BCL-2 antagonism decreases HIV replication and infected cell survival in acute *in vitro* infection.

• In all but one patient HIV has not been cured
• Two principal reasons that cure is elusive:
  – HIV persists in a latent state ($T_{CM}$, Other)
  – When HIV reactivates the reactivating cell does not die
Why don't reactivating cell die?

- Memory \( T_{CM} \) are an historic archive of immune responses, therefore programmed to resist death.
- Death resistance of \( T_{CM} \) linked to inactivation of the Foxo3A pathway


"Prime Shock and Kill"

Latently HIV Infected Memory CD4 T Cell

Apoptosis Resistant

Apoptosis Resistant Cell Produces Virus and Survives

Apoptosis Resistant Cell

HIV provirus

Viral reactivation

Eg. vorinostat

Apoptosis resistant

Apoptosis sensitization
- P53 activation
- Immune-based therapies
- Bcl2 antagonists
- Etc.

Apoptosis Sensitive

Apoptosis prone

HIV reactivation causes cell death

CART

CART

Casp8p41 pathway of infected cell killing

AIDS. 2010 Jun 1;24(9):1251-8.
Clinical Implications of Casp8p41

- Casp8p41 is only seen in HIV infected persons.
- Casp8p41 higher in viremic compared to suppressed patients.
- Casp8p41 is detected predominately in memory CD4 T cells.
- Casp8p41 expression in memory CD4 T cells is inversely correlated with absolute CD4 T cell count.
- Changes in Casp8p41 expression better predict CD4 T cell count change than changes in HIV viral load.
- Higher Casp8p41 expression is associated with future decreases in CD4 T cell counts.
- In ART-treated patients with breakthrough viremia without subsequent loss of CD4 T cells, drug resistance mutations in HIV protease are associated with decreased ability to cleave procaspase 8 to generate Casp8p41 (without effecting GagPol cleavage).

- Cummins NW et al., J Infect Dis. 2010 Aug 15;202(3):386-91
Modelling the Casp8p41:Bak interaction

- Unbiased lentiviral shRNA screen
- Previously unrecognized BH3 domain in Casp8p41, unmasked by HIV Protease cleavage.
- Casp8p41 BH3 domain binds the BH3 groove on Bak
- Confirmed by mutagenesis studies

Sainski A et al., J Cell Biol. 2014 Sep 29;206(7):867-76
BH3:BH3 binding groove interactions

Bcl-2 Family

Anti-Apoptotic
- Bcl-2
- Bcl-XL
- Mcl-1
- CED-9
- A1
- Bfl-1

Pro-Apoptotic
- Bax
- Bak
- Diva
- Bcl-Xs
- Bik
- Bim
- Bad
- Bid
- Egl-1

Pro-survival proteins
- Bcl-2
- Bcl-xL
- Bcl-w
- Mcl-1
- A1

BH3-only proteins
- Bim
- Bid
- Puma
- Bad
- Bmf
- Hrk
- Noxa
- Bik

Bax/Bak
- Bax
- Bak (and Bok?)

α1 α2 α3 α4 α5 α6 α7 α8 α9

BH4 BH3 BH1 BH2 TM

Groove

www.iasociety.org
Casp8p41 binds Bcl2.

Hypothesis:
Bcl2 levels determine if HIV infection results in death of infected cells
• ABT-199
• BH3-mimetic
• Co-developed by Abbvie and Genentech
• Approved April 11, 2016
• Patients with CLL with 17p deletion, >1 prior therapy.
• Tumor lysis syndrome / neutropenia.
Venetoclax (ABT-199), a selective Bcl-2 inhibitor reduces HIV DNA in ex vivo reactivated primary CD4 T cells from HIV infected persons.

Primary CD4 T cells from HIV infected patients on ART

→ DMSO vehicle

αCD3/αCD28

16hrs

→ ABT-199

→ TDF/RAL

→ 72hrs TDF/RAL

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Summary

- Casp8p41 is produced by HIV protease
  - only in infected but non-uninfected cells
- Binds to Bak to induce apoptosis
- Casp8p41 binds Bcl2, apoptosis prevented
- Casp8p41 produced in reactivating cells
- Bcl2 antagonist promotes death of reactivating cell
  - Death is selective for infected > uninfected cells.
Aims

• Does Bcl2 impact the outcome of acute HIV infection

• Acute HIV_{IIIb} infections Jurkat or Jurkat Bcl2.
  • HIV_{IIIb} primary CD4T cells +/- Bcl2 inhibitor
  • Monitor HIV DNA, P24, viability
Bcl2 blocks HIV infected cell killing
Bcl2 increases HIV DNA and P24 production
Bcl2 antagonist increased HIV killing

Venetoclax causes more cells to die in the infected cultures
Bcl2 antagonist decreases HIV DNA and p24

Venetoclax
- Decreases number of HIV DNA + cells
- Decreases HIV P24 in culture supernatant

P = 0.032

P = 0.021
Bcl2 antagonists

• Favor death of HIV infected reactivating cells, sparing uninfected cells
• Reduce reservoir size as measured by cell associated HIV DNA & QVOA
• Favor death of acutely HIV infected cells (prevent establishment of the reservoir)
• Reduce HIV replication by causing death of cells which produce virus.
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