with the fibrin glue
Phase II clinical trial
Design

50 patients

Randomise

25 patients
25 patients

Experimental Treatment Group
Cx401 + Fibrin glue

Control Group
Fibrin glue

24 patients (ITT)
25 patients (ITT)

Primary Outcome
Secondary Outcome

Experimental Treatment Group
Cx401 + Fibrin glue

Control Group
Fibrin glue

Cell injection: ½ cell dose in the fistula wall
½ cell dose in internal opening
Tract identification
Curetage
Internal opening closure
Tract sealant

19
Phase II clinical trial

Variables

Primary endpoint
- Proportion of patients whose fistula was healed at week 8 after last dose of study drug
  - Definition of Healing: no suppuration + complete re-epithelization of the external fistula opening assessed by an independent evaluation committee

Secondary endpoints
- Maintenance of healing at 12 months
- Time to healing
- Quality of Life evolution (SF-12 score)
- Non serious and serious adverse events incidence
# Efficacy

## Primary endpoint

<table>
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<tr>
<th>TREATMENT</th>
<th>Relative risk</th>
<th>CI (95%)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Cx401</td>
<td>Fibrin glue</td>
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<tr>
<td>(N=24)</td>
<td>(N=25)</td>
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<tr>
<td>Healing (all cases)</td>
<td>17 (71%)</td>
<td>4 (16%)</td>
<td>(1.74, 11.27)</td>
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<tr>
<td>(N=17)</td>
<td>(N=18)</td>
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<tr>
<td>Healing in non-Crohn’s population</td>
<td>12 (71%)</td>
<td>3 (17%)</td>
<td>(1.44, 12.44)</td>
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<td>(N=7)</td>
<td>(N=7)</td>
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<tr>
<td>Healing in cases of suprasphincteric fistulous tract</td>
<td>10 (71%)</td>
<td>2 (12%)</td>
<td>(1.49, 21.78)</td>
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<td>(N=14)</td>
<td>(N=16)</td>
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</table>

1 No statistical significance recognized (total sample size of Crohn’s patients = 14)
Safety
Acute phase

- Primary evaluation (eight weeks after last treatment) revealed 17 adverse events with Cx401 and 11 with fibrin glue
- Only 2 serious adverse events (SAEs) were observed with fibrin glue and 2 with Cx401
- Not a single SAEs was related to Cx401

<table>
<thead>
<tr>
<th>Group</th>
<th>Crohn’s disease</th>
<th>Description</th>
<th>Severity</th>
<th>Results</th>
<th>Causality</th>
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<tr>
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<td>Crohn’s crisis and intrabdominal abscess</td>
<td>Yes</td>
<td>In recovery</td>
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<tr>
<td>Cx401</td>
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<td>Perianal abscess</td>
<td>Yes</td>
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<td>Cholecystitis and choledolithiasis.</td>
<td>Yes</td>
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<td>Not related</td>
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<td>Choledocholothiasis after cholecystomy</td>
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<tr>
<td>Fibrin glue</td>
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<td>Perianal abscess</td>
<td>Yes</td>
<td>Recovered</td>
<td>Related</td>
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The recurrence rate for Cx401 ITT patients at 1 year follow-up was 17.6% (3 out of 17 healed Cx401 cases)

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<tr>
<td>External openings</td>
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<tr>
<td>Recurrence appearance</td>
<td>1 year</td>
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Fistula treatment in Crohn’s Disease

### INFLIXIMAB

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### ADSC

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<th>Healing 50% Placebo</th>
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<th>T to recurrence(m)</th>
<th>Abscess(%)</th>
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<td>Garcia-Olmo 2009</td>
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<td>---</td>
<td>71</td>
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Administration of Adipose Derived Stem Cells was effective in inducing healing in patients with complex fistula-in-ano including Crohn’s disease, and this procedure can be considered safe.
<table>
<thead>
<tr>
<th>Aspex Clinical Development in Fistula</th>
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<tbody>
<tr>
<td><strong>Preclinical</strong></td>
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<td><strong>Proof of Concept</strong></td>
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</tr>
<tr>
<td><strong>Phase II</strong></td>
</tr>
<tr>
<td>50 cases</td>
</tr>
<tr>
<td>3 centers</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Phase III</strong></td>
</tr>
<tr>
<td>207 cases</td>
</tr>
<tr>
<td>10 centers</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Completed</strong></td>
</tr>
<tr>
<td>Autologous</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Ongoing</strong></td>
</tr>
<tr>
<td>Allogenic</td>
</tr>
</tbody>
</table>
Phase III (Autologous ASCs):

<table>
<thead>
<tr>
<th>Non-Crohn:</th>
<th>FATT I TRIAL DESIGN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>Fistula in non-Crohn’s patients</td>
</tr>
</tbody>
</table>
| Study design | • Randomised  
• Double blind  
• Parallel Assignment |
| Recruitment: | Completed: 200 patients  
Dosed: 135 patients |
| Controlled | Yes (fibrin and placebo) |
| # of centres | 23 centres in Spain |
| Primary endpoint | Complete healing assessed clinically and through MRI |
| Secondary endpoint | Long term healing, Quality of Life parameters, surgeries avoided, etc |
| Results | Release anticipated 1 Q 2011 |

FATT1 protocol design:

- 207 patients
- Randomise
- Experimental Treatment Group (1) Cx401
  - no suppurlation from external orifice and re-epithelisation
  - MRI: absence of collections >2 cm related to fistula tract
- Experimental Treatment Group (2) Cx401 + Fibrin glue
- Control Group Fibrin glue
  - Complete closure 12 wks after last dose received (only clinical)
  - Quality of life (SF-36)
  - Complexity score
  - Incontinence (Wexner)
  - Drug-related AEs
  - Surgeries avoided
Phase III (Autologous ASCs):

<table>
<thead>
<tr>
<th>Condition</th>
<th>Fistula in Crohn´s patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study design</td>
<td>Randomised, Double blind, Parallel Assignment</td>
</tr>
<tr>
<td>Recruitment</td>
<td>156 patients</td>
</tr>
<tr>
<td>Controlled</td>
<td>Yes (placebo)</td>
</tr>
<tr>
<td># of centres</td>
<td>35 centres in EU</td>
</tr>
<tr>
<td>Primary endpoint</td>
<td>Complete healing assessed clinically and through MRI</td>
</tr>
<tr>
<td>Secondary endpoint</td>
<td>Long term healing, Quality of Life parameters, surgeries avoided, etc</td>
</tr>
<tr>
<td>Results</td>
<td>2011</td>
</tr>
</tbody>
</table>
## ASC Clinical Development in Fistula

### Timeline:

- **Preclinical**
  - 1 case
  - 1 center

- **Proof of Concept**
  - 8 cases
  - 1 center

- **Phase I**
  - 8 cases
  - 1 center

- **Phase II**
  - 50 cases
  - 1 center

- **Phase III**
  - 207 cases
  - 1 center

### Projects:

- **CX601**
  - 24 cases
  - 3 centers

- **FIS NC**
  - 10 cases
  - 1 center

- **FATT1**
  - 207 cases
  - 10 centers

- **FATT2**
  - 210 cases
  - 22 centers

### Notes:

- **Autologous**
- **Allogenic**

### Data:

- 2002: 1 case
- 2003: 1 center
- 2004: 8 cases
- 2005: 50 cases
- 2006: 207 cases
- 2007: 207 cases
- 2008: 210 cases
- 2009: 207 cases
- 2010: 210 cases
## Safety

### Adverse events related to either study procedures or treatment*

<table>
<thead>
<tr>
<th>Treatment received group</th>
<th>ASCs (N=64)</th>
<th>ASCs+Fibrin tissue adhesive (N=59)</th>
<th>Fibrin tissue adhesive (N=60)</th>
<th>Total (N=183)</th>
<th>p-value, general association</th>
<th>p-value ASCs vs Fibrin tissue adhesive</th>
<th>p-value ASCs and Fibrin tissue adhesive vs Fibrin tissue adhesive</th>
<th>p-value ASCs vs ASCs and Fibrin tissue adhesive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Related to study treatment</td>
<td>4 6.3 4 6.8 6 10.00 14 7.7</td>
<td>0.7026 0.4473</td>
<td>0.5291</td>
<td>0.9053</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Related to study procedures</td>
<td>28 43.8 24 40.7 25 41.7 77 42.1</td>
<td>0.9397 0.8147</td>
<td>0.9128</td>
<td>0.7305</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Overall, there were no significant differences among the three treatment groups in the proportion and nature of both non-serious and serious adverse events. In addition, all treatments were safe as they had a low proportion adverse events either related to study treatment or related to study procedures, and a few severe/life-threatening adverse events. In conclusion, the results of this trial indicate that the use of ASCs alone or with fibrin tissue adhesive does not increase the risk for the patients at least during the period covered in this phase III clinical trial.
FATT1 Non-Crohn Population
Efficacy 24 weeks COMMITTEE
All cases

No statistical significance
Efficacy 24 weeks COMITTEE

Why?

P<0.0001
Patient distribution by “Complexity Score” and center (Median)

La Paz

Others

P < 0.001
Efficacy 24 weeks COMITTEE

Why?
We detect a large numbers of mistakes during the surgical procedure in “others hospitals”: use of hydrogen peroxide (H$_2$O$_2$), cell shake, cells spilling.
What have we learned?

Clinical Trials Peculiarities in Cell Therapy

- Surgical procedure for Cell delivering:
  - Environment
  - Aggressiveness
  - Scrub
  - Needle
  - ...
What have we learned?

**Clinical Trials Peculiarities in Cell Therapy**

- This is a “Living medicament” and hence a careful management is a key point.
Our Sponsor lost the interest in autologous ADSCs
ASC Clinical Development in fistula

Preclinical
- 2002-2010
- 1 case 1 center

Proof of Concept
- 1 case 1 center

Phase I
- 2002-2007
- 8 cases 1 center
- CX601 24 cases 3 centers
- FIS NC 10 cases 1 center

Phase II
- 2002-2009
- 50 cases 3 centers
- FATT1 207 cases 10 centers

Phase III
- 2002-2010
- 210 cases 22 centers
- Autologous
- Allogenic

Completed
Ongoing
### Immunogenicity (ADSCs/Mesenchymal) Privileged Cells

<table>
<thead>
<tr>
<th>Other cell types</th>
<th>MSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surface antigens</strong></td>
<td><strong>Surface antigens</strong></td>
</tr>
<tr>
<td>- High levels of MHC I (HLA-A, B, C)</td>
<td>- Low levels of Mayor Histocompatibility Complex Class I (HLA-A, B, C)</td>
</tr>
<tr>
<td>- MHC II: depending on cell type</td>
<td>- Lack of Mayor Histocompatibility Complex Class II (HLA-DR, DQ, DP)</td>
</tr>
<tr>
<td>- Co-stimulatory molecules</td>
<td>- Lack of co-stimulatory molecules</td>
</tr>
<tr>
<td>- Depending on cell type</td>
<td>- CD40 (TNFR), CD80 (B7-1), CD86 (B7-2)</td>
</tr>
<tr>
<td>- CD55 and CD59: depending on cell type</td>
<td>- High levels of CD55 (DAF) and CD59 (Protectin) =&gt; protectors of complement associated lysis</td>
</tr>
<tr>
<td><strong>Other Factors</strong></td>
<td><strong>Other Factors</strong></td>
</tr>
<tr>
<td>- Lack of IDO induction</td>
<td>- Strong IDO induction</td>
</tr>
</tbody>
</table>
Allogenic ASCs

- 2 PHASE I/IIa Allogenic
  - Crohn recto-vaginal fistula
  - Crohn perianal fistula

Starting: December 2009
Results: December 2011
How does it work?

• An immunomodulator effect of adipose derived stem cells has been recently described.
• These immunosuppressive properties are the same as MSCs (mesenchymal stem cells derived from bone marrow)
Mechanism of Action of ASCs

- ASCs are activated in an inflamed environment.
- Activated ASCs suppress the proliferation of lymphocytes and suppress the inflammation.
- Local treatment of inflammatory diseases with tissue damage/ wounds: ASCs act at the source of the inflammation and establish an environment that will permit a healing.
- Systemic treatment of diseases with acute inflammatory component: ASCs migrate to the inflammatory environment and suppress inflammation, avoiding tissue damage.
eASCs inhibit T cell proliferation in a dose dependent manner

The antiproliferative effect is found:

• In activated PBMCs
• In an allogeneic system (no HLA matched)
• In a dose dependent manner (ratio 1:25—1:200)
• Both in contact and transwell conditions

(DelaRosa, Lombardo, Tissue Engineering 2009)
**IDO enzyme is involved in the antiproliferative property of eASCs**

Indoleamine 2,3 dioxygenase activity: a Trp catabolizing enzyme

(IFN-γ) eASC

IDO enzyme is involved in the antiproliferative property of eASCs

(Trp) (Kyn) (HAA)

( DelaRosa, Lombardo, Tissue Engineering 2009)
How many times are ADSCs alive?

- Durability
- Long-term adverse events...
Long Term Efficacy (Fistula Closed)

No statistical significance
Injection of Adipose Derived Stem Cells in fistula are safe...at least during 10 years.
Where do we go?

• Safety Profile Excellent
  ▪ DOSE INCREASE

• Cell Mortality
  ▪ IMPROVING METHODS FOR DELIVERING

• Soft effects
  ▪ CELL INGENIERING
    ▪ CELL IMPROVING WITH STIMULATING FACTORS (BMPs,...)
Thank you!