Discovery of Next-Generation CD4 Mimetic-Based Long-Acting Entry Inhibitors

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BACKGROUND

- CD4 mimetics have shown promise for HIV prevention and treatment because of their broad HIV neutralization profiles and high barrier to virus escape stemming from the essential role of CD4 in HIV entry.
- Development has been limited, however, by rapid in vivo clearance, possibly related to major histocompatibility complex (MHC) class II binding.
- An optimal CD4 mimetic construct would retain neutralization breadth and have a long in vivo half-life requiring infrequent administration.
- To this end, we have engineered CD4 mimetics to reduce/abrogate MHC II binding, increase neutralization potency and prolong in vivo half-life

METHODS

- Engineering of candidate CD4 mimetic constructs exhibiting increased stability by:
  - *In silico* screening
  - DNA mutagenesis
  - Phage display selection.
- Promising variants were assessed for:
  - MHC Class II binding by biolayer interferometry
  - Thermostability by temperature ramping CD spectrum
  - Antiviral potency by TZM-bl neutralization assays
  - Plasma PK following intravenous injection in wild type Balb/c mice.

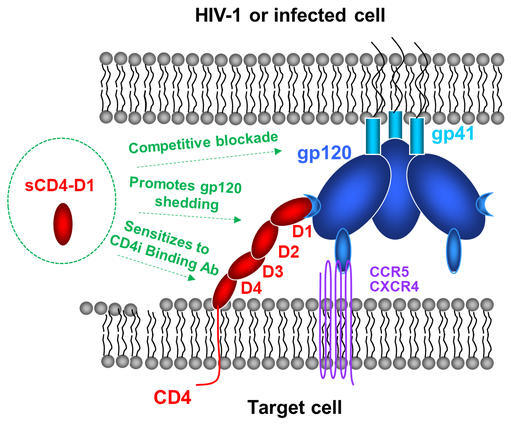
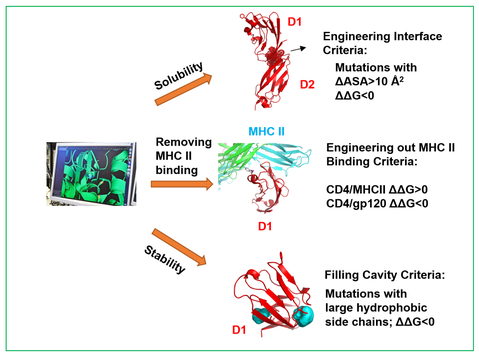


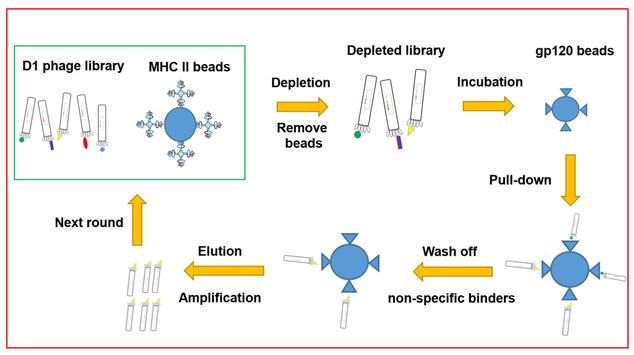
Figure 1. Schematic of CD4 mimetic. Soluble CD4-D1 domain inhibits HIV entry through versatile mechanisms.

We have engineered CD4 mimetics to prolong their *in vivo* half-life without affecting antiviral potency. These new constructs are promising candidates as long-acting entry inhibitors for HIV prevention and treatment.

***In silico* screening**

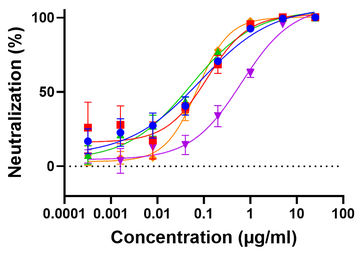


***Phage display* screening**



RESULTS

Neutralization of 3BNC117-Resistant HIV by *1A2-4*



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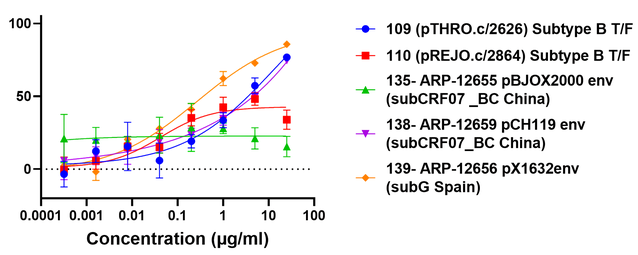
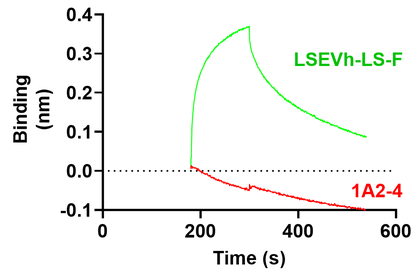
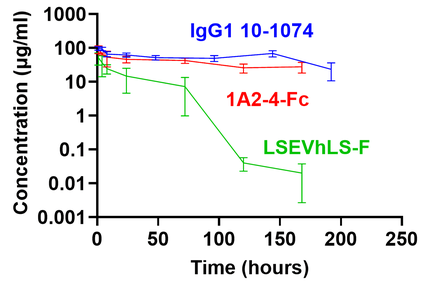


Figure 2. 1A2-4 neutralizes HIV-1 more broadly than 3BNC117. The new CD4-D1 variant, 1A2-4 efficiently neutralizes 3BNC117-resistant HIV strains in the TZM-BL assay.

1A2-4 does NOT bind to MHC II



1A2-4 exhibits PK comparable to IgG1 10-1074 in mice



Summary of PK calculation

	IgG1 10-1074	1A2-4-Fc	LSEVhLS-F
Cmax (μg/ml)	97.4	91.5	80.3
$AUC_{0\text{-last}}$ (μg h/ml)	12497.6	6375.6	1334.3
$AUC_{0\text{-}\infty}$ (μg h/ml)	12810.6	12985.7	1334.7
Tmax (h)	1	1	/
λz (/h)	0.00389	0.00419	0.052
t1/2 (h)	174	165.3	13.3

Figure 3. Characterization of the optimal CD4-D1 clone, 1A2-4. 1A2-4 shows abrogated binding to recombinant human MHC II, as tested using biolayer interferometry. By contrast, LSEh-LS-F showed robust binding to MHC II. 1A2-4 exhibits prolonged half-life in balb/c mice. A dose of 25 mg/kg proteins was injected through i.v. and blood was sampled for one week

CONCLUSIONS

- By combining in silico structural modeling with phage display technology, we identified a set of CD4-D1 variants with abrogated MHC II binding.
- The best clone, 1A2-4, exhibits enhanced thermostability, broad neutralization and prolonged PK comparable to the clinical benchmark IgG1 10-1074.
- 1A2-4 can be engineered as a long-acting CD4 mimetic with applications in HIV functional cure.

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