alpha-1 biologics

Cynthia L. Bristow, Ph.D.

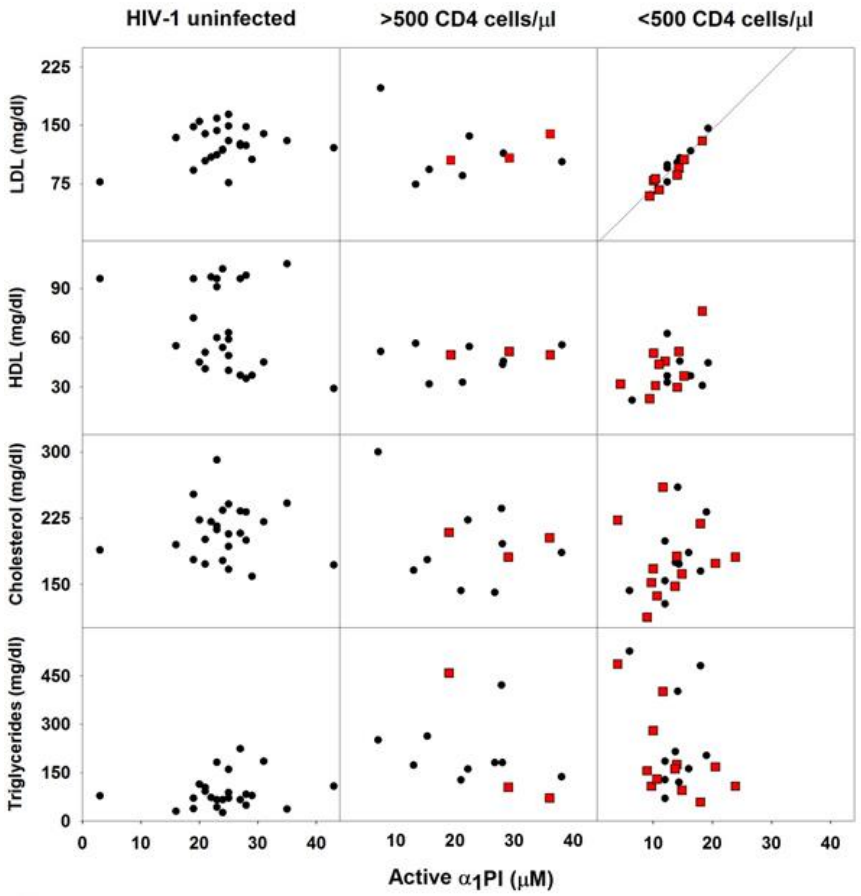
Using Flow Cytometry to Measure the Effects of HIV Receptor Expression and Signaling on HIV Endocytosis and Infectivity

The Clinical Observation

α1 proteinase inhibitor (α1PI, α1 antitrypsin) is the most abundant proteinase inhibitor in our bodies. It is present in several forms, but the important forms are Active and Inactive.

Active α1PI binds to a plasma membrane protein human leukocyte elastase (HLE-CS).

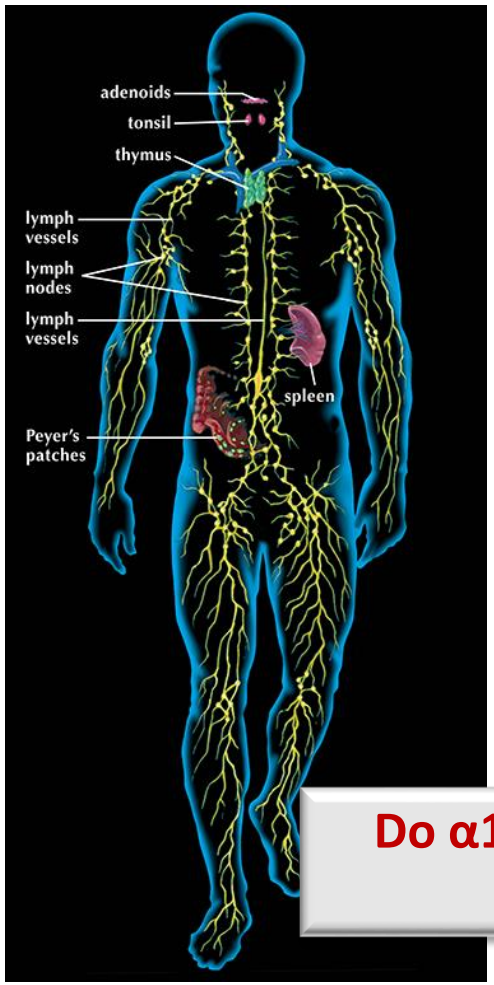
Inactive α1PI occurs when α1PI binds to HLE-CS. It also occurs by oxygenation or when cleaved by host or pathogen proteinases.



● not on HIV protease inhibitor therapy
■ on HIV protease inhibitor therapy

Bristow CL, et al. 2013 Discovery Medicine 16: 201-218.

The Question



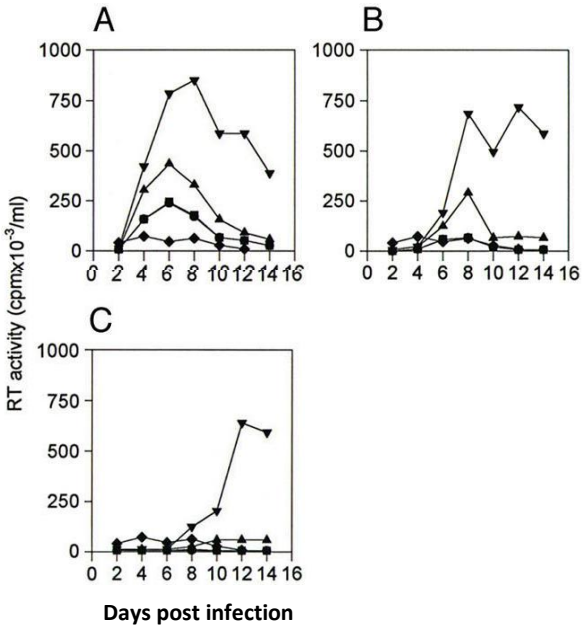
- Dietary fats bind to apolipoproteins and are transported from the gut through lymph to blood <u>by lymphocytes</u>.
- Lipoproteins (HDL, LDL, etc.) bind to members of the LDL receptor family (LDL-RFMs).
- So do proteinase inhibitors including α1PI.
- Our group showed that α1PI regulates the number of $CD4^+$ T cells in blood by inducing cellular locomotion (Bristow CL, et al. 2012 PLoS ONE e31383).

Do α1PI and $CD4^+$ T cells regulate lipoprotein levels?

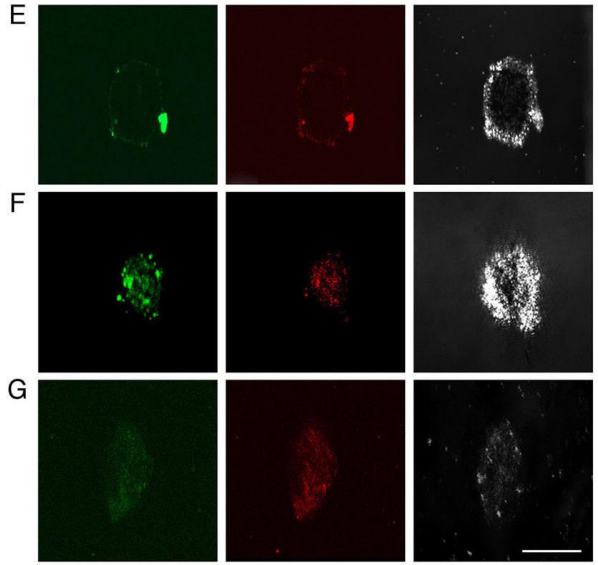
The Complication

Our group showed that active α1PI is <u>required</u> for HIV infectivity.

(Bristow CL., et al. 2003 Blood 102: 4479-4486)



A. 10^{-2} MOI
B. 10^{-3} MOI
C. 10^{-4} MOI
using U937 Clone 10



Green – HLE-CS
Red – CD4
Silver – HIV NL4-3

Green – HLE-CS
Red – CXCR4
Silver – HIV NL4-3

Green – HLE-CS
Red – CD4
Silver – Absence of HIV NL4-3

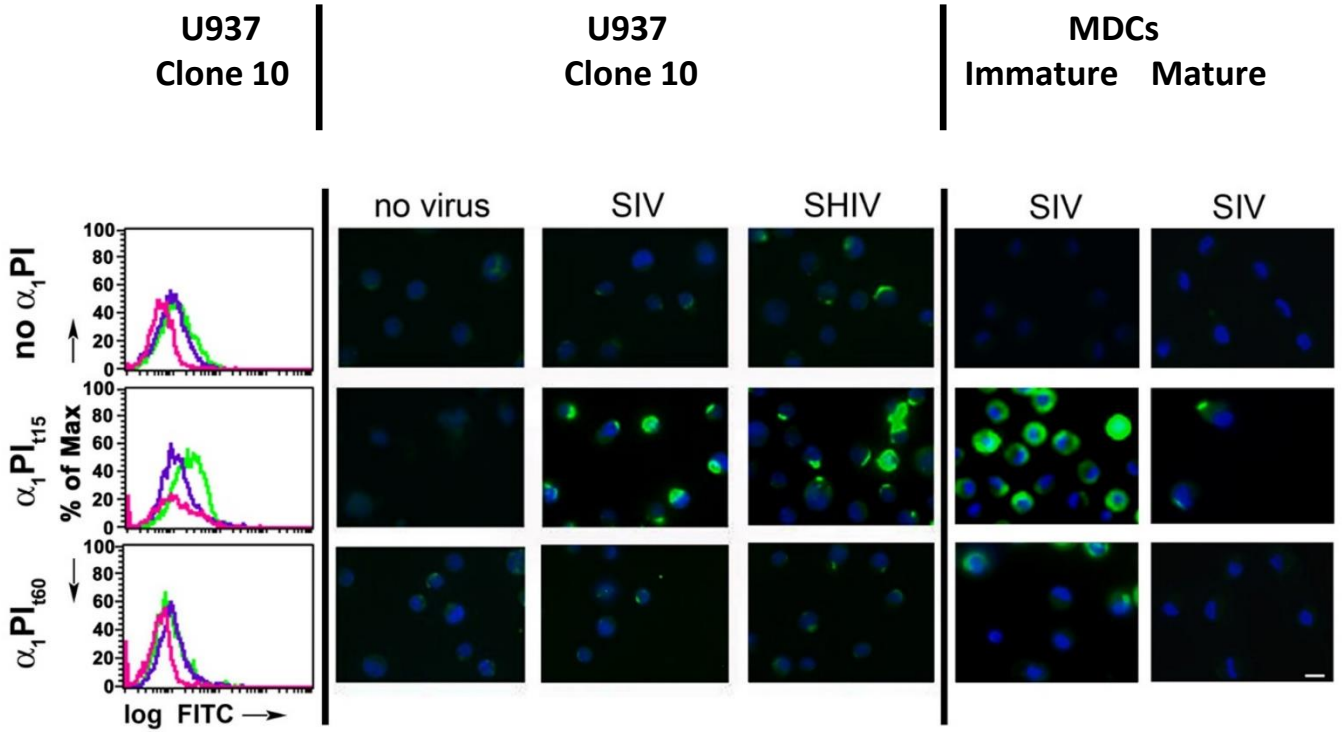
Another group showed that inactive α1PI <u>blocks</u> HIV infectivity following 60 min incubation.

(Munch J., et al. 2007 Cell 129: 263-275)

But they didn't look at the cells and they didn't consider the effect of active α1PI .

Bristow CL, et al. 2003 Blood 102: 4479-4086.

Kinetic effect of α1PI on HIV binding

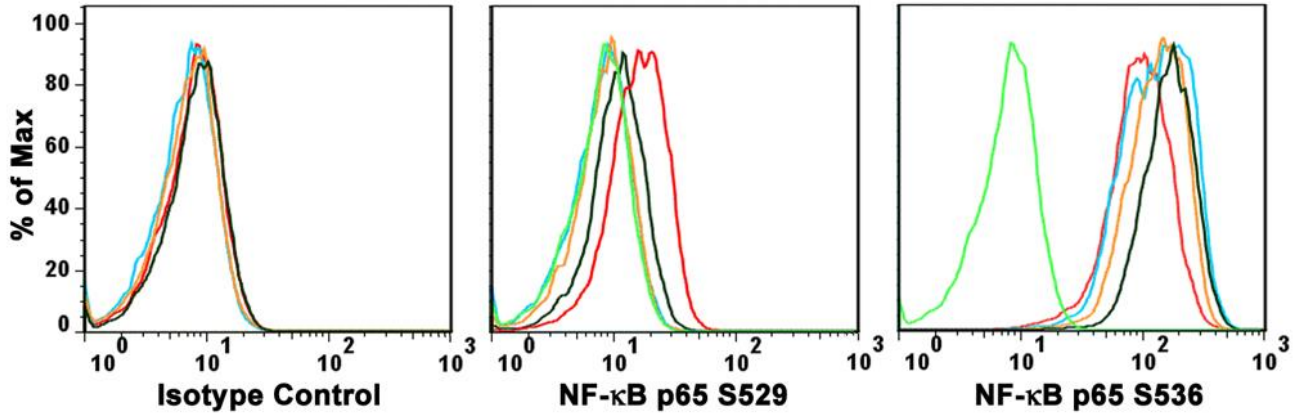


Green: SHIV (AT-2 inactivated)
Purple: Buffer
Pink: Isotype control

Blue: DAPI nuclear staining
Green: virus (AT-2 inactivated)

Bristow CL, et al. 2013 Discovery Medicine 16: 201-218.

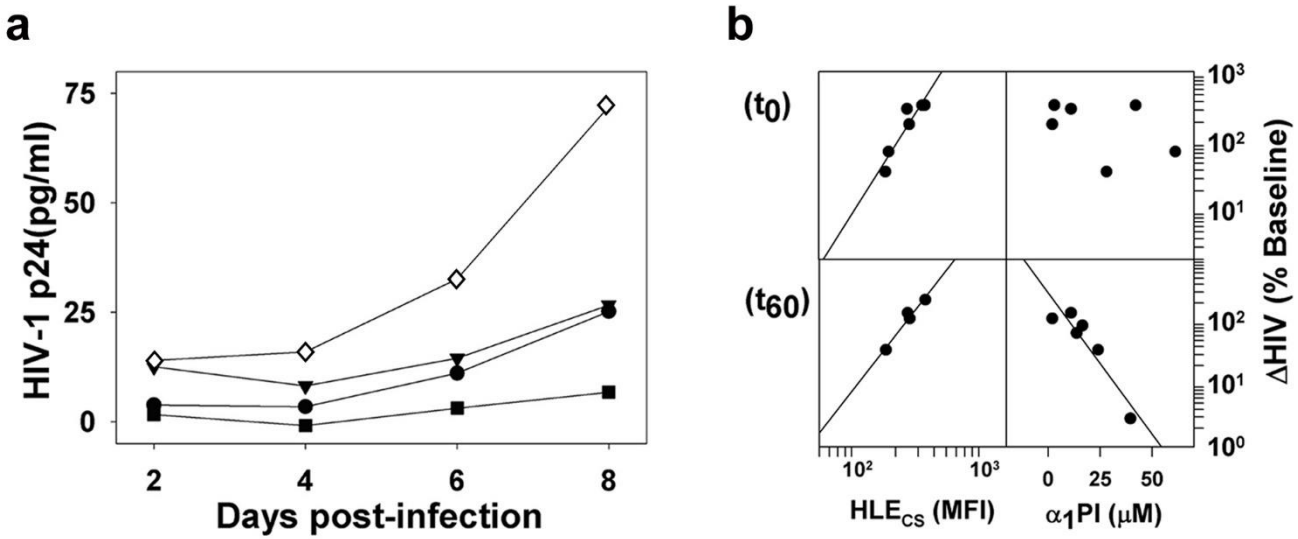
Kinetic effect of α1PI on NF-κB activation



Red: α1PI t0
Light blue: α1PI t15
Orange: α1PI t60
Dark green: α1PI t120
Light green: isotype control t0

Bristow CL, et al. 2013 Discovery Medicine 16: 201-218.

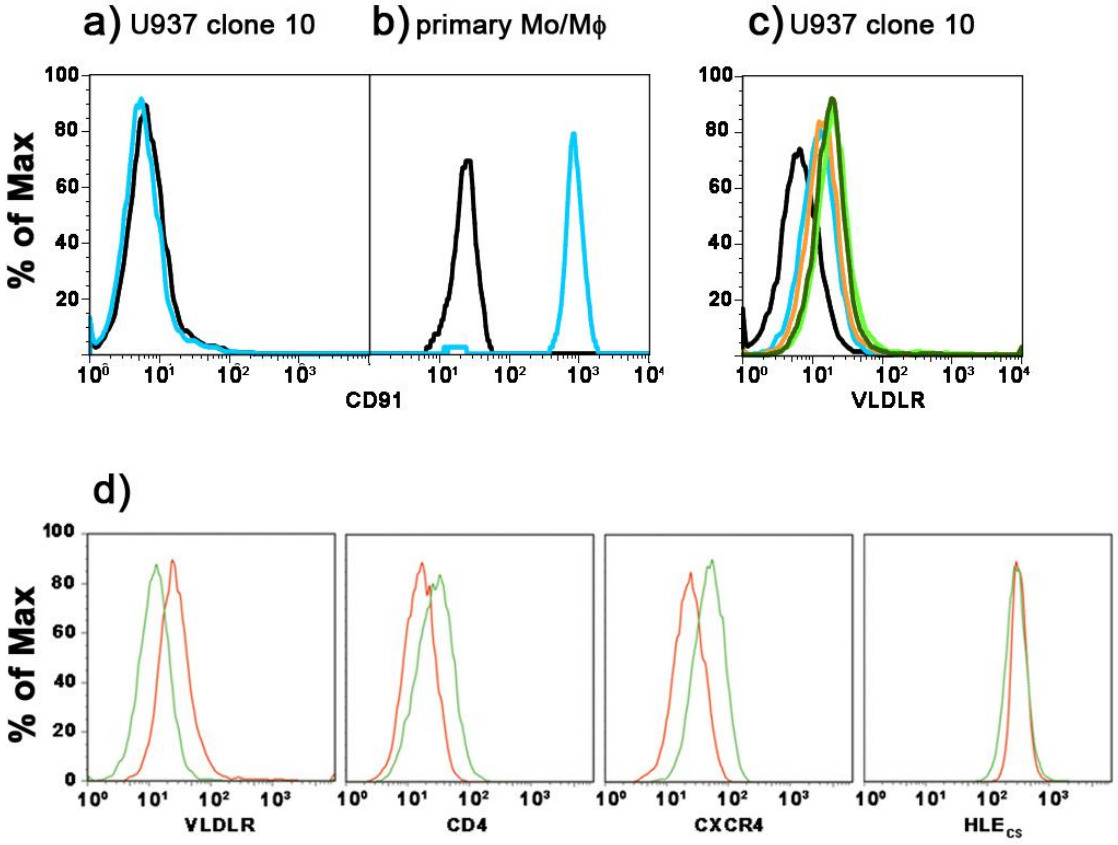
Kinetic effect of α1PI on HIV infectivity



▼: α1PI t0
◇: α1PI t15
■: α1PI t15 + T20 fusion inhibitor
●: α1PI t60

Bristow CL, et al. 2013 Discovery Medicine 16: 201-218.

Receptor recycling (HIV uptake) controlled by LDL-RFMs



a and b
Light blue: CD91 (LRP1)
Black: Isotype control

c
Dark green: 0.05µM siRNA
Light green: 0.1µM siRNA
Orange: 1µM siRNA
Blue: 10µM siRNA
Black: Isotype control

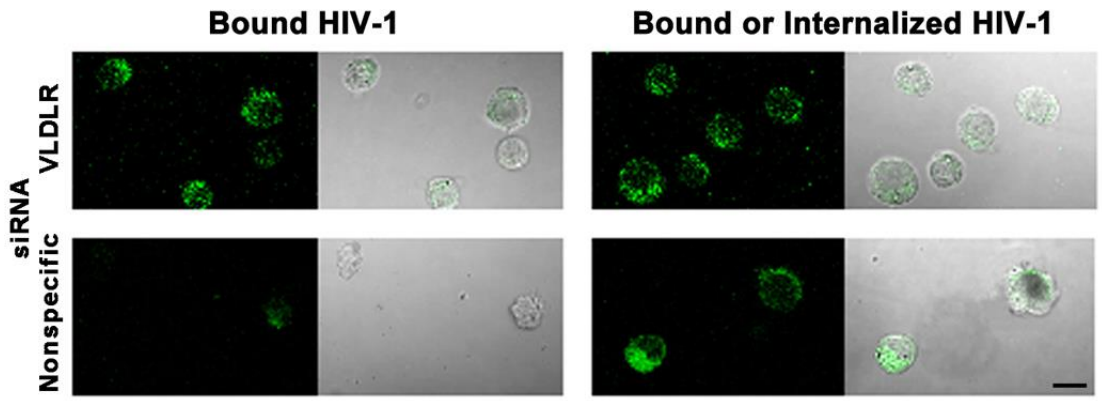
Light green: VLDLR siRNA
Red: nonspecific siRNA

Bristow CL, et al. 2013 Discovery Medicine 16: 201-218.

HIV uptake and infectivity controlled by VLDLR

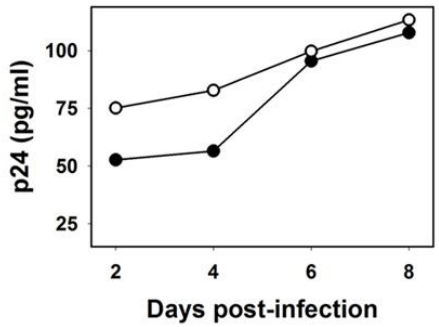
Non-permeabilized cells

Permeabilized cells

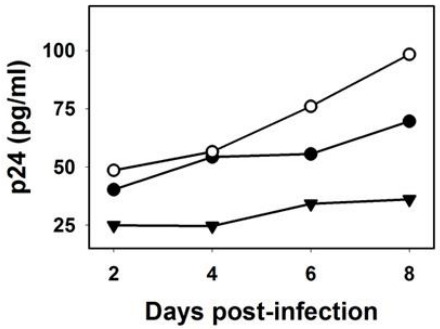


Green: infectious HIV NL4-3

VLDLR siRNA



RAP

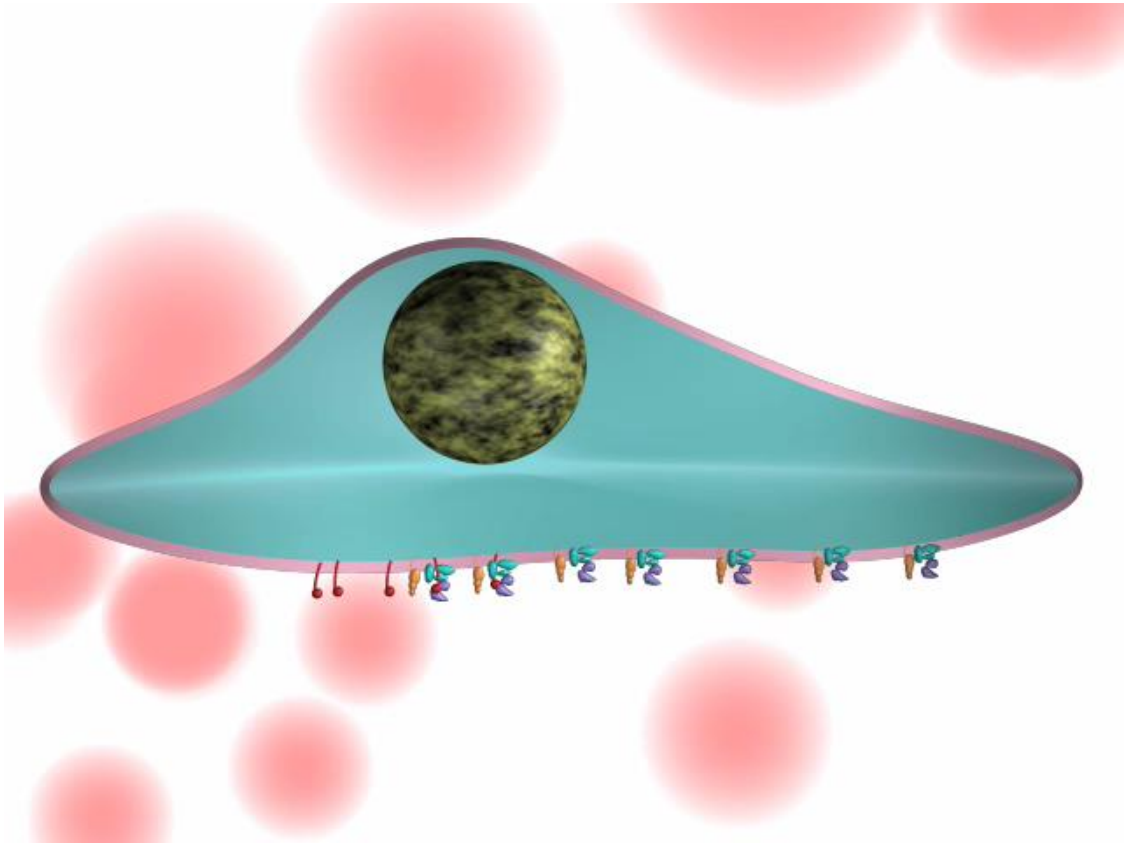


VLDLR siRNA
●: VLDLR siRNA
○: nonspecific siRNA

RAP
○: α1PI t15
●: RAP + α1PI
▼: RAP t15 followed by α1PI t15

Bristow CL, et al. 2013 Discovery Medicine 16: 201-218.

LDL receptor-mediated recycling of receptors

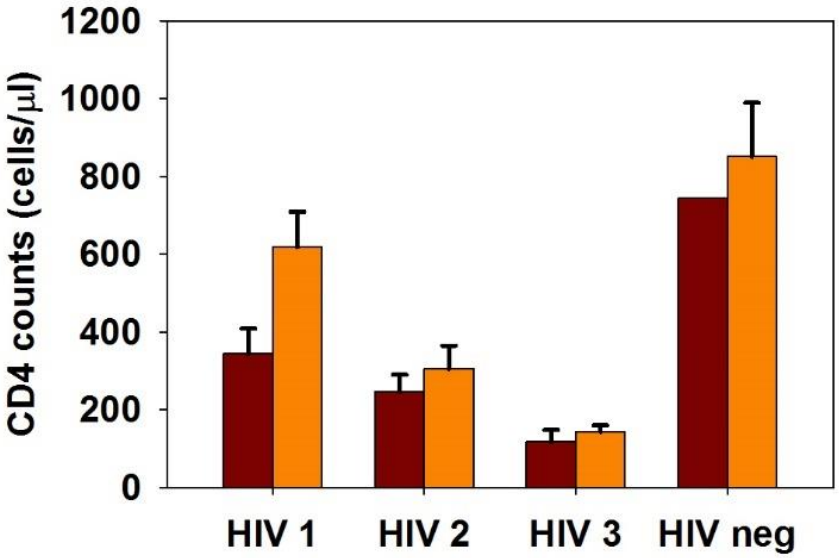


Purple: α1PI
Green: HLE-CS
Orange: CD4
Red: LDL receptor
Pink spheres: RBCs

The Clinical Trial Evidence

α1PI is an FDA – approved biological treatment for emphysema in patients with the inherited deficiency

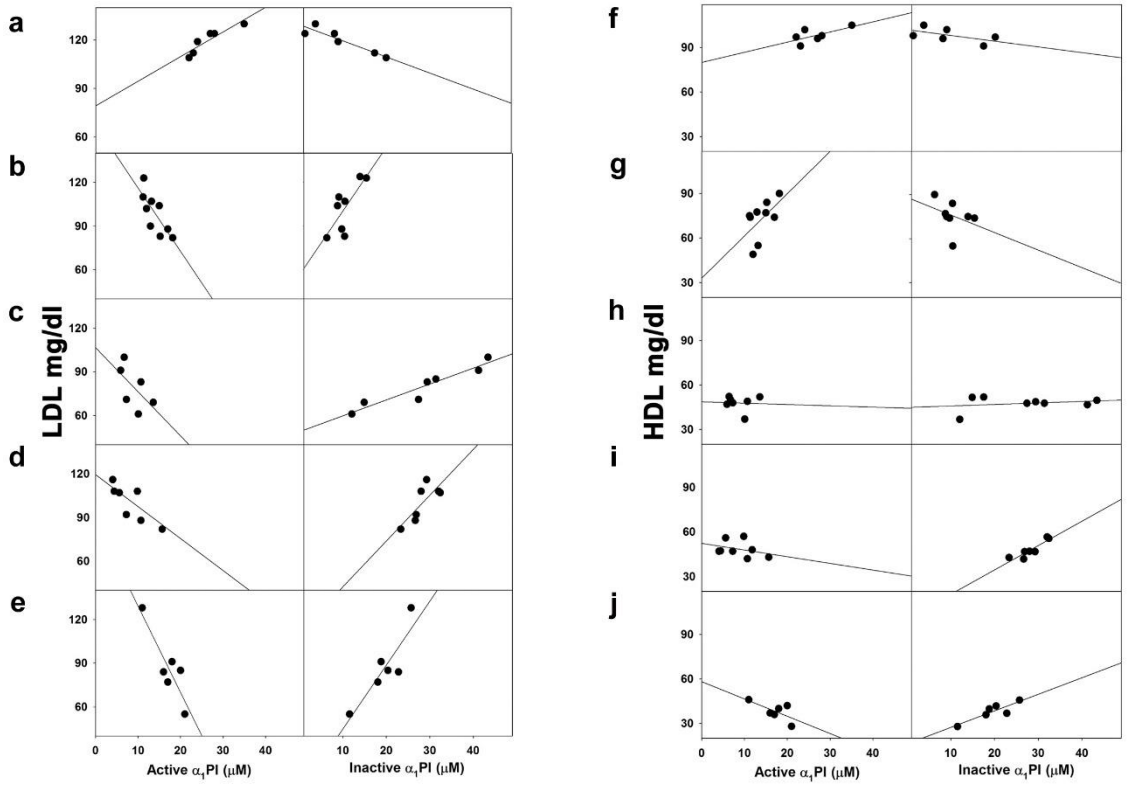
1 in 1,500 people of European ancestry



Maroon: Baseline
Orange: Following weekly α1PI therapy

Bristow CL, et al. Soluble Factors Mediating Innate Immune Responses to HIV Infection (2010) p. 102-110.

The Clinical Trial Evidence



Untreated control

α1PI therapy subject 1

α1PI therapy subject 2

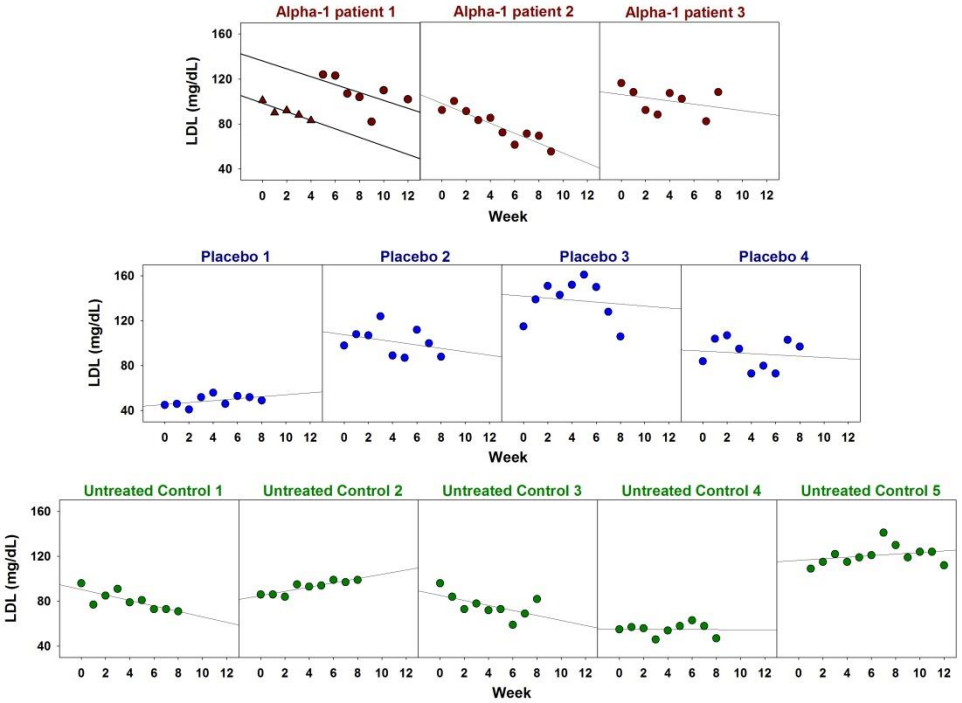
α1PI therapy subject 3

α1PI therapy subject 4

Bristow CL, et al. 2013 Discovery Medicine 16: 201-218.

The Clinical Trial Evidence

α1PI treatment decreased LDL levels in **HIV patients** as compared with **Placebo** and **Untreated Uninfected Controls**.



Mean = -3.39 ± 1.3, n=3

Mean = -0.19 ± 0.93, n=4

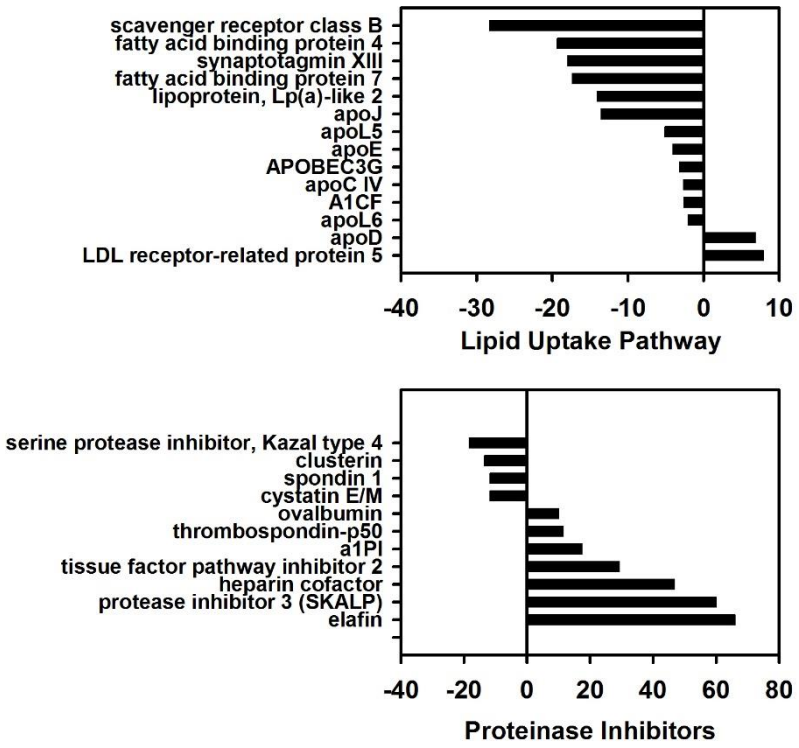
Mean = -0.22 ± 2.07, n=5

* Mean decreases (slopes) in LDL levels for Alpha-1 patients 1-4 were greater than for placebo treatment (***P*=0.04**). Alpha-1 Patient 1 was treated for 12 weeks, and untreated control 5 was monitored for 12 weeks. Alpha-1 patient 1 stopped antiretroviral medication for 1 week at week 5 of α1PI treatment. Alpha-1 patient 4 was receiving Lipitor treatment to lower LDL levels prior to initiating α1PI treatment and continued Lipitor throughout the study. Excluded from the LDL analysis were 2 of 6 patients on treatment and 1 on placebo who exhibited abnormally elevated markers of inflammation (CRP, IL-2).

Bristow CL, et al., unpublished results.

alpha-1 biologics

cDNA Microarray analysis



Mo/MØ were harvested from 1 uninfected and 2 HIV-1 infected individuals on ritonavir therapy. The gene expression ratio of HIV-1 infected to uninfected cells was calculated. All of the genes with lipoprotein and proteinase inhibitor functions that changed more than 3-fold are depicted.

Bristow CL, et al. 2013 Discovery Medicine 16: 201-218.

Summary

1) Using VLDLR siRNA and flow cytometry, recycling receptors are not able to internalize without the activity of VLDLR.

2) Using phosphoflow, α1PI induces NFκB signaling.

3) Using flow cytometry, HIV does not internalize or infect cells in the absence of α1PI-induced, VLDLR-mediated endocytosis.

4) Weekly α1PI therapy lowers LDL levels in individuals with low levels of α1PI.

5) Using gene expression ratios, α1PI and LDL are in negative feedback regulation.

We showed that α1PI and $CD4^+$ T cells DO regulate lipoprotein levels.

We are currently developing a small molecule to act as a proxy for α1PI to restore the immune system and lower LDL levels.

Conclusion

We need (and love) our Fats

and fats helped us understand HIV-1 disease.

Contact

Cynthia L. Bristow, PhD
Chief Executive Officer
Alpha-1 Biologics
25 Health Sciences Drive, Suite 110
Stony Brook, NY 11790
Mobile: 917 301 3292
Office: 631 444 6238
FAX: 631 444 8825
cynthia.bristow@alpha1biologics.com
cynthia.bristow@stonybrook.edu
www.alpha1biologics.com