T cell therapy of inflammation with regulatory cells

Arnaud Foussat, VP Research & New Products
ISCT Annual Meeting- 23-26 April 2014, Paris
Teaching your cells to treat your disease

**Cellular immunotherapy**

- **Stimulate immunity**
  - Dendritic cells
  - Effectors T cells
    - CD8+
    - CD4+
    - γδ+
    - CAR-T cells
  - NK cells
  - Activated PBMCs
    - Provenge

- **Induce immune tolerance**
  - Regulatory T cells
  - Tolerogenic Dendritic cells

**Cancer & infectious diseases**

**Inflammation, autoimmunity & transplantation**
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• **Thymic derived Treg** (*nTreg/tTreg*)

CD25⁺Foxp3⁺
CD25⁺CD127$^{\text{neg}}$
CTLA-4⁺GITR⁺PD-1⁺CD62L⁺
Naïve CD45RA⁺ vs Memory CD45RO⁺

Specific for self
→ Autoimmunity & transplant tolerance

• **Peripheral derived Treg** (*iTreg/pTreg*)

Ex Vivo IL2 + TGF-β
Induced Treg
TGF-β producing Th3 Treg
IL-10 producing Type 1 Treg

Specific for self & non-self
→ Autoimmunity & transplant tolerance
→ Chronic inflammation & allergy

Multiple sub-populations with different biology,
Repertoire, MoAs, Migratory & Proliferative capacity…..
Treg cells: Multi-target, multi-MoA approach

- Is there in vivo a dominant mechanism of action? Is a multi-MoA required for Treg activity in vivo?
- MoA might be dependent on disease, disease state, disease history, previous treatments.
- Difference in MoA can lead to differences in main cellular and molecular targets
- How to set-up a potency assay with a Treg cell product?
- Potential less patient refractoriness to multiple target approaches.

Sub-population differs in inhibitory pathways
(Cytokine driven inhibition, contact molecules, ICOS, PD-1, GITR, Granzyme A/B, CD39)

Antigen-specific activation but bystander activity

From Nature Immunology review, Ethan Shevach & Todd Davidson
Teaching your cells to treat your disease

- **Isolation of Natural pre-existing Tregs**
  - Mandatory cell surface markers,
  - Polyclonal Treg populations
  - Expansion prior to administration
  - Functionality of cells required

- **Ex vivo differentiation of Tregs**
  - Regimen of stimulation is key
  - % of Treg differentiation to control effective standard doses
  - Polyclonal & antigen-specific Treg populations
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25 Treg clinical studies referenced

- 13 in HSCT
- 8 autoimmunity & chronic inflammation
  - Diabetes
  - Crohn’s Disease
  - Asthma
  - Uveitis
- 4 in transplantation
  - Liver transplantation
  - Kidney transplantation
- 5 completed
- 7 Industry sponsored
- All Phase I & Phase II

<table>
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<tr>
<th>Sponsors/Collaborators</th>
<th>Phase</th>
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Teaching your cells to treat your disease

Published data

Freshly isolated donor derived
CD4+CD25+ Treg
(Di Anni et al, Blood, 2011)

In vitro expanded third party
UCB derived CD4+CD25+ Treg
Anti-CD3+anti-CD28 Beads + IL-2
(Brunstein et al, Blood, 2011)

In vitro expanded family donors
derived CD4+CD25+CD127neg
Anti-CD3+anti-CD28 Beads + IL-2
(Trzonkowski et al, clinical Immunology, 2014)

IL-10 anergized allo-specific
donor T cells
Type 1 Treg cells induced upon MLR + IL-10
(Bachetta et al, Frontiers in Immunology, 2014)

13 Treg in HSCT

13 Phase I to phase II clinical trials referenced

- Prevention & treatment
- Polyclonal vs allo-specific
- Tolerability demonstrated
  - No increase in infection
  - Fast immune reconstitution
- Preliminary efficacy data
  - Less high grade GVHD
  - Alleviation of symptoms
- No controlled study yet
- Issue of Immunosupression
- Fine tuning of the dose

Expansion

Prevention & treatment
Polyclonal vs allo-specific
Tolerability demonstrated
- No increase in infection
- Fast immune reconstitution
Preliminary efficacy data
- Less high grade GVHD
- Alleviation of symptoms
No controlled study yet
- Issue of Immunosupression
- Fine tuning of the dose

MLR
4 Treg in Solid Organ Transplant

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*Planned or ongoing*

**Autologous allo-Ag-specific CD4+CD25+ Treg**

*IL-2+TGFbeta + recipient’s DC*

(NCT01624077)

**Autologous allo-specific CD4+CD25+ Treg**

*MLR with CTLA-4 Ig*

(NCT02091232)

**Polyclonal autologous expanded CD4+CD25+CD127neg Treg**

(NCT02088931)

**Polyclonal autologous expanded CD4+CD25+CD127neg Treg**

(NCT01446484)

4 Phase I to phase II clinical trials referenced in liver (1) and kidney (3) transplantation

- No results published yet
- Polyclonal vs allogeneic vs Allo-Ag-specific approaches
- Issue of immunosuppressive regimen
- Dose ≈ 10^6/kg with Ag-specific Tregs
- Dose ≈ 2.10^8 to 10^9 with autologous
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**Published data**

**Expanded Ag-specific Type 1 Treg in Crohn’s Disease**
*Ova-specific, in vitro expanded*  
(Desreumaux et al, Gastroenterology 2012)

**Autologous polyclonal**

*CD4+CD25+CD127* Treg in T1D  
*Anti-CD3+anti-CD28 Beads + IL-2*  
(Marek, Diabetes care 2012)

**Ongoing**

**Autologous polyclonal**

*CD4+CD25+Treg in Uveitis*  
*Anti-CD3+anti-CD28 mAb + IL-2*  
(La Pitié Salpetrière Hospital, Paris)

**Autologous polyclonal**

*CD4+CD25+CD127* Treg in T1D  
*Anti-CD3+anti-CD28 Beads + IL-2*  
(NCT01210664)

**8 Phase I to phase II clinical trials referenced**

- Polyclonal vs Ag-specific
- Tolerability demonstrated
- Preliminary efficacy data
- No controlled study yet
- Fine tuning of the dose
- Dose ≈ 10^6 to 10^9
Teaching your cells to treat your disease

**Isolation of a subpopulation**
- GMP sorting (Flow cytometry vs immuno-affinity)
- Single marker or gene expression profile
- Purity of the isolated populations (first Treg populations < 60% Foxp3+ cells)

**Potency assay**
- Issue of multiple putative mechanisms of action
- Phenotypic evaluation of different inhibitory molecules (cytokines, surface markers, enzymes, cytolytic granules….)
- In vitro assays (cell contact inhibition, inhibition with soluble factors)
- In vivo/in vitro blocking experiments
- Potency can include migration/proliferative activity/cell adhesion

**T-cell therapy/cell therapy challenges**
- GMP manufacturing, closed system & automation
- Scale-up for late stage clinical trial
- Batch-to-batch comparability (identity/potency/purity)
Potential toxicity of Treg cells could be related to:

- **Purity**
  - Control of proinflammatory cell content (Th17…)
  - Patients with inflamed conditions may have circulating pro-inflammatory cells

- **Tumorigenicity/uncontrolled proliferation**
  - Karyotyping, in vitro persistence, dependence to TCR stimulation & growth factors, clonogenicity assays, telomere shortening capacity
  - In vivo long-term studies with cell tracking

- **Plasticity/de-differentiation**
  - In vitro & in vivo plasticity assays (Th17, Th1)
  - Stability of phenotype, potency and epigenetic markers in proinflammatory promoting culture conditions
  - Tregs from inflammatory patients might be more prone to plasticity

- **Indiscriminate immunosuppression**
  - In vivo toxicology studies
  - Patients clinical monitoring especially in patients with a depressed immune system
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### Alteration of nTregs in chronic inflammation & autoimmunity

**CD25+ FoxP3+ Treg polyclonal deficiency in autoimmune & chronic inflammatory diseases**

- Cause or consequence ➔ Treg are normalized in remitting patients
- Issues of CD25, Foxp3 & Treg markers also expressed by proinflammatory cells

### Defect in Ag-specific cell number, differentiation pathway and/or molecular signalling

- **Alteration of CD46 mediated IL-10 Treg differentiation in MS patients**
  Alterations in CD46-mediated Tr1 regulatory T cells in patients with multiple sclerosis (Astier AL, J Clin Invest)

- **Alteration of Desmoglein-3 specific Tr1 cells in pemphigus vulgaris**
  Type I regulatory T cells specific for desmoglein-3 are more frequently detected in healthy individuals than in patients with pemphigus vulgaris (Veldman C, J Immunol. 2004)

- **Alteration of CTLA-4 intracellular signalling in Rheumatoid Arthritis patients**
  Defects in CTLA-4 are associated with abnormal regulatory T cell function in rheumatoid arthritis. (Flores-Borja F, PNAS, 2008)

- **Enhanced plasticity in Tregs from Rheumatoid Arthritis patients**
  Regulatory T cells in rheumatoid arthritis showed increased plasticity toward Th17 but retained suppressive function in peripheral blood. (Wang T, Ann Rheum Dis, 2014.)

 ➔ **Autologous re-infusion of patients un-manipulated Treg cells might not be an ideal approach**
Understanding the Treg compartment defects in patients can help to define the better strategy

- Antigen specific versus polyclonal Tregs
- Natural versus induced Treg cells
- Gene engineering of Treg cells

Regeneration of a defective compartment

- Induction of tolerance
  - Persistence or induction of memory

Pharmacological inhibition of inflammation

- Inhibition of inflammation
- Induction tolerance
  - Persistence or induction of memory
Safety and Efficacy of Antigen-Specific Regulatory T-Cell Therapy for Patients With Refractory Crohn’s Disease

PIERRE DESREUMAUX,* ARNAUD FOUSSAT,† MATTHIEU ALLEZ,§ LAURENT BEAUGERIE,* XAVIER HÉBUTERNE,* YORAM BOUHNIK,* MARIA NACHURY,‡ VALÉRIE BRUN,‡‡ HERVÉ BASTIAN,‡ NATHALIE BELMONTE,* MICHEL TICCHIONI,* AGNÈS DUCHANGE,* PATRICIA MOREL-MANDRINO,* VIRGINIE NEVEU,‖ NATHALIE CLERGET-CHOSSAT,*‡‡ MIGUEL FORTE,*‡ and JEAN-FRÉDÉRIC COLOMBEL*

Ovasave® development

Ovasave®: autologous ovalbumin-specific type 1 regulatory cells

Percentage of Patient in CDAI response/remission (all doses, n=20)

- Week 5: 40 Response, 15 Remission
- Week 8: 40 Response, 10 Remission

Percentage of patients in CDAI Response or Remission at 10^6 (n=8)

- Week 5: 75 Response, 38 Remission
- Week 8: 75 Response, 25 Remission

Mean (±SEM) CDAI variation at 10^6 (n=8)

Analysis of 8 patients receiving 10^6 cells

CDAI response = decrease 100, CDAI remission <150
Biomarker of clinical response: PBMC proliferative activity in response to Ovalbumin

10^6 cohort

CDAI vs Ova-response

Supports efficacy and mechanism of action of Ova-Treg cells in CD patients

Phase IIb to start in H2 2014 in refractory Crohn’s Disease patients
Treg cells represent a novel therapeutic approach for treatment of chronic inflammation, autoimmunity and transplantation.

- Natural anti-inflammatory activity
- Multiple MoA and multiple cellular and molecular targets

- Early development stage
- Tolerability of the approach demonstrated, encouraging preliminary efficacy observed
- Need fine tuning of doses and comparative analysis of different sub-populations and strategies