350 - SIV PERSISTS IN LYMPHOID TISSUES DESPITE ALEMTUZUMAB-INDUCED CD4+ T CELL DEPLETION

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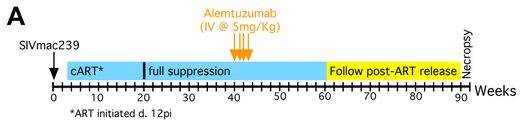
Background

Alemtuzumab (ATM) is a licensed humanized anti-CD52 monoclonal antibody used for pre-transplant conditioning, cutaneous T cell lymphoma, and relapsing-remitting multiple sclerosis. The CD52 antigen, a cell surface antigen of unknown function, is expressed at different densities on all T cell subsets, NK cells, B cells and myeloid cells[1]. ATM depletes CD52-positive cells via antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity[1]. In humans, ATM leads to preferential depletion of naïve (TN) and central memory T cells (TCM) with minimal change in myeloid cells and effector memory T cells (TEM)[2-4]. The preservation of TEM has been linked to low incidence of opportunistic infections following ATM for multiple sclerosis[5]. Here, we evaluated whether ATM can reduce the viral reservoir by depletion of long-lived latently infected CD4$^+$ memory T cells in SIV-infected rhesus macaques (RM) on suppressive combination antiretroviral therapy (cART). We hypothesized that CD4+ T cell depletion by ATM treatment, followed by reconstitution will reduce the reservoir significantly and potentially lead to sustained virus remission after cessation of cART.

References

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Experimental Protocol



- *Rhesus macaques (RM) were intravenously inoculated with SIVmac239 and placed on cART (TDF/FTC/DTG) 12 days later.*
- *At 42 weeks post-infection (pi), 6 RM received 4 intravenous doses of alemtuzumab @ 5mg/kg on 294, 301, 308 and 323 days pi.*
- *PBMC, lymph node and small intestinal biopsies were obtained before and after alemtuzumab to determine changes in cell-associated SIV DNA/RNA.*

Figure 1: (**A**) Schematic representation of the animal protocol. (**B**) Plasma viral load profiles of RM after intravenous inoculation with SIVmac239 and cART administration 12 days post-infection. At 42 weeks post-infection, 6 RM received 4 intravenous doses of ATM at 5mg/kg. Three SIV-infected RM received cART but no ATM.

Alemtuzumab-depleted CD4+ T cells in cART suppressed RM

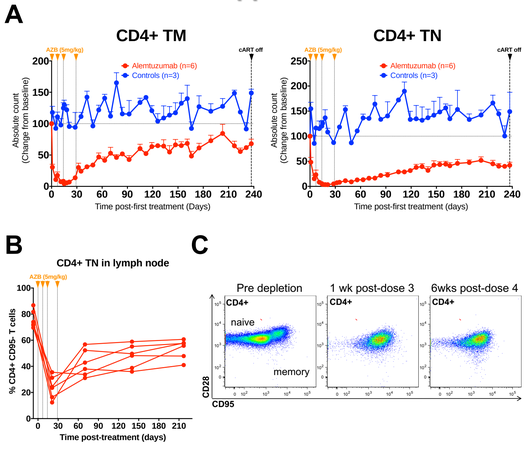


Figure 2: (**A**) Showing mean (+SEM) change in the absolute number of CD4+ total memory (TM) and CD4+ naïve (TN) T cells in blood of SIV-infected RM on cART treated with ATM (n=6) or controls (n=3). Note that ATM induced near complete (>95%) depletion of all circulating CD4+ T cell subsets. (**B**) Percentage of CD4+ CD95- TN in lymph nodes of 6 ATM-treated RM. (**C**) Flow cytometric plots of a representative ATM-treated RM gated on CD3+ CD4+ CD8- small lymphocytes in lymph node.

Depletion was accompanied by massive proliferation of CD4+ memory T cells

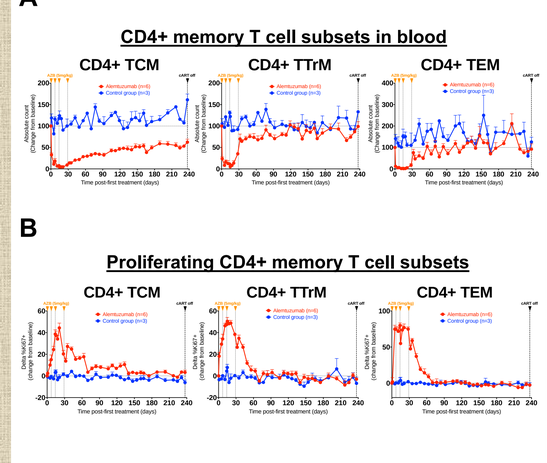


Figure 3: (**A**) Showing mean (+SEM) change in the absolute number of CD4+ central memory (TCM), transitional memory (TTrM) and effector memory (TEM) cells in blood of RM treated with ATM (n=6) or controls (n=3). (**B**) Mean (+SEM) change in the percentage of proliferating CD4+ TCM, TTrM and TEM cells in blood as measured by change in expression of the proliferation antigen, Ki-67.

SIV DNA declined in PBMC but remained stable in lymph node and small intestine

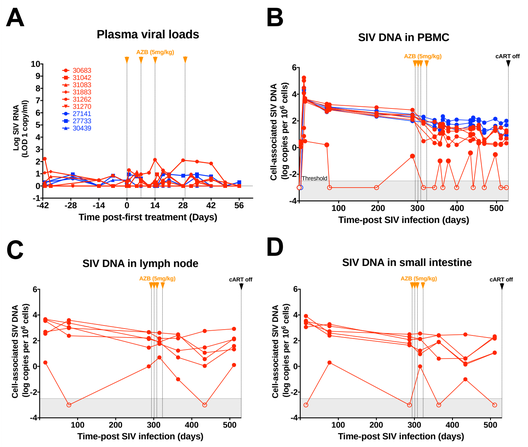


Figure 4: (**A**) Showing high sensitivity plasma viral loads (1 RNA copy/ml) of RM 6 weeks before and 8 weeks post-ATM. ATM-treated RM are shown in red and controls in blue. (**B**) Longitudinal quantification of cell-associated SIV DNA in PBMC of ATM-treated RM and controls. Note the ~0.5-1 log decline in SIV DNA in blood just prior to cART release. (**C-D**) Longitudinal quantification of SIV DNA in lymph node and small intestine of the 6 ATM-treated RM.

Decline in SIV DNA in PBMC was associated with the loss of CD4+ T cells

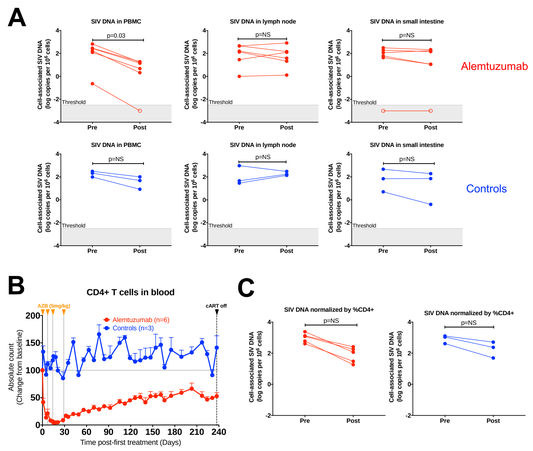


Figure 5: (**A**) Comparison of SIV DNA in PBMC, lymph node and small intestine measured 1 week before and 31 weeks after the first dose of ATM. (**B**) Mean (+SEM) change in the absolute number of CD4+ T cells in blood of SIV-infected RM on cART treated with ATM (n=6) or controls (n=3). (**C**) Comparison of SIV DNA in PBMC after normalization by the frequency of CD4+ T cells in blood.

Alemtuzumab did not alter kinetics of virus rebound

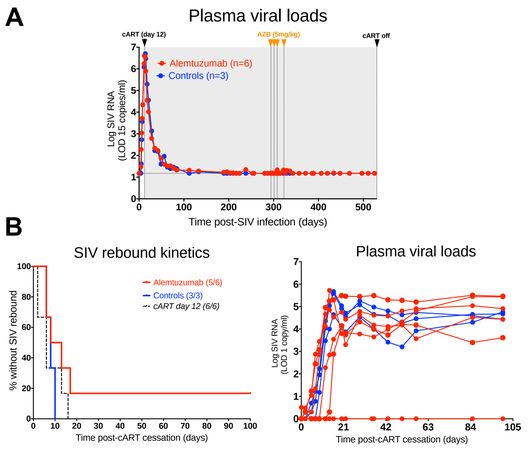


Figure 6: (**A**) Showing mean (+SEM) plasma viral load profiles of RM treated with ATM (n=6) or controls (n=3) through 518 days of SIV infection and just prior to cART release. (**B**) Kaplan-Meier curve of time to SIV rebound after cART release. Rebound kinetics of 6 RM placed on cART 12 days post-SIV infection and treated for a similar length of time are shown for comparison. (**C**) Individual plasma viral load profiles of ATM treated (n=6) and control (n=3) RM after cART release. Note 1 RM in the ATM-treated group with no viral rebound up to 100 days after stopping cART.

Summary

- ATM is a pan-lymphocyte depleting monoclonal antibody that effectively depleted >95% of CD4+ TM and TN in the blood of SIV-infected RM on cART. CD8+ T cells, NK cells, B cells and CD4+ T regulatory cells were also depleted.
- ATM-mediated CD4+ T cell depletion was accompanied by massive homeostatic proliferation of CD4+ TM, likely cells in tissues that survive the depletion. This proliferative response may induce the clonal expansion of residual CD4+ T cells carrying replication-competent proviruses.
- Although total SIV DNA in PBMC declined post-treatment, total SIV DNA in lymph node and small intestine remained stable.
- ATM did not alter the kinetics of virus recrudescence after cART release.
- This data suggests that depletion of circulating lymphocytes is not sufficient to reduce reservoir size and highlights the importance of targeting tissue-resident CD4+ T cells in HIV eradication strategies.

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