OHTN 2013 RESEARCH CONFERENCE
NOVEMBER 17-19, 2013
CHANGING THE COURSE OF THE HIV PREVENTION, ENGAGEMENT AND TREATMENT CASCADE

HIV Research
UNIVERSITY OF TORONTO
Department of Medicine

A TRIMERIC HIV-1 GP140-BAFF FUSION CONSTRUCT ENHANCES MUCOSAL ANTI-TRIMERIC HIV-1 GP140 IGA IN MICE

Jun Liu [1], Kiera Clayton [2], Yu Li [2], Matthew Haaland [2], Jordan Schwartz [2], Hampavi Sivanesan [2], Aamir Saiyed [2], Wei Zhan [2], Feng Yun Yue [2], James Rini [3], Mario Ostrowski [1, 2]

1 Division of Clinical Sciences, University of Toronto;
2 Department of Immunology, University of Toronto;
3 Department of Biochemistry, University of Toronto;

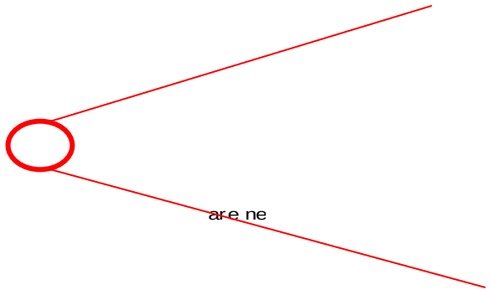
Background

- A safe and effective HIV-1 vaccine is needed to ultimately control HIV-1 pandemic

  1. Fauci, AS et al. Nat Immunol, 2013; 14: 1104-1107
- Broadly neutralizing antibodies(bNAbs) can prevent HIV-1 infection (sterilizing immunity) and thus are the holy grail for HIV-1 vaccine development

  1. Mascola JR et al. J. Virol. 1999, 73:4009-4018. 2. Baba TW et al. Nat. Med. , 2000, 6:200-206. 3. Parren PW et al. J. Virol. 2001, 75:8340-8347. 4. Hessell AJ et al. Plos Patho, 2009, 5:e1000433. 5. Hessell AJ. J. Virol. 2010, 84:1302-1313
- Non-broadly neutralizing antibodies(nbNAbs) can also prevent HIV-1 infection

  1. Burton DR et al. PNAS, 2011, 108:11181-11186. 2. Haynes BF et al. NEJM, 2012, 366, 1275-1286. 3. Rolland M et al. Nature, 2012, 490:417-420
- To elicit protective antibodies (bNAbs and nbNAbs), the target antigen, HIV-1 Env, should mimic the native trimer conformation.
- 1. Mattias F, et al. Curr Opin HIV AIDS, 2009, 4:380-387. 2. Sundling C et al. J Exp Med, 2010, 207:2013-2017. 3. Kovacs JM, et al. PNAS, 2012, 109:12111-12116. 4. Burton DR et al. Cell Host Microbe, 2012: 12:396-407



QuickTime™ and a
decompressor
are needed to see this picture.

Klein JS et al. Plos Patho, 2010, 6:e1000908
Zhu P et al. Nature, 2006,441:847-852
https://www.aidsreagent.org/program_info.cfm
Haynes, BF et al. Nat. Biotech. 2012, 30: 423-433

Background

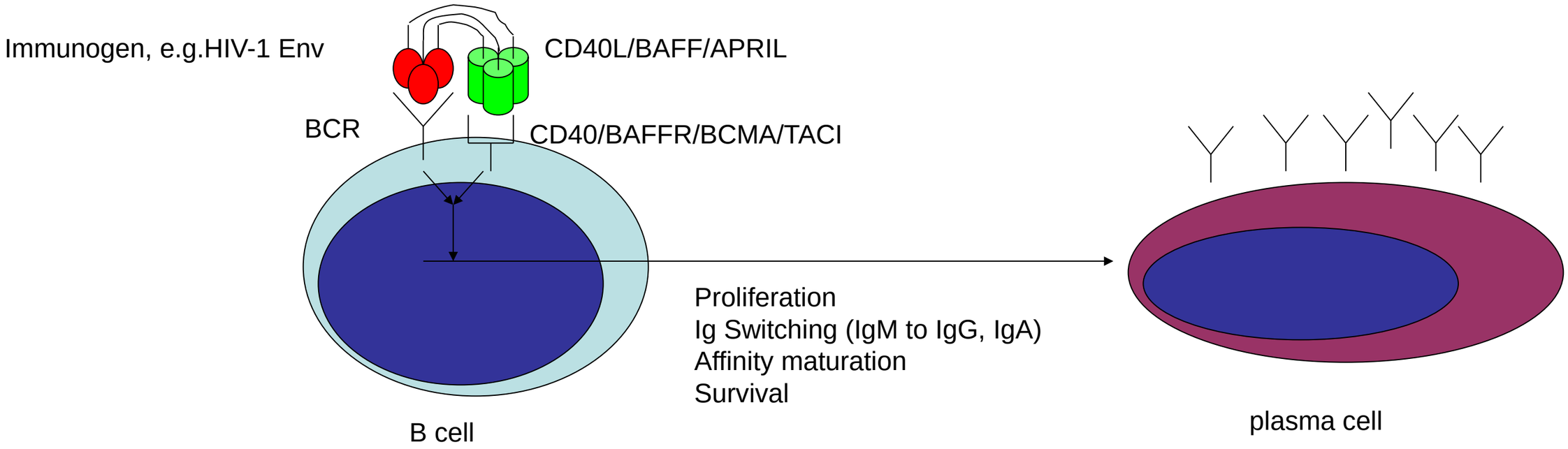
- HIV-1 predominantly transmits through genital/rectal mucosa.

  1. Hladik, et al. Nature Rev Immunol, 2008, 8:447-457
- Mucosal IgA is the dominant Ig subtype at most mucosal surface (except genital mucosa) and is vital for prevention of microbial transmucosal infections, including HIV-1.

  1.Mestecky J et al. Am J Reprod Immunol, 2011, 65:361-367. 2. Bomsel M, et al. Immunity, 2011, 34:269-280. 3. Choi RY, et al. AIDS, 2012, 26:2155-2163
- HIV-1 Env is weak in immunogenicity and needs potent adjuvants to elicit strong and long-lasting Ab responses.

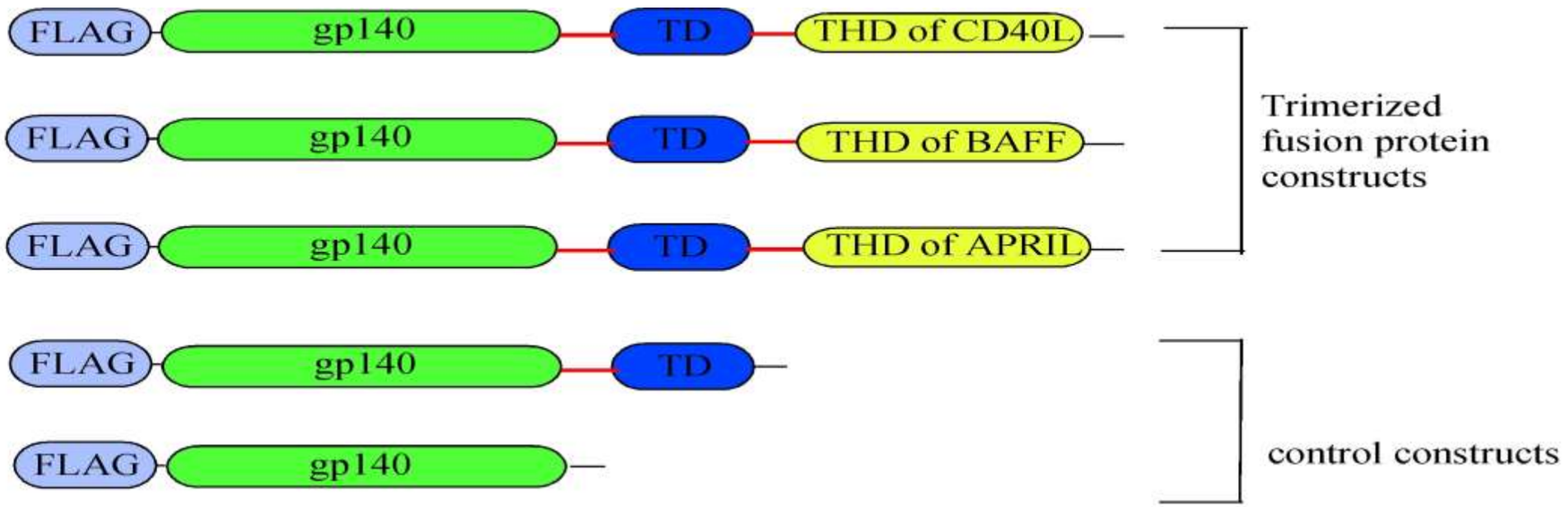
  1. McElrath MJ et al. Immunity, 2010, 33:542-554. 2. Bonsignori M et al. J Immunol, 2009, 183:2708-2717
- Three TNFSF members, CD40L, BAFF (B cell activating factor of the TNF family), APRIL (a proliferation-inducing ligand) , are costimulatory molecules for antibody responses through promoting B cell proliferation and survival, Ig isotype switch (IgM$\rightarrow$IgG and IgA), and somatic hypermutation (affinity maturation).

  1. Bossen C. Seminar Immunol, 2006, 18: 263-275. 2. Elgueta R. Immunol Rev, 2009, 229:152-172



Hypothesis

A trimeric fusion construct of HIV-1 Env and APRIL/BAFF/CD40L (Env-A/B/C trimer) can improve anti-HIV-1 Env antibody responses.



: flexible GGGSGGG linker

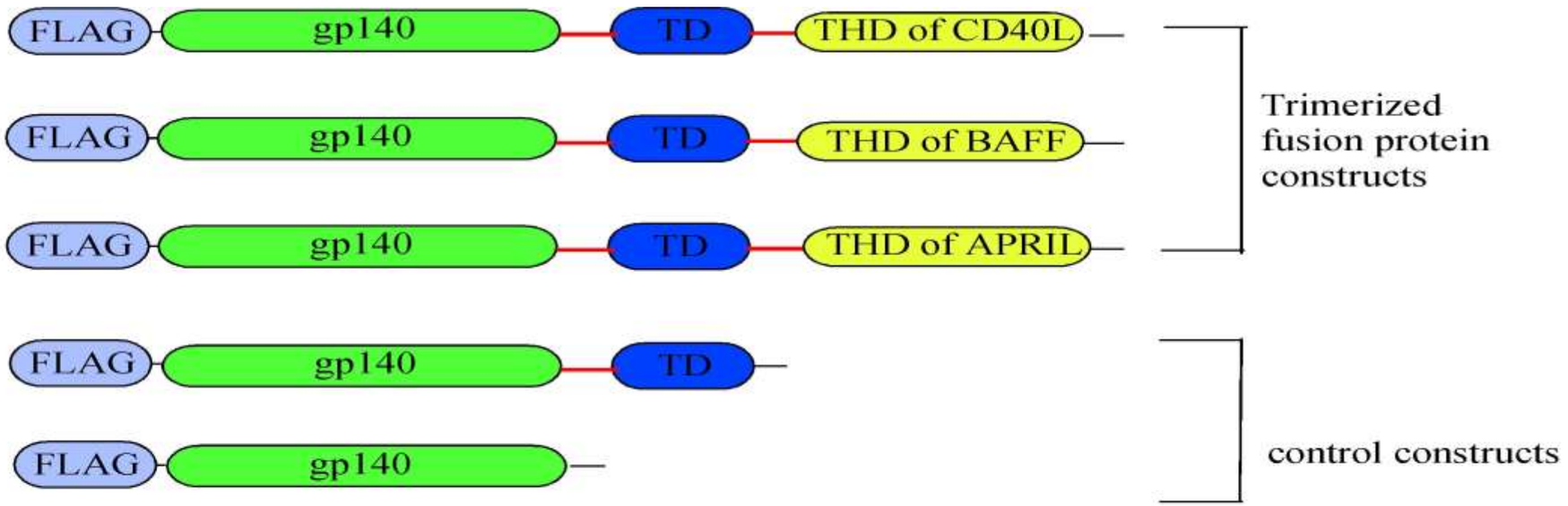
FLAG: 3xFLAG tag

TD : trimerization domain

THD : TNF homology domain

Questions

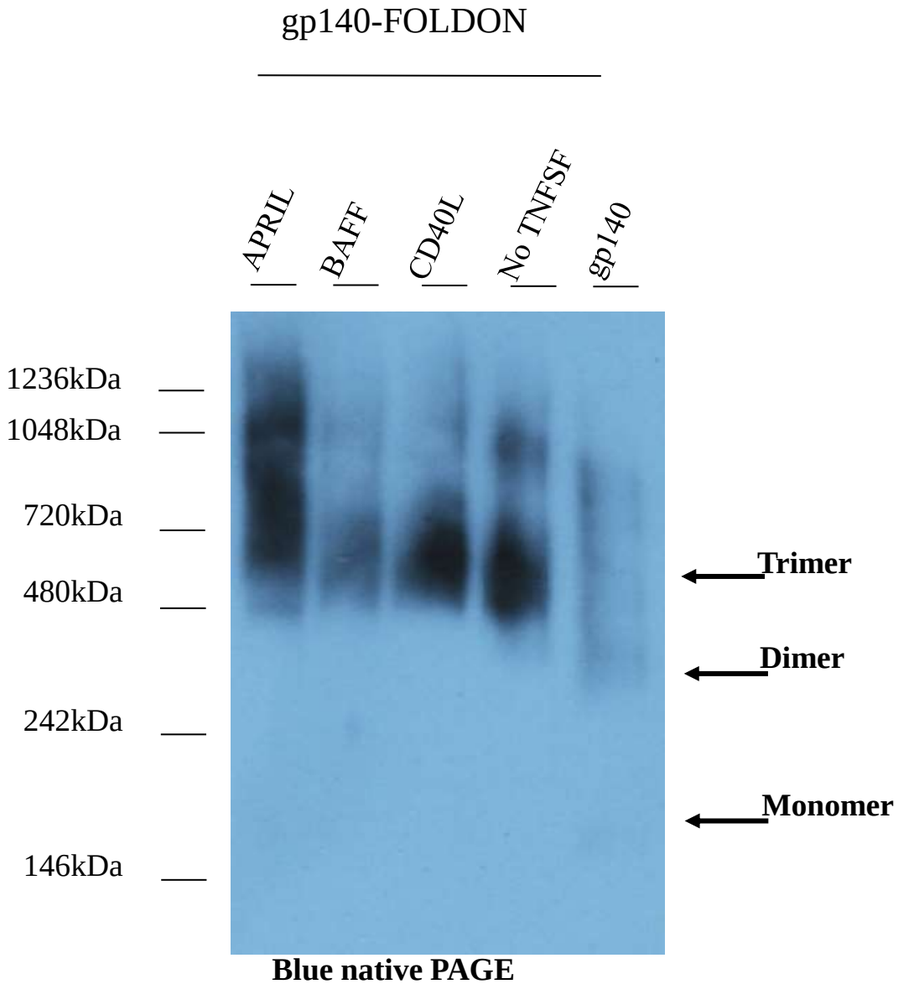
- Will the fusion constructs form trimer?
- Will the fusion constructs keep the native conformation of HIV-1 Env?
- Can the fusion constructs enhance antibody responses against HIV-1 Env, esp. at mucosal surface?

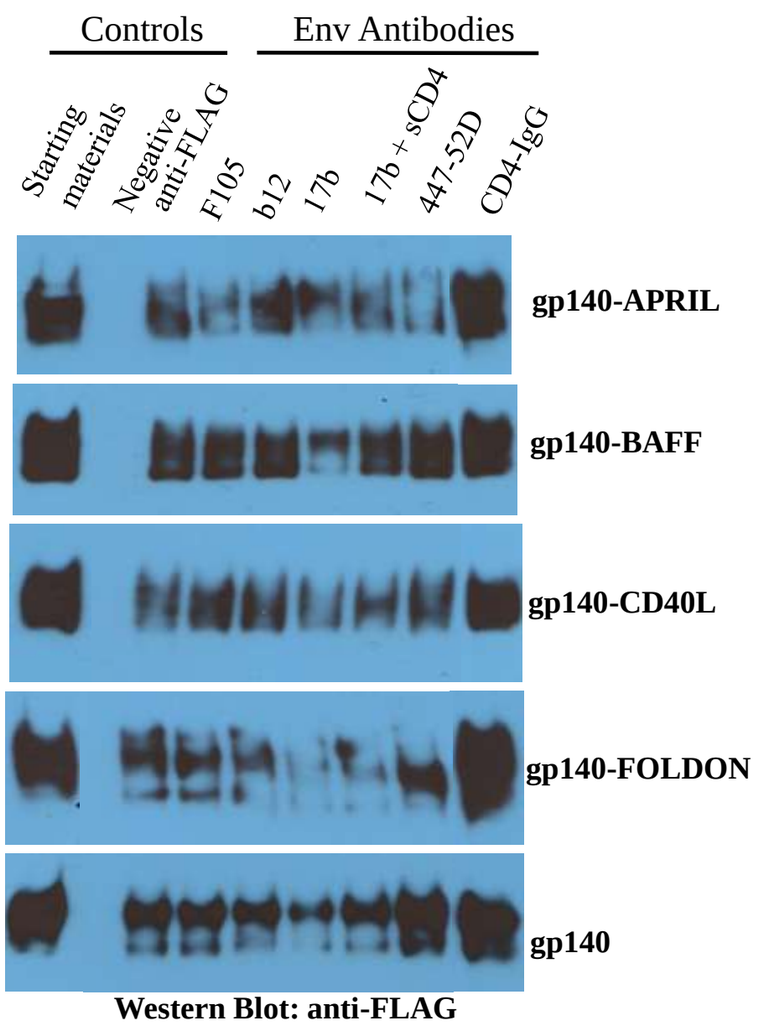


Transfect HEK293T cells

Supernatant subjected to SDS-PAGE, Blue native PAGE, immunoprecipitation followed by Western blot

Fusion constructs form trimers and keep HIV-1 Env native conformation





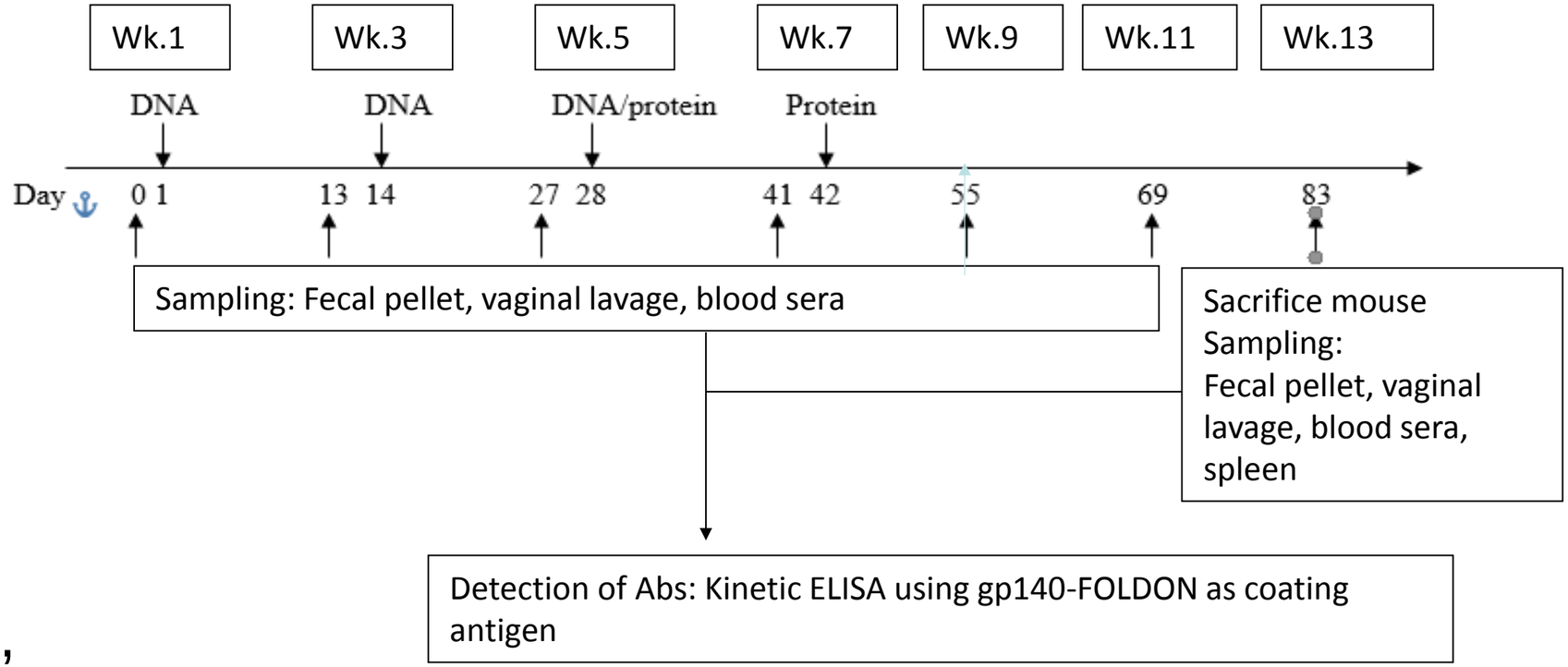
Credit: Clayton K, et al.

Questions

- Will the fusion constructs form trimer?
- Will the fusion constructs keep the native conformation of HIV-1 Env?
- Can the fusion constructs enhance antibody responses against HIV-1 Env, esp. at mucosal surface?

Vaccination regimen

- 6 groups (4 mice/group):
  - gp140
  - gp140-FOLDON
  - gp140-CD40L
  - gp140-BAFF
  - gp140-APRIL
  - Naïve (PBS)
- DNA: 100 µg in 100µl PBS/mouse/vaccination, 50µg(µl) per hing leg muscle per mouse
- Protein: 20µg in 100 µl PBS mouse/vaccination, i.p.



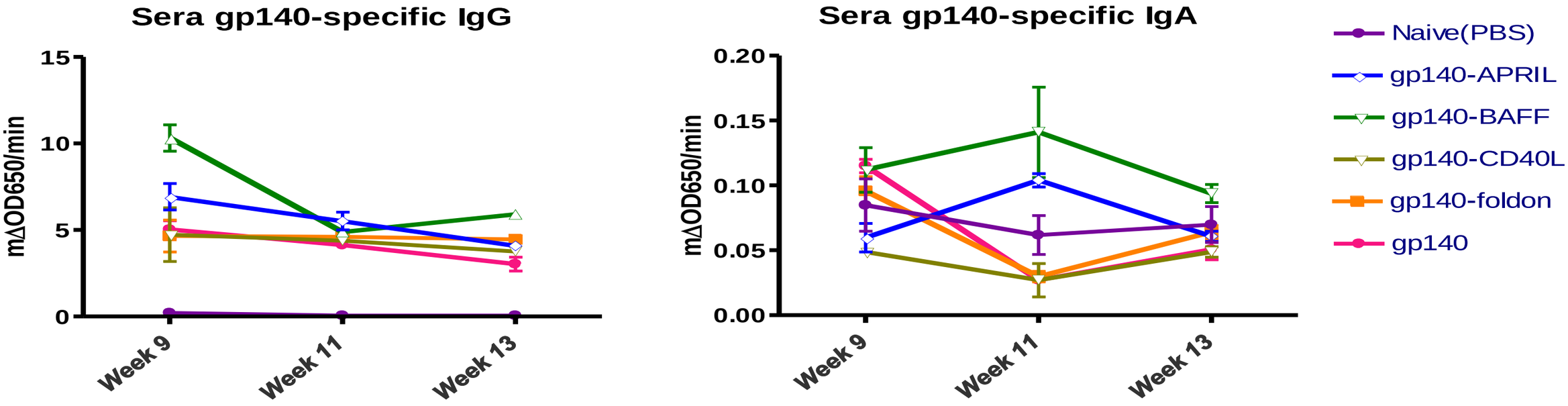
Kinetic ELISA

- Take OD_{650} reading every 15s for the first 3 min after adding substrate, and calculate slope (ΔmOD_{650}/min) from linear regression.
- More accurate in Ab quantification than endpoint ELISA: slope is proportional to concentration of antigen/antibody during initial stage of reaction.

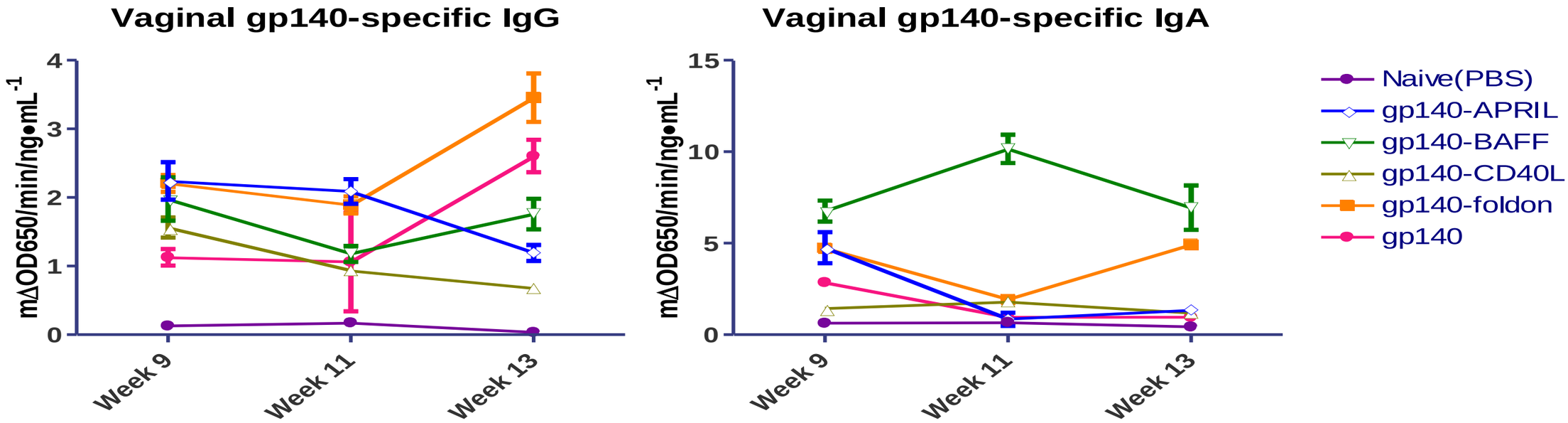
  1. Tsang VCW et al. Clin Chem, 1980, 26:1255-1260.
- No need to do serial dilutions of samples.

  1. Tsang VCW et al. Clin Chem, 1980, 26:1255-1260. 2. Snyder MH et al. J Clin Microbiol, 1988, 26:2034-2040
- Standardization of mucosal gp140-specific Ab: ΔmOD_{650}/min/total IgG or IgA (ΔmOD_{650}/min/ng•mL^{-1}).

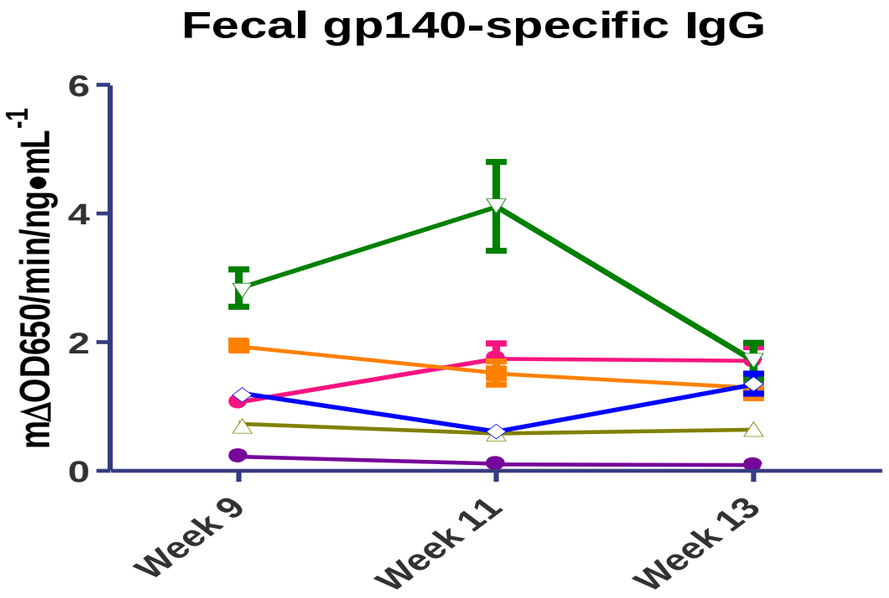
HIV-1 trimeric gp140-specific antibody responses-sera

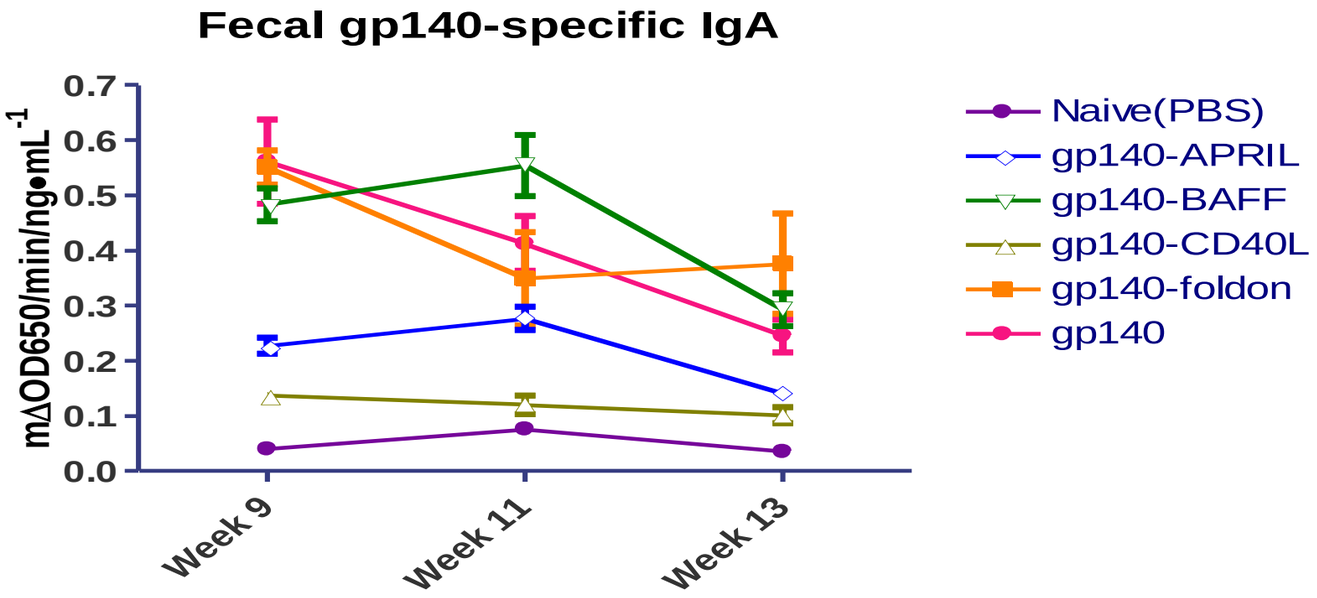


HIV-1 trimeric gp140-specific antibody responses-vaginal lavage



HIV-1 trimeric gp140-specific antibody responses-fecal pellet





Conclusions

- Fusion constructs, gp140-ARPIL/BAFF/CD40L, form trimers and keep native conformation of HIV-1 Env.
- gp140-BAFF can enhance trimeric HIV-1 Env-specific antibody responses, esp. mucosal IgA responses.
- gp140-APRIL and gp140-CD40L can not enhance trimeric HIV-1 Env-specific antibody responses.

Ongoing experiments and future directions

- HIV-1 neutralization
- Other vaccination platforms using gp140-BAFF as immunogen? (microneedle, nanoparticles, etc.)

Acknowledgement

Dr. Ostrowski Lab (UofT)

Kiera Clayton

Yu Li

Matthew Haaland

Jordan Schwartz

Hampavi Sivanesan

Aamir Saiyed

Wei Zhan

Dr. Elizabeth Yue

Dr. Sonya MacParland

Dr. Nur-ur-Rahman

Wade Coutinho

Shariq Mujib

Wayne Zhao

Ali Fawaz

Ardalan Bozorgzad

Matthew Douglas-Vail

Dr. Mario Ostrowski

Dr. James Rini (UofT)

Dr. Kelly MacDonald Lab(UofT)

Dr. Rupert Kaul Lab (UofT)

Funding Agency:

QuickTime™ and a
decompressor
are needed to see this picture.