Developing Strategies To Image HIV In Vivo: Combining The Sarcophagine Chelator MeCOSar To 3BNC117 Does Not Affect HIV Binding Or Neutralisation

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ASHM 2018

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Broadly Neutralizing Antibodies (bNAbs)

- Derived from a people that develop potent cross-neutralizing antibodies to many different HIV strains
  - International HIV Controller study[1]
- Bind envelope protein (gp 120 / gp 41) expressed on HIV or the surface of infected cells
  - Neutralise free virus → can't go on to infect other cells
  - Clear infected cells → Fc receptor-dependent mechanisms (binding to Fc receptors on cytotoxic / phagocytic cells) e.g. ADCC, facilitate antigen presentation
- Can be produced in larger quantities with new Ab cloning techniques

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1 Pereyra, Science, 2010

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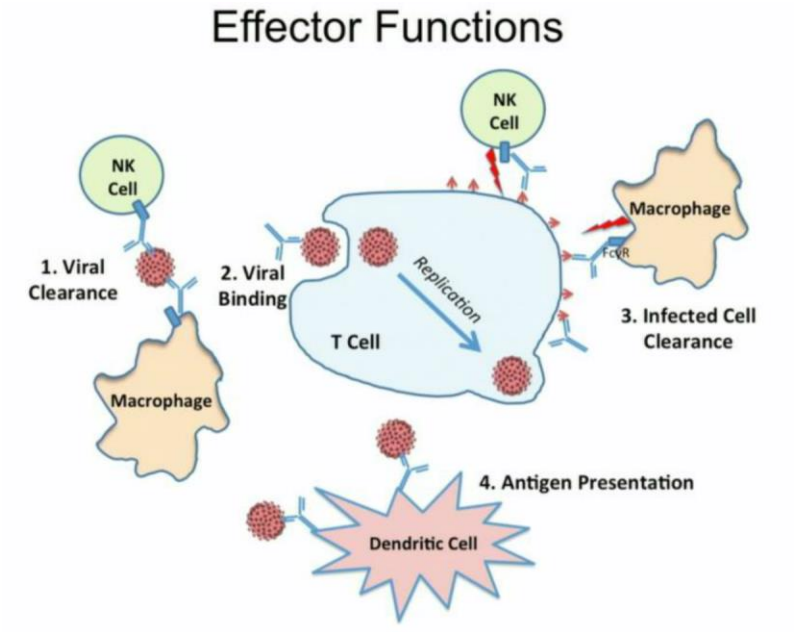

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Nussenzweig CROI 2017

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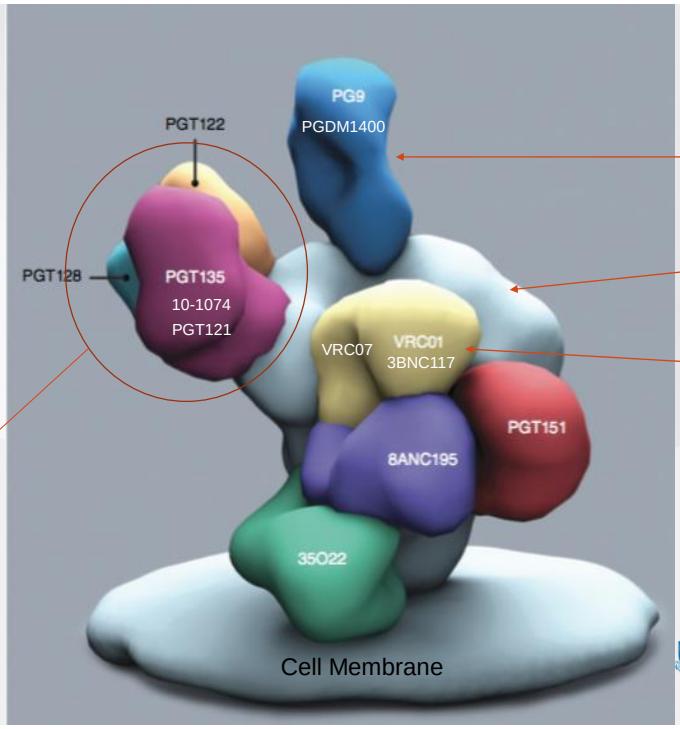
Broadly Neutralizing Abs (antigen-binding (Fab) fragments) bound to the HIV Envelope trimer



High Mannose Patch / V3 loop / N332 Glycan Supersite

Adapted from Burton Nat Immunol 2015

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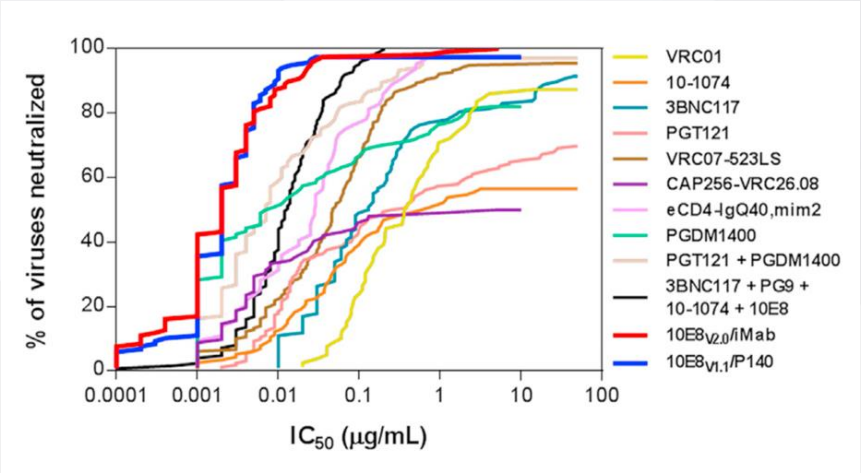
bNAb potency and breadth



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Huang, Cell 2016.

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PET/CT imaging with bNAb in Macaques

- Conjugate SIV Gp120–specific antibody with ^{64}Cu isotope[1]
  - SIV Env protein–specific monoclonal antibody 7D3
- SIV Macaque model
  - 3 'ART macaques' → scanned prior (viremic) and post ART
  - 2-4 uninfected controls
  - 4 elite controllers
- Identified SIV in GI tract, lymphatic tissue and nasal mucosa
- ^{64}Cu isotope safely used in human cancer studies [2]

1 Santangelo, Nat Methods, 2015 2 Mortimer, J Nuc Med, 2014. Tamura, J Nuc Med, 2013

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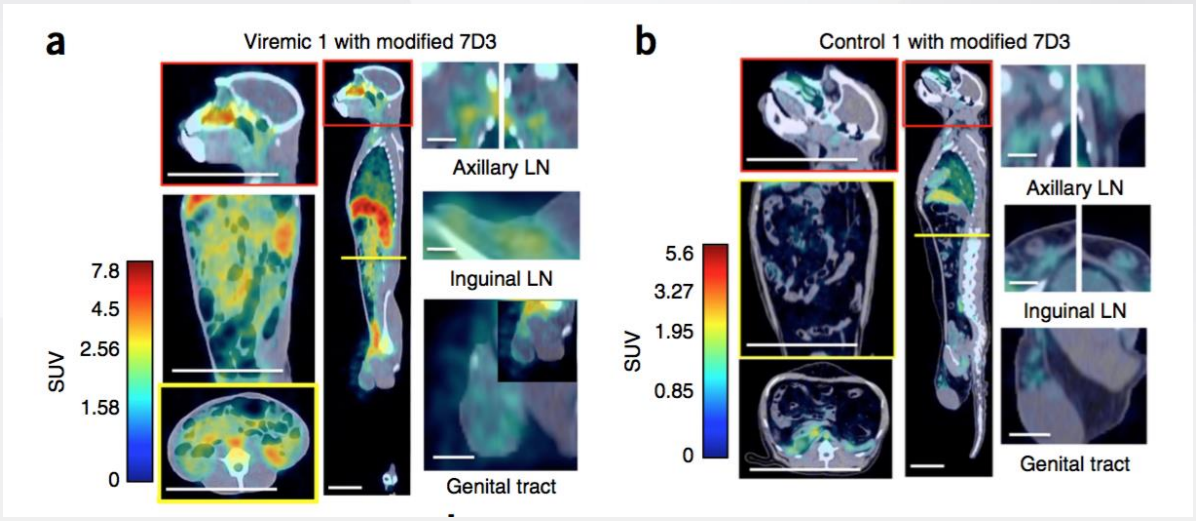

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Santangelo, Nat Methods, 2015

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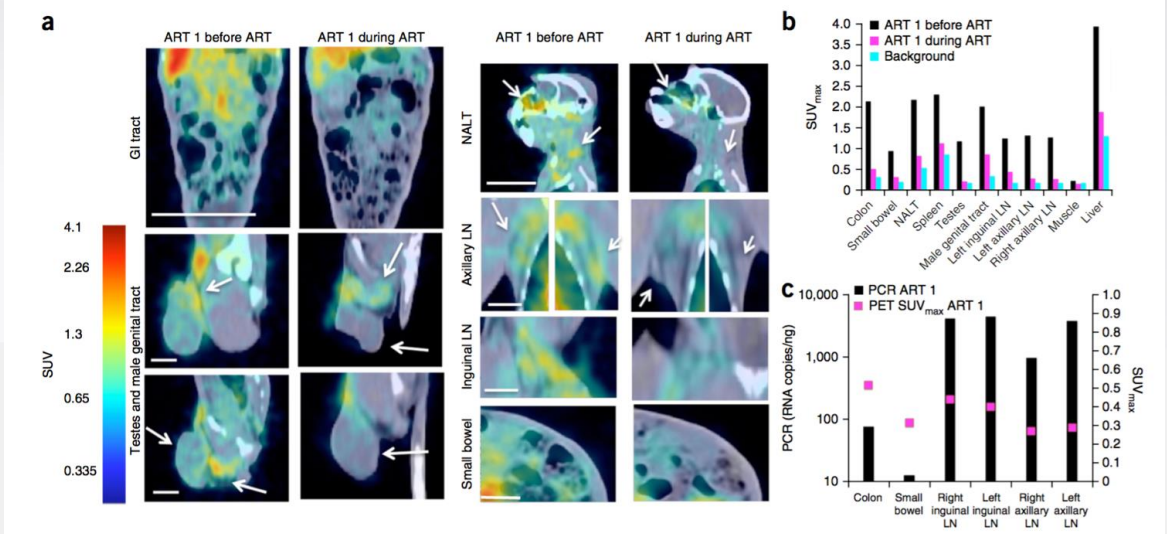
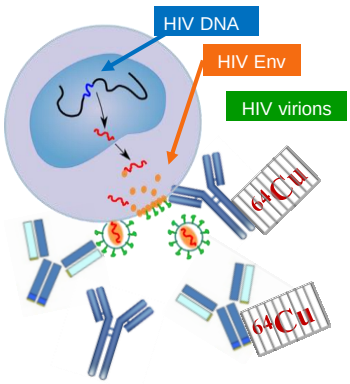


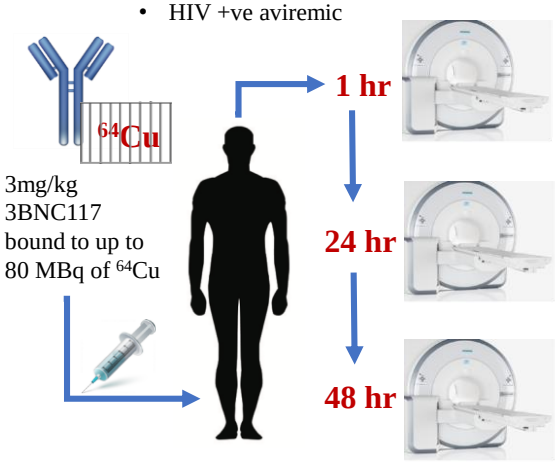
Figure 2 | PET/CT results from a chronically infected macaque, before and at 5 weeks of ART. (**a**) Standard uptake value (SUV) maps of GI tract, lymph nodes (LN), genital tract, spleen and small bowel. NALT, nasal-associated lymphoid tissue. Arrows indicate areas for which specific PET signals decreased during ART. Scale bars: frontal view of torso, 100 mm; sagittal view of head, 50 mm; LN and genital tract, 20 mm; small bowel, 15 mm. (**b**) SUV_{max} values before and after 5 weeks of ART compared with background uptake in 2 uninfected animals. (**c**) qRT-PCR verification of residual virus compared with SUV_{max} PET data at 5 weeks of ART in one representative treated macaque.

Imaging Persistent HIV with radioisOTOpe and 3BNC117 (IPHOTO3)

Examine binding and neutralisation of unlabelled or modified 3BNC117

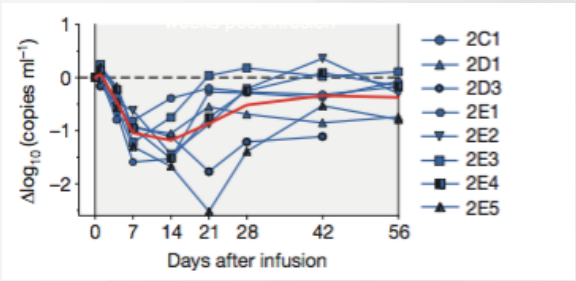


3 sequential cohorts
- HIV -ve
- HIV +ve viremic
- HIV +ve aviremic

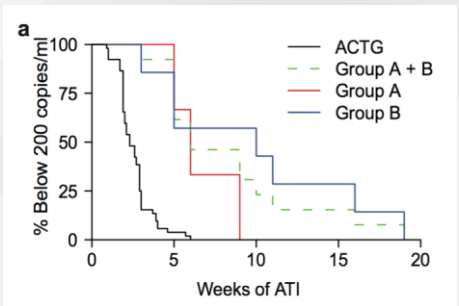


3BNC117

Single infusion 30 mg/kg lowers HIV RNA (mean 1.48 $\log_{10}$) in viremic participants



- Interrupting ART then 2 or 4 infusions of 30 mg/kg
- Delay in rebound 6.7 (2 infusions – Group A) and 9.9 weeks (4 infusions – Group B) compared with 2.6 weeks for historical controls



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Caskey Nature 2015; Scheid, Nature, 2016

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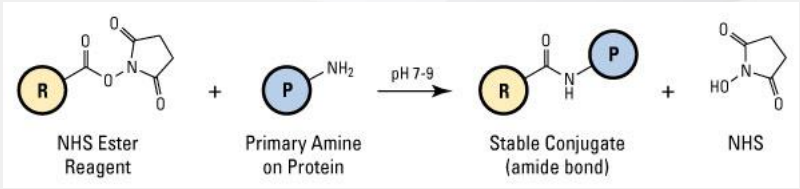
MeCOSar-NHS

- Chelates ^{64}Cu with targeting antibodies
- Next generation cage amine sarcophagine chelator
- Superior complexation compared to commercially available copper ligands[1]
- Binds copper irreversibly



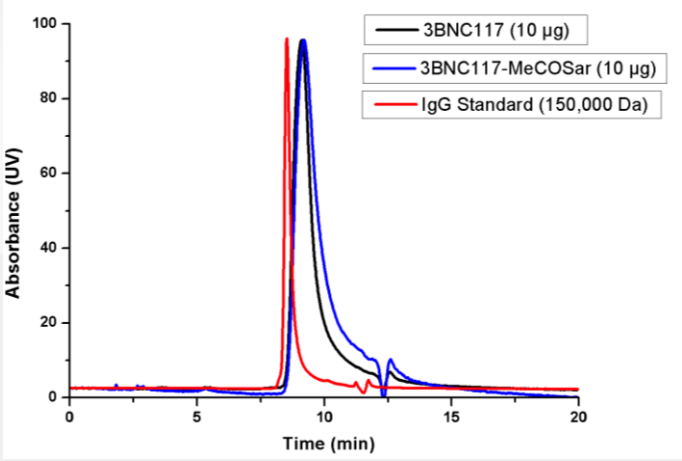
Alt, Mol Pharm, 2014

Prior to a clinical trial:

Characterise extent of chelation to 3BNC117 and select optimal ratio of MeCOSar to 3BNC117

3BNC117 binding to its Env target with the addition of MeCOSar needs to be confirmed *in vitro* → 3 assays developed

Size Exclusion Chromatography (SEC)



Similar elution of Unlabeled and MeCOSar-3BNC117

Therefore no antibody breakdown post conjugation

Graph is for 10x molar ratio of MeCOSar

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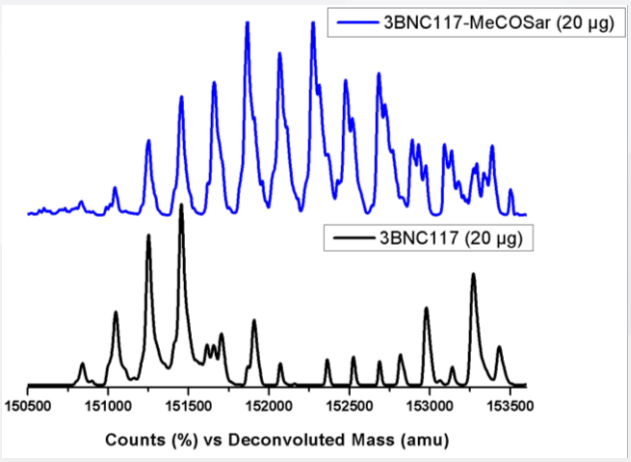
Liquid chromatography-mass spectrometry (LC-MS)



Predominant peak for 3BNC117 mass on LC-MS was 151467 Dalton (Da) (bottom)

When combined with 10x molar ratio revealed addition of 1, 2 and 3 MeCOSar (410 Da each) per 3BNC117 (top)

10x molar ratio selected going forward

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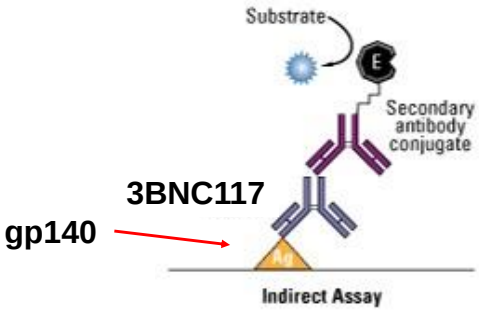
1) ELISA Binding Assay

Serial antibody dilutions (Unlabelled, MeCOSar-3BNC117 and ^{64}Cu-MeCOSar-3BNC117) added to gp140 coated wells then addition of a secondary horse radish peroxidase-conjugated anti-Fc antibody

Can be done with radioactively labeled antibody. Doesn't need PC2/PC3



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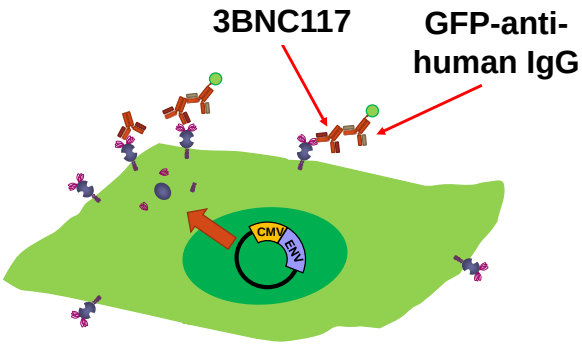
2) Cell Binding Assay

Env presented on surface of human embryonic kidney cells transfected with Env expression plasmids for HIV strains AD8 and NL4.3.

Then incubated with Unlabelled and MeCOSar-3BNC117 followed by incubation with a green fluorescent protein (GFP)-conjugated rabbit anti-human IgG. GFP expression was measured by flow cytometry

Most closely recapitulates binding to HIV infected tissues

Needs PC2 (not suitable for radiolabeling lab). Some expressed Env are not trimers



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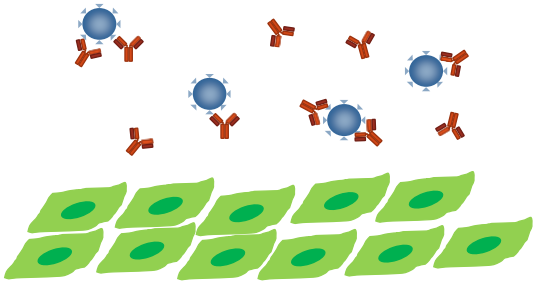
3) Neutralisation Assay

Unlabelled and MeCOSar-3BNC117 assessed for neutralisation capability of reporter viruses pseudotyped with 3 subtype B Env strains (AD8, NL4.3 and TRO.11) in JC53 cells

Requires PC3 (not suitable for Nuclear Medicine lab)

Measures binding to Env trimers and does not require secondary antibody



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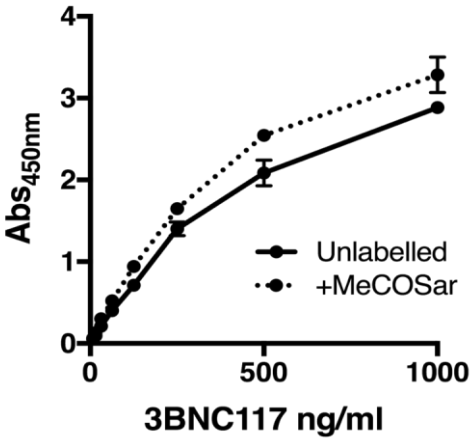
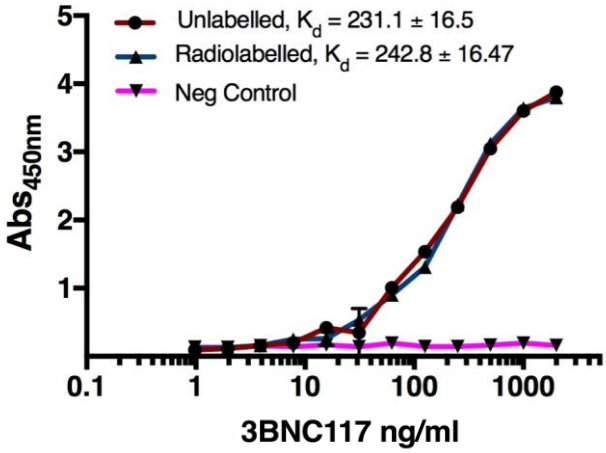
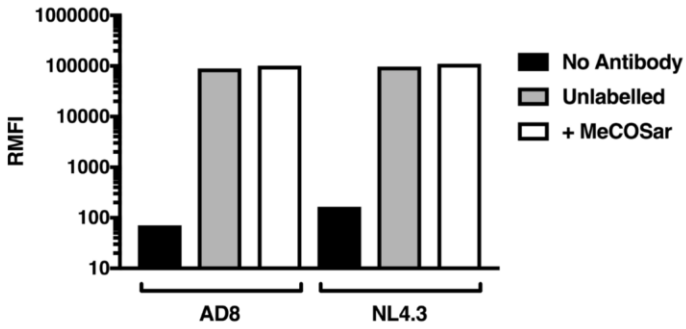
ELISA – comparable binding to gp140

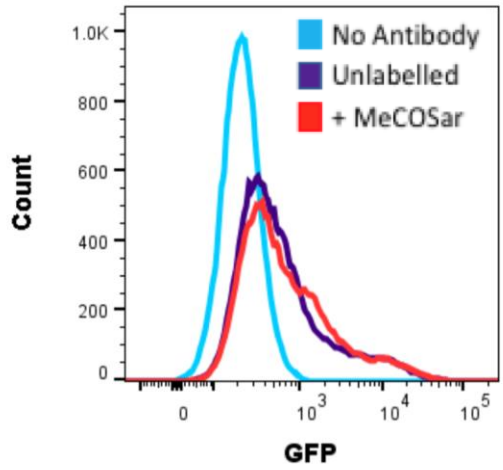




Cell Binding Assay

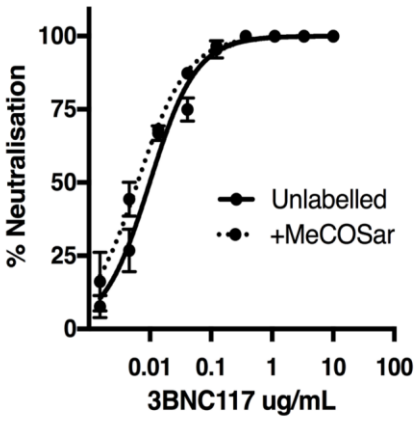


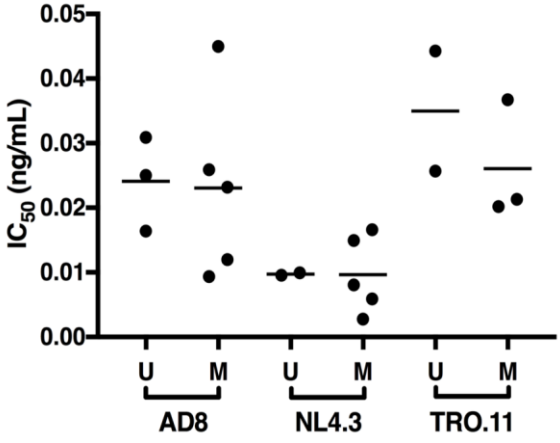
No differences in binding for either HIV strain (RMFI = Relative Mean Fluorescence Intensity).



Clear binding of 3BNC117 with and without MeCOSar; (red and purple plots,

Neutralisation Assay





No difference in 50% inhibitory concentration (IC_{50}), between 3BNC117 and MeCOSar-3BNC117 for Env strains with high (NL4.3 and AD8) and low sensitivity (TRO.11) to neutralising antibodies

Conclusions

- The sarcophagine copper chelator MeCOSar conjugates to 3BNC117 and does not interfere with binding to Env or neutralisation *in vitro*
- In addition ^{64}Cu radiolabeling of 3BNC117 does not interfere with binding to Env as assessed by an ELISA binding assay
- ^{64}Cu radiolabelled 3BNC117 is ideally suited for use in a clinical trial of a single infusion followed by serial PET/MRI scans to detect sites of HIV persistence on ART

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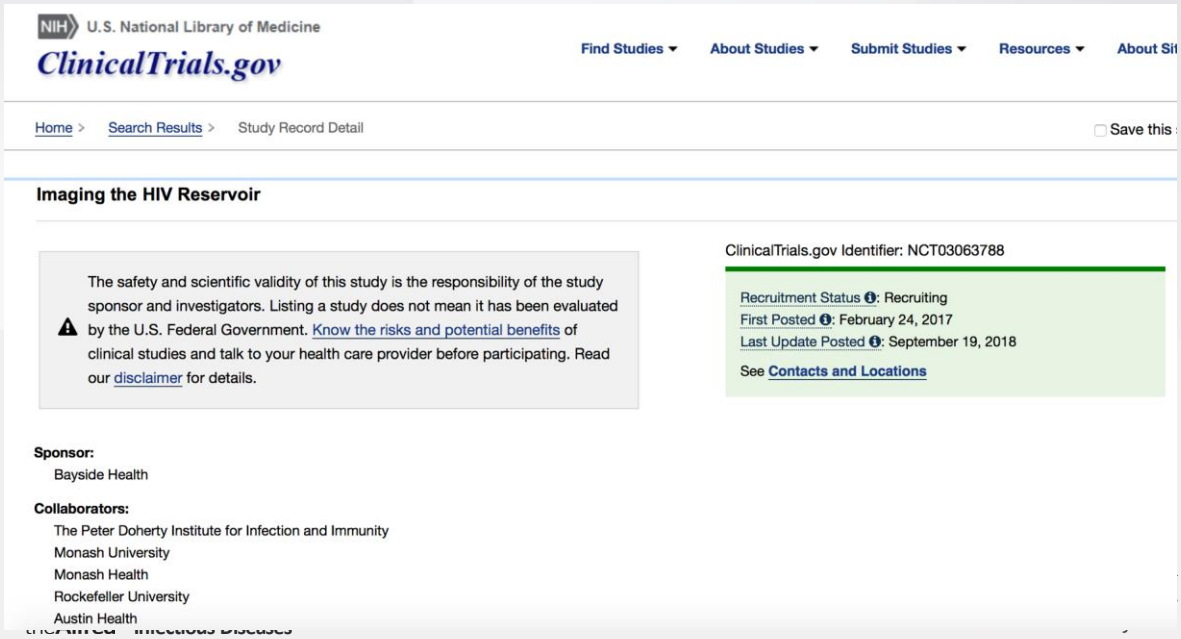

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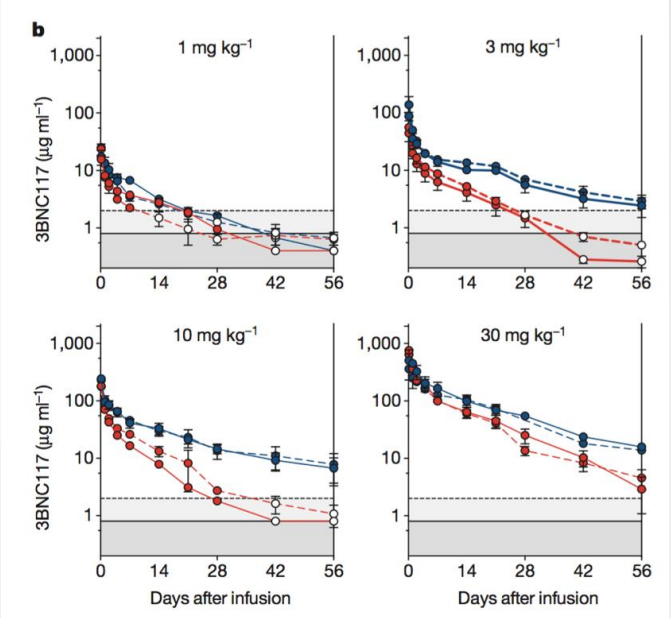
Dosing of 3BNC117

- Factors considered for IgG dosing
  - Lowest possible dose
  - Dose with linear clearance of IgG
    - Suggests saturation of antibody to target (Env)
- PK of 3BNC117 in HIV infected viremic (red) and HIV uninfected (blue)[1]
  - $t_{1/2}$ at 3mg/kg dose 18-18.7 days in HIV uninfected and 9.2-11.2 days HIV infected



1 Caskey Nature 2015

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Dosing of isotope

- Balance benefits of long $t_{1/2}$ to optimise imaging with risks of increased radiation
- $t_{1/2}$ of candidate isotopes
  - ^{64}Cu – 12.8 hours
  - ^{89}Zr – 78.4 hours
- Typically require 1-2 millicurie (37-74 megabecquerel) to image
- Safety is assessed by the effective dose in Sieverts
  - Effective dose for ^{89}Zr is 0.5 – 0.6 mSv/MBq[1]
  - Effective dose for ^{64}Cu is 0.06 mSv/MBq

1 Borjesson JNM 2009, Jauw FIP 2016

Dosing

- Single IV infusion of 3mg/kg 3BNC117 bound to 40 to 55 MBq (no more than 80 MBq) of ^{64}Cu
- Provides interpretable images for 48-60 hrs ($t_{1/2}$ of 12.8hrs)
  - Gives an estimated effective radiation dose of 4.8 mSv
  - 3BNC117 has $t_{1/2}$ of 9-11 days in blood, in 48-60 hrs will only see initial distribution and equilibrium phase
- Federal guidance of effective dose in medical research for healthy volunteers is ≤ 5 mSv per year
  - > 60 years dose constraint is 8 mSv/year, > 70 years is 12 mSv/year

Analysis

- Primary endpoint
  - Comparisons of PET SUVs in ROIs (GIT, LN groups, genital tract, spleen) between the three groups

- In addition
  - Global comparison of PET SUVs (measures all organs) across three groups
  - Compare ROIs within each individual to radioactivity levels in a homogenous area of muscle (e.g. thigh) to generate target to muscle ratios
    - Organ specific ^{64}Cu-3BCN117 uptake to muscle ratio assesses organ specific binding
  - Comparison of PET-SUVs within groups at the three scan timepoints to examine the distribution and elimination over 48 hours

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