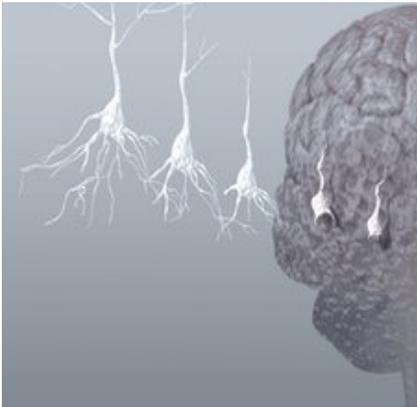


Peptide T & Dallas Buyers Club

What the film got right, What the Washington Post got wrong.

By Michael Ruff, February 2016



"The stuff he was importing from Mexico was almost all useless," Engel says. A case in point is the experimental drug **Peptide T**.

"[Woodruff] did become a proponent of **Peptide T**, and he used it himself, and believed it himself," Staley says. "It never panned out. It's a useless therapy, and it never got approved, and nobody uses it today, but the film implies that it helped him."

The above quotes are from an article in the Washington Post by Dylan Matthews, December 10, 2013

[Dylan Matthews Washington Post](#) ,

“Staley” is **Peter Staley** an [American HIV/AIDS-LGBT rights activist](#), known for founding the [Treatment Action Group](#) (TAG) (Wiki entry)

Re Peptide T, Staley says:

1. “ It never panned out.
2. It's a useless therapy,
3. it never got approved,
4. nobody uses it today,
5. but the film implies that it helped him."

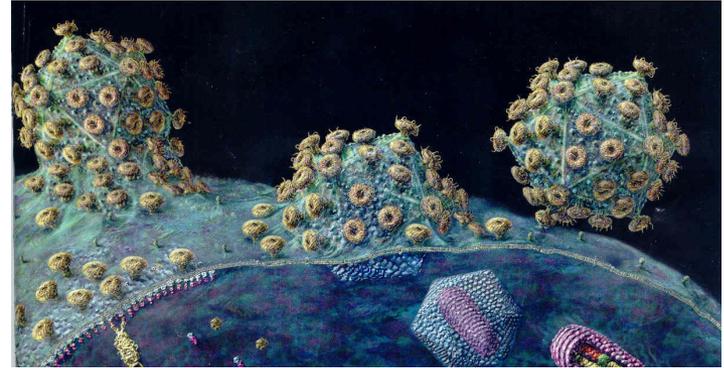


Illustration of HIV viruses emerging from an infected cell

Response (by Michael Ruff, co-inventor Peptide T, with Dr Pert)

- 1) Wrong
- 2) Wrong
- 3) True
- 4) Wrong, but irrelevant. In 2016 the unmet medical need in HIV is Neuro-AIDS.
- 5) True, Woodroof himself was interviewed for [Dallas Life Magazine](#) Aug 9, 1992: “Look at Peptide T. It is the only line I have to staying alive. When I stop it, I start dragging my leg. I urinate on myself, I can’t speak. I slobber all over the place”.

There is a scene in the film where the cops father used it for Alzheimers, another neurodegenerative brain disease which shares brain inflammation with HIV.

The focus of the information provided on the next few slides is to address #2, the view that Peptide T is “a useless therapy”. What does the published data show.

What is Peptide T? and Dala₁-peptide T-amide (“DAPTA”)?

A small peptide from the HIV envelope protein discovered in 1986 by Dr Candace Pert and colleagues. The concept was to find a peptide from the virus that would block its binding to its cellular receptor (which would not be discovered for another ten years) , and thereby block infection. Peptide T wouldn't be a good drug, as it was easily broken down in the body. Hence modifications were made to Peptide T to arrive at DAPTA, the clinical use compound. When people refer to Peptide T they really mean DAPTA.

- [Octapeptides deduced from the neuropeptide receptor-like pattern of antigen T4 in brain potently inhibit human immunodeficiency virus receptor binding and T-cell infectivity.](#)

Pert CB, Hill JM, Ruff MR, Berman RM, Robey WG, Arthur LO, Ruscetti FW, Farrar WL.

Proc Natl Acad Sci U S A. 1986 Dec;83(23):9254-8.

PMID: 3097649 [Free PMC Article](#)

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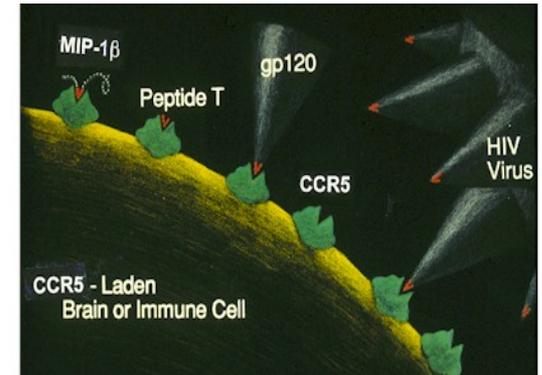


Illustration of the Peptide T peptide (red) at the “tip” of gp120, Where it binds to its receptor, and how this synthetic peptide can block virus binding by occupying the chemokine co-receptor.

By now it is well appreciated that viral envelope proteins encode chemokine analogs, and you could design drugs around these viral sequences.

At the time of the Pert paper (1986) this was a very advanced idea, she was first to the party, but later it became well understood that “Numerous viruses, including members of the herpesvirus, poxvirus, and lentivirus families encode peptides that block innate immunity, presumably to help them overcome immune responses” (Murphy, 2001)

[Curr Top Microbiol Immunol. 2006;303:121-54.](#)

A viral conspiracy: hijacking the chemokine system through virally encoded pirated chemokine receptors.

[Vischer HF¹, Vink C, Smit MJ.](#)

How Does DAPTA Work? Short answer: As knowledge accumulated, especially once the viral chemokine receptors were discovered in 1996, it was demonstrated that DAPTA blocked CCR5 mediated HIV infection and prevented GP120 binding to CCR5. All the early “replications” used “X4” viruses, not “R5” viruses. This is important as only the R5 viruses initiate infection, and those are the viruses most people have for most of their life. DAPTA also protects neurons from dying so has brain benefits on cognition and neuropathies, eg in Neuro-AIDS.

Dala₁-peptide T-amide (DAPTA):

Functional antagonist of a cluster of chemokine receptors that mediate innate immunity causing inflammation in the brain and body

Blocks HIV infection at CCR5

Reduces viral levels in people

Blocks HIV gp120 killing of brain neurons

Improves cognition in HIV brain infection

Improves brain scans

Lowers the inflammatory cytokines that drive HIV replication

Blocks, reverses neuropathic pain by blocking inflammation

- 1) Ruff, M.R., M. Polianova, C.B. Pert, and F.W. Ruscetti. 2003. Update on D-Ala¹-Peptide T-Amide (DAPTA): A Viral Entry Inhibitor that Blocks CCR5 Chemokine Receptors *Curr HIV Res.* 1:51-67
- 2) Ruff, M.R., L.M. Melendez-Guerrero, Q.E. Yang, W.Z. et al. 2001. Peptide T inhibits HIV-1 infection mediated by the chemokine receptor-5 (CCR5). *Antiviral Res.* 52:63-75
- 3) Polianova, M.T., F.W. Ruscetti, C.B. Pert, and M.R. Ruff. 2005. Chemokine receptor-5 (CCR5) is a receptor for the HIV entry inhibitor peptide T (DAPTA). *Antiviral Res.* 67:83-92
- 4) Padi, S.S., X.Q. Shi, Y.Q. Zhao, M.R. Ruff, N. Baichoo, C.B. Pert, and J. Zhang. 2012. Attenuation of rodent neuropathic pain by an orally active peptide, RAP-103, which potently blocks CCR2- and CCR5-mediated monocyte chemotaxis and inflammation. *Pain.* 153:95-106

Does DAPTA have any benefits in People?

There are many publications, and I will provide some of the bibliography at the end. There has been only one major Phase 2 clinical trial in HIV, and that was conducted by the NIH, I will focus on those data.

Two publications appeared, and I am not an author on either of them. I will summarize those two studies, and below provide the citations to the full articles.

Heseltine, P.N., K. Goodkin, J.H. Atkinson, B. Vitiello, J. Rochon, R.K. Heaton, E.M. Eaton, F.L. Wilkie, E. Sobel, S.J. Brown, D. Feaster, L. Schneider, W.L. Goldschmidts, and E.S. Stover. 1998. Randomized double-blind placebo-controlled trial of peptide T for HIV-associated cognitive impairment. *Arch Neurol.* 55:41-51

Goodkin, K., B. Vitiello, W.D. Lyman, D. Asthana, J.H. Atkinson, P.N. Heseltine, R. Molina, W. Zheng, I. Khamis, F.L. Wilkie, and P. Shapshak. 2006. HIV-1 viral load in a multisite, randomized, double-blind, placebo-controlled trial of DAPTA for HIV-1-associated cognitive-motor impairment. *J Neurovirol.* 12:178-189

Some background re the clinical research on DAPTA

Peptide T/DAPTA was developed by the National Institute of Mental Health (NIMH), home to Dr Pert's research team.

The focus of NIMH was Neuro-AIDS, while the virology focus was out of the National Institute of Allergy & Infectious Diseases (NIAID).

The early anti-viral effects of Peptide T/DAPTA, circa 1987, before the chemokine receptors were known, was questioned. Most people used so-called lab adapted or "X4" HIV isolates, that used the chemokine CXCR4 receptor. Pert studied natural patient isolates, that later turned out to use the CCR5 receptor. DAPTA had high activity against these strains. (Citations slide 14)

NIMH was interested in HIV brain disease, so the focus of the research turned to Neuro-AIDS, and the clinical use of DAPTA to protect neurons from dying and to improve brain cognitive function.

Studies in the late 1980's examined DAPTA effects on brain scans, Those improvements are shown in the next slide.

DAPTA Human Benefits: Normalized Brain Scan in HIV Dementia, Three Studies

- A 39-year old man with AIDS Dementia Complex received 12 weeks of intranasal DAPTA (0.4 mg TID)
- Thirty-four of 35 brain regions having high Z-scores (reds and yellows) showed remission after therapy

Villemagne VL, Phillips RL, Liu X, et al. Peptide T and glucose metabolism in AIDS dementia complex. *J Nucl Med.* 1996 Jul;37(7):1177-80.

DAPTA Human Studies in Neuro-AIDS

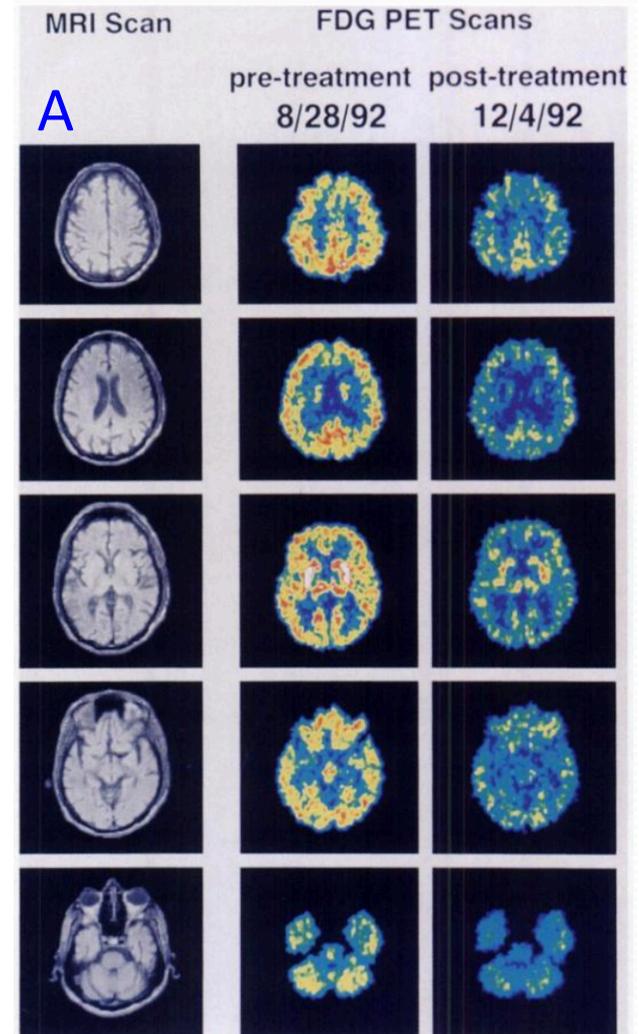
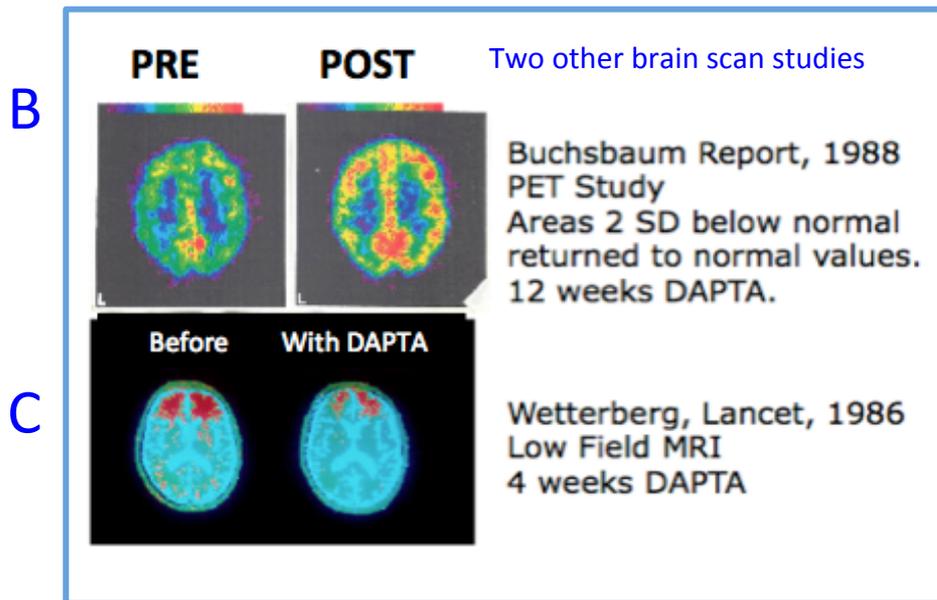


FIGURE 1. MRI and color-coded transforms of FDG-PET scans at five different levels of the brain of the ADC patient showing the baseline study and the study after a 12-wk treatment protocol with 1.2 mg/kg of intranasal peptide T.



Cognitive Improvement with DAPTA in HIV Cognitive Deficits

Positive Results from Three Controlled Trials Conducted by the NIH

Bridge, T.P., P.N. Heseltine, E.S. Parker, E.M. Eaton, L.J. Ingraham, M.L. McGrail, and F.K. Goodwin. 1991. Results of extended peptide T administration in AIDS and ARC patients. *Psychopharmacol Bull.* 27:237-245

Kosten, T.R., M.I. Rosen, T.L. McMahon, T.P. Bridge, S.S. O'Malley, R. Pearsall, and P.G. O'Connor. 1997. Treatment of early AIDS dementia in intravenous drug users: high versus low dose peptide T. *Am J Drug Alcohol Abuse.* 23:543-553

Heseltine, P.N., eal. 1998. Randomized double-blind placebo-controlled trial of peptide T for HIV-associated cognitive impairment. *Arch Neurol.* 55:41-51

Those Preceding Studies Informed a Data Review/Decision in 1990 for Phase II Testing with NIAID Approval

NIAID *Aids* AGENDA

National Institute of Allergy and Infectious Diseases January 1990

NIMH, NIAID Suggest Controlled Double Blind Peptide T Study

Page 7

On November 9, 1989, the National Institute of Mental Health (NIMH) convened a special committee to review data generated from initial Phase I clinical testing of Peptide T sponsored by the NIMH Intramural Research Program and to make recommendations regarding further clinical development of this agent.

The committee included six scientists proposed by NIMH and four scientists nominated by the National Institute of Allergy and Infectious

ORIGINAL CONTRIBUTION

Randomized Double-blind Placebo-Controlled Trial of Peptide T for HIV-Associated Cognitive Impairment

Peter N. R. Heseltine, MD; Karl Goodkin, MD, PhD; J. Hampton Atkinson, MD; Benedetto Vitiello, MD; James Rochon, PhD; Robert K. Heaton, PhD; Elaine M. Eaton, PhD; Frances L. Wilkie, PhD; Eugene Sobel, PhD; Stephen J. Brown, MD; Dan Feaster; Lon Schneider, PhD; Walter L. Goldschmidts, PhD; Ellen S. Stover, PhD

NIMH Multi-site 24-Week Phase 2 Testing for Cognitive Improvements

Heseltine, P.N., et.al. 1998. Randomized double-blind placebo-controlled trial of peptide T for HIV-associated cognitive impairment. *Arch Neurol.* 55:41-51

- Three-site DBPC trial of DAPTA (“Peptide T”) given intra-nasally at a dose of 2 mgs, three times per day for 6 months
- 457 persons screened - 205 men and 10 women were randomized; 106 to DAPTA and 109 to placebo
- The efficacy analysis was conducted on N=143
- The primary outcome measurement was a global neuropsychological NP score derived from 23 standardized scores
- The efficacy endpoint was change in NP score at 6 months compared to baseline
- Additional planned analyses were conducted based on baseline NP deficit

Results of NIMH Multi-Site NP DBPC Trial

- Analysis of the primary efficacy endpoint, neuropsychological (NP) global change score failed to show a difference between DAPTA and placebo, $P=0.18$
- However, 2 of 7 domains, abstract thinking and speed of information processing, **did show improvement** in the DAPTA group ($p<.05$)
- **Also, twice as many DAPTA-treated patients improved, whereas twice as many placebo patients deteriorated, $P=.02$**
- 62% of the efficacy sample did not display global impairment (although they met the inclusion standard) on the NP battery.
- The cohort was minimally cognitively impaired. Mini-Mental score of 28/30, v mild.
- **Among the more cognitively impaired patients, DAPTA was associated with improved performance, while deterioration was more common in the placebo group** (ie global NP deficit ≥ 0.5 , 54 of the 143 patients, $P=.02$).
- This sub-group had a CLINICALLY SIGNIFICANT level of impairment, means this is the group you would want to treat. For the others, any benefit wouldn't be noticed.

Summary of NIMH Multi-Site NP DBPC Phase 2 Trial

- DAPTA benefits were significant for patients with greater deficit
- The NP deficit ≥ 0.5 global Z-score is a clinically significant level of impairment
- DAPTA was well tolerated and no clinically significant toxic effects emerged

Summary of 2 Pilot Phase I Studies

- Both studies tested patients with cognitive global deficit scores > 0.5
- Both studies reported statistically significant drug effects for DAPTA improvements

DAPTA has cognitive benefits only in patients
who are at least > 0.5 NP score impaired
CERTAINLY NOT A “USELESS THERAPY”

Neuro-AIDS affects close to 30% patients today and brain
neurons are dying in the first months after you get infected.

DAPTA Outperforms 2014 Maraviroc Clinical Trial for Neuro-AIDS

Ndhlovu, L.C. 2014. Treatment intensification with maraviroc (CCR5 antagonist) leads to declines in CD16-expressing monocytes in cART-suppressed chronic HIV-infected subjects and is associated with improvements in neurocognitive test performance: implications for HIV-associated neurocognitive disease (HAND). *J Neurovirol.* 20:571-582

RESULTS

- “There was no significant change from entry (week 0) to week 24 in global composite NP scores with Maraviroc. The median change in NP score in the entire group from weeks 0 to 24 was 0.13 ($p=0.27$). However, some significant neuropsychological improvement was evident when the six subjects who entered the study with impairment (NP Z global ≤ -0.5) were analyzed separately.”
- ClinTrials.gov lists a phase 2 trial for Maraviroc, NCT02159027 based on this study. NIAID funded?
- **DAPTA performed BETTER than Maraviroc in Ndhlovu, L.C.. 2014,**
- **Ndhlovu, L.C.. 2014, also only showed benefit with a CCR5 Blocking Drug in patients**
- **with NP Z global score ≤ -0.5 , just like the DAPTA trial**
- **DAPTA study was DBPC and had N=54 with NP Z score < -0.5 , (P=.02) (vs. N=6 in the open label study of Ndhlovu et al., 2014)**

What About Antiviral Effects?

Pert, C.B., J.M. Hill, M.R. Ruff, R.M. Berman, W.G. Robey L.O. Arthur, F.W. Ruscetti, and W.L. Farrar. 1986. Octapeptides deduced from the neuropeptide receptor-like pattern of antigen T4 in brain potently inhibit human immunodeficiency virus receptor binding and T-cell infectivity. *Proc Natl Acad Sci U S A.* 83:9254-8.

Ruff, M.R., P.L. Hallberg, J.M. Hill, and C.B. Pert. 1987. Peptide T[4-8] is core HIV envelope sequence required for CD4 receptor attachment [letter]. *Lancet.* 2:751

Ruff, M.R., L.M. Melendez-Guerrero, Q.E. Yang, W.Z. Ho, J.W. Mikovits, C.B. Pert, and F.A. Ruscetti. 2001. Peptide T inhibits HIV-1 infection mediated by the chemokine receptor-5 (CCR5). *Antiviral Res.* 52:63-75

Polianova, M.T., F.W. Ruscetti, C.B. Pert, R.E. Tractenber, G. Leoung, S. Strang, and M.R. Ruff. 2003. Antiviral and immunological benefits in HIV patients receiving intranasal peptide T (DAPTA). *Peptides.* 24:1093-1098.

Ruff, M.R., M. Polianova, Q.E. Yang, G.S. Leoung, F.W. Ruscetti, and C.B. Pert. 2003. Update on D-alanine peptide T-amide (DAPTA): a viral entry inhibitor that blocks CCR5 chemokine receptors. *Curr HIV Res.* 1:51-67

Pollicita, M., M.R. Ruff, C.B. Pert, M.T. Polianova, D. Schols, A. Ranazzi, C.F. Perno, and S. Aquaro. 2007. Profound anti-HIV-1 activity of DAPTA in monocytes/macrophages and inhibition of CCR5-mediated apoptosis in neuronal cells. *Antivir Chem Chemother.* 18:285-295

Goodkin, K., B. Vitiello, W.D. Lyman, D. Asthana, J.H. Atkinson, P.N. Heseltine, R. Molina, W. Zheng, I. Khamis, F.L. Wilkie, and P. Shapshak. 2006. HIV-1 viral load in a multisite, randomized, double-blind, placebo-controlled trial of DAPTA for HIV-1-associated cognitive-motor impairment. *J Neurovirol.* 12:178-189

DAPTA Reduced Plasma/Serum Viral Load

(Goodkin et al, J Neurovirol, 2006)

Sub-group analysis from the NIH Sponsored Multi-Site Trial Phase 2 Trial for Cognitive Endpoints

N=57 samples from the Univ Miami site



DAPTA proven to have an anti-viral effect in a double-blind controlled trial.

DAPTA Anti-viral Effects in People

Data are from the NIMH phase 2 trial, the Univ of Miami cohort.

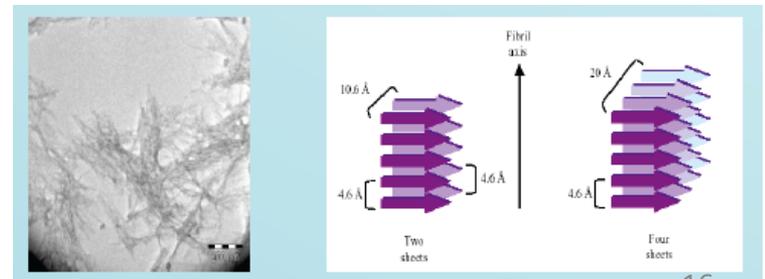
This study never even measured viral load! Those data were captured after the fact (post hoc analysis of BLINDED samples) by Goodkin et al., 2006

The antiviral effect is clinically significant, and statistically significant, but quite modest by current standards.

DAPTA wouldn't be used as monotherapy now but as this study was done pre protease inhibitors it was essentially a DAPTA monotherapy trial, that wasn't designed to detect antiviral effects, yet it did

The NIMH multi-site DBPC study was severely flawed, yet still showed multiple DAPTA benefits !

- NIMH changed both the drug manufacturer and the study formulation from the successful phase I tests.
- They identified a “cheaper” drug vendor, and they doubled the concentration of the DAPTA in the nasal sprayers to save costs of formulation (vials) and shipping.
- They also prepared large batches of study drug and stored them for >2 years without ever determining the effects of storage on biopotency!
- These modifications destroyed the study medication AS IT FORMED A GEL IN THE NASAL SPRAYERS.
- The notes/instructions to the PI’s from the NIH Pharmacy provide the evidence. “Drug may thicken, warm in your hands and shake”. Unacceptable!
- Formation of a gel indicates the peptide aggregated, and it aggregated because they changed the manufacturer and 2x the concentration to save formulation and shipping costs.
- EM images of the NIMH formulation
 - Shows fibrils. X-ray crystal confirms beta-sheet fibrils
 - Fibril formation loses biopotency



What Would a Proper DAPTA Study in 2016 Look Like?

Focus on Neuro-AIDS

- Use non-aggregating formulation of DAPTA nasal spray, use original GMP manufacturer (Bachem, a major US peptide company)
- Select cognitively impaired patients >0.5 mean deficit by FUNCTIONAL NP assessment
- DAPTA 6 mgs per day vs placebo
- Endpoints
 - *Morphometric, such as Ann Ragan (Northwestern). Determination if stop/reverse loss of dendritic arbor IN FIRST 6 MONTHS post infection*
 - *Microglial activation brain scan*
 - *Change in inflammatory biomarkers, IL-1, 6, TNF α*
 - *Change in HIV DNA, monocytes (CD14 and CD16 subsets)*
 - *Neurocognitive tests of abstract thinking and speed of information processing*

Treatment Goals for Neuro-AIDS in 2016

- *Protect neurons from dying*
- *Stop, reverse cognitive declines*
- *Stop, reverse motor declines*
- *Reverse neuropathies*
- *Reduce burden of infected monocytes*

DAPTA Will Meet These Treatment Goals

- ✓ Blocks HIV mediated neurotoxicity, eg block gp120
- ✓ Blocks microglial activation that causes neuronal bystander death
- ✓ Blocks innate immune pathways, cytokine release
- ✓ Blocks HIV replication. Blocks monocytes to enter the CNS
- ✓ Enters the brain
- ✓ Reversed cognitive deficits, even with a flawed formulation, in a clinically impaired cohort
- ✓ Blocks neuropathic pain, reverses neuropathies. Spares synapses
- ✓ Safe. Oral analogs now exist

DAPTA: Partial List of Pre-Clinical Benefits Relevant to Neuro-AIDS

A. First identification of gp120 as a neurotoxin

- 1) Brenneman et al., 1988, Nature 335: 639-42

B) DAPTA blocks gp120 neurotoxicity, developmental delays, loss of dendritic arbor, brain inflammation, microglial activation, excitotoxicity, and neuropathic pain

- 1) Brenneman, et al. Peptide T prevents gp120 induced neuronal cell death in vitro: Relevance to AIDS dementia. *Drug Dev. Res.* 15: 361-369, 1988.
- 2) Hill, J.M., R.F. Mervis, R. Avidor, T.W. Moody, and D.E. Brenneman. 1993. HIV envelope protein-induced neuronal damage and retardation of behavioral development in rat neonates. *Brain Res.* 603:222-233
- 3) Brenneman, D.E., J. Hauser, C.Y. Spong, T.M. Phillips, C.B. Pert, and M. Ruff. 1999. VIP and D-ala-peptide T-amide release chemokines which prevent HIV-1 GP120-induced neuronal death. *Brain Res.* 838:27-36.
- 4) Rosi, S., C.B. Pert, M.R. Ruff, K. McGann-Gramling, and G.L. Wenk. 2005. Chemokine receptor 5 antagonist D-Ala-peptide T-amide reduces microglia and astrocyte activation within the hippocampus in a neuroinflammatory rat model of Alzheimer's disease. *Neuroscience.* 134:671-676.
- 5) Pollicita, M., M.R. Ruff, C.B. Pert, M.T. Polianova, D. Schols, A. Ranazzi, C.F. Perno, and S. Aquaro. 2007. Profound anti-HIV-1 activity of DAPTA in monocytes/macrophages and inhibition of CCR5-mediated apoptosis in neuronal cells. *Antivir Chem Chemother.* 18:285-295
- 6) Bachis, A., F. Biggio, E.O. Major, and I. Mocchetti. 2009. M- and T-tropic HIVs promote apoptosis in rat neurons. *J Neuroimmune Pharmacol.* 4:150-160.
- 7) Lee, Y.K., D.Y. Choi, Y.Y. Jung, Y.W. Yun, B.J. Lee, S.B. Han, and J.T. Hong. 2013. Decreased pain responses of C-C chemokine receptor 5 knockout mice to chemical or inflammatory stimuli. *Neuropharmacology.* 67:57-65
- 8) Padi, S.S., X.Q. Shi, Y.Q. Zhao, M.R. Ruff, N. Baichoo, C.B. Pert, and J. Zhang. 2012. Attenuation of rodent neuropathic pain by an orally active peptide, RAP-103, which potently blocks CCR2- and CCR5-mediated monocyte chemotaxis and inflammation. *Pain.* 153:95-106.
- 9) Di Prisco, S., M. Summa, V. Chellakudam, P.I. Rossi, and A. Pittaluga. 2012. RANTES-mediated control of excitatory amino acid release in mouse spinal cord. *J Neurochem.* 121:428-437.
- 10) Di Prisco, S., E. Merega, and A. Pittaluga. 2014. Functional adaptation of presynaptic chemokine receptors in EAE mouse central nervous system. *Synapse.* 68:529-535.
- 11) Many other citations, probably over 50 on benefits in patients for psoriasis, MS, mechanisms of action etc.