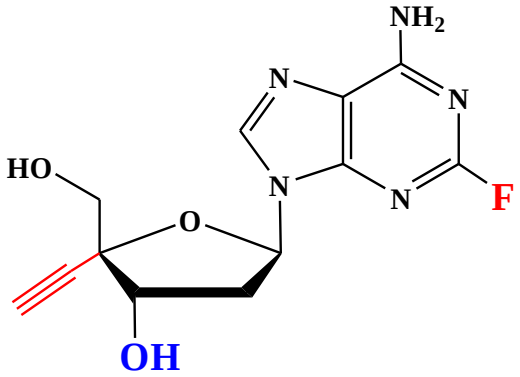
An Extremely Excellent Anti-HIV Active Modified Nucleoside, EFdA, Focused on its Design



EFdA(*4'-C-Ethynyl-2-Fluoro-2'-Deoxyadenosine*)

Hiroshi Ohrui
Yokohama University of Pharmacy

EFdA has attracted much attention due to its extremely excellent anti-HIV activity and physical properties, and is now under clinical investigation by Merck & Co. as MK-8591.

Brief Summary of Phase 1 and 1b reported by Merck:

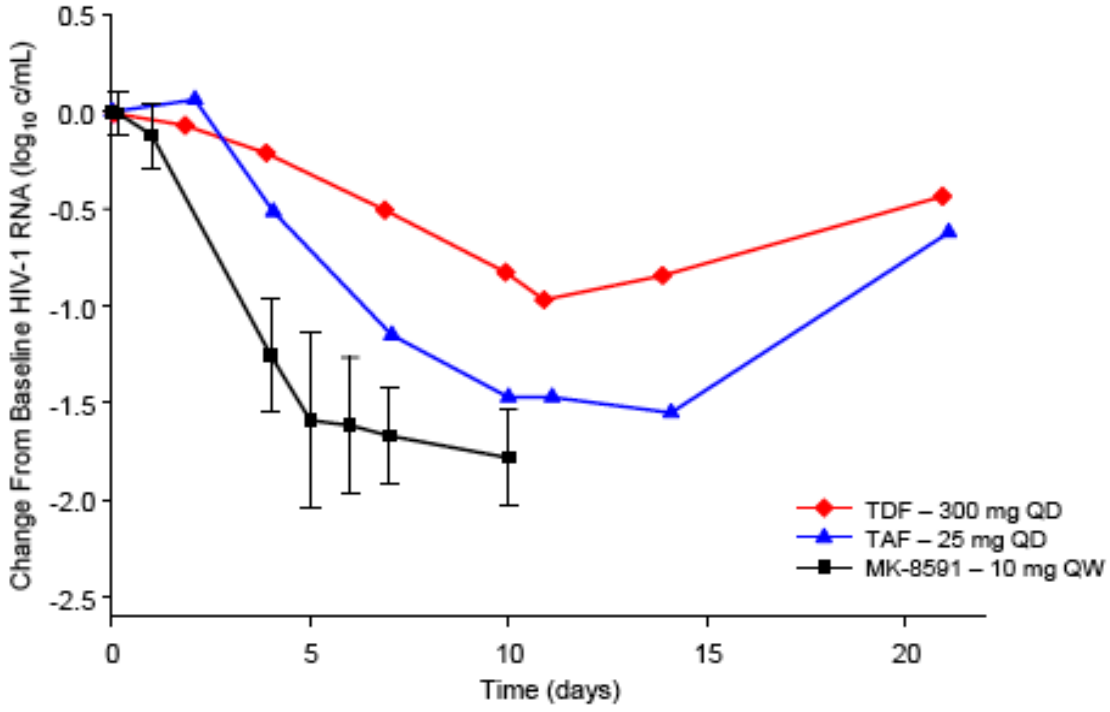
MK-8591(EFdA) suppressed HIV replication for at least 7 days when administrated as a single dose as low as 0.5mg.

No evidence of the emergence of resistant HIV variant.

All doses (0.5-30mg) were well tolerated with mild/moderate adverse experiences.

The antiviral potency, human pharmacokinetics (PK), and physical properties of MK-8591(EFdA) have the potential to open new paradigms for extended duration HIV treatment and prophylaxis approaches.

Comparison of Single Once-Weekly Dose of MK-8591 and Once-Daily Dosing of TDF and TAF



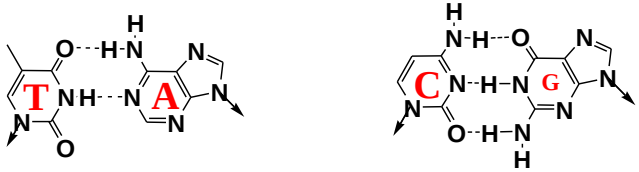
Adapted from:
Ruane PJ, DeJesus E, Berger D, et al. *J Acquir Immune Defic Syndr*. 2013;63(4):449-455

Mean and standard deviation MK-8591 data from: PN003, Panel A (10 mg).

MERCK

A General Idea for the development of Antiviral Modified Nucleosides

Viruses adapt themselves to the environmental change by mutation.
Mutation causes drug-resistant variants and makes the treatment of viral infectious disease very difficult. Therefore, mutation has been taken for only the cause of the problems of the treatment of viral infectious disease.
However, I think that mutation is the heaven-sent opportunity for the development of antiviral modified nucleosides, for the following reasons.



Mutation is that viruses change their genes by taking not-programmed nucleosides into their genes by ignoring A:T, G:C pairing. This indicates that the substrate selectivity of viral nucleic acid polymerases is not strict.
On the other hand, human beings do not accept the incorrect nucleosides into their genes. This indicates that the substrate selectivity of human nucleic acid polymerases is very strict.

Therefore, by taking advantage of the difference of the substrate selectivity between viral and human nucleic acid polymerases, it is possible to develop antiviral modified nucleosides which are selectively accepted by viruses (selectively active to viruses) and not accepted by human beings(not toxic to human beings).

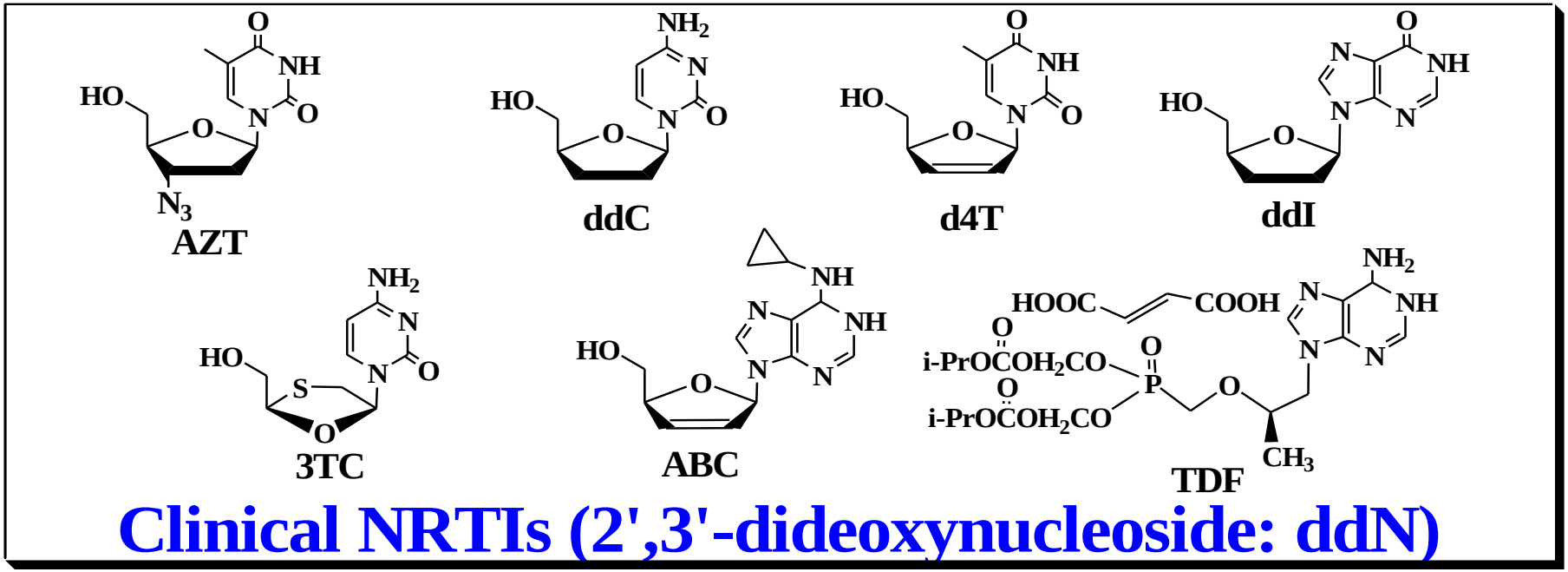
Critical Problems in the treatment of HIV-Infection

1. **Emergence of Drug-resistant HIV-mutants.**
2. **Side Effects of Drugs which must be taken every day in one's life.**

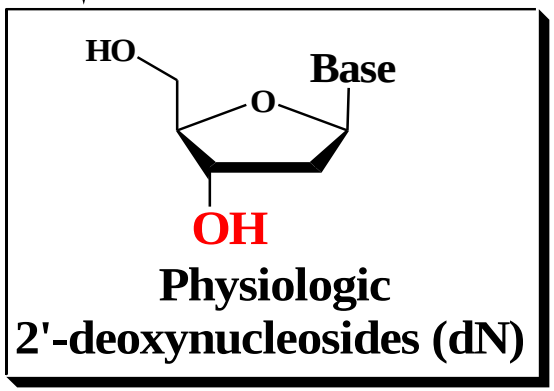
To solve these problems, I have proposed the following four working hypotheses.

1. **The way to prevent the emergence of drug-resistant HIV-mutants.**
2. **The way to decrease the toxicity of nucleosides.**
3. **The Substrate Selectivity of RT is different from that of human DNA-polymerases.**
4. **The way to make nucleoside-drugs long-Acting.**

1. The Way to Prevent the Emergence of Resistant HIV-mutants

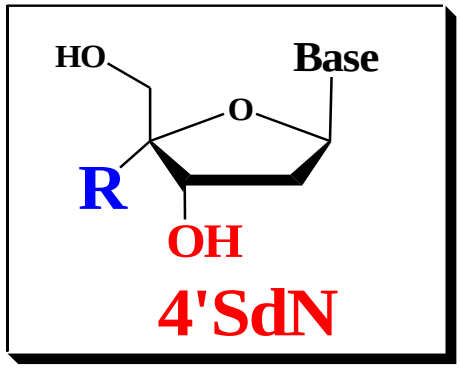


HIV-mutants have obtained the ability to distinguish

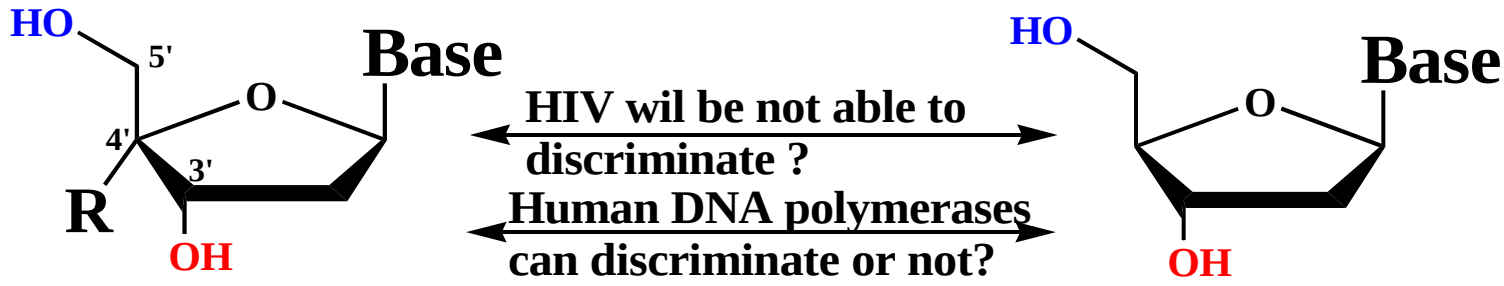


HIV will not be able to discriminate

Human DNA polymerases can distinguish or not?



Neopentyl alcohol is a primary alcohol, but the hydroxy group is not reactive because the hydroxymethyl group is bonded to a tertiary carbon.



4'SdN
(4'-*C*-substituted-2'-deoxynucleoside)

physiologic 2'-deoxynucleoside

4'SdN has two neopentyl-type hydroxy groups, 5'-OH (primary), and 3'-OH (secondary).

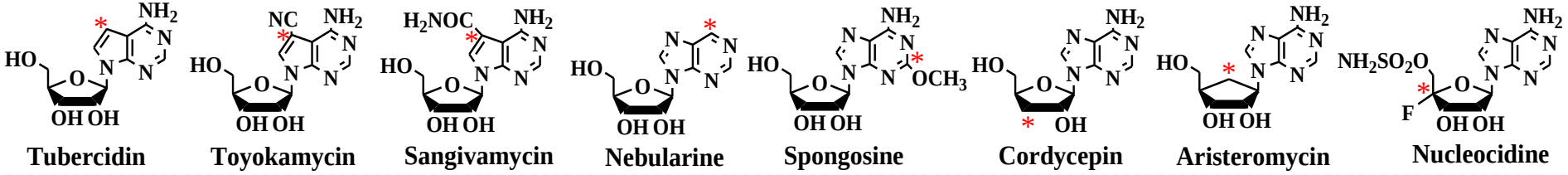
I expected that the 5'-OH could be phosphorylated by cellular kinase and the 3'-OH could be used for mistaking 4'SdN for physiologic 2'-deoxy-nucleoside by RT, but the 3'-OH could not be used for the elongation of pro-viral DNA chain due to its unreactivity.

Thus, 4'SdN could be the chain terminator of RT.

However, if human DNA-polymerases can't discriminate 4'SdN should be highly toxic!

2.The Way to Decrease the Toxicity of Nucleosides

Structures of Nucleoside Antibiotics (Antibacterial, Antitumor ative)

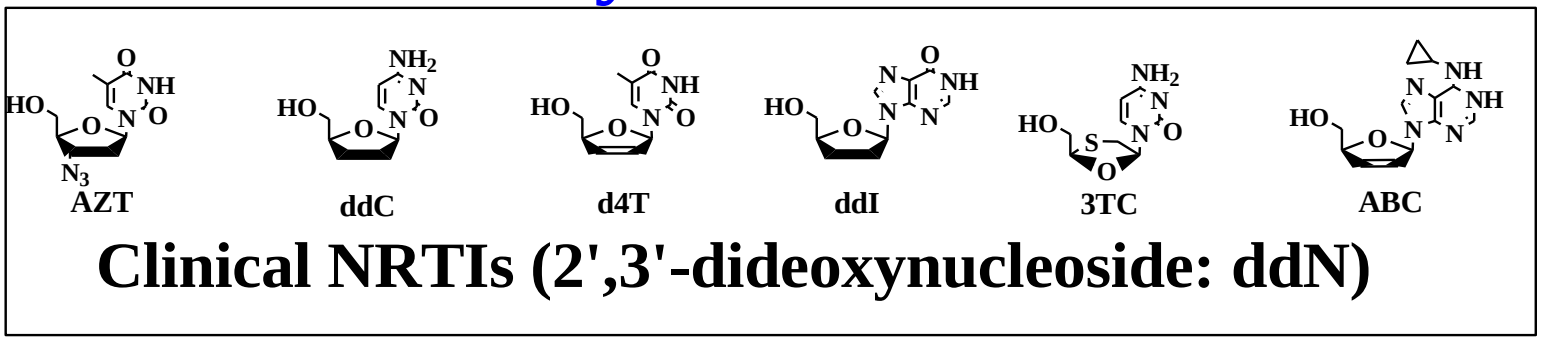


Most of the nucleoside antibiotics are one site modified physiolgic nucleiside and highly antimicrobial and antitumor active but highly toxic, too.

Modification of these antibiotics resulted in the loss of antibiotic activities.
Synthetic modified nucleosides gave the same results.
Many chemists said "No Future in Nucleoside Chemistry"
and left nucleoside chemistry in 1960s and 1970s.

Loss of antibiotic activity means the loss of toxicity!

3. The Substrate Selectivity is Different between HIV's Reverse Transcriptase(RT) and Human DNA-Polymerases

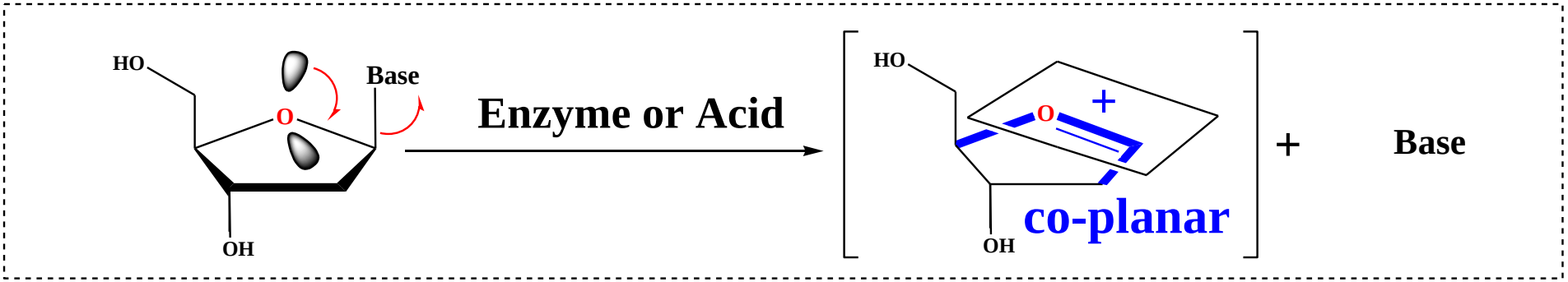


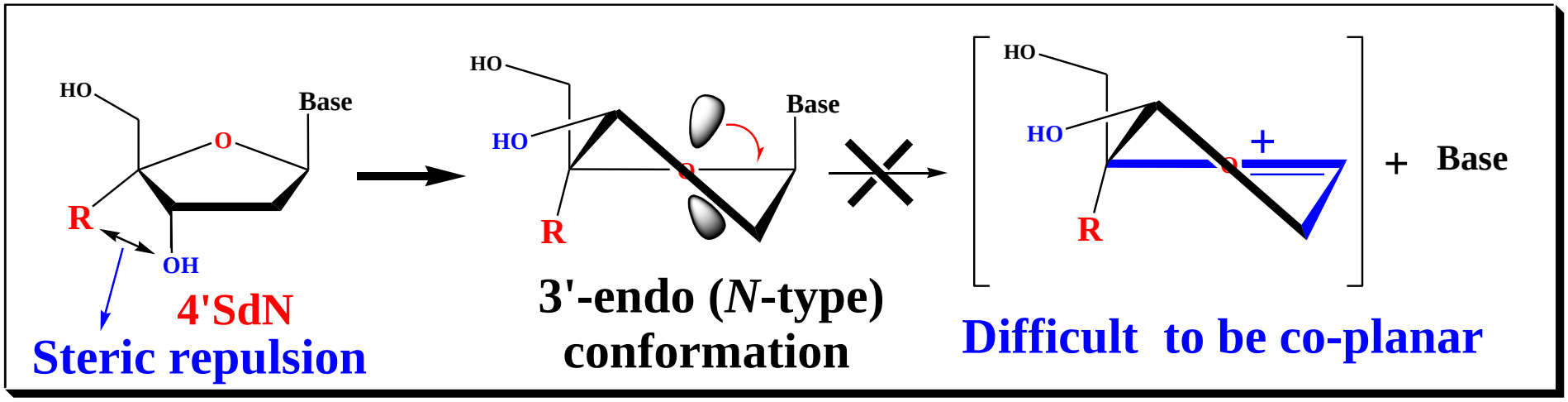
Clinical NRTIs (2',3'-dideoxynucleoside: ddN)

Sanger Method tells us that ddNs are the chain terminators of Human DNA-polymerases and therefore these clinical ddNs will be highly toxic. However, they have been and being clinically used by using limitted amounts of them. These facts indicate that the activity of these drugs to RT and DNA-Polymerases is different. Thus, the Substrate Selectivity between HIV's RT and Human DNA-polymerases will be different.

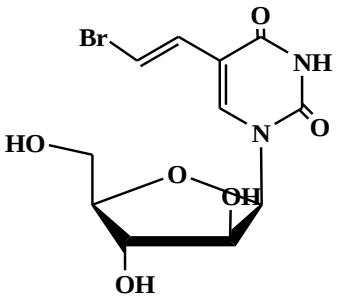
Therefore, we have the chance to develop the modified nucleosides that are much more active to RT and much less toxic to human beings than the clinical drugs.

4. 4'-Substituents Provide Nucleoside with Stability to both Enzymatic and Acidic Glycolysis and make 4'SdNs Long Acting.



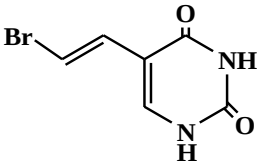


Sorivudine Affair



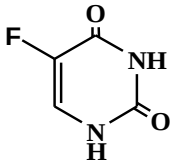
Sorivudine
(herpes zoster)

Glycolysis
in body

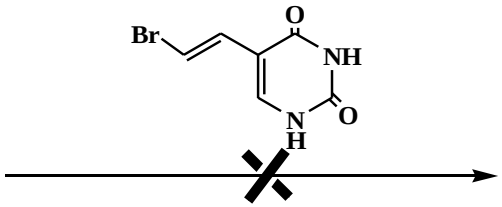


5-bromovinyl uracil

+ Sugar



5FU

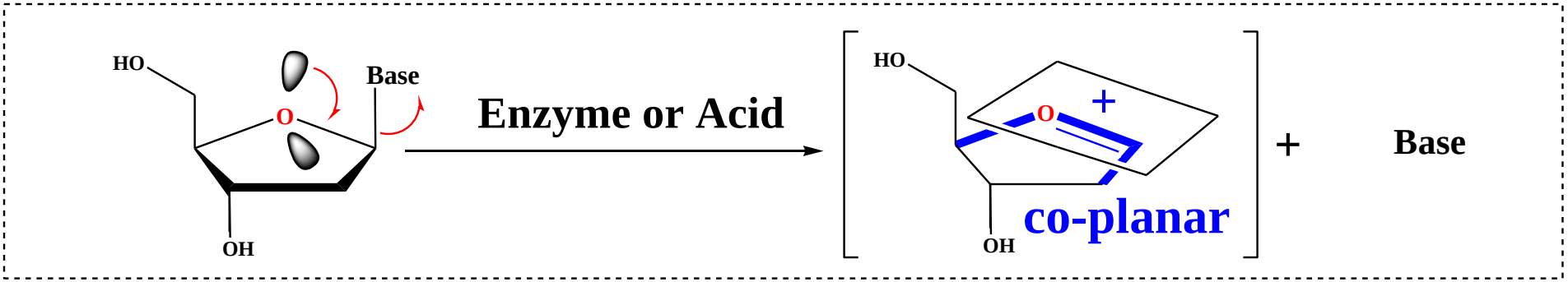


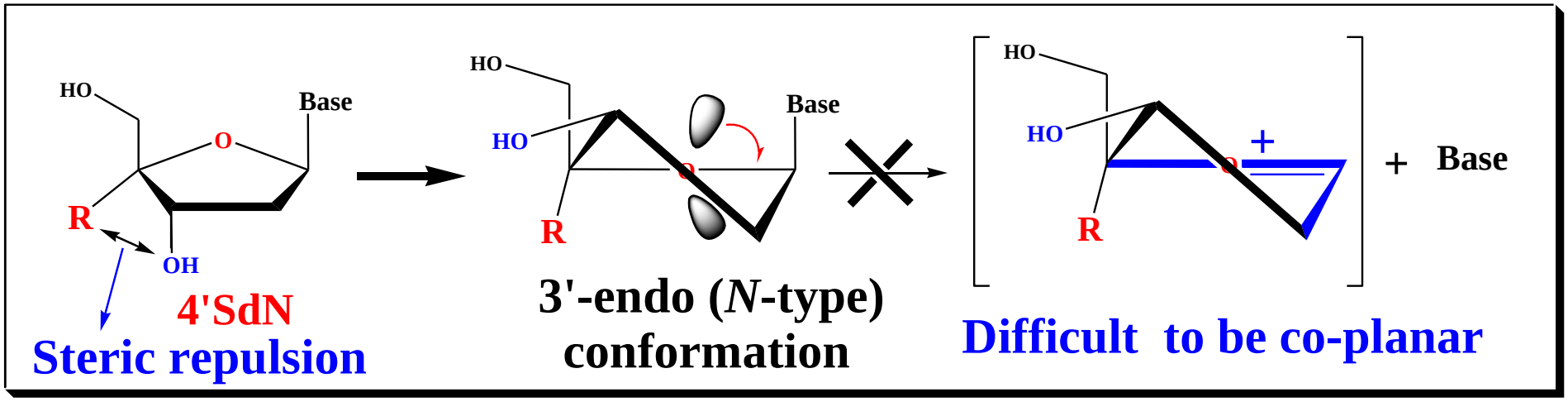
decompose 5FU to
nontoxic compounds

Several cancer patients who took 5FU died by taking Sorivudine due to the toxicity of the accumulated 5FU

Sorivudine was developed by Yamasa Corporation.
However,Yamasa had a very bitter experience with Sorvudine in developing antiviral drugs.This is a big Trauma for Yamasa in developing antiviral drugs.
This is the reason why the clinical development of EFdA was delayed.

4. 4'-Substituents Provide Nucleoside with Stability to both Enzymatic and Acidic Glycolysis and make 4'SdNs Long Acting.





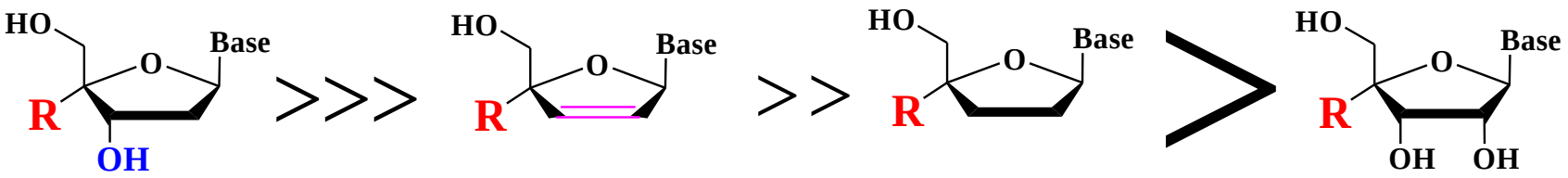
This study was started as the collaboration with Asahi Breweries LTD, because I must ask someone the biological evaluation of my 4'-C-substituted-2'-deoxy nucleosides. Since Asahi Breweries did not have the system of the evaluation of anti-HIV activity, the anti-HIV activity was evaluated by Dr. Masanori Baba of Fukushima Medical University (now he is Professor of Kagoshima University).

However, soon after Asahi Breweries quitted the development of pharmaceutical compounds.

Therefore, next , I asked Yamasa Corporation the biological evaluation of my modified nucleosides.

Then, Yamasa asked Dr. Hiroaki Mitsuya the evaluation of anti-HIV activity of my modified nucleosides. Thus, the collaboration between I, Yamasa, and Dr. Mitsuya has started.

The Anti-HIV Activity of 4'SNs



4'SdN **d4** **dd** ***D-ribo***

No activity

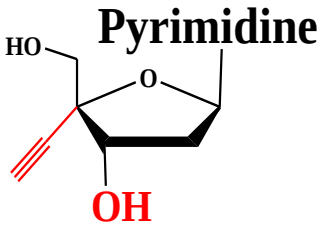
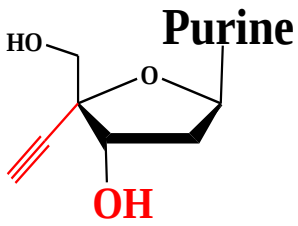
3'-OH plays a very important role for anti-HIV activity, as expected.
makes the 5'-OH easily phosphorylated by kinase.

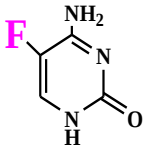
R: -C≡CH ≥ -CN > -CH_3 ~ -CH_2CH_3> -$CH{=}CH_2$
>> -CH_2OH >>> -$CH_2CH_2CH_3$

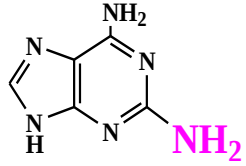
The sterically less demanding group (the smaller A factor) is superior.

Base: Purine >>> Pyrimidine

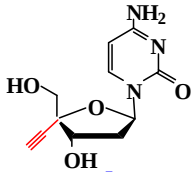
Anti-HIV Activity of 4'EdN

Structure	Base	$EC_{50}(\mu M)$	$CC_{50}(\mu M)$	SI
	T	0.61	>380	>623
	5I-U	0.34	>260	>765
	5Me-C	0.011	0.70	63
	(Ara)-C	0.0048	1.74	363
	C	0.0048	0.92	192
	5F-C*	0.030	>100	>3333
	Ad	0.012	16	1333
	I	0.15	216	1440
	2AA**	0.0003	0.82	2733
	G	0.0014	1.36	971
AZT		0.01	>20	>2000

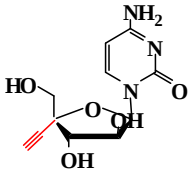
5F-C* 

2AA** 

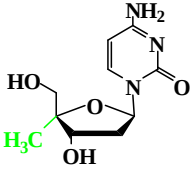
Anti-HIV Activity of Selected 4'SdNs against resistant HIV-mutants



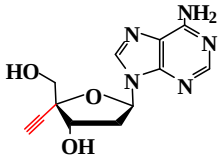
4'EdC



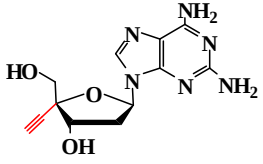
4'EaraC



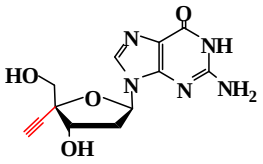
4'MedC



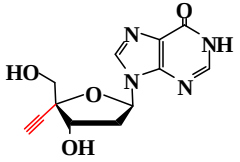
4'EdA



4'Ed2AA



4'EdG



4'EdI

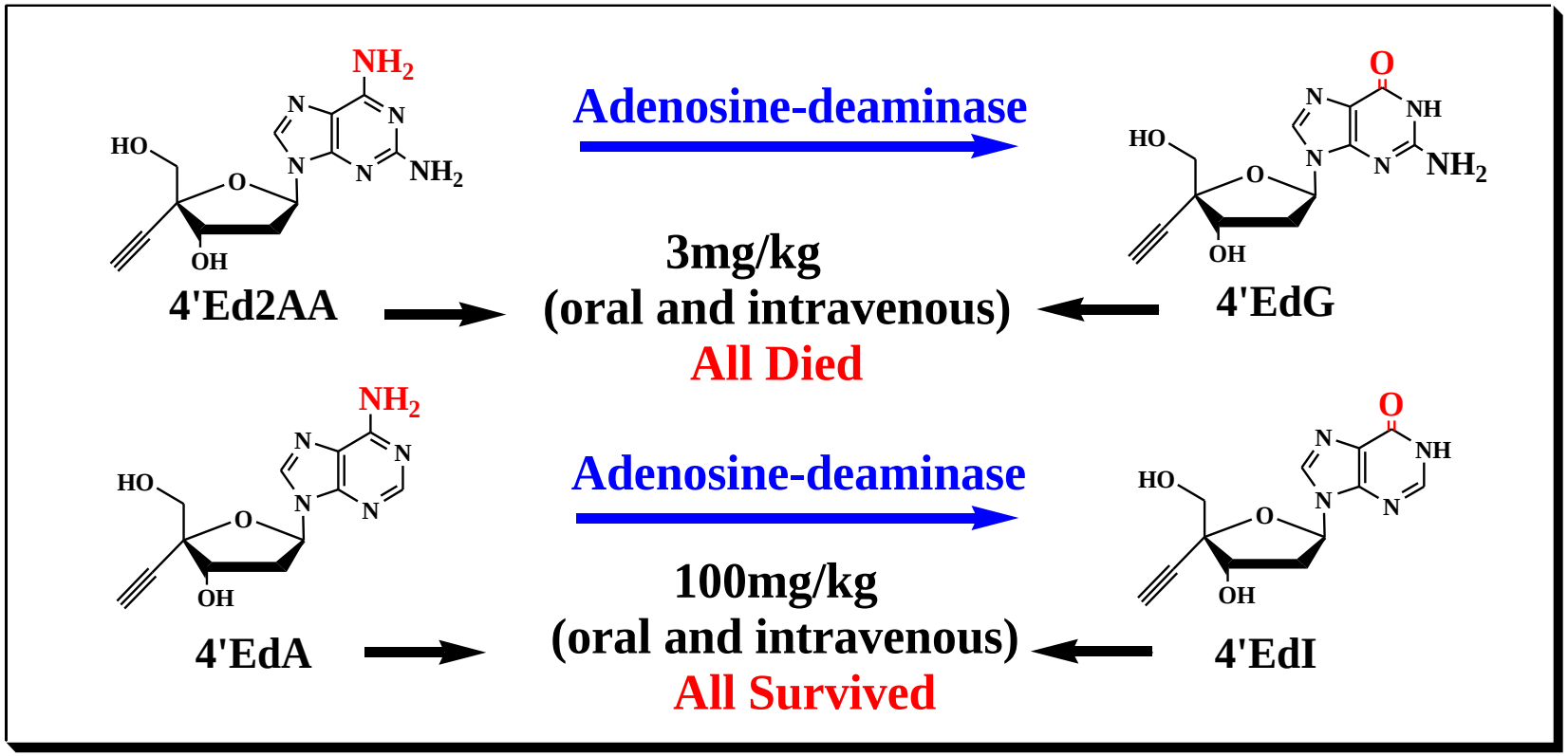
Compound	EC50 (μM) HXB2[a)]	KH65R	L74V	41/215	M184V	M184I	125/SG	MDR	Y181C	CC_{50} (μM)
4'EdC	0.0012	0.0008	0.0013	0.006	0.0024	0.0026	0.015	0.0012	0.0021	>200
4'EaraC	0.0071	0.015	0.026	0.026	0.71	0.0026	0.48	0.17	0.0079	>200
4'MedC	0.0058	0.0071	0.0052	ND	0.2	0.74	ND	0.0033	ND	>200
4'EdA	0.008	0.0033	0.004	0.012	0.047	0.022	0.065	0.0062	0.011	>200
4'Ed2AA	0.0014	0.00035	0.0007	0.0017	0.0059	0.0027	0.0041	0.001	0.0008	>200
4'EdG	0.007	0.001	0.0012	0.019	0.008	0.0041	0.0068	0.0048	0.01	52
4'EdI	0.81	0.25	0.61	1.3	1.6	1.5	2.2	0.51	ND	>200
AZT	0.022	0.02	0.02	0.3	0.01	0.07	1.6	15.7	0.014	>100
3TC	0.71	ND	ND	ND	>100	>100	9.9	1.1	ND	>100
ddC	0.2	3.0	1.5	ND	2.2	ND	1.3	5.5	ND	>100
ddI	3.9	12.7	19.5	3.6	10.1	ND	12.2	25	ND	>100

[a)] Wild-type HIV

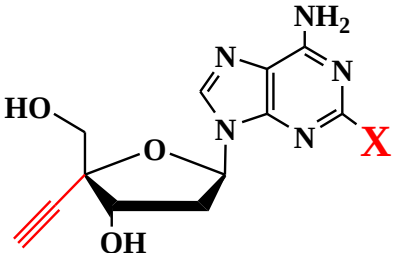
Anti-HIV activity was determined with MAGI assay.

ND=not determined

Mouse Toxicity of 4'-*C*-ethynyl-2'-deoxypurines



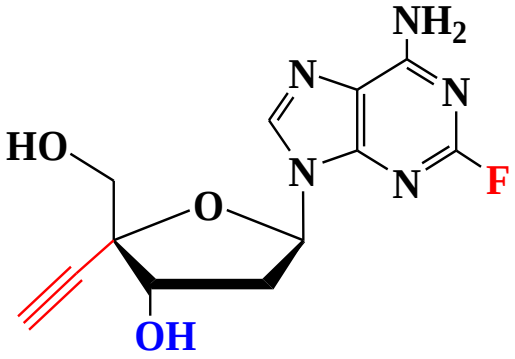
Problem: Adenine derivatives are deaminated by Adenosine Deaminase



2-Haloadenosines are stable to adenosine deaminase!

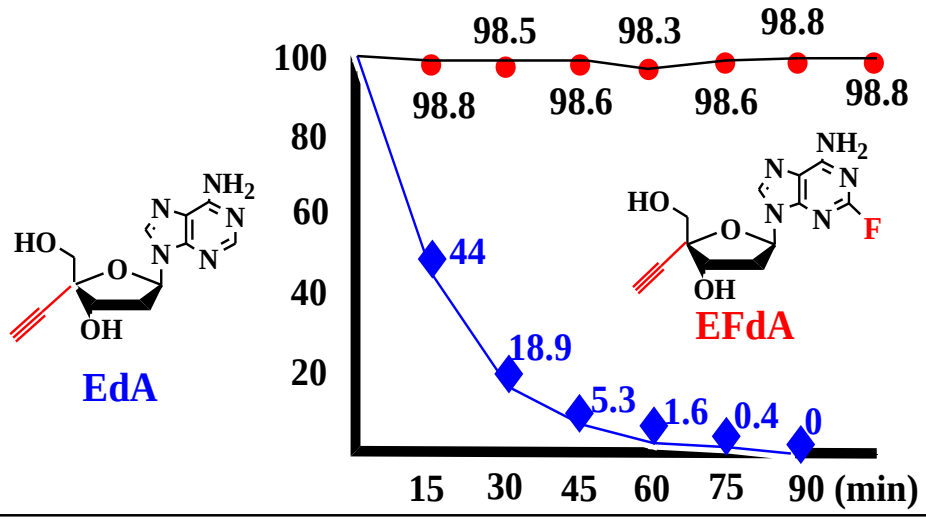
Montogomery, J.A., et al *J. Med. Chem.*, 12, 498, 1969

Stability of EFdA against Adenosine deaminase and under acidic conditions

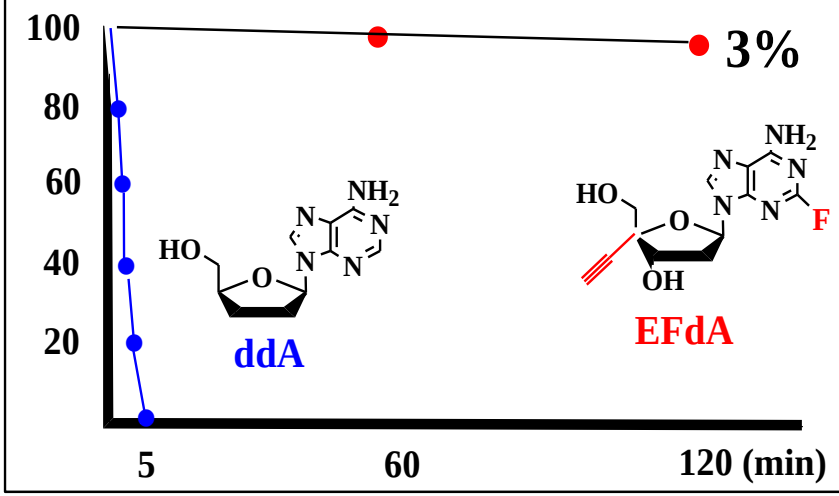


2'-Deoxy-4'-*C*-ethynyl-2-fluoroadenosine(EFdA)

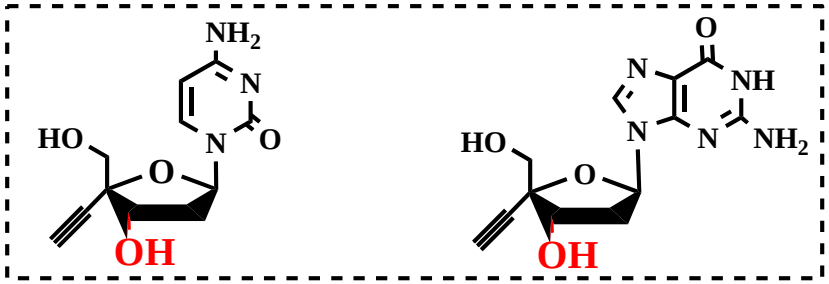
Stability of EFdA to Adenosine Deaminase



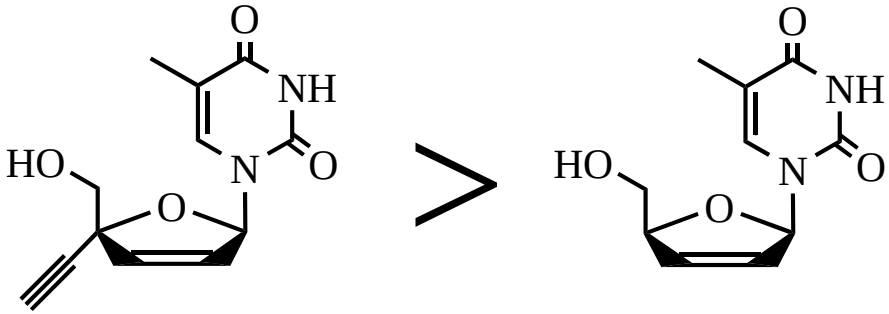
Stability of EFdA at pH 1.06, 36 °C



Appearance of Two Papers Claiming that the 3'-OH is the Cause of the Toxicity of EdNs!!



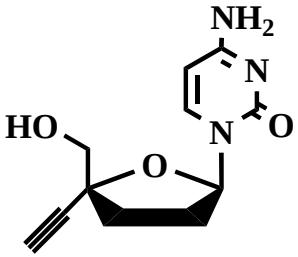
1. **Haraguchi K, Takeda S, Tanaka H, Nitanda T, Baba M, Dutschman G. E, and Cheng Y-C,** ***Bioorg. Med. Chem. Lett.*** **13, 3775-3777 (2003)**



Ed4T **d4T**

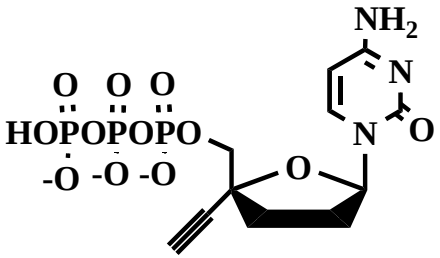
Ed4T is More active than d4T and less toxic than d4T

2. Siddiqui M. A, Hughes S. H, Boyer P. L, Mitsuya H, Van Q. N, Geroge C, Sarafianos S. G, and Marquez V. E. A, *J. Med. Chem.*, 47, 5041-5048(2004).

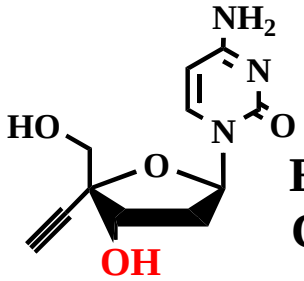


EddC

not active in cellular system



EddCTP

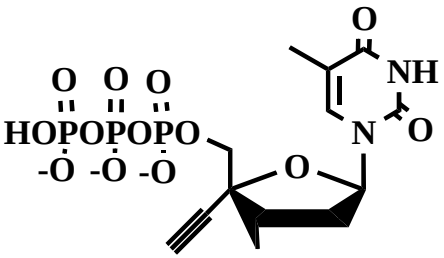


EdC

EC_{50}=0.0048 μ M

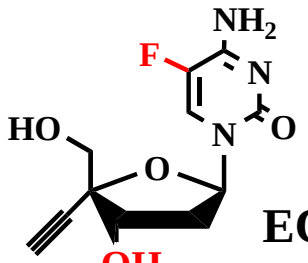
CC_{50}=0.92 μ M

highly active in cellular system but higly toxic



N_3

AZTTP



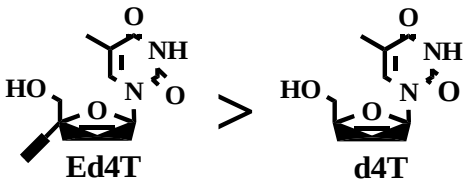
EFdC

EC_{50}=0.038 μ M

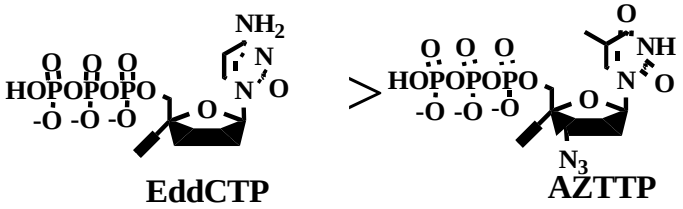
CC_{50}>100 μ M

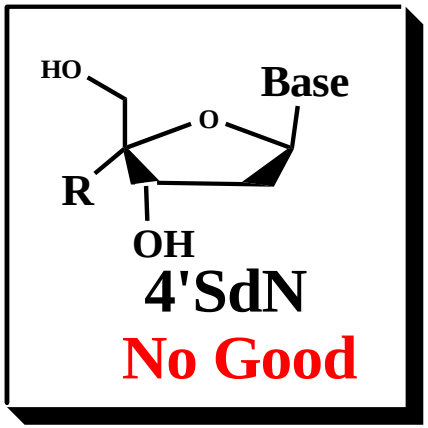
much less toxic than EdC

1. **Haraguchi K, Takeda S, Tanaka H, Nitanda T, Baba M, Dutschman G. E, and Cheng Y-C,** ***Bioorg. Med. Chem. Lett.*** **13, 3775-3777 (2003)**

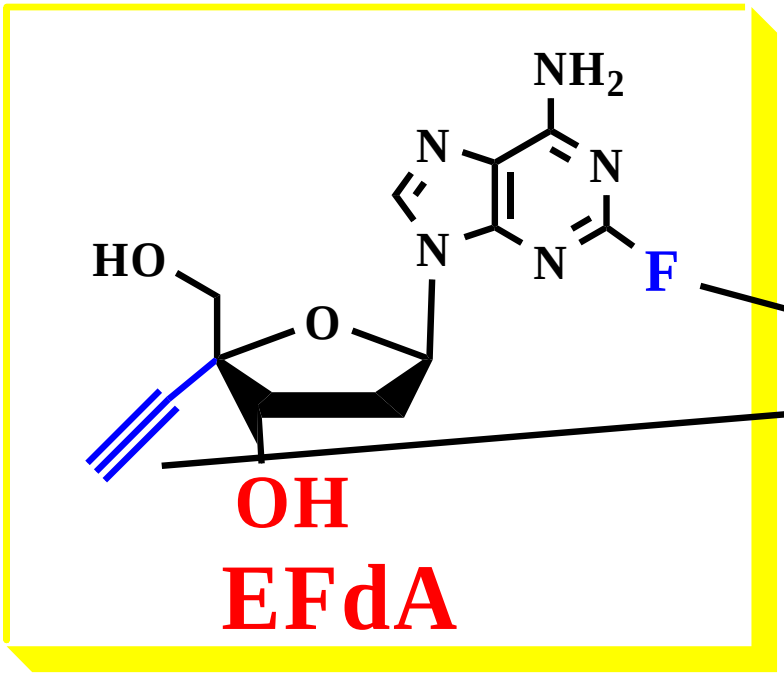


2. **Siddiqui M. A, Hughes S. H, Boyer P. L, Mitsuya H, Van Q. N, Geroge C, Sarafianos S. G, and Marquez V. E. A,** ***J. Med. Chem.*****, 47, 5041-5048(2004).**





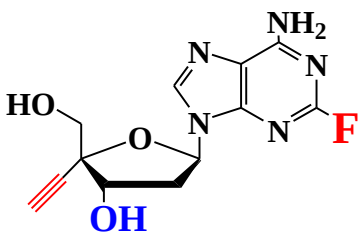
Since these two studies were carried out by the colaboration of the world-leading nucleoside chemists and virologists, many scientists have come to recognize that 4'SdN will be not good for anti-HIV drug due to its toxicity.



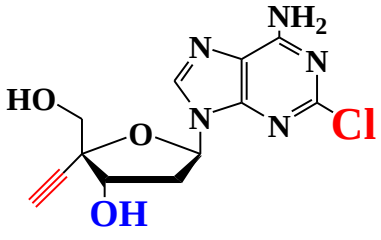
two position modification

However, in my study, 3'-OH is essential to prevent the emergence of resistant HIV mutants, and EFdA is two position-modified nucleoside and therefore expected to be low toxic.

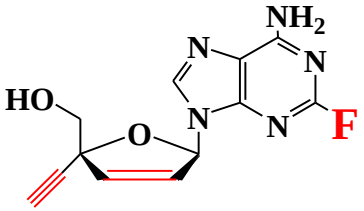
Anti-HIV Activity of 4'-E-2-halo-dA



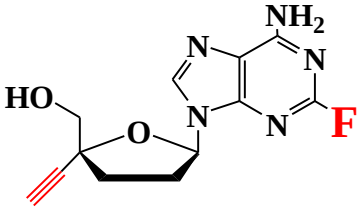
EFdA



ECldA



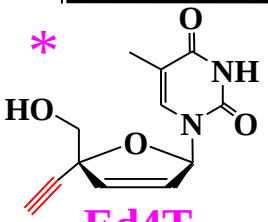
EFd4A



EFddA

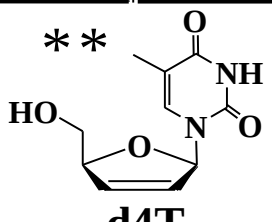
Compound	CE_{50}(MAGI assay, μM)			
	$HIV\text{-}1_{wild}$	$HIV\text{-}1_{MDR}$	$HIV\text{-}1_{M181V}$	SI
EFdA	0.00020	0.00014	0.0031	110,000
ECldA	0.0019	0.0084	0.01	330,000
EFd4A	0.80	0.15	1.8	
EFddA	0.94	8.7	97	
AZT	0.17	74.3	0.13	
3TC	1.0	2.8	>100	
Ed4T*	1.5	1.1	17	>50,000
d4T**	7.6	64	5.6	

*



Ed4T

**



d4T

*K. Haraguchi, et al,
Bioorg. Med. Chem. Lett., 13, 3775 (2003)

K. Maeda, D. V. Desai, M. Aoki, H. Nakata, E. Kodama, H. Mitsuya, *Antiviral Therapy*, 19, 179-189 (2014).

"Delayed emergence of HIV-1 variants resistant to 4'-ethynyl-2-fluoro-2'-deoxyadenosine: comparative sequential passage study with lamivudine, tenofovir, emtricitabine and BMS-986001"

IC_{50} of EFdA agaist $HIV_{11MIX}^{EFdA\text{-}p17}$ = 0.15 μ M

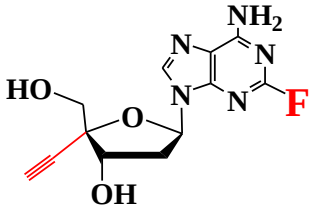
IC_{50} of EFdA agaist $HIV_{11MIX}^{TDF\text{-}p14}$ = 0.10 μ M

These mutants are resistant to 3TC, Ed4T and FTC

(IC_{50} > 10 μ M)

Toxicity and Stability of EFdA and its Triphosphate

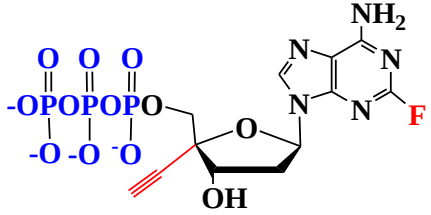
- **Mouse Toxicity of EFdA:**
  No Acute Toxicity up to 100mg/kg
- **EFdA did not show toxicity to Macaques**



EFdA

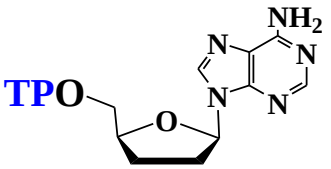
(M.A. Parniak. et al, Antimicrobial Agents and Chemotherapy, 2012, 56, 4707-4712)

- **Inhibition of DNA-polymerases by EFdA-TP**



EFdA-TP

	IC_{50}
DNA polymerase α	>200μM
DNA polymerase β	>200μM
Mitochondria DNA polymerase γ	>200μM



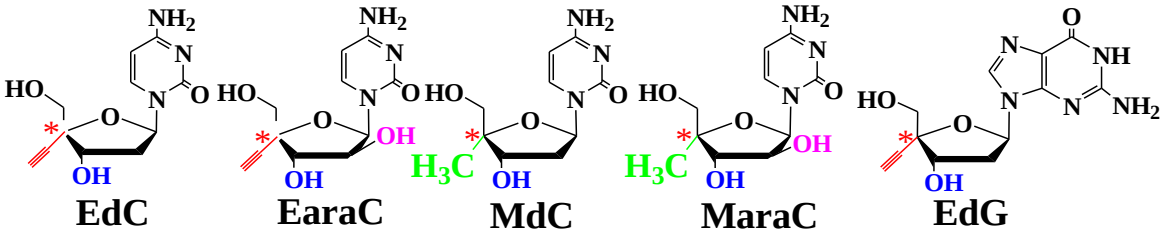
ddA-TP

0.2μM

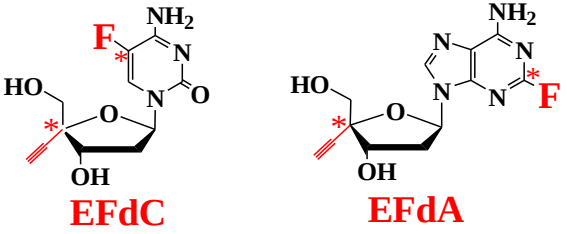
- **Half life ($T_{1/2}$) of EFdA-TP in plasma: > 100 h**

AZT-TP: $T_{1/2}$: ~2.8 h

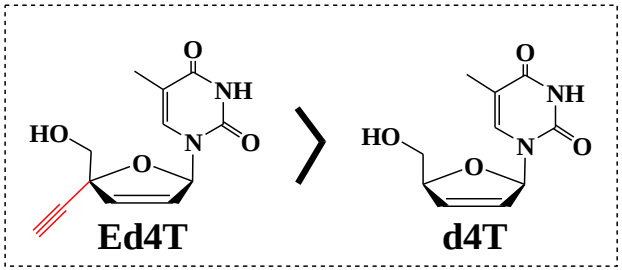
Rationalization of the inhibition of RT and DNA-polymerases by 4'-*C*-substituted-2'-deoxynucleosides

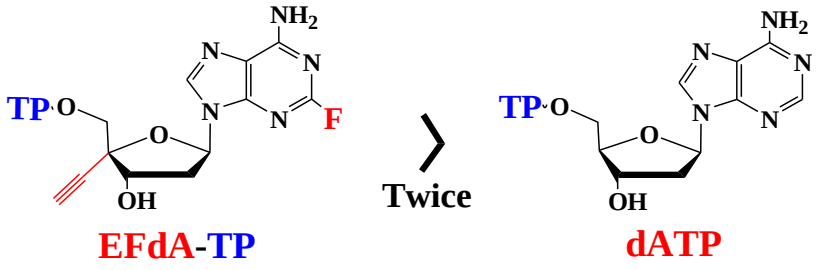


One position Modified Nucleosides, Neopentyl type secondary OH



Two positions Modified Nucleosides, Different Substrate Selectivity between RT and DNA polymerase

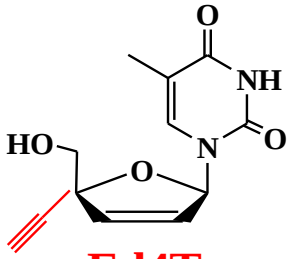




Michailidis, E., et al. *J. Biol. Chem.*, 284, 35681-35691 (2009)

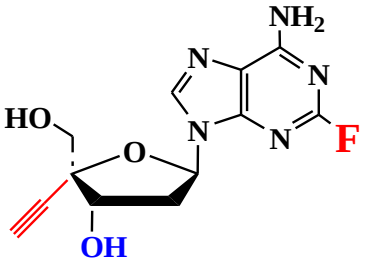
4'-Ethynyl group has special affinity to RT

4'-Ethynyl group fits into a hydrophobic poket defined by RT residues Ala-11S, Try-115, Phe-160, and Met-184 and the aliphatic chain of asp-185



Ed4T

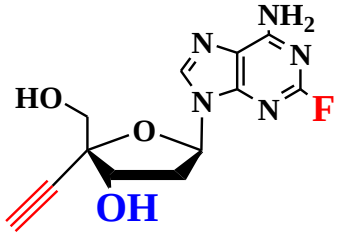
Yang, G., Tanaka, H., Baba. M., et al. *Antimicrobial Agents & Chemotherapy*, 52, 2035-2042 (2008)



EFdA

Michailidis, E., et al. *J. Biol. Chem.*, 18, 35681-35691 (2009)

Translocation-Defective Reverse Transcriptase Inhibitor (TDRTI)



EFdA

The affinity of EFdA by both 4'-ethynyl and 3'-OH groups to RT is so strong that the 3'-EFdA-MP-terminated primer strand on the RT does not translocate from the pre-translocation site (N-site) to the post-translocation site (P-site) to accept the next dNTP. Therefore, the next dNTP can't react with the 3'-EFdA-MP-terminus.

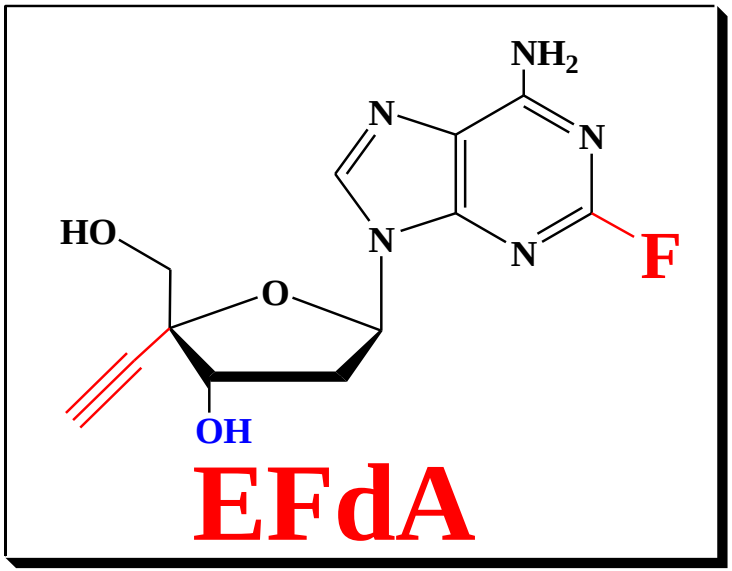
Michailidis, E., et al., J. Biol. Chem., 18, 35681-35691 (2009)

This is an extraordinary big luck!

(No one could predict it! Synthesis has made it)

Chance Favors the Prepared Mind!

The Validity of All my Hypotheses has been verified!



EFdA

(1) Prevents the emergence of HIV-mutants,
(2) 400 times more active than AZT and sevearal ordesrs of magnitude more active than the other clinical dideoxynucleoside drugs,
(3) very low toxic,
(4) very long acting

Acknowledgement

I would like to dedicate all my studies to late Prof. Masanao Matsui. I have learned his Geometrical Organic Chemistry.

Biological evaluation:

Prof. Dr. Hiroaki Mitsuya (NIH, Kumamoto University)
Prof. Dr. Stefan G. Sarafianos (Missouri University)
Prof. Dr. Michael A. Parniak (University of Pittsburgh)
Prof. Dr. Masanori Baba (Kagoshima University)
Prof. Dr. Mineo Saneyoshi (Teikyo Science University)

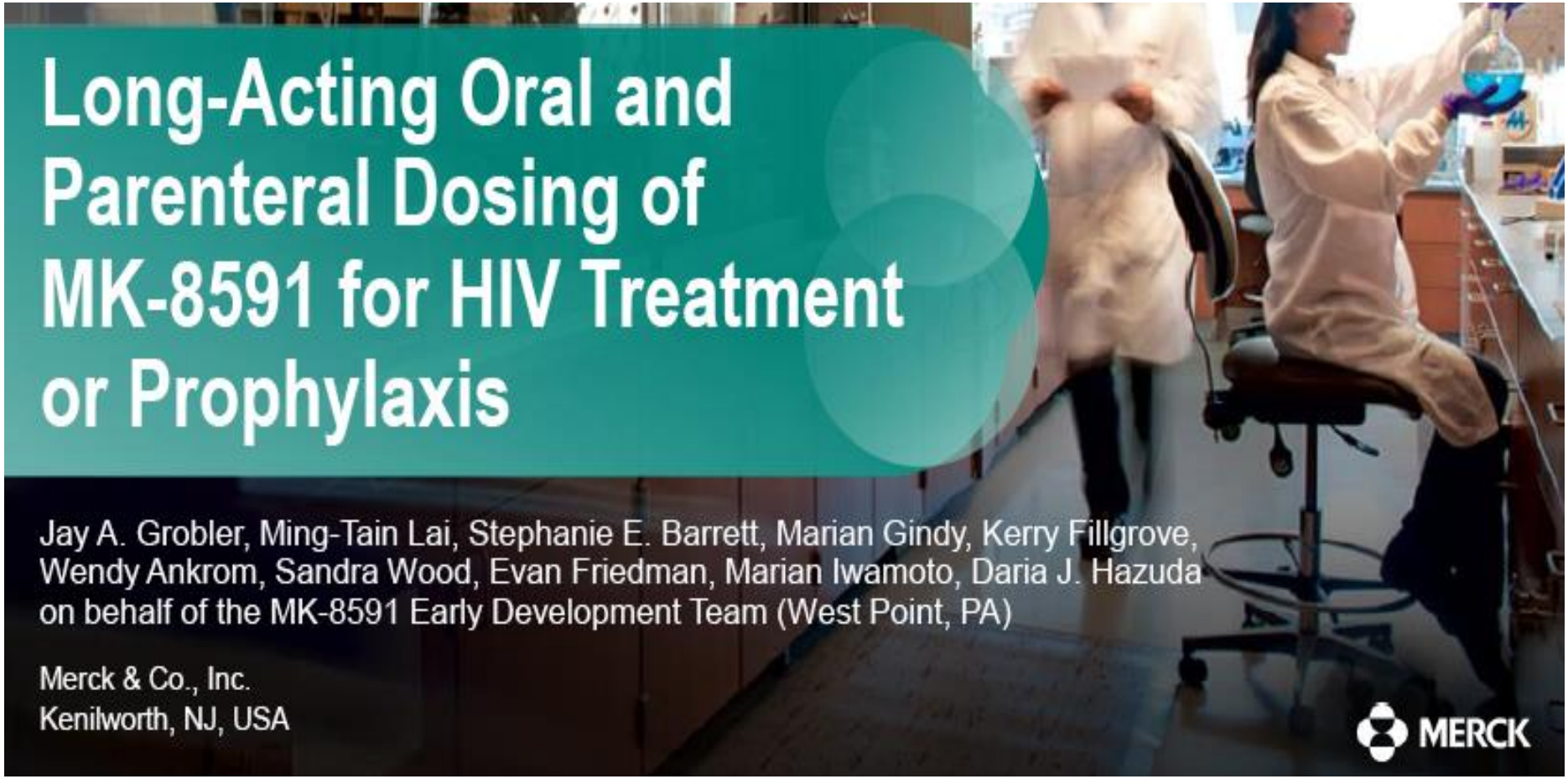
Financial Support:

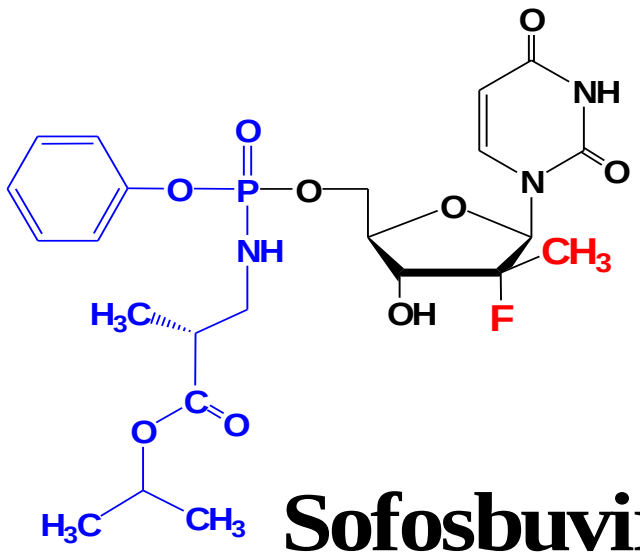
1. **Grant-in-Aid from Ministry of Education, Science, Sport, and Culture of Japan,**
2. **Asahi Beer, Breweries Ltd,**
3. **Yamasa Corporation**
4. **Sankyo**

Improvement of Synthesis:

Prof. Dr. Shigenobu Kuwahara (Tohoku University)

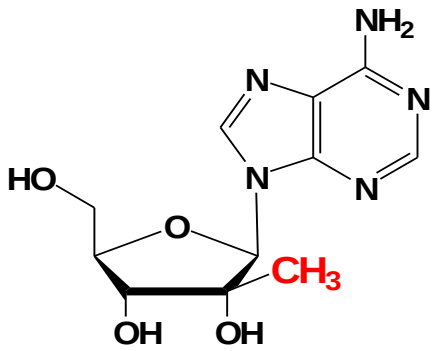
CROI, 2016, Boston, Phase 1 and Phase 1b



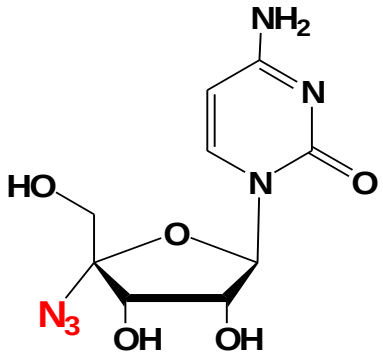


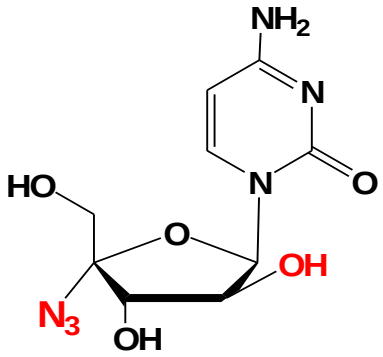
Sofosbuvir

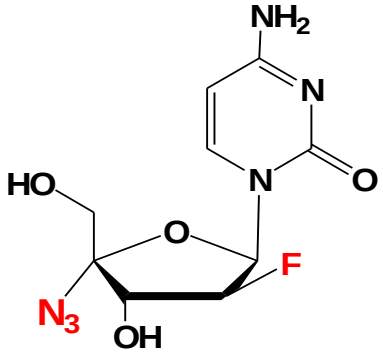
(Kyouichi. A. Watanabe)



Highly Anti-HCV Active, but Highly Toxic

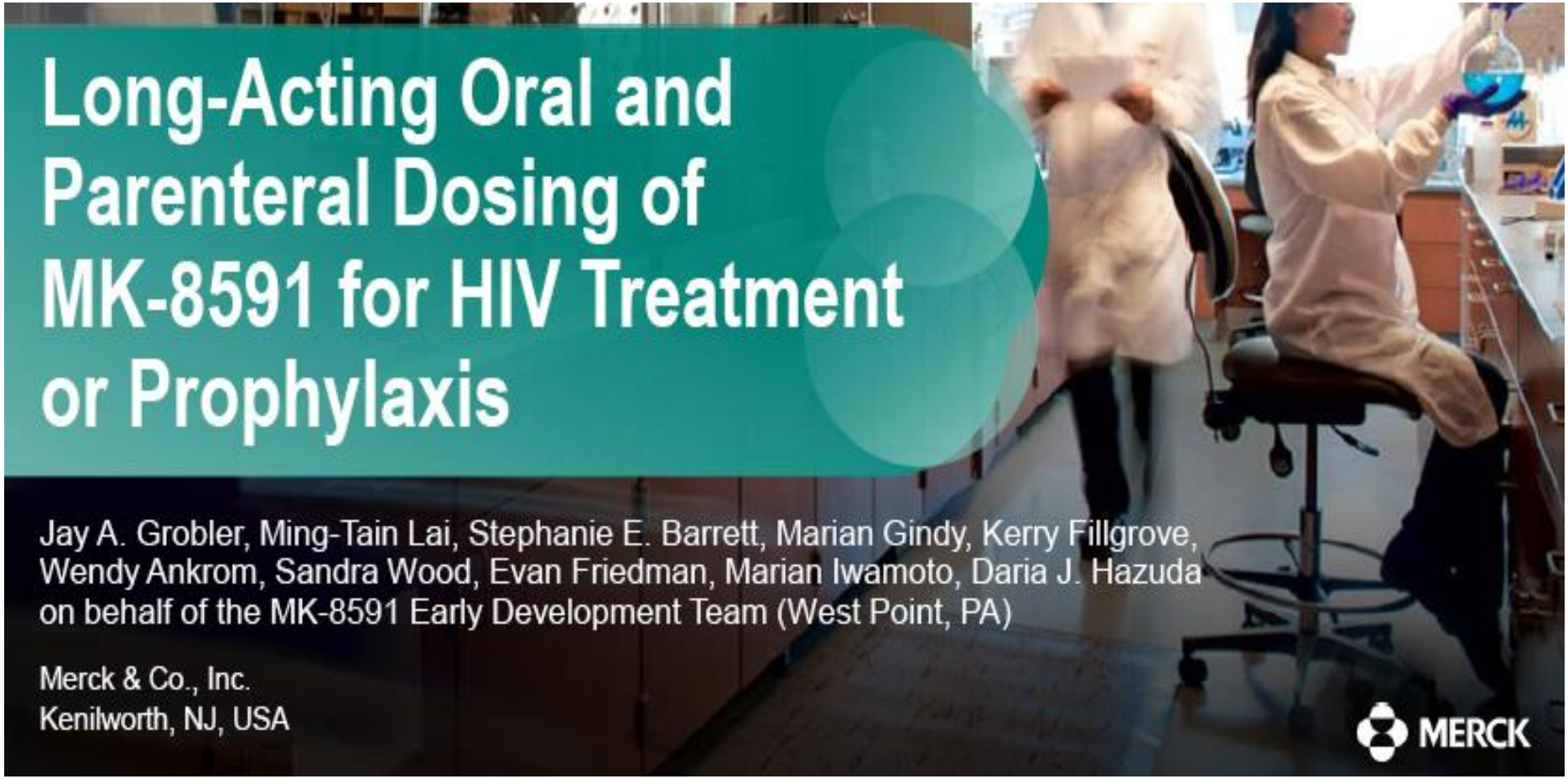




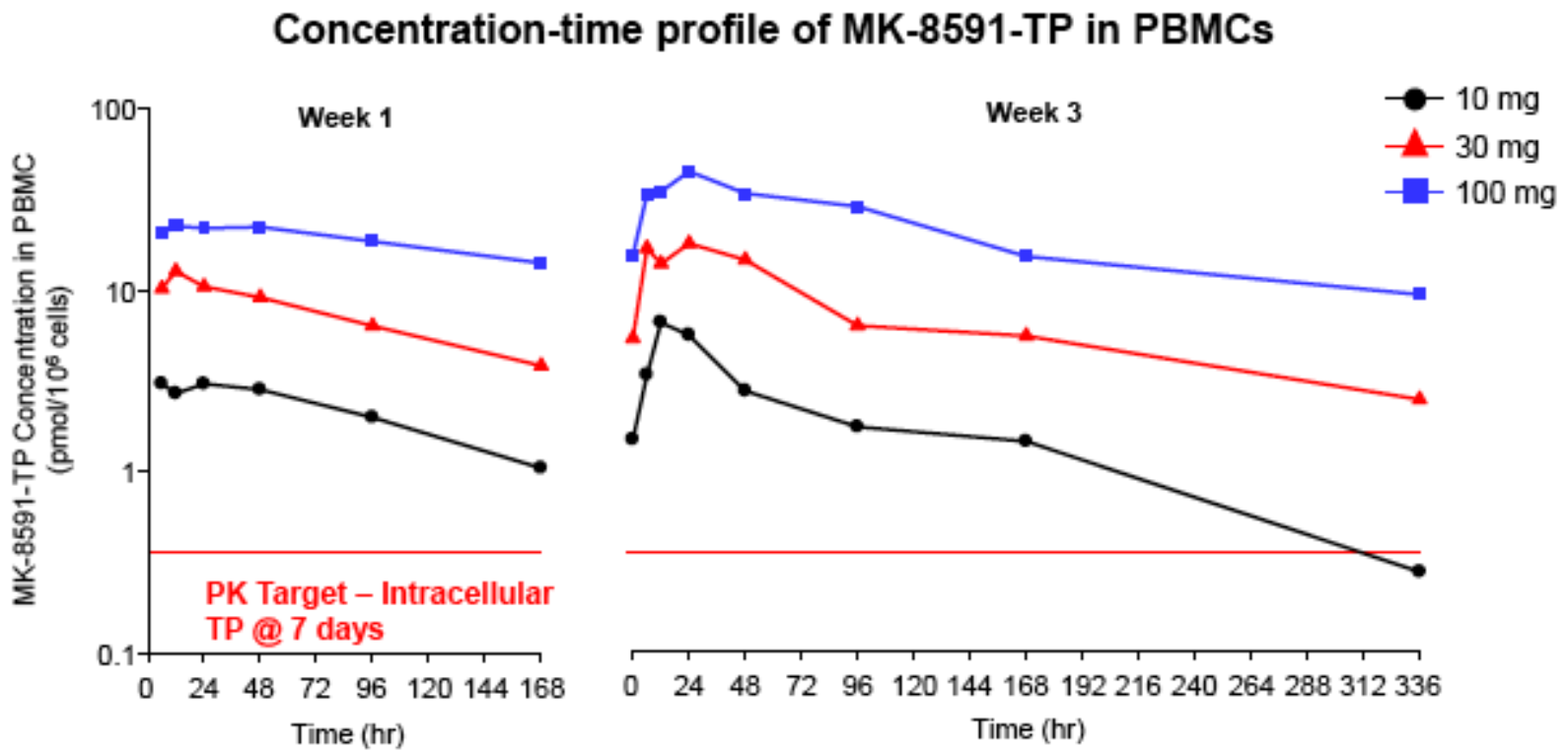


Hoffman-Rosche, Paroalto

CROI, 2016, Boston, Phase 1 and Phase 1b



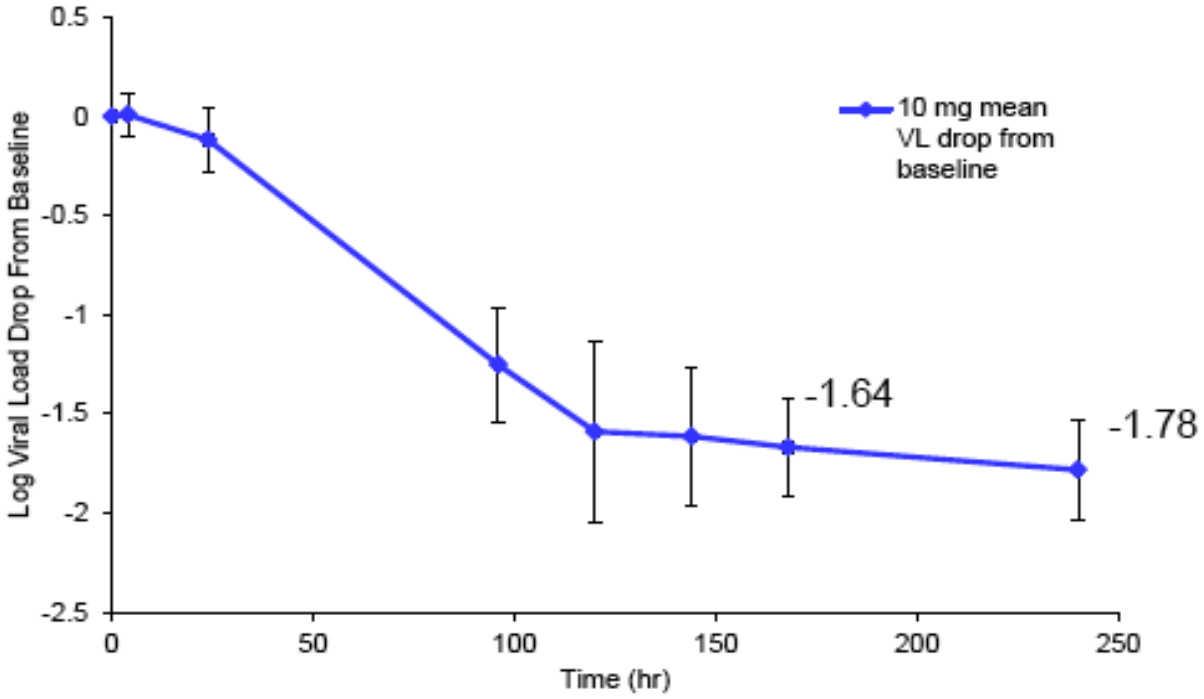
MK-8591 Human Phase 1 PK Confirms QW Potential



- Well tolerated in healthy adult subjects（**up to 400mg**）
- Intracellular MK-8591-TP C_{168hr} target concentration exceeded with 10 mg dose for > 7 days

MK-8591 is Effective in HIV patients when Dosed Once-Weekly: Results from ongoing Ph1b study

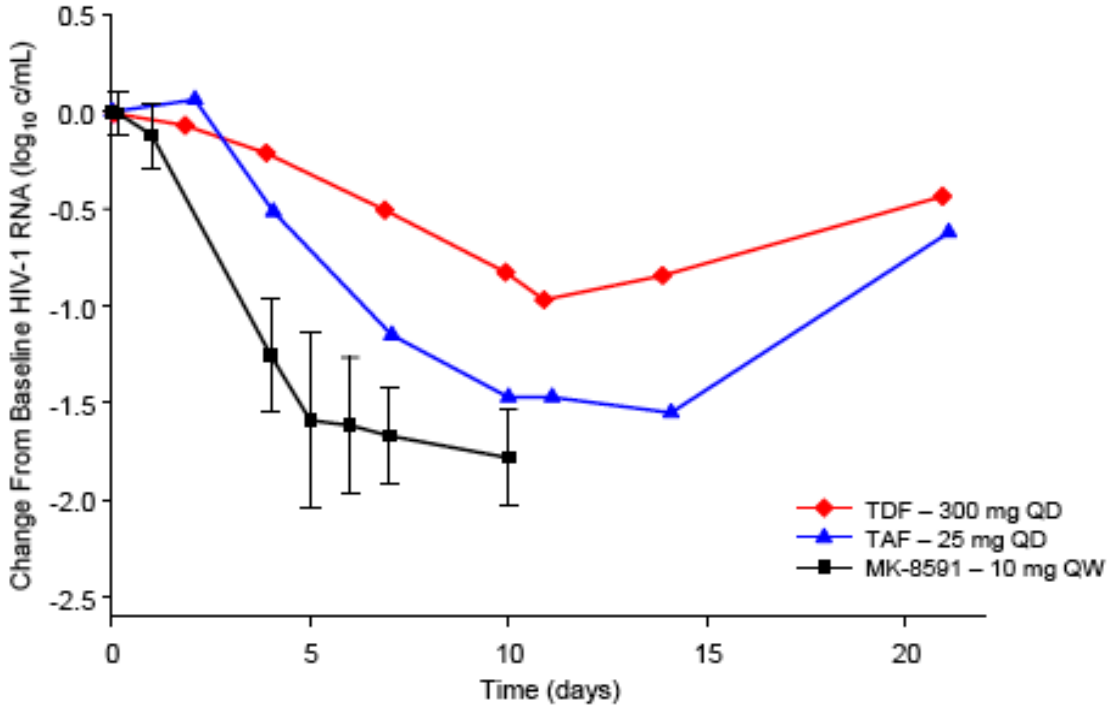
Friedman, et al., Poster 437LB



- A single 10 mg oral dose in HIV-infected patients results in 1.6 log decrease in viral load at day 7-10
- Intracellular MK-8591-TP $t_{1/2}$ = 103 hr
- No evidence of resistance out to Day 10

MERCK

Comparison of Single Once-Weekly Dose of MK-8591 and Once-Daily Dosing of TDF and TAF



Adapted from:
Ruane PJ, DeJesus E, Berger D, et al. *J Acquir Immune Defic Syndr*. 2013;63(4):449-455

Mean and standard deviation MK-8591 data from: PN003, Panel A (10 mg).

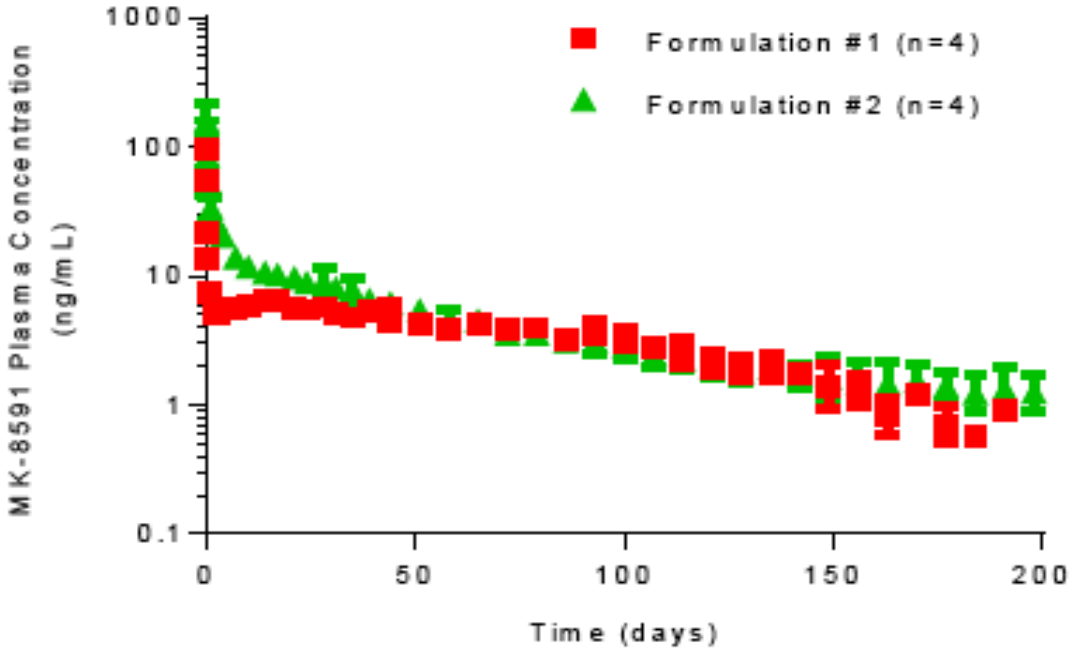
MERCK

AE Summary

Adverse Experience†	Incidence
Acne	1
Abdominal pain	1
Anal warts	1
Apathy	1
Diarrhea	1
Dizziness	1
Headache	6
Joint pain	1
Nausea	1
Sore throat	1

†AE are represented as number of events, in some cases multiple events were reported by one subject.
Note: N = 6 subjects.

MK-8591 Parenteral Formulations Release Effective Drug Levels for >180 days



- Low dose amenable to extended-duration parenteral formulation
- >180-day extended release from solid state formulations after a single injection in rat
- Data suggest the potential to provide coverage for durations up to 1 year

Acknowledgement

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