Predictive in vivo Pharmacology

Full Functional Human Immune System Mouse Models

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Who We Are and What We Do

Expertise
• Full reconstitution of the human immune system/function in mouse models
• Protocol designs, tailor-made

Application
• Preclinical Candidate Selection
• Efficacy models for ONCO, HIV, IBD, Lupus, full Ag/Immune Responses
• Immunosafety
• Combination strategy

About us
• European CRO located in Archamps, France near Geneva (Switzerland)
• Team of 8 FTE/experts (5 PhD)
• Bio Safety Level BSL-2 and BSL-3 animal and lab. certified facilities
• High quality and flexibility
• Fee for Services
• International recurring customers (Pharma, Biotech)
2013-2016

- 25 Accounts in USA, EUROPE & JAPAN
- 45 projects executed
- > 1’600 huMice used for R&D projects
- huMouse Models (number of mice):
  - HIV >250
  - Inflammatory Bowel Disease >300
  - Cancer >550
  - Lupus >40
  - Vaccination and full Immune Responses: new >30
  - Accredited R&D Crédit Impôt Research (CIR)
Full Fee-for-Services

Study Design
- Study: Pilot, Main
- Tailor-made protocols
- SOP driven
- Clear Role and Responsability
- Quotes
- Timelines

Protocol Execution
- BSL2&3 animal facility
- State of the art cytometry and cell biology

Data Analysis
- Raw data collection
- Data Analysis
- Draft Report

Final Reporting
- Final Report
- Data transfer
- Feedback collection

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The Team

- **Patrick Nef, PhD, Founder and CEO**, has over 25 years of experience in R&D, and Business Development. He was Global Business Director, Vice-President & Disease Area Head for CNS at F. Hoffmann-La Roche Ltd., CEO, CSO or CBO in several biotech. companies, and earlier Patrick was Assistant Professor in the Department of Biochemistry at the University of Geneva. He holds a PhD in neurosciences from the University of Geneva, Switzerland.

- **Sébastien Tabruyn, PhD, Chief Scientific Officer**, has 12 years of international experience in Oncology, Molecular Biology and in vivo models. Project leader at UCSF (California, USA), the Center for Cancer Biology (Adelaide, Australia) and the University of Liege (Belgium), he has more than 30 publications in the field of Tumor, Vascular biology and Inflammation. Sébastien holds a PhD in molecular biology from the University of Liege, Belgium.

- **Stéphane Legastelois, PhD, President**, has over 20 years experience with the management of Service and Product companies in Life Sciences. He began his carrier as a R&D Manager in a start-up company, before joining Merck KGaA as Sales & Marketing Manager. Stéphane is also Founder & President of the CMO Indicia Production, and President at Mabdesign, a non-profit organization dedicated to the therapeutic antibody industrial sector. Stéphane holds a Ph-D in Immunology from the University of Lyon, France.
For best Clinical Candidate Selection

Hematopoietic Stem Cells (HSC) from human cord blood

+/− growth factor expression

Predictive Drug Testing

Humanized Immune System Mouse

NOG/NSG/BRG

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CD34+ Stem Cell Origin for Humanization

- From EFS (Etablissement Français du Sang)
- Informed consent signed by the parents
- Anonymization
- Maximum 48h after delivery
- Volume: +/- 100ml
- Total Number of cells (around 2x10^9)
- % of CD34: around 0.5%

Affinity Separation

between 5x10^5 and 3x10^6
Purity: >90%
Haplotype Selection
Hematopoietic Reconstitution

- T cells
- B Cells
- Myeloid Cells
- NK
- Dendritic Cells

Time (Weeks)

% among hCD45
Optimized Protocol for Humanization

- High rate of humanization (20-90%)
- Lifelong stability
- Reconstitution kinetics fully established @14 weeks
- Functional B & T-cells
Humanization Process and Quality Control

- Chemoablation (DX)
- CD34 injection (DX)

4 week old NOG

- Quality Control
- (Flow Cytometry)

- Only mice with hRate > 25% enter the Study

Time (Week)

0

14

Customer Study

Mouse CD45

Human CD45

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Flow Cytometry Characterization at W15

B cells 39%

Monocytes 10%

NK cells 1.5%

T cells CD3+

CD8+ 71%

CD4+ 20%

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# Human Cells in Peripheral Blood

<table>
<thead>
<tr>
<th>Human Cell type</th>
<th>Expressed in HIS</th>
<th>Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytes</td>
<td>✔</td>
<td>CD45</td>
</tr>
<tr>
<td>T-Lymphocytes</td>
<td>✔</td>
<td>CD3, CD4, CD8, CD25</td>
</tr>
<tr>
<td>B-Lymphocytes</td>
<td>✔</td>
<td>CD19</td>
</tr>
<tr>
<td>NK cells</td>
<td>✔</td>
<td>CD16, CD56</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>✔</td>
<td>CD1c, CD303, CD141, CD11c</td>
</tr>
<tr>
<td>Monocytes</td>
<td>✔</td>
<td>CD14</td>
</tr>
<tr>
<td>Macrophages</td>
<td>✔</td>
<td>CD11b</td>
</tr>
<tr>
<td>Activated T and/or NK cells</td>
<td>✔</td>
<td>HLA-DR, CD69</td>
</tr>
</tbody>
</table>

Detected by Flow cytometry

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# Human Cytokines Expressed in hu-Mice

<table>
<thead>
<tr>
<th>Human Cytokines</th>
<th>Expressed in HIS</th>
<th>Method of Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>huTNF-α</td>
<td>✔</td>
<td>IHC</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>✔</td>
<td>FC, RT-PCR</td>
</tr>
<tr>
<td>IL-2</td>
<td>✔</td>
<td>FC</td>
</tr>
<tr>
<td>IL-4</td>
<td>✔</td>
<td>FC</td>
</tr>
<tr>
<td>IL-17</td>
<td>✔</td>
<td>FC, RT-PCR</td>
</tr>
<tr>
<td>IL-23</td>
<td>✔</td>
<td>RT-PCR</td>
</tr>
</tbody>
</table>

IHC (Immunohistochemistry), FC (Flow Cytometry), qRT-PCR (Real Time TaqMan)
Multiple Indications

**Infectious Diseases**
Ex: HIV, Dengue,...

**Inflammation/Autoimmune Diseases**
Ex: IBD, RA, Lupus, SLE,...

**Cancer:**
**Immunotherapy**
Ex: Solid tumors, leukemia, PDX.

**Vaccines & ImmunoSafety**
100% human IgG, prophylaxis,
Inflammatory Bowel Disease (IBD)
Inflammatory bowel disease (IBD)

- Complex multigenic inflammatory gastrointestinal disease
- Include 2 major phenotypic forms, Crohn’s disease and ulcerative colitis
- 1 to 1.5 million patients in the US
- Current Treatment: biologic inflammatory mediators such as tumor necrosis factor (TNF) inhibitor
- Unmet medical need particularly in patients with severe or complicated clinical manifestations
- Need for appropriate animal models for preclinical efficacy testing
IBD: Symptoms

- Persistent diarrhea
- Abdominal pain or cramps
- Rectal bleeding
- Weight loss
- Fatigue
- Joint, skin, or eye irritations
IBD: a disease involving several tissues

NOG Humanized Mice

- Bowel Infiltrated Human Leukocytes
- Human Macrophages
- Human Cytokines
- Human Splenic Leukocytes
IBD characteristics

**Ulcerative colitis**
- Most common form of IBD
- Continuous mucosal inflammation
- Affecting rectum and progressing up the colon
- Inflammatory infiltrates in the mucosa: Lymphocytes, granulocytes, plasma cells

**Crohn’s Disease**
- Discontinuous inflammation
- Affect any part of the gastrointestinal tract
Murine Models of Inflammatory Bowel Disease

- Chemically induced: DSS, TNBS, oxazolone, ...
- Gene-manipulated: IL-2-/-, IL-2R-/-, and IL-10-/-
- Bacteria-infected models

These models were performed in immunocompetent mice: C57BL/6, BALB/c, ...

Difficult to test drug acting on human-specific cells/cytokines
Hu-IBD Models

Acute or Chronic Model of Inflammatory Bowel Diseases

Chemical Inducers
- DSS
- TNBS

Endpoints or Disease Progression
- Survival
- Weight loss
- IBD TCS Scoring (body weight/diarrhea/bleeding)
# IBD TCS Scoring

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>Description</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td><strong>Diarrhea</strong></td>
<td>Normal stool</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Softer stool</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Unformed stool</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Watery stool</td>
<td>3 (severe)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 (very severe)</td>
</tr>
<tr>
<td><strong>Bleeding</strong></td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Weak appearance of blood</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Visual blood in stool</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Fresh rectal bleeding</td>
<td>3 (severe)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 (very severe)</td>
</tr>
<tr>
<td><strong>Body weight loss</strong></td>
<td>X&lt;2%</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2%&lt;X&lt;5%</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5%&lt;X&lt;10%</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10%&lt;X&lt;15%</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>15%&lt;X&lt;20%</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>20%&lt;X&lt;25%</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>25%&lt;X&lt;30%</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>X&gt;35%</td>
<td>7</td>
</tr>
</tbody>
</table>

**MAXIMUM CUMULATIVE SCORE**

15
TNBS Model: Acute Colitis HuIBD

- **Presensitization**: Subcutaneous injection of TNBS
- **Colitis Induction**: Intra rectal administration of TNBS
- **Monitoring and IBD Scoring**
- **Sacrifice and IHC**
TNBS Model: Optimised protocol

- Importance of Presensitization: 100 µl of TNBS 1% (Subcutaneous injection)
- Concentration of TNBS for rectal administration: 0.20% to 0.50% (100 µl)
- TNBS formulation and Vehicle: 40% Ethanol (no side effects of vehicle)
- No anaesthesia: anti-inflammatory properties of isoflurorane
- CsA validated in hu-NOG (DSS Model)
- Number of TNBS-induced colitis mice: 25
DSS Model: Acute Colitis HuIBD

D-5
Acclimations

D1
DSS

Body Weight
Diarrhea Score
Bleeding Score

D8
Recovery

D13

Sacrifice
Colon collection
Data Analysis Report

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Diminution of Colitis Symptoms

Therapeutic huMAB reduces inflammation in IBD models
DSS-induced huIBD Colitis

Ulceration and extensive damage of the mucosa

Histological Score = 3
Human Leukocyte Infiltration

Human leukocytes infiltration at the site of inflammation
(ALU staining and huTNF-α secretion)

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Human Mast Cells Infiltration in healthy Colon in hu-Mice

Tryptase staining

ALU staining

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DSS Model: huIBD Chronic Colitis

**DSS Induction**

- **D-5**: Acclimation
- **D1**: DSS Induction
- **D6 - D12**: Recovery
- **D17**: DSS Induction
- **D23 - D28**: Recovery
- **D35**: Sacrifice

**Body Weight**

**Diarrhea Score**

**Bleeding Score**

Sacrifice
Colon collection
Data Analysis
Report

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Effects of Cyclosporine-A (CSA)

- Cyclosporine-A decreased DSS induced chronic colitis severity
- Implication of T-Cells in DSS induced chronic colitis
hCD3 infiltration in inflamed colon

CD3 labeling

Negative Ctrl
hCD4 infiltration in inflamed colon

B

CD4 labeling

C

Negative Ctrl
2- Effects of Anti-TNF-α

- Anti-TNF-α decreased DSS induced chronic colitis severity
- Implication of human cytokines in DSS induced chronic colitis
Strength of IBD huMouse Models

- Acute and chronic models
- Infiltration of human leukocytes in inflamed colon
- Lymphoid and Myeloid compartments implicated
- Efficacy evaluation and bioequivalence of human specific monoclonal antibodies (anti-hTNFa)
## R&D Partnerships for Grants

<table>
<thead>
<tr>
<th>Grant</th>
<th>Subject</th>
<th>Partner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eurostars 2013</td>
<td>AML</td>
<td>Advanced biodesign (FR), CNRS (FR)</td>
</tr>
<tr>
<td>Eurostars 2014</td>
<td>DC Based Vaccin: Glioblastoma</td>
<td>Amal Therapeutics (CH)</td>
</tr>
<tr>
<td>Eurostars 2015</td>
<td>DC Based Vaccin: Breast Cancer</td>
<td>DCPrime (NL)</td>
</tr>
</tbody>
</table>
Customer’s Advantages

- Save Time and Budget
- Focus on the most promising clinical candidates
- HuData useful for IND filling
- One stop shop for *in vivo* pharmacology expertise
- Derisking for Head of Translational Projects
- Flexibility and Adaptability
Q&A

Happy to answer your questions/concerns

Patrick and Sebastien

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