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In vivo T-depleted reduced intensity transplantation for GATA2-related immune dysfunction.

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Key points
• Fludarabine-based reduced intensity transplantation with T depletion is safe and effective
• Severe infection or respiratory compromise are indications for transplantation, even in the absence of myeloid neoplasia

Running Title
Transplant for GATA2 immunodeficiency

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Germline heterozygous GATA2 mutation causes failure of mononuclear cell development, immune dysfunction, and evolution to myeloid neoplasia\textsuperscript{1-4}. Symptoms may arise at almost any age, reaching a penetrance of 90% in the seventh decade\textsuperscript{3}. Fatal complications include mycobacterial infection, uncontrolled herpesvirus infection, HPV-associated carcinoma and hematological transformation\textsuperscript{1-4}. Bone marrow transplantation is curative but the optimal timing and protocol remain undefined\textsuperscript{4-11}. Patients who developed GATA2-related MDS or AML have been successfully transplanted with myeloablative and reduced intensity conditioning, following standard clinical algorithms for hematological malignancy\textsuperscript{2-4}. However, 21-60% of GATA2 patients do not present with MDS or AML\textsuperscript{2,3} and may suffer severe infectious complications, including significant pulmonary dysfunction. The optimal therapy for this group remains undefined\textsuperscript{8,9}.

Here we present a retrospective analysis of four patients, successfully transplanted with a T-cell depleted reduced intensity regimen for predominantly infectious and respiratory complications of GATA2 mutation. They include consecutive patients in our cohort transplanted at two UK centers, Manchester and Newcastle, with at least 2 years of follow up (2008-2012). Their laboratory indices have been previously described\textsuperscript{2,12,13} and are summarized in Table S1. Patients 1-3 had grossly elevated fms-like tyrosine kinase-3 (flt3) ligand and a severe DCML deficiency phenotype with relatively well preserved hemoglobin, neutrophils and platelets. Patient 4 had monocytopenia in the context of more conventional MDS with anemia, thrombocytopenia and mildly elevated flt3 ligand. GATA2 mutation and clinical features are summarized in Table 1. The interval between presentation and transplant was 1-8 years and the age at transplantation, 16-35 years. Patients 2-4 had a family history. Patients 1-3 had experienced severe infection: Patient 1 with BCG; Patient 2 with pulmonary P. aeruginosa and Methicillin resistant S. aureus and with genital HPV infection, causing vulval intra-epithelial neoplasia (VIN) grade III; Patient 3 with M. malmöense (Figure 1). Patients 2-4 had moderate to severe respiratory compromise due to pulmonary alveolar proteinosis (PAP) or emphysema. At the time of transplantation, patients 1 and 3 were receiving anti-mycobacterial therapy. Patient 2 required continuous oxygen and non-invasive ventilation at night and had been scheduled for radical surgical resection of advanced VIN. Patient 3 had a history of granulomatous hepatitis and Addison’s disease that may have been attributable to GATA2 mutation\textsuperscript{14}. Bone marrow examination was relatively unremarkable with normal cytogenetics and no excess of blasts in all patients. Patients 1-3 were hypocellular with small hypolobulated megakaryocytes, mild increases in reticulin and scattered non-caseating granulomata (Figure 1A). Only Patient 4 had clear evidence of trilineage dysplasia but no excess of blasts. A BM examination 8 years previously had been reported as normal. Both 2 and 4 had acquired ASXL1 mutation at the time of transplantation. Patient 2 had delivered her first child prematurely at 28 week’s gestation.

Transplantation is summarized in Table 1. All patients received five doses of fludarabine 30mg/m\textsuperscript{2} (day -7 to -3 or day -6 to -2) and an alkylating agent: melphalan 140mg/m\textsuperscript{2} day -2; or busulfan IV 3.2mg/kg day -7 to -6; or treosulfan 14mg/m\textsuperscript{2} day -6 to -4. Choice of alkylator reflected unit practice and was not patient-specific.
in vivo T cell depletion with alemtuzumab was given at a total dose of 0.7 mg/kg to 1.2mg/kg in 2-5 divided doses. Mobilized PBSC grafts were infused at a dose of 1.9 – 8.0 x10^6 CD34^+ cells/kg supported with G-CSF from day +6. All patients received ciclosporin GVHD prophylaxis; Patients 1-3 with unrelated donors also received mycophenolate mofetil 1g twice daily for 30-60 days post transplant. All patients were discharged from hospital between 14 and 33 days post transplant. Three patients experienced grade I delayed acute GVHD (skin rash) during immunosuppression withdrawal but none required systemic therapy and none went on to develop chronic GVHD (Table 1). Patient 1 experienced an immune reconstitution syndrome of non-GVHD rash and fever in the first month, a common complication of BMT during active mycobacterial infection^15, and required a short course of systemic corticosteroids. ITP was observed in patient 2 and haemolytic anaemia in patient 3, at 7 and 9 months post transplant, respectively. These complications are observed in other patients transplanted with fludarabine and Alemtuzumab-based conditioning and resolved within 4 weeks after initiation of treatment with Rituximab (375mg/m^2 per week for 4 weeks). High levels of myeloid and T cell chimerism were observed in all patients by three months(Figure 1B). B cell chimerism was not routinely monitored. Patients 2-4 with significant respiratory compromise showed a marked and sustained improvement within weeks of transplant (Figure 1C). Continuous oxygen and non-invasive ventilation at night was rapidly withdrawn from patient 2 in association with dramatic radiological improvement (Figure 1D). Anti-mycobacterial drugs were discontinued post-transplant in patients 1 and 3, within 1 year, when CD3 T cells had increased above 200/µl. Patient 3 and his donor were both seropositive for CMV and a short reactivation was treated uneventfully with oral valganciclovir. The other 3 transplants were matched CMV negative. Patient 2 was vaccinated with bivalent HPV type 16/18 vaccine at 20, 21 and 26 months post-transplant and strikingly, her HPV-associated VIN III completely regressed by 36 months. She subsequently had two successful full-term pregnancies. Patient 1 was diagnosed with malignant melanoma 3 years after transplant but this was fully resected without further evidence of disease. All patients are alive between 4 and 8 years post-transplant with Karnofsky Performance Score 90-100%.

These cases illustrate that fludarabine-based reduced intensity transplantation with alemtuzumab in vivo T depletion is acceptable for patients with severe infectious and respiratory complications of GATA2 mutation. Alemtuzumab-based conditioning provided excellent control of graft versus host disease (GVHD) even with mismatched donors. Although it is surprising that GVHD occurs at all in the absence of recipient dendritic cells, monocytes and B cells, it has been reported as a lethal complication in other series^6,8,9,11. T cell depletion was not problematic and the rapid restoration of donor DCs, monocytes, B cells and NK cells led to a full recovery.

Life-threatening infection, severe respiratory compromise due to PAP, or both, were the major triggers for transplantation. In retrospect, it was discovered that 2 of the 4 patients had developed clonal hematopoiesis with ASXL1 mutation. Patients with both severe infection and myeloid neoplasia are especially challenging as reductions in conditioning intensity for infection-related co-morbidity may compromise the
durability of remission from MDS or AML. Relapses were observed in a previously reported cohort following non-myeloablative conditioning with fludarabine and 2Gy total body irradiation. Fludarabine combined with moderate dose alkylating agents, as employed here, has a track record in the transplantation of adults with MDS or AML. Our results suggest that this approach is a useful compromise for patients with uncontrolled infection and clonal hematopoiesis or early MDS. In females of reproductive age, fertility may already be compromised by pre-term labor and cervical or vulval intra-epithelial neoplasia. Transplantation may indeed improve these prospects, as in the case of patient 2.

In summarizing this experience, we propose that life-threatening complications of GATA2-related disease including PAP, atypical mycobacterial infection and advanced HPV-associated dysplasia should be considered indications for transplantation, even in the absence of overt myeloid neoplasia. PAP responds poorly to conventional therapy with whole lung lavage and GM-CSF, but resolves within weeks of transplantation. Recurrent mycobacterial infection may exacerbate respiratory decline beyond the point of salvage if immune reconstitution is not provided. HPV-related intra-epithelial neoplasia can lead to disfiguring surgery which may prove ineffective at preventing lethal metastatic disease. Detection of the accessory cellular phenotype of DCML deficiency is critical in identifying these patients, who present to a wide range of specialist services but rarely to hemat-oncology. Once identified, thorough hematological evaluation is mandatory to exclude myeloid neoplasia and annual BM surveillance for clonal evolution is prudent. Finally, owing to the highly variable nature of GATA-2 associated syndromes, it is unlikely that a single approach to transplantation will suit all patients.


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Authorship contributions
Eleni Tholouli identified patients, provided clinical care and reported clinical data
Katherine Sturgess reported clinical data, analyzed data and wrote the manuscript
Rachel Dickinson performed sequencing and molecular analysis
Andrew Gennery provided clinical care and reported clinical data
Andrew Cant provided clinical care and reported clinical data
Graham Jackson provided clinical care and reported clinical data
Jim Lordan identified patients, provided clinical care and reported clinical data
Sophie Hambleton identified patients, provided clinical care and reported clinical data
Mary Slatter provided clinical care and reported clinical data
Venetia Bigley identified patients, reported clinical data, analyzed data and wrote the manuscript
Matthew Collin reported clinical data, analyzed data and wrote the manuscript

Disclosure of conflicts of interest
The authors have no conflict of interest to disclose
Table 1. GATA2 mutation, clinical features, transplantation and outcome

GATA mutation at DNA (for insertion) or protein coding level. Relevant family history was confirmed by GATA2 sequencing in at least 1 first degree relative. The total doses of drugs administered for transplant conditioning is indicated. HLA allele match is described with mismatched alleles in parenthesis. All patients are currently alive. Abbreviations: HPV: human papilloma virus; VIN: vulval intra-epithelial neoplasia; PAP: pulmonary alveolar proteinosis; MRSA: methicillin-resistant S. aureus; Flu: fludarabine; Mel: melphalan; Bu: busulfan; Treo: treosulfan; Alem: Alemtuzumab; MUD: matched unrelated donor; Sib: sibling; Ciclo: ciclosporin; MMF: mycophenolate mofetil; ITP: idiopathic thrombocytopenic purpura; AIHA: autoimmune haemolytic anemia; aGVHD acute graft versus host disease; KPS: Karnofsky performance score; My: myeloid; T: T cell; CMV (D/ R): cytomegalovirus serostatus (donor / recipient).
<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation Family history</th>
<th>Age at onset M/F</th>
<th>Age (year) transplanted Days to discharge</th>
<th>Conditioning Graft GVHD prophylaxis CMV (D / R)</th>
<th>Survival % donor (My / T))</th>
<th>Significant post-transplant events</th>
<th>KPS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.599_600insG De novo</td>
<td>12 M</td>
<td>16 (2008) +33</td>
<td>Flu 150 mg/m² Mel 140 mg/m² Alem 0.2mg/kg -8 to -4 (1mg/kg) 9/10 (C) MUD Ciclo MMF NEG / NEG</td>
<td>Alive</td>
<td>8 years 100 / 85</td>
<td>Immune reconstitution syndrome</td>
</tr>
<tr>
<td></td>
<td>- Recurrent BCG-osis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Melanoma 100%</td>
</tr>
<tr>
<td>2</td>
<td>R398W Family history chest/MDS</td>
<td>20 F</td>
<td>23 (2010) +28</td>
<td>Flu 150 mg/m² Bu 6.4 mg/kg, Alem 30mg x 2 -4; -2 (60mg) 8/10 (A,DQ) MUD Ciclo MMF NEG / NEG</td>
<td>Alive</td>
<td>6 years 100 / 96</td>
<td>ITP (Rituximab) At 7 months</td>
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<tr>
<td></td>
<td>- HPV 16/18 with VIN III</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Two viable pregnancies 100%</td>
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<td></td>
<td>- PAP</td>
<td></td>
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<td></td>
<td>- MRSA and P. aeruognosa</td>
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<tr>
<td></td>
<td>- ASXL1 mutation</td>
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<td></td>
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</tr>
<tr>
<td>3</td>
<td>R398Q Family history MDS/AML</td>
<td>34 M</td>
<td>35 (2012) +18</td>
<td>Flu 150 mg/m² Treo 42 mg/m² Alem 10mg/day -7 to -3 (50mg) 10/10 MUD Ciclo MMF POS / POS</td>
<td>Alive</td>
<td>4 years 100 / 95</td>
<td>Grade I aGVHD No chronic GVHD</td>
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<tr>
<td></td>
<td>- Mycobacterium malmoense</td>
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<td></td>
<td>AIHA (Rituximab) at 9 months</td>
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<td></td>
<td>- granulomatous hepatitis</td>
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<td></td>
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<td>90%</td>
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<td>- Addison’s</td>
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<td>- Emphysema</td>
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<tr>
<td>4</td>
<td>G200fs; 390delIK Family history MDS/AML</td>
<td>21 M</td>
<td>29 (2012) +14</td>
<td>Flu 150 mg/m² Treo 42 mg/m² Alem 10mg/day -7 to -3 (50mg) Matched sib Ciclo NEG / NEG</td>
<td>Alive</td>
<td>4 years 100 / 100</td>
<td>Grade I aGVHD No chronic GVHD</td>
</tr>
<tr>
<td></td>
<td>- PAP</td>
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<td>100%</td>
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<td>- Trilineage dysplasia</td>
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<td>- ASXL1 mutation</td>
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Figure 1. Bone marrow morphology, infection, post transplant chimerism and recovery of respiratory function

A. (i-iii) patient 1; (iv-vi) patient 2. Panels depict: (i, iv) Gomori reticulin stain; (ii, v) megakaryocyte morphology by H and E staining; (iii) acid fast bacilli in a skin biopsy of patient 1; (vi) HPV 16 and 18 RNA by in situ hybridization in a cervical biopsy of patient 2.

B. Percentage donor myeloid and T cell chimerism in each patient as indicated with time after transplantation.


D. Chest radiographs of patient 2 pre transplant with bilateral central symmetrical lung opacities, relative sparing of the apices and costophrenic angles, extensive diffuse and military opacities, which had significantly resolved 6 months post-transplant.
Figure 1

A

B

C

D

Patient 2

Pre

Post