Title: Autologous Hematopoietic Stem Cell Transplantation in Refractory Celiac Disease with aberrant T-cells

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Abstract

Autologous Hematopoietic Stem Cell Transplantation (ASCT) is an increasingly accepted treatment for refractory autoimmune diseases. Refractory Celiac Disease with aberrant T cells (RCD-type II) is unresponsive to available therapies and carries a high risk of transition into Enteropathy Associated T-cell Lymphoma (EATL). This study reports on the feasibility, safety and efficacy of ASCT in RCD type II.

Thirteen patients with RCD-II were evaluated. Seven patients [4M, 3 F, mean age 61.5 years (range 51-69 years)] were transplanted. After conditioning with fludarabine and melphalan, ASCT was performed. Patients were monitored for response, adverse effects and hematopoietic reconstitution.

All 7 patients completed the mobilization and leucopheresis procedures successfully and subsequently received conditioning and transplantation. Engraftment occurred in all patients. No major non-hematological toxicity or transplantation-related mortality was observed. There was a significant reduction in the aberrant T-cells in duodenal biopsies associated with improvement in clinical wellbeing, and normalization of hematological and biochemical markers (mean follow-up 15.5 months, range 7-30 months). One patient died 8 months post-transplant form progressive neuroceliac disease.

These preliminary results showed that high-dose chemotherapy followed by ASCT seems feasible and safe, and might result in long-term improvement of RCD II patients whose condition did not respond promptly to available drugs.

Keywords:

Introduction

Autologous Hematopoietic Stem Cell Transplantation (ASCT) is an increasingly accepted effective treatment option for patients with severe autoimmune diseases refractory to conventional treatment\(^1\) and has been used successfully in patients with multiple sclerosis,\(^2\) rheumatoid arthritis,\(^3\) systemic sclerosis,\(^4\) systemic lupus erythematosus,\(^5\) and Crohn’s disease.\(^6\) The rationale for this strategy is based on the concept of immunoablation by intense immunosuppression using high dose chemotherapy, with subsequent regeneration of naïve T lymphocytes derived from reinfused hematopoietic progenitor cells.\(^7\)

In celiac disease (CD), HLA-DQ molecules bind and present gluten peptides to antigen-specific T-cells. These HLA-DQ-peptide complexes induce inflammatory responses in the small intestine consisting of lymphocytic infiltration of the lamina propria, expansion of the intraepithelial lymphocyte population, hyperplasia of the crypts and atrophy of the villi.\(^8\) In a small percentage (2-5%) of celiac disease patients diagnosed as adults a refractory state develops despite strict adherence to a gluten-free diet (GFD).\(^9\) In refractory celiac disease (RCD) the number of intraepithelial lymphocytes (IEL) is markedly raised and it is from these IEL’s that enteropathy associated T cell lymphoma (EATL) may arise.\(^9,10\)

Immunophenotyping of the IEL's identifies two groups of RCD patients: those with normal IEL’s (RCD I) and those with aberrant IEL's, lacking surface expression of CD3 and CD8 (RCD II).\(^10,11\) RCD II can be regarded as a ‘cryptic’ lymphoma.\(^9\) There is now strong molecular and immunophenotypic evidence showing that a monoclonal neoplastic T-cell population may emerge from IEL’s in RCD. Clonal expansion of this monoclonal T-cell population eventually leads to frank EATL. The genesis and expansion of these monoclonal T-cells involve both inappropriate immune responses to gluten and acquisition of genetic abnormalities. Although the monoclonal IEL’s in patients with RCD are neoplastic, they are not cytologically abnormal and do not form tumour masses which differentiate these patients
from EATL patients, in addition to the absence of radiological and bone marrow evidence of lymphoma.\textsuperscript{10,12,13,14}

RCD II is usually resistant to any known therapy, including azathioprine/prednisone, cyclosporine and IL-10 therapy\textsuperscript{15-18} and has a high risk of developing EATL (60-80\% within 5 years).\textsuperscript{10,19} This specific type of peripheral T-cell lymphomas has a very poor outcome with 1- and 5-year survival rates in the range of 31-39\% and 11-20\% respectively.\textsuperscript{19,20,21} In a prospective multicenter study of 35 patients with EATL treated with six cycles of CHOP (Cyclophosphamide, Doxorubicine, Vincristine and Prednisone), the cumulative 2-year survival was only 28\%.\textsuperscript{11} Therefore, new treatment strategies for patients with “premalignant” CD (RCD-II) are urgently needed to improve their clinical condition with the ultimate goal of resetting the immune response which might prevent or delay development of overt EATL. This study reports on the feasibility, safety and efficacy of high dose chemotherapy followed by ASCT in patients with RCD type II.

**Methods**

**Patients**

Between March 2004 and March 2006 thirteen patients were evaluated for ASCT. Seven patients [4 males, 3 female, mean age 61.5 years (range 51-69 years)] with RCD-II underwent ASCT. Six other patients were excluded because of the presence of coexistent coronary artery disease and heart failure (NYHA classification III) (two patients), EATL found on pre-transplantation evaluation (3 patients), and low performance status (one patient). One patient could not be treated due to unsuccessful leucopheresis; he developed EATL and died subsequently despite chemotherapy and immunotherapy with antiCD52 (Alemtuzumab).\textsuperscript{22}

The 2 patients with congestive heart failure died from: progressive disease, cachexia (one patient) and bronchiectasis (second patient). The 3 patients with EATL all died within few months, while the patient with low performance status died from cachexia.
The baseline characteristics of the patients are shown in table 1. All patients received therapy with prednisone and cladribine (2-CDA) several months before receiving ASCT (not within 6 months of transplantation). The first 3 patients (patients A, B and C) were diagnosed with CD at relatively advanced age, had persistent diarrhea, weight loss and failed to respond to GFD, steroids and immunosuppressives. Because of the presence of active disease and high percentage of aberrant T cells in the small bowel mucosa, they were included in this study protocol. Patient D was diagnosed with CD at the age of 48 years in association with dermatitis herpetiformis. Furthermore, he had a clinical picture of neuroceliac disease with ataxia. After exclusion of structural brain and infectious disorders, he received ASCT at the age of 63.5 years.

Patient E has, in addition to CD with ulcerative jejunitis, Hashimoto’s thyroiditis, while patient F has CD with ulcerative jejunitis. One patient (patient G) was included because of the presence of very extensive ulcerative jejunitis with multiple small bowel strictures necessitating repeated resections although initially biopsies showed a low percentage of aberrant T cells. He had clinically short bowel syndrome (remaining small bowel approximately 100-150 cm) requiring total parenteral nutrition.

Criteria for diagnosis of RCD

Patients with CD were considered to be refractory when symptoms of malabsorption due to villous atrophy persisted or recurred after a former good response despite strict adherence to a GFD for at least one year. Furthermore, possible underlying diseases such as autoimmune enteritis, bacterial overgrowth, giardiasis, amyloidosis, intestinal lymphangiectasia, Whipple’s disease, hypogamma-globulinemia, eosinophilic enteritis, EATL and inflammatory bowel disease were excluded. The diagnosis of RCD was established as type II when ≥ 20% aberrant T-cells were present.
<table>
<thead>
<tr>
<th>Patients</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
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<td>IIIA</td>
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<td>Diarrhea, Weight loss, Hypocalcemia</td>
<td>Diarrhea, Weight loss, Dermatitis herpetiformis, neurological symptoms (ataxia)</td>
<td>Weight loss, skin rash, Hashimoto’s thyroiditis</td>
<td>Weight loss, diarrhea</td>
<td>Diarrhea, Hypocalcemia, weight loss, extensive small bowel resection</td>
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<td>EMA+, Anti-TTG+</td>
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<td>EMA+, anti-TTG+</td>
<td>EMA+, anti-TTG+</td>
<td>EMA+, anti-TTG+</td>
<td>EMA+, anti-TTG+</td>
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<td>Nodular mucosa</td>
<td>Mosaic mucosa, erosions and ulcerations</td>
<td>Mosaic mucosa, visible vessels, no ulcerations Nodular mucosa, disappearance of folds, erosions Ulcerative jejunitis Ulcerative jejunitis Ulcerative jejunitis with multiple stenosis</td>
<td></td>
<td></td>
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<td>Splenic atrophy, thickened SI wall</td>
<td>Thickened SI loops</td>
<td>Splenic atrophy, dilated SI loops</td>
<td>Splenic atrophy</td>
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<td>No abnormality</td>
<td>Small intestine ileus</td>
</tr>
<tr>
<td>PET scan</td>
<td>Increased uptake in SI</td>
<td>Increased uptake in SI</td>
<td>Increased uptake in SI</td>
<td>No abnormality</td>
<td>No abnormality</td>
<td>No abnormality</td>
<td>No abnormality</td>
</tr>
</tbody>
</table>

Table 1 Baseline characteristics of the patients. M=male, F=female, Pred=prednisone, 2-CDA=Cladribine, SI= small intestine, GDS=Gastroroduodenoscopy VCE=Video capsule enteroscopy, DBE= double balloon enteroscopy
Inclusion criteria

Patients were only included when the diagnosis of true RCD with aberrant T cells was confirmed (except one patient (patient G) was included on the basis of extensive ulcerative jejunitis with short bowel syndrome despite having only 10% aberrant T cells), after verifying their strict adherence to a GFD, performance status according to the WHO criteria needed to be 0-2, if there was no severe concomitant cardiac, pulmonary, renal or hepatic disease. EATL was excluded by endoscopic examination with multiple biopsies, CT-scan, PET and a trephine bone marrow biopsy. Furthermore, neither active uncontrolled infection nor HIV positivity was permitted.

Evaluation

Before proceeding to ASCT, the patients were extensively evaluated as to their performance status, the presence of concomitant diseases and extraintestinal disease or EATL. This evaluation included:

- Clinical assessment noting particularly signs and symptoms of malabsorption, body mass index (BMI) and performance according to the WHO score,23
- Evaluating the adherence to a GFD: Frequent consultation with dietician (advice and follow up); in addition to checking serology (anti-endomysium (EMA) and anti-tissue transglutaminase-antibody (anti-tTG), both of which usually revert to negative after strict adherence to the GFD,
- Endoscopic evaluation by upper gastrointestinal (UGIE), video capsule endoscopy (VCE) and double balloon enteroscopy (DBE). Duodenal biopsies (4 biopsies) were classified according to the modified Marsh criteria.24,25 T-cell Receptor (TCR-) gene rearrangement study,12,13,14 T-cell flow-cytometry and IEL phenotyping were performed,15,26,27
- Laboratory evaluation: whole blood cell counts, serum levels of creatinine, bilirubin, liver enzymes, lactate dehydrogenase, albumin, electrolytes, iron, ferritin, folic acid and vitamin B12. Anti-endomysium (EMA) and anti-tissue transglutaminase-antibody (anti-tTG) assays,
HLA-DQ typing, thyroid function tests, stool examination for Giardia and other parasites and HIV serology were also performed.²⁸

- Radiological evaluation: the patients underwent whole body computed tomography (CT-scan), whole body positron emission tomography (PET) to exclude intestinal and extra-intestinal localization of EATL.²⁹,³⁰

**Immunophenotyping of IEL’s**

IEL’s were isolated from 3 duodenal biopsies by passing them through nylon filters (1x100µm, 1x 40µm, BD Falcon). Cells were stained with fluorescent labeled monoclonal antibodies to CD3, 7, 8, 45, 103, and TCRγδ, as well as with relevant isotype controls. All monoclonal antibodies were from BD Falcon (BD biosciences, CA USA), except for CD103, which was from IQ-products, Groningen, The Netherlands) and analyzed by 4-color flow-cytometry (FACS-Calibur, BD). Leucocyte common antigen (CD45) was always included to identify the lymphocyte population. In some tubes cell surface CD3 staining (anti-CD3-APC) was followed by permeabilization (Cytofix /cytoperm, BD Biosciences Pharmingen, CA USA) and subsequent cytoplasmic staining with anti-CD3-FITC or isotype control. Aberrant T cells were defined either as CD7+ surface CD3− cells (expressed as % of CD103+ lymphocytes) or as cytoplasmic CD3+, surface CD3 negative cells (expressed as % of CD103+ lymphocytes).¹²,²⁶

All flow-cytometry analyses were performed by an analyst and interpreted by the same medical immunologist, while histopathology was performed by the same pathologist to ensure uniformity, reproducibility and consistency of results.

**Assessment of TCR gene rearrangement by Polymerase Chain Reaction (PCR)**

T-cell receptor-gamma (TCR-γ) gene rearrangements studies were performed in separate three-four duodenal specimens that were preserved on histocon and frozen at −20°C. DNA was extracted from cryosections of duodenal specimens by a standard procedure using
proteinase-K digestion and ethanol precipitation of the genomic DNA. TCR-\(\gamma\) gene rearrangements were analyzed by multiplex polymerase chain reaction (PCR) amplification under standardized conditions. A monoclonal and polyclonal control was included in each experiment. Clonality assessment for TCR-\(\gamma\) gene rearrangements was done using the BIOMED-2 multiplex TCR PCR protocol\(^{12,13,14}\).

**Peripheral blood stem cells mobilization and collection**

Mobilization of hematopoietic progenitor cells from the bone marrow into the peripheral blood was achieved using granulocyte-colony stimulating factor (G-CSF) \(2 \times 5 \, \mu\text{g/m}\text{kg}\) by subcutaneous injection for at least four days. Hematopoietic stem cells were harvested from the peripheral blood by leucapheresis and kept frozen until ASCT. The target CD34+ count was \(> 2 \times 10^6/\text{kg}\).

**Conditioning and ASCT**

The conditioning regimen consisted of fludarabine given orally for five days (40 mg/m\(^2\)/day) and melphalan (intravenous, two days 70 mg/m\(^2\)/day) (Figure 1). At day 0, the frozen stem-cell suspension was thawed and reinfused. The rationale for this conditioning regimen was based on T cell depletion by a purine analogue combined with a modified dose of melphalan (total dose 140 mg/m\(^2\)) for myeloablation.

<table>
<thead>
<tr>
<th>Fludarabine 40 mg/m(^2)/day orally</th>
<th>Melphalan 70 mg/m(^2)/day i.v.</th>
<th>Reinfusion of Stem Cells</th>
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<tr>
<td>Days -8(^#)</td>
<td>-7 - 6 - 5 - 4 - 3(^*)</td>
<td>-2 -1 0</td>
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<tr>
<td>#Baseline</td>
<td>*Admission</td>
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</table>

*Figure 1:* Scheme of transplantation protocol
Supportive care

Patients A, C and D were supported with parenteral feeding during the two week period of oral mucositis after ASCT, while patient G was receiving parenteral nutritional support before receiving the transplant. After discharge, all patients except one (patient G) were able to be fed enterally. Patient G was supported to gain weight for several months with a duodenal feeding tube and limited TPN (twice a week). During admission, all patients received standard antibacterial and antifungal prophylaxis. Pneumocystis jiroveci pneumonia prophylaxis was initiated (Trimethoprim-sulphamethoxazole gluten-free syrup 480-960 mg daily) until six months posttransplant. No patient received antidiarrheal or narcotic medications in the peri-transplantation period. Blood and platelet transfusions were given as indicated.

Follow - up and criteria of response

During follow up, WHO performance, nutritional status, changes in weight and stool frequency were noted, as well as relevant biochemical markers. An endoscopic and histological examination of the small intestine was performed (3, 12 and 24 months post ASCT). From the second part of duodenum, 4 biopsies were taken for histological assessment and 4-6 for T-cell flow-cytometry study. Hematological data [Hemoglobin, White cell count (WBC), differential and platelets] were registered before inclusion, after preconditioning and after transplantation until recovery. The nadir WBC, duration of neutropenia, infectious complications, bleeding tendency, and the need for supportive therapies such as blood and platelet transfusions were documented.

Ethical approval and Informed consent

Approval of the medical ethics committee was obtained and all treated patients signed an informed consent.
Results

Table 1 summarizes the demographic and clinical characteristics of the patients before ASCT. The mean age at diagnosis of CD was 52.5 years (range 47-62 years) and for RCD-II was 59 years (range 51-64 years). Four patients were DQ2 homozygous and three were heterozygous. The mean follow-up is 15.5 months (range 7-30 months). All patients had a WHO performance status of one except patient G (WHO performance 2). Four patients (patients B, E, F and G) had ulcerative jejunitis. Three patients (patients A, C and D) had splenic atrophy on CT scan. PET scan showed an increased uptake in the small intestine in 3 patients (patients A, B and C). At the time of diagnosis of CD, all patients were positive for anti-tTG and EMA, but all reverted to negative after GFD. Before and after ASCT all patients remained negative for anti-tTG and EMA. There was no transplant related mortality. The conditioning regimen seems feasible in this group of patients. The mean duration of hospitalization was 19.5 days (range 18-22 days). ASCT-related toxicity was relatively mild. Patient B had transient diarrhea and fever of undetermined origin which was treated with intravenous antibiotics. Three weeks after discharge from the hospital, he suffered from a transient visual disturbance caused by minor retinal bleeding, which was not related to thrombocytopenia. Patient D experienced fever of undetermined origin and recovered after intravenous antibiotics. One month after ASCT, patient E developed self-limiting erythematous plaque skin lesions with central necrosis. Detailed histopathological tests excluded EATL and showed aberrant T lymphocyte infiltration (CD8- CD7+ CD30+).

The mean time from the day of transplantation to neutrophil recovery was 17.8 days (range 10-21 days). Only one patient (patient B) had a transient a 5 days period of severe thrombocytopenia of 5×10⁹/l, while all other patients had nadir platelets counts between 17-32 ×10⁹/l without need for platelet transfusions.

Clinical and laboratory tests before and after ASCT are shown in table 2. Patients A, C and D were supported with parenteral feeding during the period of oral mucositis. No patient
received antidiarrheal or long term narcotic medications. Within 3 months after ASCT, all patients showed impressive clinical improvement with normalization of stools frequency, disappearance of abdominal pain and improvement of biochemical markers. Also improvement of BMI was documented [from mean 20.2 at baseline to 24.1 after ASCT]. Mean serum albumin level increased from 29 g/l to 40.7 g/l. Patient G showed a remarkable clinical improvement 3-4 months after ASCT and was able to be fed partly enterally with parenteral nutritional support twice a week.

Table 3 shows the endoscopic and immunological results. All patients were monoclonal for the TCR-γ. Endoscopically there was disappearance of erosions and ulcerations in the jejunum in all patients (patients B, E, F and G) who had ulcerative jejunitis before ASCT, and histology of the small intestine showed significant regeneration as documented by down-staging of the Marsh class (patients A, B, C, E, F and G).

Overall, the aberrant [CD7+CD3-] T-cell percentage of CD103+ lymphocytes decreased from a mean of 63% (range 11-95%) at baseline to 38% (range 7-68%) three to four months post-transplantation. Aberrant cytoplasmic CD3+ surface CD3- T-cell percentage of CD103+ lymphocytes has decreased from a mean of 61% (range 10-94%) to 42% (range 2-89%). Furthermore, the mean percentage of CD8+ cells has increased from 23% to 30% after ASCT. This was particularly noticeable in the first 3 patients. Patient D did not show a significant increase in CD8+ cells and the last 3 patients have not yet shown a significant change. Individual responses to ASCT differed from each patient as shown in table 3. Patient B showed the most impressive response with a virtual complete disappearance of aberrant T cells. The fluorescent-activated cell-sorting (FACS) data form patient B is shown in figure 2.

Figure 3 shows the trend of aberrant T cells and body weight for the first 4 patients who have a follow-up period of at least one year. Follow up of patients E, F and G is yet limited. Two years after transplantation, our first patient (patient A) is showing further improvement in his immunopathology status as demonstrated in further decline in the percentage of aberrant T cells to 3% and histologically improved from Marsh III-A to Marsh-I and the second patient
(patient B) still showing persistent complete clinical and histological response. Patient D had no significant change in the percentage of aberrant T cells, showed no histological improvement and also no significant improvement in CD8+ percentage and he died 8 months post-transplantation. After ruling out structural and infectious (bacterial and viral) causes, we assumed that progressive disease of RCD-II with oligoclonal T lymphocytes infiltrating the brain was the cause of death in this particular patient. EATL could not be detected. Autopsy confirmed the presence of chronic encephalitis of the right temporal lobe with T-lymphocytes infiltration. Immunohistochemistry showed that the lymphocyte infiltrate was CD3 positive and the majority of cells expressed CD8 positivity. TCR gene analysis showed the T-cells to be oligoclonal.
Table 2 Clinical and laboratory tests before and at the last follow up after ASCT. Normal range: Albumin (34-50 g/l), iron (10-32 uM0l/l), calcium (2.20-2.60 mmol/l), folic acid (>5.9 nmol/l), B12 (156-672 pMol/l)
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**Table 3:** Histological and phenotypical flow-cytometric analysis of IEL’s in duodenal biopsies before (1-6 months) and after (3, 12, and 24 months) ASCT. ly = lymphocytes. Normal range for Cyt CD3’surf CD3’ % of lymphocytes ≤ 10%. TCRγ-PCR analysis = T cell receptor γ–polymerase chain rearrangement. Mean* = calculated for values at 3 months post ASCT.
Figure 2: Flow-cytometric analysis of duodenal cells obtained from patient B, showing the change in the percentage of aberrant T-cell population pre- and post- ASCT. Aberrant population is shown as CD7+CD3- within CD103+ lymphocytes *(left hand side)* or as cytoplasmic CD3+ surface CD3- within lymphocyte gate *(right hand side).*

Normal range for Cyt CD3+surf CD3- % of CD103+ lymphocytes ≤10%
**Figure 3**: The trend of aberrant T cells and body weight per patient. Ly= lymphocytes. Before = 1-3 months. Normal range for Cyt CD3+surf CD3- % of lymphocytes ≤ 10%
Discussion

In this pilot study ASCT in RCD II patients was shown to be feasible. The conditioning regimen was well tolerated in all patients and there was a substantial clinical improvement. The rapid initial response (within 3 months) and the duration (2 years in patient A and B and 14 months in patient C) of the remission up to now are promising. Complications included the occurrence of neutropenic fever in 2 patients and retinal bleeding in one patient not related to thrombocytopenia, all with full recovery. The nadir leucocytes and platelets counts and the duration of leucopenia and thrombocytopenia were comparable to our experience in patients with Non-Hodgkin lymphomas and multiple myeloma receiving ASCT after a combination of carmustine, etoposid, cytarabine, and melphalan (BEAM) or high dose melphalan (HDM,200 mg/m²). As there is no standard conditioning regimen for ASCT used in autoimmune disease, a standard regimen used in our institution was used. Fludarabine induces T cell depletion while the alkylating agent melphalan was used to achieve myeloablation. One patient was excluded due to unsuccessful leucopheresis. Although we could achieve successful leucopheresis in all patients despite earlier 2-CDA therapy, it is possible that the reason for failure of stem cell mobilization in one particular patient might be related to the use of 2-CDA. T cells play an essential role in the pathogenesis of CD and RCD-II / EATL.

Through the activity of the enzyme tissue transglutaminase (tTG) glutamine residues in gluten are converted into glutamic acid. Subsequently a multitude of gluten-derived peptides is generated, that, when bound to either HLA-DQ2 or DQ8 can induce T-cell responses in CD patients. A particular gluten and proline rich 33-mer α-gliadin peptide that contains 6 different T-cell stimulatory sequences and is resistant to gastric and duodenal proteolysis might be the primary initiator of the inflammatory response to gluten. In the large majority of patients, even in children with CD, inflammatory T-cell responses to other gluten peptides are also observed, implicating multiple gluten peptides in the disease process.

The definition of RCD I/II has undergone refinement in recent years. It seems that the most
reliable available method to differentiate between RCD I and RCD II is flow-cytometry of intestinal biopsies revealing the presence of aberrant T cells. Detection of a clonal T cell population by testing for TCR rearrangement was thought to be highly predictive of EATL development. However, oligo- or monoclonal IEL’s populations can be detected in the large majority of both RCD I and RCD II patients, also in patients that do not develop an EATL. Clonality is therefore of limited use in establishing the diagnosis of RCD and to predict the development of EATL.\(^{14,37,38}\)

RCD II is usually resistant to any known immunosuppressive therapy, including azathioprine/prednisone\(^{15}\), cyclosporine\(^{16}\) and IL 10 therapy.\(^{17}\) Recently, we treated 17 patients with 2-CDA on intention to induce remission. Within a mean follow up period was 22 months (range 7- 67 months) 47% had a significant decrease in aberrant T-cell percentages with or without clinical response.\(^{39}\) However, another 41% did not respond clinically, histologically nor immunopathologically and subsequently died from EATL.

Remissions of autoimmune diseases have been described in adults after both allogenic and autologous ASCT\(^{1-7}\) most probably due to the extreme immunosuppressive effects of these strategies\(^1\), resulting in immunoablation with subsequent regeneration of naïve T lymphocytes derived from reinfused hematopoietic progenitor cells.\(^7\) Furthermore, recently, interesting insights into possible unsuspected mechanisms by which stem cell transplantation could affect the gut have emerged. In both animal and patient studies, sex mismatched allogeneic stem cell transplants have shown in both mice and women that a population of myofibroblast derived from the donor populates the intestinal mucosa. Given the importance of myofibroblasts in orchestrating the function of epithelial cells, these data suggest a mechanism other than one targeted at immunosuppression that could beneficially reset patient functions, for example enhancing barrier function following stem cell transplantation.\(^{40}\)

These positive results, the high risk of transforming into EATL and the absence of effective therapy for RCD with aberrant T-cells led us to introduce this new strategy with the ultimate goal of resetting the immune response which might prevent or delays development of overt
EATL. On follow up, our patients showed improvement in the small intestinal histology, together with impressive clinical improvement as demonstrated by disappearance of diarrhea and abdominal pain, normalization of serum albumin, electrolytes and hemoglobin, increase in BMI and improvement of the performance status. Two years after transplantation, our first patient is showing further improvement in his immunopathology status as demonstrated in further decline in the percentage of aberrant T cells to 3% and histologically improved from Marsh III-A to Marsh-I. We propose that enhanced apoptosis of activated but aberrant T cells has led to this late but remarkable decline.\(^{41}\) One patient died 8 months post ASCT from progressive neurological manifestations in association with CD. Autopsy had excluded any structural or infectious cause. One patient had developed self-limiting erythematous plaque skin lesions with central necrosis two months post ASCT. Detailed analysis had excluded the presence of EATL. Our most recent patient with clinically short bowel syndrome is showing remarkable clinical, endoscopic and immunological improvement. All our patients had negative serology before inclusion confirming their strict adherence to GFD and after ASCT all patients remained negative for anti-tTG and EMA. Furthermore, the first 3 patients showed a significant increase in the percentage of CD8+ lymphocytes, which is seen as a marker of lymphocyte regeneration after ASCT.\(^{42}\) Patient D did not show a significant increase in CD8+ cells and the last 3 patients have not yet shown a significant change. Absence of a demonstrable improvement in the surface expression of CD8 on the IEL might be regarded as a poor prognostic indicator of response; this is only to be proved or disproved on longer term follow up. Although the short term results in these patients are promising, follow up at present is too short to permit firm conclusions as to efficacy. The selection of patients for this treatment should be restricted to those patients with a substantial population of aberrant T cells, even after therapy with 2-CDA who have a greater tendency to progress to highly lethal EATL. High-dose chemotherapy followed by ASCT seems feasible and safe, and might result in long-term improvement of disease activity in RCD patients with aberrant T cells whose
condition previously did not respond to available treatments. Longer-term follow up and additional pilot studies with larger groups of patients are needed to confirm the efficacy of this therapy.

References


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Autologous hematopoietic stem cell transplantation in refractory celiac disease with aberrant T-cells