# STEM CELL THERAPY PROTECTS THE RENAL ALLOGRAFT FROM IMMUNE INJURY SINGLE CENTRE EXPERIENCE


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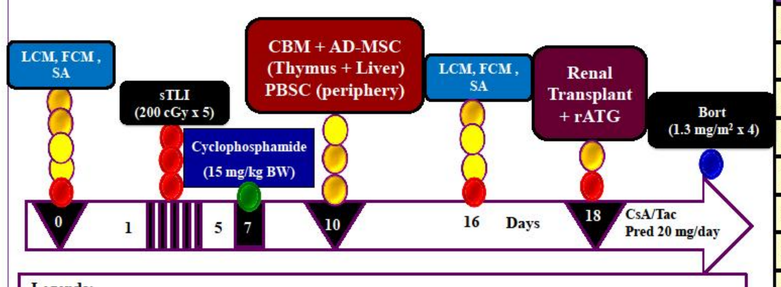
## BACKGROUND AND OBJECTIVES

- Donor specific antibodies (DSA) are believed to cause immune injury to renal allografts eventually leading to graft loss.
- Stem cell therapy (SCT) holds promise of allograft protection from immune injury.
- We present a cohort of renal transplant (RT) patients subjected to SCT, having stable graft function in spite of de novo DSA and compare them with RT patients without DSA.

## METHODS

**Retrospective study of 271 Patients Subjected To Pre-transplant SCT**

**Ahmedabad Tolerance Induction Protocol**




Legends:
LCM : Lymphocytotoxicity cross-match
SA : Single antigen
r-ATG : rabbit Anti-thymocyte globulin (1.5 mg/ kg BW)
CBM : Cultured donor bone marrow
AD-MSC : (Donor) adipose tissue derived mesenchymal stem cells
PBSC : Donor-derived peripheral blood stem cell infusion
FCM : Flow cross-match
Bort : Bortezomib
sTLI : Sub total lymphoid irradiation

IRB Approved

DSA (Luminex assay) and T-regulatory cells (Tregs) ($CD127^{low/neg}CD25^{high}CD4^{+}$) repeated every 6 months

**INCLUSION CRITERIA**
- RT Patients subjected to SCT
- S. Creatinine (SCr) <2 mg/dL

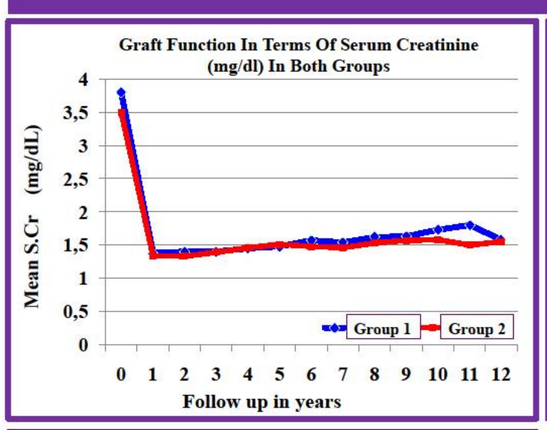
**EXCLUSION CRITERIA**
- HBsAg/HCV/HIV Positive
- Malignancy

**Patient Demographics**

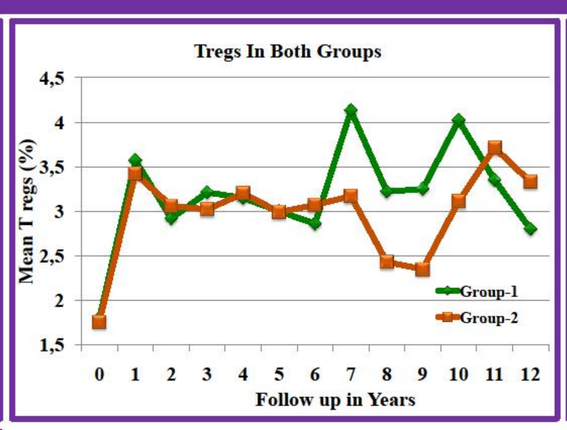
| Groups | Group-1 ( DSA Present ) | Group-2 ( DSA Absent ) |
|---|---|---|
| Number (males: females) | 153 (137: 16) | 118 (102: 16) |
| Age (years) | 31.5 9.98 | 33 10.4 |
| Mean HLA match | 2.43 1.3 | 2.84 1.47 |
| Donor age (years) | 44.6 11.3 | 45.4 9.8 |
| Males: females | 46: 107 | 30: 88 |
| Relation: Parents | 36 (23.5%) | 25 (21.2%) |
| Spouse | 77 (50.3%) | 65 (55.1%) |
| Siblings | 22 (14.4%) | 21 (17.8%) |
| Others | 18 (11.8%) | 07 (5.9%) |
| Commonest original disease: CGN | 82 (53.6%) | 65 (55.1%) |
| Chronic tubulointerstitial nephritis | 25 (16.3%) | 12 (10.2%) |
| Hypertensive nephropathy | 17 (11.1%) | 06 (5.1%) |
| Diabetic nephropathy | 05 (3.3%) | 06 (5.1%) |
| Others | 24 (15.7%) | 29 (24.6%) |

All variables were statistically similar except for HLA match with $p < 0.05$, better in group-2

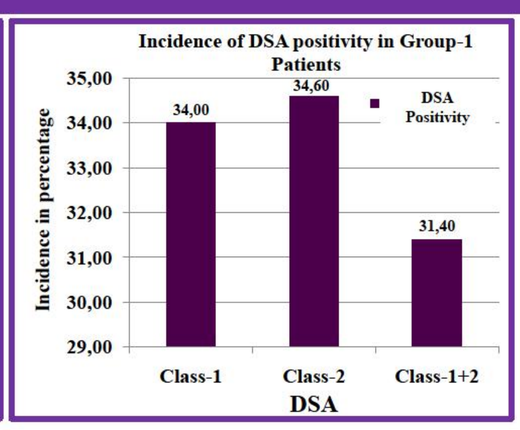
## RESULTS




Over mean follow-up of 7.26 years in group-1 and 6.55 years in group-2, graft function was similar in both groups; with S. Cr. of 1.59 mg/dl in gp-1 and 1.53 mg/dl in gp-2 .




Mean T-regs ([$CD127^{low/neg}CD25^{high}CD4^{+}$] in both groups remained between 2.4% to 4%



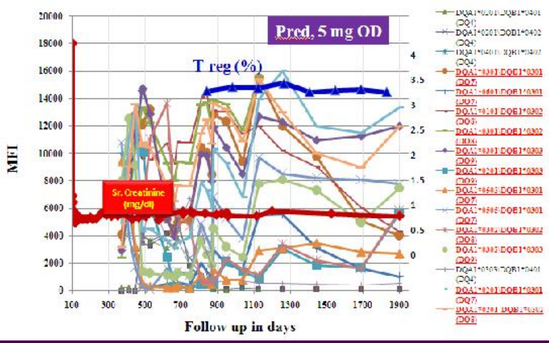

DSA class-1 were persistently observed in 34% , class-2 in 34.6% and both class 1+2 in 31.4% post-Tx.

| Groups | Group-1 ( DSA Present ) | Group-2 ( DSA Absent ) |
|---|---|---|
| Mean follow-up (years) | 7 .26 3.37 | 6.55 3.54 |
| Mean S. Creatinine (mg/dl) | 1.59 0.53 | 1.53 0.54 |
| Mean Tregs (%) ($CD127^{low/neg}CD25^{high}CD4^{+}$) | 2.81 1.27 | 3.01 1.47 |
| Normal protocol biopsies | 36/60 (60%) | 26/46(56.5%) |
| Suspicious B and/ or T-cell mediated rejections* (Banff score <ag1 at1 av1 ai1) | 15/60 (25%) | 12/46 (26%) |
| Drug toxicity | 6/60 (10%) | 4 /46 (8.8%) |
| Recurrence | 1/60(1.6%) | 1 /46 (2.2%) |
| Others | 2/60 (3.4%) | 3/46 (6.5%) |
| **Immunosuppression** | | |
| No Drug | 9/153 (5.8%) | 6/118 (5%) |
| Single drug | 27/153 (17.6%) | 24/118 (20.3%) |
| Two Drug | 89/153 (58.1%) | 58 /118 (49.1%) |
| Triple Drug | 28/153 (18.3%) | 30/118 (25.4%) |

None of the patients had severe acute rejections

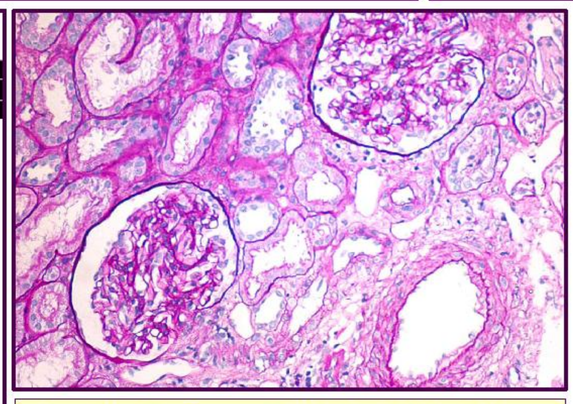
**SH, 53/F, Donor : Son, Tx dt: 26 Nov '08** | 10 Feb '14 | POD: 1987

| | A | | B | | C | | DRB1 | | DQ | | DRB345 | | Bw | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PT | 2 | 11 | 8 | 40 | 7 | 15 | 17(3) | 15(2) | 2 | 6 | 51 | 52 | 6 | - |
| DN | 11 | 24(9) | 18 | 40 | 12 | 15 | 11 | 15(2) | 3 | 6 | 51 | 52 | 6 | - |

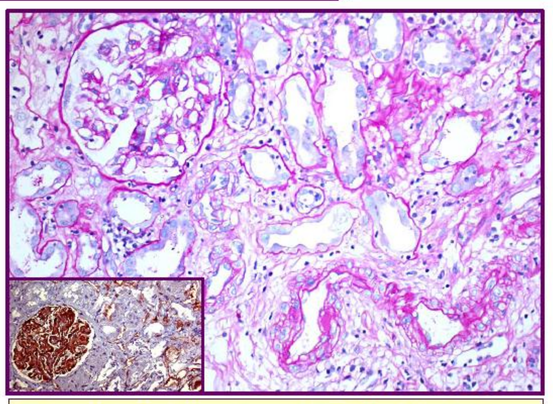



Example : Graph showing the complete status of a patient from Group-1; on top is initials , age, and in the box is the date when this graph was prepared. Right side shows days since induction of protocol. HLA status of patient (PT) & donor (DN) are shown on the top.

- X - axis : Follow up in days since the patient was inducted in protocol
- Y - axis : Left : Antibody measurement ( By Luminex assay) in mean fluorescent intensity (MFI)
  Right : SCr (mg/dL) & peripheral Tregs in percentage.
- Figure shows that SCr & Tregs has remained fairly stable in spite of persistent high level of DSA.
- On right corner is photomicrograph showing renal allograft biopsy performed at 3 years post-transplant showing normal glomeruli, medium calibre artery and surrounding tubules, PAS x 200.



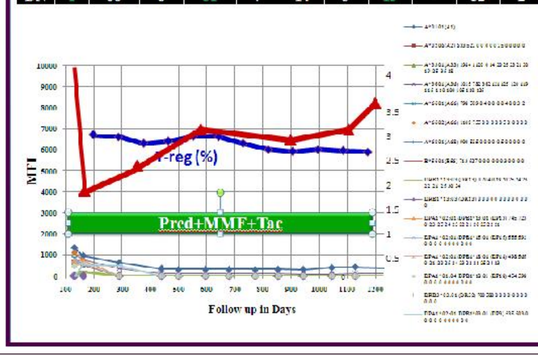
3 yrs PostTx, Unremarkable Graft Morphology Banff update 2007,Type I; (PAS X 200)



3 yrs PostTx, Chronic activeT +B cell rejection Banff Update 2007, Type II + IV; (PAS, x 200) ( inset-C4d Positive, x 200)

**IR, 39/M, Donor: Mother ; Tx dt: 1 Nov'10** | 16 Mar'14 | POD: 1231

| | A | | B | | Cw | | DRB1 | | DRB345 | | DQ | | Bw | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PT | 30 | 68 | 8 | 49 | 7 | | 3 | 8 | | 52 | 2 | 7 | 4 | 6 |
| DN | 1 | 68 | 8 | 51 | 7 | 14 | 3 | 13 | - | 52 | 2 | 6 | 4 | 6 |




Example : Graph showing the complete status of a patient from Group-2; on top is initial, age, the date when this graph was prepared & the right side shows days since induction of protocol. HLA status of patient (PT) & donor (DN) are shown on the top.

- X - axis : Follow up in days since the patient was inducted in protocol
- Y - axis : Left : Antibody measurement in MFI
  Right : SCr (mg/dL) and Tregs (%)
- The different colored spikes are for antibodies shown by asterix in right side, red line is SCr & blue line for Tregs.
- Figure shows that Tregs have dropped with rising SCr although he never had DSA.
- On left side is photomicrograph showing renal allograft biopsy performed at 3 years showing chronic active T+ B-cell mediated rejection (PAS, x 200) with C4d deposits on the peritubular capillary membranes (inset). C4d stain with background of hematoxylin, x 200.

## DISCUSSION

- DSA are believed to be detrimental to graft survival by causing antibody mediated injury.[1]
- SCT is believed to induce chimeric tolerance.[2]
- We have been using SCT (HSC +AD-MSC) to achieve stable graft function with minimization of immunosuppression and rejection since 1998.[3]
- MSC exhibit their genetically unrestricted immunosuppressive effects by inhibition of proliferation and function of T-cells, B-cells and NK cells in a dose-dependent manner. [3-5]
- MSCs also have tolerogenic effect by which they prolong survival of organ grafts and prevent GVHD.[5]
- MSCs avoid allogenic rejection by being hypoimmunogenic, modulating T-cell phenotype and by creating an immunosuppressive local milieu.[5-7]
- Generation of Tregs from SCT helps in sustaining tolerance through "linked suppression" and "infectious tolerance". [5-8]

## CONCLUSIONS

- Stem cells can protect the graft from immune injury despite DSA.
- Tregs [$CD127^{low/neg}CD25^{high}CD4^{+}$] help in sustaining stable graft function by preventing injury caused by DSA through the phenomenon of "linked suppression" and "infectious tolerance"

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51ERA-EDTA 606--MP

N) Renal Transplantation. N1) Transplantation basic science and immune-tolerance of allogenic and xenogenic transplants.
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DOI: 10.3252/pso.eu.51era.2014

Poster presented at: 51 ERA-EDTA CONGRESS AMSTERDAM THE NETHERLANDS MAY 31 JUNE 3 2014