Potential Rebalancing of the Immune System by Anti-CD52 Therapy

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Alemtuzumab / Lemtrada

- Humanized monoclonal antibody
- Targets CD52 antigen present at high levels on the surface of B and T lymphocytes and at lower levels on innate immune cells
- Mediates lymphocyte lysis through antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)
- Administered to multiple sclerosis patients as 5 daily IV infusions at start of treatment and 3 daily IV infusions 1 year later
- Submitted for approval to FDA and EMA. Decision expected in the second half of 2013.
Multiple Sclerosis

- MS is a neurodegenerative autoimmune disease characterized by inflammation leading to demyelination and axonal loss.
- Affects >1 million people worldwide (US ~400,000). More common in women vs men (2:1). Typically diagnosed at the age of 20-40 years old.
- Most patients initially present with a relapsing/remitting form of the disease and the majority (~65%) enter a secondary progressive phase.
Autoimmune Demyelination in MS

Cells involved:
- Neuron
- T cell
- B cell
- Macrophage
- Oligodendrocyte

Cytokines:
- IL-2
- IL-17
- IFN-γ

Other components:
- CD8
- Antibodies
- Myelin
- Enzymes
- O2 radicals

Locations:
- Blood
- CNS
Alemtuzumab – Clinical Trial Design

- Phase 2 trial in treatment naïve RRMS patients (N = 334, 3yrs)
- Phase 3 trial in treatment naïve RRMS patients (N = 581, 2 yrs)
- Phase 3 trial in RRMS patients who relapsed on prior therapy (N = 840, 2yrs)
- Extension study ongoing
Alemtuzumab (Lemtrada) vs Interferon-β in Relapsing-Remitting MS

Phase 2 Trial (CAMMS223)

Extension study: Sustained accumulation of disability still reduced by 72% and rate of relapse by 69% compared to IFNβ-1a out to four years after the last alemtuzumab treatment

Alemtuzumab Treatment in MS Patients

- Alemtuzumab selectively depletes circulating T and B lymphocytes; other leukocytes are minimally affected.
- A distinctive pattern of T and B cell repopulation begins within weeks after treatment with alemtuzumab:
  - B cells return to baseline levels within 6 months.
  - T cells counts rise more slowly, generally approaching the lower limit of normal by 12 months.
Alemtuzumab Treatment in MS Patients – Mechanism of Action

- **Infection**
  - Marked depletion of circulating lymphocyte counts and prolonged period of repopulation raised concerns around susceptibility to infection
  - Only moderate increase in overall rate of infection seen in MS patients (66-77% alem vs 45-66% IFN-β across Phase II, III trials)
  - Infections predominantly mild to moderate in severity
  → Assess immune status post-alemtuzumab treatment in human CD52 transgenic mice

- **Durability of clinical benefit**
  - Mechanism explored in EAE mouse model, patient sample analysis

- **Secondary autoimmunity**
  - Thyroid disorder in ~30%, ITP ~3% at 5 yrs
  - Isolated cases of Goodpasture’s disease
  - Detected early through monitoring and managed through conventional therapy
  - Etiology unclear: Lymphopenia + Genetic/environmental factor
  - Efforts underway to identify predictive biomarkers
Human CD52 Transgenic Mouse

Alemtuzumab epitope

Human CD52: -GQ----------NDTSQTSSPS- GPI anchor

Mouse CD52: LGQATTAASGTNKNSTSTKTPPLKSG- GPI anchor

- Alemtuzumab does not cross-react with mouse CD52 thus precluding in vivo studies in wild-type mice

- Human CD52 transgenic (huCD52) mouse model
  - Created on an outbred mouse strain (CD1)
  - Expresses human CD52 under control of the human CD52 promoter
  - Tissue distribution and levels of CD52 on mouse lymphocytes comparable to that of humans

Hu et al. Immunology. 2009
Leukocyte Depletion in huCD52 Transgenic Mouse is Greater in Peripheral Blood vs Lymphoid Organs

Alemtuzumab 1 mg/kg, day 3
Lymph nodes: inguinal, axillary, brachial, cervical, mesenteric

Turner et al, submitted for publication
Immune Status Post-Alemtuzumab in huCD52 Transgenic Mice - Summary

- Remaining immune cells are fully functional
- Transient decrease in primary immune responses with little impact on memory responses
- Largely intact innate immunity (in vitro and in vivo)

=> Overall, the results suggest that the selective nature of immune cell depletion and preservation of functional activity following alemtuzumab treatment may contribute to the low incidence of serious infection seen in MS patients
Studying Durability of Effect – Anti-CD52 Therapy in EAE

- Mouse monoclonal antibody against murine CD52
- Studies performed in MOG peptide-induced EAE in C57BL/6 mice
  - Impact on disease course
  - Frequency of MOG-reactive cells after anti-CD52 treatment
  - Analysis of CNS infiltration, histopathology

M Turner et al, ECTRIMS 2012
Spleen repopulated to normal levels by end of study
Anti-muCD52 Reduces the Number of Autoreactive T cells

MOG-specific T cells in the spleen

T cells in the CNS
Anti-muCD52-Treated Mice Show Reduced CNS Inflammation

M Turner et al. ECTRIMS 2012
Relative Sparing of T Cells with a Regulatory Phenotype in huCD52 Transgenic Mice and MS Patients

HuCD52 transgenic mouse

Blood  Spleen

1 mg/kg alemtuzumab, day 3

Genzyme unpublished data: M Turner, B Siders et al

CARE MS-I

'Treg' phenotype: CD4+CD25int/br+CD127lo-

- Increased percentage of cells with Treg phenotype in MS patients post-alemtuzumab also observed by: Cox et al, Zhang et al, Durelli et al.

Cox EJI 2005; Zhang AAN 2012; Durelli ECTRIMS 2012
Shift in CD4+ T Lymphocyte Cytokine Pattern in MS Patients Post-Alemtuzumab

Increased percentage of TGF-β and IL-10 producing CD4 T cells

Decreased percentage of IFN-γ and IL-17-producing CD4 T cells

- Zhang et al, AAN 2012
- Similar findings by Jones et al, Brain 2010
Increased *in vitro* Production of Neurotrophins by Lymphocytes from Alemtuzumab-Treated Patients

**BDNF secretion**  
(Brain derived neurotrophic factor)

**CNTF secretion**  
(Ciliary neurotrophic factor)

Neurotrophin secretion after alemtuzumab is specific to stimulation with myelin basic protein

**p<0.01**

Jones *et al.* *Brain* 2010
What May Account for the Reported Durability of the Clinical Response in Alemtuzumab MS Studies?

- Depletion of CD52+ autoreactive lymphocytes and subsequent repopulation may induce rebalancing of the immune system

- Alterations in the number, proportion and properties of lymphocyte subsets post-alemtuzumab:
  - Increased percentage of T cells with regulatory activity post-alemtuzumab may contribute to reduction in MS disease activity
  - Shift in the T cell cytokine profile may lead to reduced inflammation
  - Lymphocyte production of neurotrophic factors post-alemtuzumab may promote neural repair
Use of Anti-CD52 in Non-MS Indications

- Ability to simultaneously target both T and B lymphocytes could be applied to other autoimmune diseases

- Preliminary evidence of alemtuzumab efficacy in small investigator-sponsored trials and case reports
  - Rheumatoid arthritis (RA) (n>100)
  - Vasculitis (n=71)
  - Myositis
  - Uveitis
  - Autoimmune cytopenias (hemolytic anemia, ITP, autoimmune neutropenia)
  - Scleroderma

- Variable dosing regimens and variable duration of effect. Several courses of treatment often necessary.
Anti-CD52 for Lupus

- SLE is an autoimmune disease characterized by the production of autoantibodies causing chronic inflammation in multiple organs including the skin, kidneys, CNS and cardiovascular system

- Existing drugs have limited efficacy and are associated with significant toxicity -> high unmet medical need

- Dysregulated immune responses in SLE involve T and B lymphocytes
  - Therapeutics aimed at targeting either B (e.g. Rituxan) or T (e.g. Abatacept) lymphocytes have demonstrated little or no efficacy in clinical trials
  - Cyclophosphamide which is cytotoxic for proliferating B and T lymphocytes provides a therapeutic benefit but is associated with significant toxicity
  - Potential for anti-CD52 to modulate T and B cell responses with improved safety profile

Lupus nephritis
T/B cell infiltrate
Efficacy of Anti-Mouse CD52 in the NZB/NZW-F1 Lupus Mouse Model

- Mice given two therapeutic cycles of anti-mCD52 (25 mg/kg, 3x/wk for 2 wks, n=30/group)

- Treated mice showed:
  - Improved kidney function (decreased proteinuria) and reduced kidney pathology
  - Survival benefit
  - Reduced anti-nuclear IgM antibodies, reduced urinary markers of damage (KIM-1, NGAL)

![Reduced kidney pathology](image)

![Improved survival](image)

Rao et al, AAI 2012
Summary

- Treatment of MS patients with alemtuzumab results in lymphocyte depletion followed by a repopulation process that may lead to a rebalancing of the immune system and durability of effect.

- Testing of anti-CD52 in a lupus mouse disease model provided evidence for a therapeutic benefit and suggests a potential application to human SLE.