Generation of the Bispecific antibody to cross the Blood Brain Barrier in SHIV infected Rhesus Monkey

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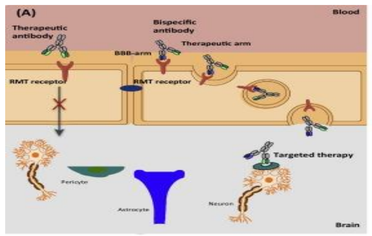
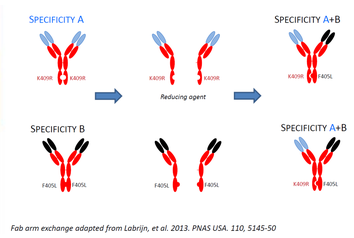
INTRODUCTION

PGT121, a broadly neutralizing antibody (bnAb) against the gp120 region of HIV envelope is in clinical trials for suppressing the progression of viral infection. However, in our earlier study, the PGT121 had low penetrance into the CNS, where concentrations in CSF of rhesus macaques infused intravenously with PGT121 was <0.2% compared with the plasma concentration. To improve upon this, we engineered a bispecific antibody of PGT121 so that one arm of the antibody binds to the human transferrin receptor (HtfR) and consequently taken up across the blood brain barrier through receptor mediated transcytosis.

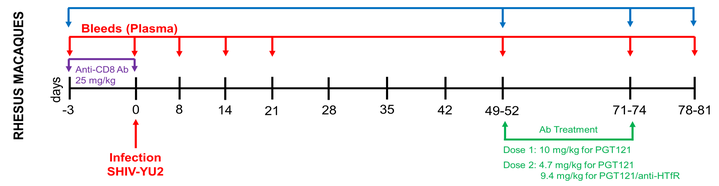




Fab arm exchange adapted from Labrijn, et al. 2013. PNAS USA. 110, 5145-50

METHODS

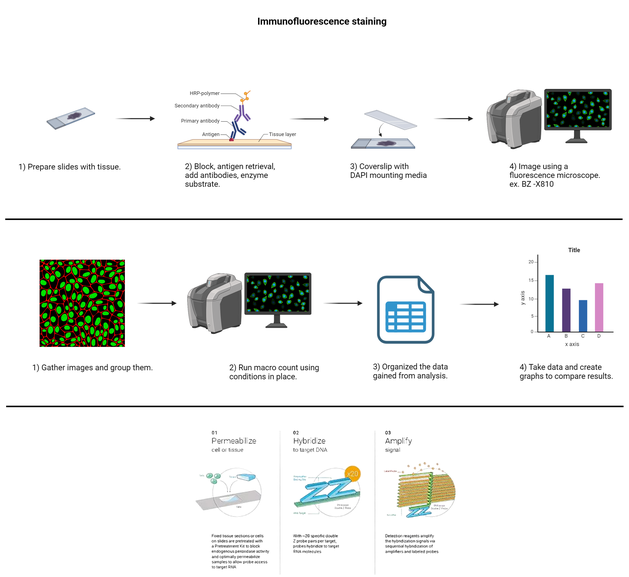
Using a Fab arm exchange methods, we generated a bispecific antibody with one arm binding the HIV envelop and the other arm binding the human transferrin receptor. Viral neutralization efficiency, binding properties, and in vivo toxicity of bispecific antibody were tested. Organ distributions of both parent (PGT121) and bispecific (PGT121/anti-HtfR) antibodies were also studied though administration of fluorophore tagged antibodies (Cy3, intrathecal administration; Cy5, intravenous administration). Efficiency against SHIV virus was studied in two group of macaques infected with SHIV-YU2 and administered with PGT121 for 1st dose, and the experimental group received PGT121/anti-HTfR for the 2nd dose, whereas control group received another dose of PGT121. One week later, animals were necropsied and RNA (plasma, CSF) and DNA (tissues) viral loads were determined using qPCR.



RESULTS

In vitro analysis showed that bispecific antibody neutralized most of the SIV and SHIV strains with one log higher in titer when compared with parent PGT121. Infusion into healthy rhesus macaques did not cause any significant clinical effects. Peripherally administered bispecific antibody was found to be present in CNS and peripheral tissues more than the parent antibody. Although no significant difference in plasma and CSF SHIV viral load was found between parent and bispecific antibody groups, bispecific antibody administered animals had low viral load in brain and peripheral tissues.

IMMUNOHISTOCHEMISTRY AND MACRO ANALYSIS AND RNAscope



HIGHLIGHTS OF THE FINDINGS

Figure 1. Bispecific antibody was safe in healthy macaques and detectable in CSF

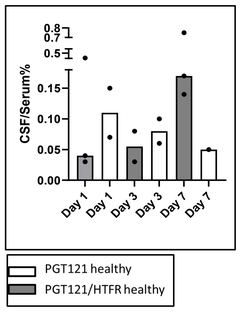


Figure 2. PGT121/anti-HTfR bispecific antibody neutralizes multiple strains of SHIV and HIV at a reduced potency

	Titer in TZM.bl cells (ug/ml)					
	PGT121 WT			PGT121 x 5G11		
Virus ID	IC50	IC80	MPI*	IC50	IC80	MPI
SHIV-SF162P3	0.015	0.040	100	0.120	0.320	100
SC422661.8	0.039	0.120	100	0.213	1.486	100
RHPA4259.7	0.008	0.029	100	0.040	0.202	100
CNE52	1.551	20.187	86	8.892	>25	62
CNE17	1.878	20.757	80	>25	>25	50
Du156.12	0.003	0.017	100	0.040	0.098	100
T251-18	18.419	>25	53	>25	>25	31
0260.v5.c36	0.046	0.157	100	0.465	1.545	100
Q842.d12	0.009	0.025	100	0.082	0.363	100
235-47	0.249	1.149	97	1.624	9.305	90

*Maximum percent inhibition

Figure 3. Peripheral viral load of SHIV-YU2 in rhesus macaques after one dose of PGT121 and second dose of either PGT121 or PGT121 anti-HTfR

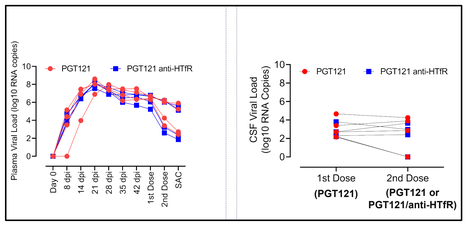


Figure 4. SHIV-YU2 DNA quantities in peripheral and CNS associated tissues after one dose of PGT121 and second dose of either PGT121 or PGT121 anti-HTfR

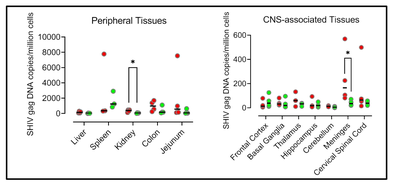
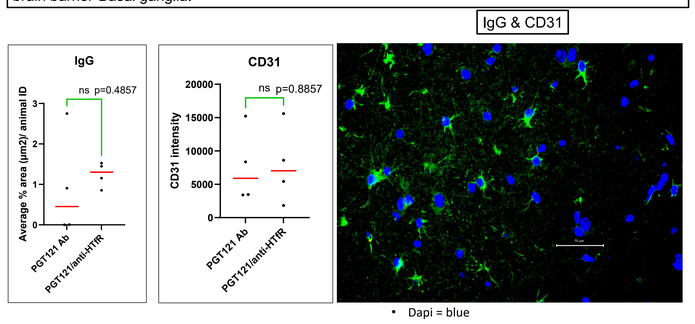
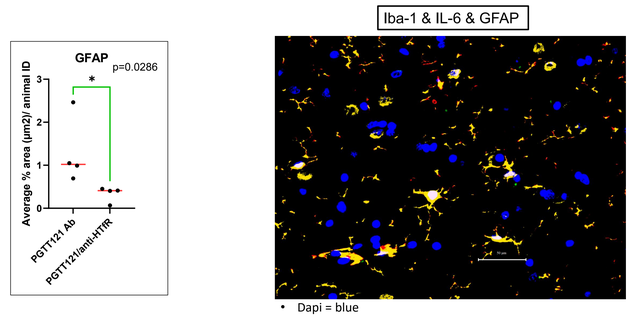


Figure 5. Comparison of the group which received the broad neutralizing antibody and the group that received the engineered bispecific antibody showed no significant change in the integrity of blood brain barrier-Basal ganglia.

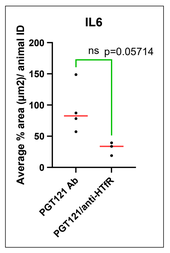


- Dapi = blue
- IgG = red
- CD31 = green

Figure 6. Activation of astrocytes (GFAP) is significantly lower in the bispecific group in Frontal cortex. With less astrocytes active, there is less expression of pro-inflammatory responses.



- Dapi = blue
- Microglia (Iba-1) = red
- Astrocytes (GFAP) = green
- IL-6 = Yellow



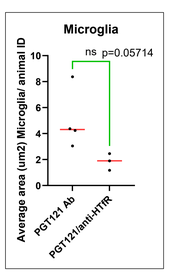
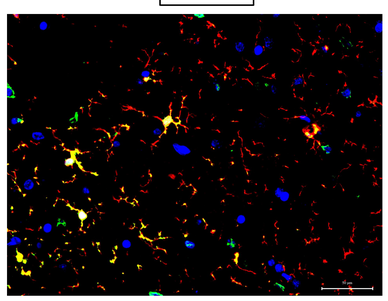
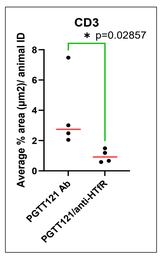


Figure 7. The activation of microglia (Iba-1) and proinflammatory cytokine IL-6 are lower in the bispecific group in Basal ganglia.



- Dapi = blue
- Microglia (Iba-1) = red
- IL-6 = green



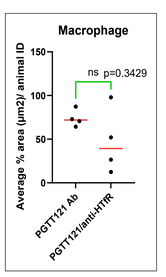
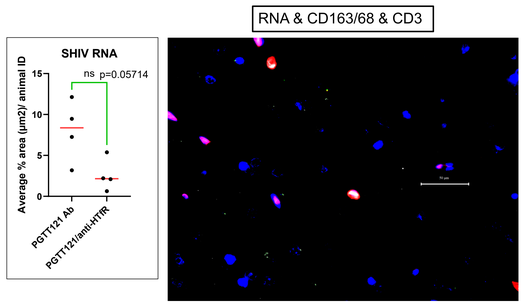


Figure 8. The presence of T-cells (CD3), are significantly lower in the bispecific group in Basal ganglia. Also, decreased macrophage and SHIV RNA is observed.



- Dapi = blue
- Macrophage (CD163/68) = red
- CD3 = green
- RNA = white

Conclusions:

- Bispecific antibody designed to improve CNS concentration of broadly neutralizing antibodies is safe in rhesus macaques.
- The integrity of the blood brain barrier is not compromised by the increased transcytosis of the bispecific antibody.
- The reduced activation of glial cell also reduced the release of proinflammatory cytokines.
- Brain tissues treated with the bispecific antibody showed reduced SIV RNA as well as reduced T cells presences.
- Although PTG121/anti-HTfR only has one arm instead of two arms to neutralize, the bispecific antibody reduced virus burden and inflammation in the brain.

References

[1] Liu et al. *Science*. 2016.
[2] Labrijn et al. *Proc. Natl. Acad. Sci. U.S.A.* 2013.
[3] Li et al. *J Virol*. 1991.

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