Weill Cornell Medicine

REACH For the Cure
Research Enterprise to Advance a Cure for HIV

HIV-Persistence During Therapy
Miami - December 14th, 2022

The EZH2 Inhibitor Tazemetostat Increases MHC I Antigen Presentation In Vitro And In Vivo, Enhancing Antiviral Activities Of HIV-specific CTLs

Brad Jones' Laboratory

Andrea Gramatica, PhD - Assistant Professor of Immunology Research

Background

HIV-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) responses partially control viral replication during untreated infection

In rare individuals, CTL responses are implicated in 'Elite control' of HIV

Combination of LRAs and autologous CTLs can reduce cell-associated HIV DNA, but fail to deplete cells with replication-competent virus

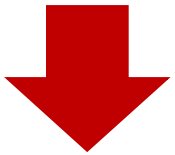
**Despite their known role in controlling HIV replication,
CTLs are not able to clear all infected cells from an individual**

Objectives:

- Enhance CTL-mediated control of viral replication in absence of ARVs
- Enable CTLs to eliminate reservoirs of infected cells

Hypothesis:

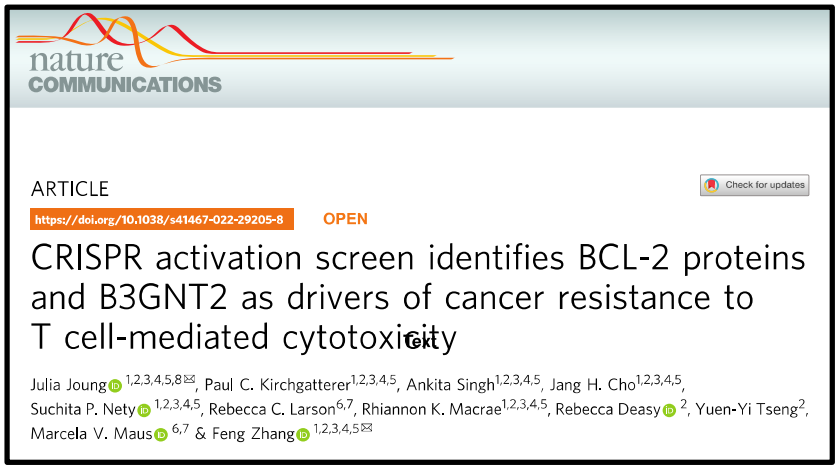
Modulation of host factors in infected cells may increase their susceptibilities to elimination by CTL



Can We Target These Intrinsic Resistance Mechanisms To Enhance Infected Cell Elimination By CTL?

Inhibition Of Anti-apoptotic Proteins In HIV-infected Cells Improves Their Elimination By CTLs

Some similarities with the cancer field:



nature COMMUNICATIONS

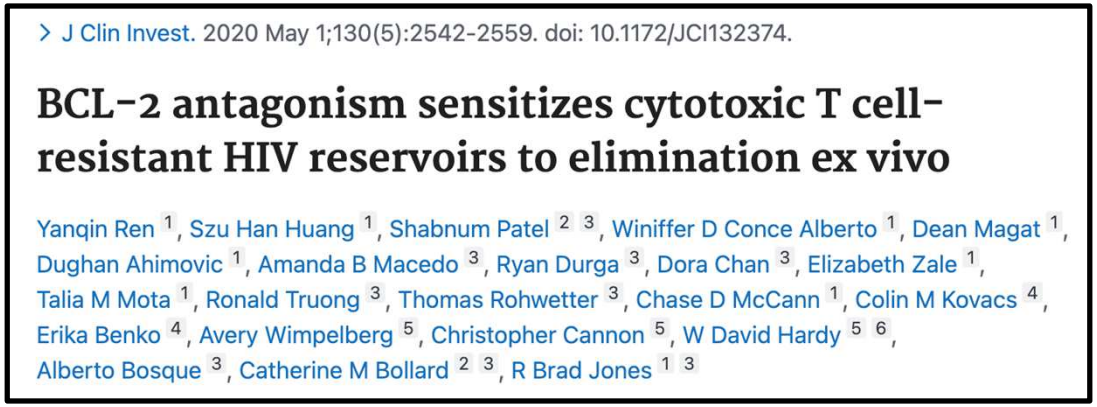
ARTICLE

https://doi.org/10.1038/s41467-022-29205-8 OPEN

CRISPR activation screen identifies BCL-2 proteins and B3GNT2 as drivers of cancer resistance to T cell-mediated cytotoxicity

Julia Joung[1,2,3,4,5,8], Paul C. Kirchgatterer[1,2,3,4,5], Ankita Singh[1,2,3,4,5], Jang H. Cho[1,2,3,4,5], Suchita P. Nety[1,2,3,4,5], Rebecca C. Larson[6,7], Rhiannon K. Macrae[1,2,3,4,5], Rebecca Deasy[2], Yuen-Yi Tseng[2], Marcela V. Maus[6,7] & Feng Zhang[1,2,3,4,5]

Approach: **Inhibition of BCL-2 in HIV infected cells increases their elimination by CTL**



J Clin Invest. 2020 May 1;130(5):2542-2559. doi: 10.1172/JCI132374.

BCL-2 antagonism sensitizes cytotoxic T cell-resistant HIV reservoirs to elimination ex vivo

Yanqin Ren [1], Szu Han Huang [1], Shabnum Patel [2 3], Winiffer D Conce Alberto [1], Dean Magat [1], Dughan Ahimovic [1], Amanda B Macedo [3], Ryan Durga [3], Dora Chan [3], Elizabeth Zale [1], Talia M Mota [1], Ronald Truong [3], Thomas Rohwetter [3], Chase D McCann [1], Colin M Kovacs [4], Erika Benko [4], Avery Wimpelberg [5], Christopher Cannon [5], W David Hardy [5 6], Alberto Bosque [3], Catherine M Bollard [2 3], R Brad Jones [1 3]

Do HIV-infected Cells Have A CTL-resistance Signature?

Research work led by **Louise Leyre**

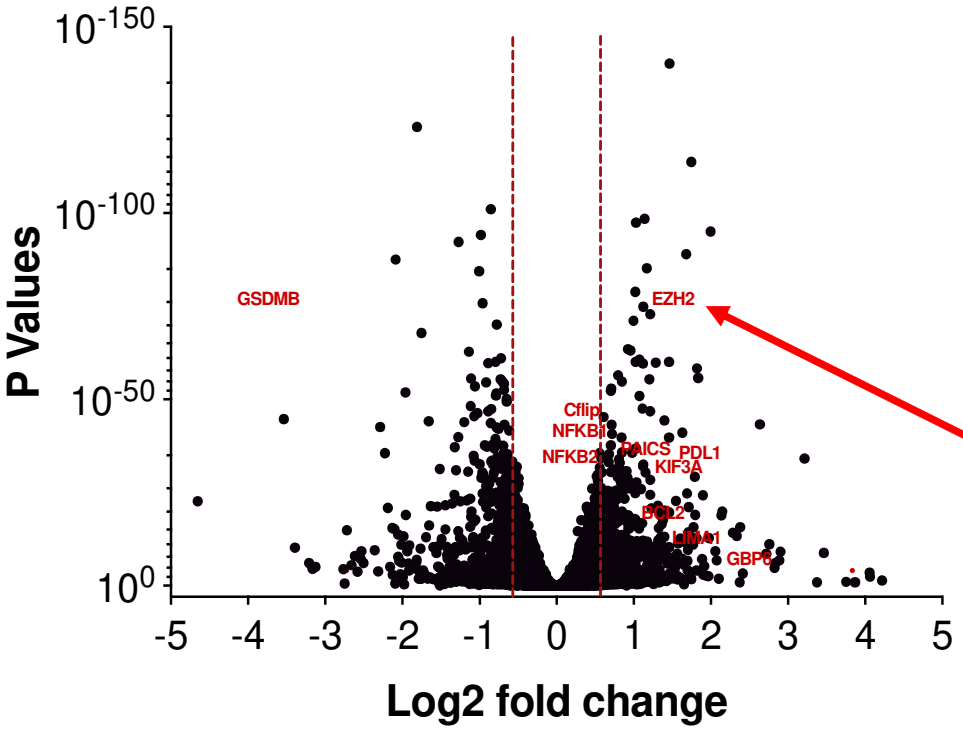
Poster: PP 4.15 #**00181**

Infected **CD4⁺ T cells** + Autologous HIV-specific **CTLs**

Infected survivors

RNA sequencing

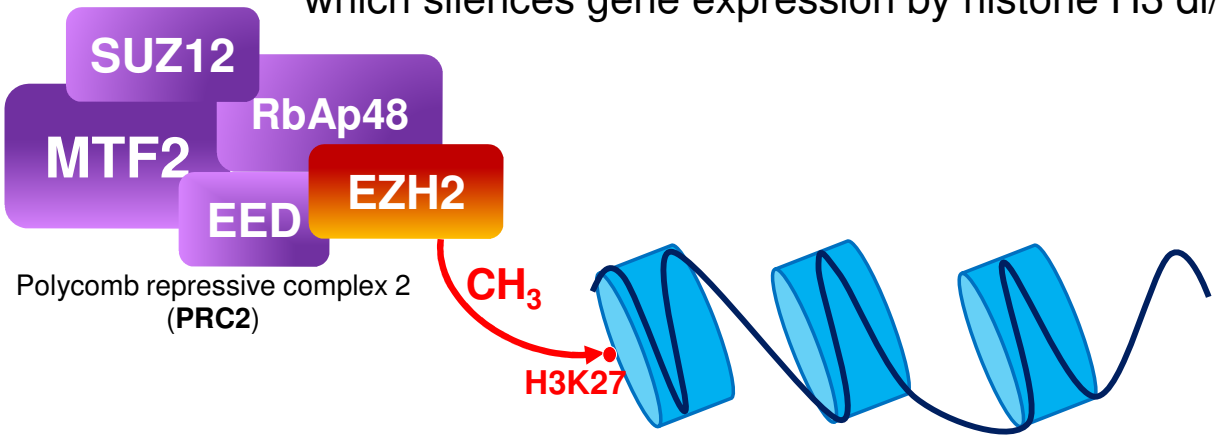
Differentially expressed genes



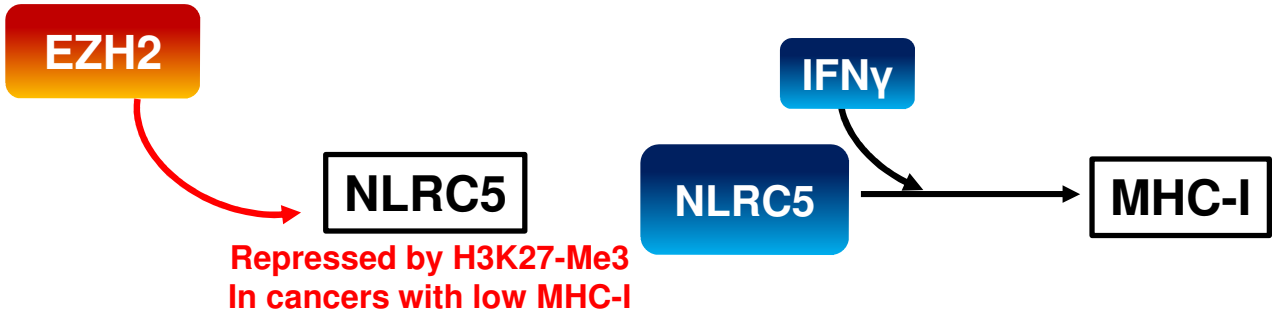
Among the strongest hits of our RNA-seq analysis:
'Enhancer of Zeste Homolog 2' (EZH2)

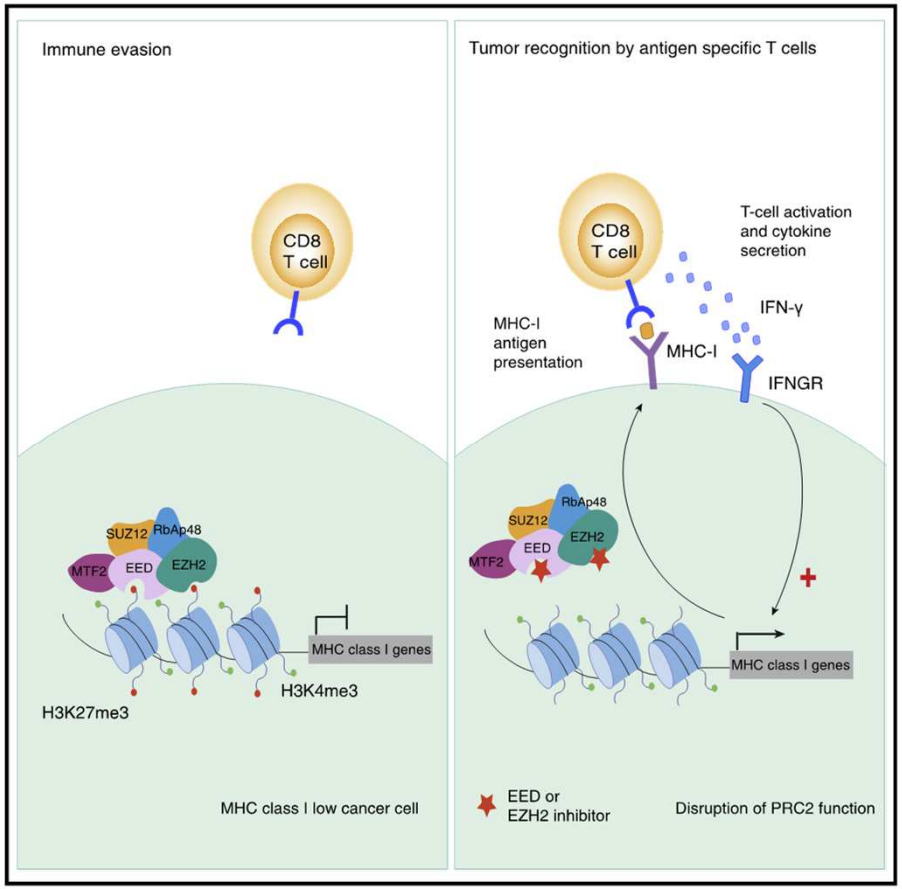
EZH2: Background Information

EZH2 is a **histone-methyltransferase**, part of the polycomb repressive complex 2, which silences gene expression by histone H3 di/tri-methylation at lysine 27 (**H3K27**)



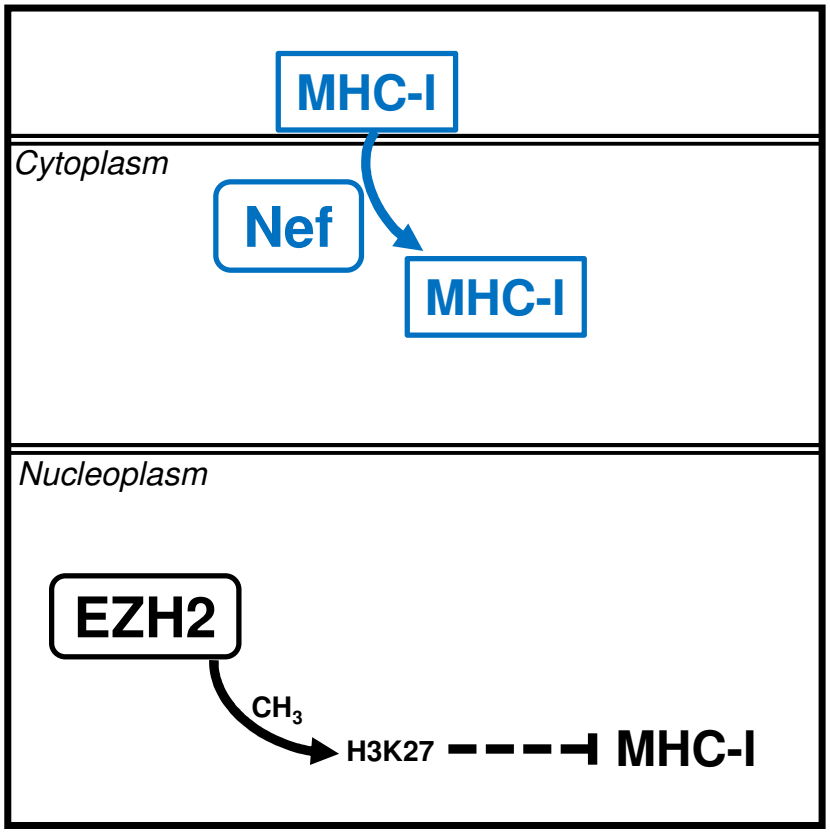
EZH2 overexpression in cancer => cancer cell survival
Mutation or over-expression of EZH2 has been linked to many forms of cancer





From: Burr ML, et al. Cancer Cell. 2019

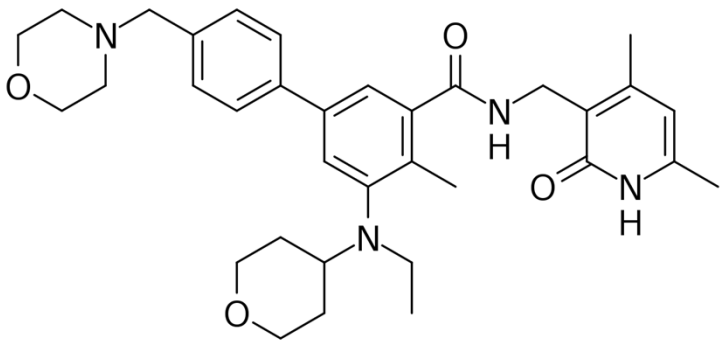
EZH2 and HIV-Nef: A “Collaboration” To Evade CTL-recognition?



If EZH2 overexpression is part of the "resistance signature" that protects a subset of infected cells from CTL-mediated killing,

Can we use EZH2 inhibitors to reverse this resistance effect?

EZH2 Inhibitor Selected: Tazemetostat (EPZ-6438), an FDA-approved compound



- Orally available
- Typically used to treat **B-cell lymphoma**, epithelioid sarcoma, mesothelioma
- Typical dosage:
  - Humans: 800mg twice daily
  - Mice: 500-600mg/kg twice daily

Completed clinical trials testing Tazemetostat for treatment of cancer (partial list)

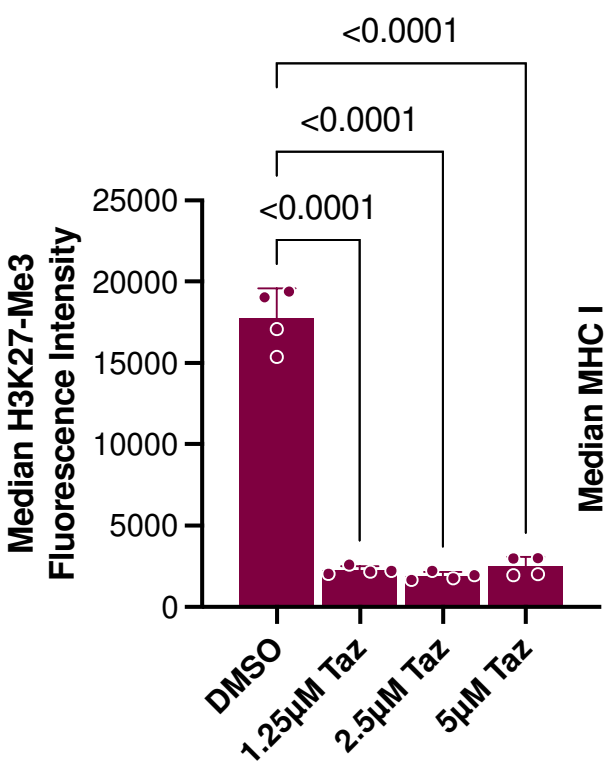
Selected (0) | Download | Manage Columns

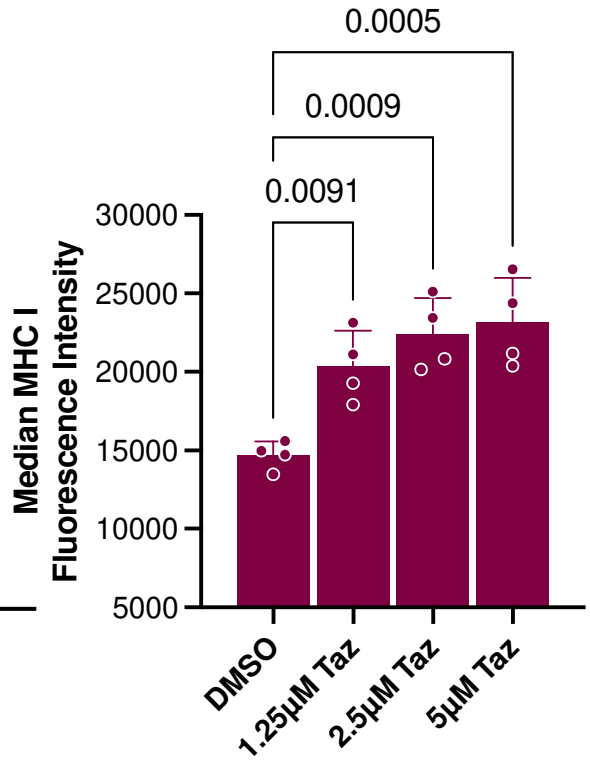
	Study Title	NCT Number	Status	Conditions	Interventions	Sponsor	Study Type
1	Study of the EZH2 Inhibitor **Tazemetostat** in Malignant Mesothelioma	NCT02860286	Completed	• Mesothelioma • BAP1 Loss of Function	• Drug: **Tazemetostat**	Epizyme, Inc.	Interventional
2	A Study of **Tazemetostat** in Participants With Relapsed or Refractory B-cell Non-Hodgkin's Lymphoma	NCT03009344	Completed	• Relapsed or Refractory B-cell Non-Hodgkin's Lymphoma	• Drug: **Tazemetostat**	Eisai Co., Ltd.	Interventional
3	Open-Label, Multi-Center, Two-Part, Ph1 Study to Characterize the PKs of an Intravenous Micro-Dose of [14C]-**Tazemetostat** (EPZ 6438) and the ADME of an Oral [14C]-Labeled Dose of **Tazemetostat** in Subjects With B-Cell Lymphomas or Adv Solid Tumors	NCT03010982	Completed	• Diffuse Large B Cell Lymphoma • Primary Mediastinal Lymphoma • Mantle-Cell Lymphoma • 3 more	• Drug: **Tazemetostat** and [14C] **Tazemetostat**	Epizyme, Inc.	Interventional
4	Open-Label, Multicenter, Phase 1/2 Study of **Tazemetostat** (EZH2 Histone Methyl Transferase [HMT] Inhibitor) as a Single Agent in Subjects With Adv. Solid Tumors or With B-cell Lymphomas and **Tazemetostat** in Combination With Prednisolone in Subjects With DLBCL	NCT01897571	Completed	• B-cell Lymphomas (Phase 1) • Advanced Solid Tumors (Phase 1) • Diffuse Large B-cell Lymphoma (Phase 2) • 3 more	• Drug: **Tazemetostat**	Epizyme, Inc.	Interventional
5	A Phase 1 Study of the EZH2 Inhibitor **Tazemetostat** in Pediatric Subjects With Relapsed or Refractory INI1-Negative Tumors or Synovial Sarcoma	NCT02601937	Completed	• Rhabdoid Tumors • INI1-negative Tumors • Synovial Sarcoma • 1 more	• Drug: **Tazemetostat**	Epizyme, Inc.	Interventional
6	Study of **Tazemetostat** in Participants With Relapsed or Refractory B-cell Non-Hodgkin's Lymphoma With EZH2 Gene Mutation	NCT03456726	Completed	• Relapsed or Refractory B-cell Non-Hodgkin's Lymphoma	• Drug: **Tazemetostat**	Eisai Co., Ltd.	Interventional
7	Open-Label, Multicenter, Two-Part, Phase 1 Study to Characterize Effects of a Moderate CYP3A Inhibitor on PK of **Tazemetostat**, Effects of **Tazemetostat** on PK of CYP2C8 and CYP2C19 Substrates, and Effect of Increased Gastric pH on PK of **Tazemetostat** in B-cell Lymphoma or Advanced Solid Tumor Patients	NCT03028103	Completed	• Diffuse Large B Cell Lymphoma • Primary Mediastinal Lymphoma • Mantle Cell Lymphoma • 2 more	• Drug: **Tazemetostat** • Drug: Fluconazole • Drug: Omeprazole	Epizyme, Inc.	Interventional

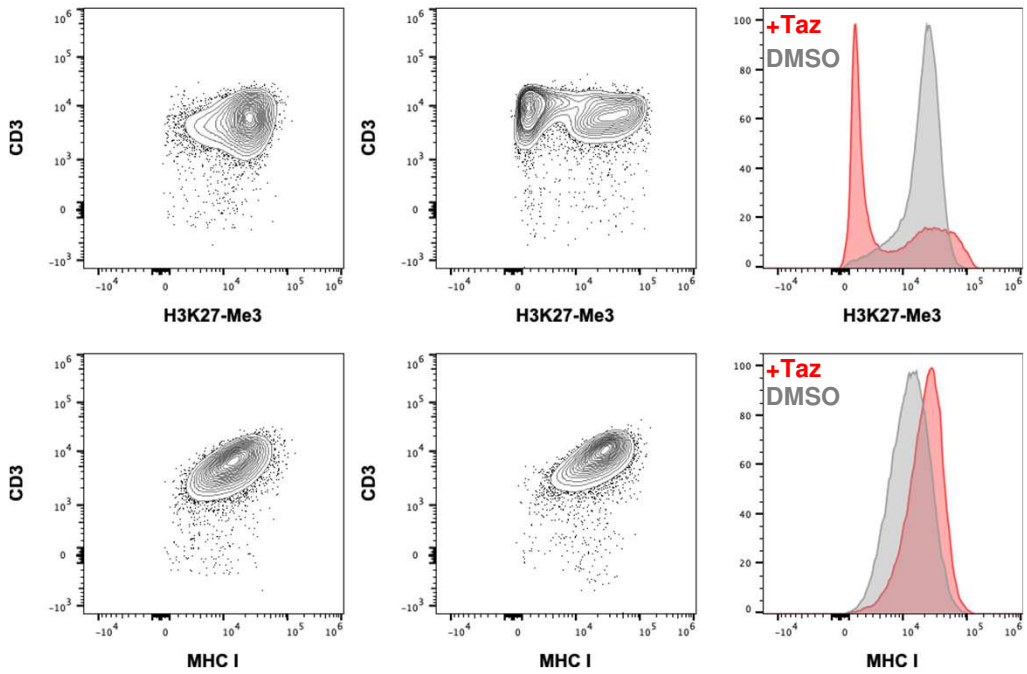
Is Tazemetostat Biologically Active In $CD4^+$ T Cells?

Tazemetostat Decreases H3K27 Tri-Methylation And Increases Surface MHC-I Expression on CD4+ T Cells

CD4+ T cells isolated from 4 independent donors were treated for 5 days with increasing concentrations of Tazemetostat

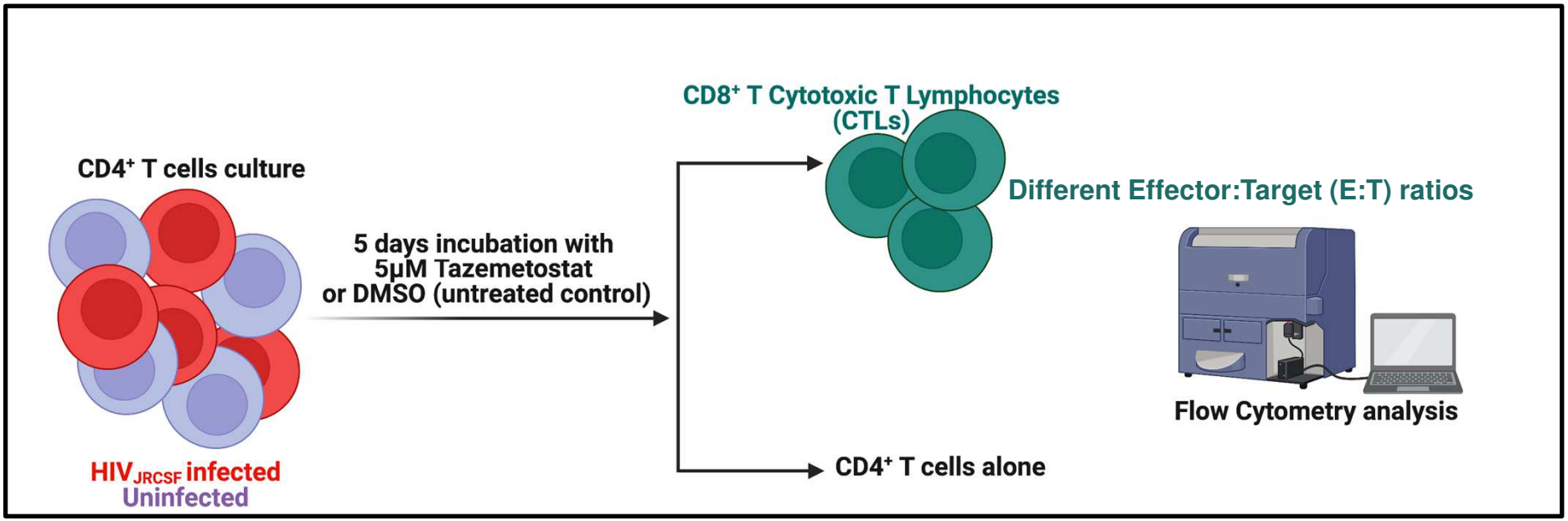


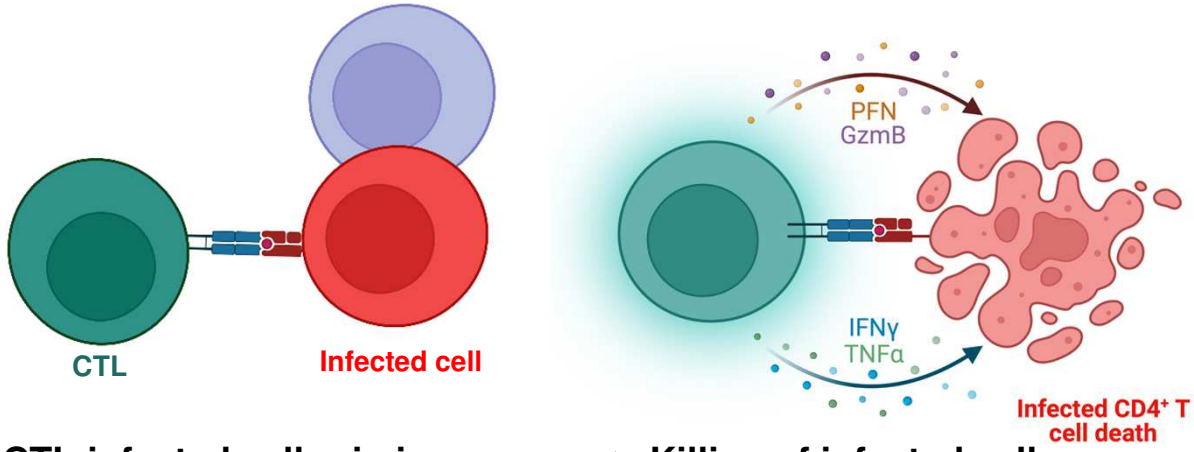




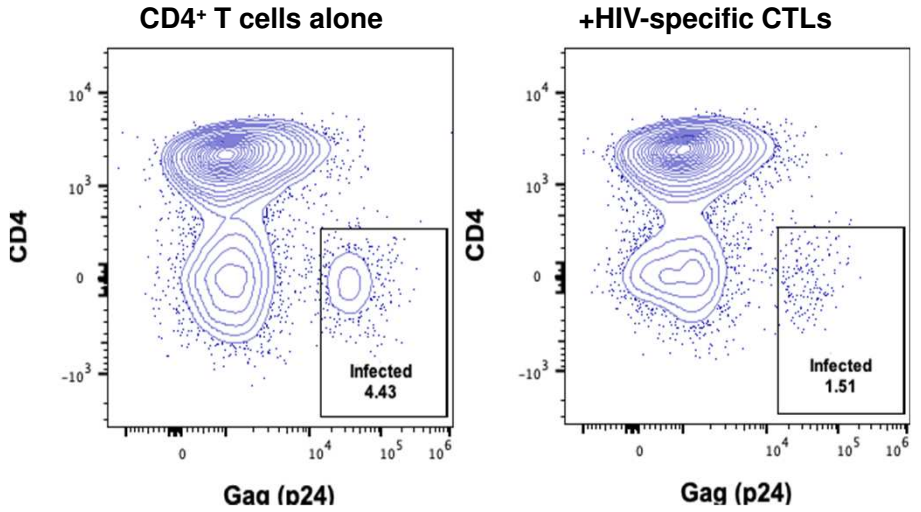
Samples from n=4 independent donors. Statistical significance determined by 1-way ANOVA. Error bars represent SD.

In Vitro Infected-cell CTL Killing Assay

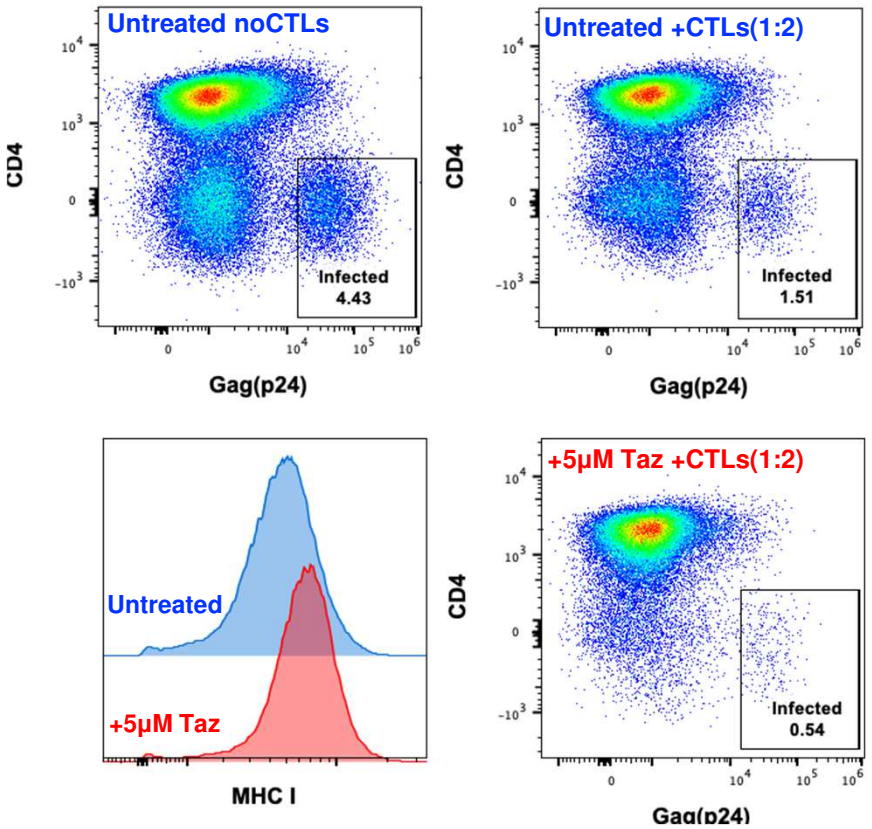


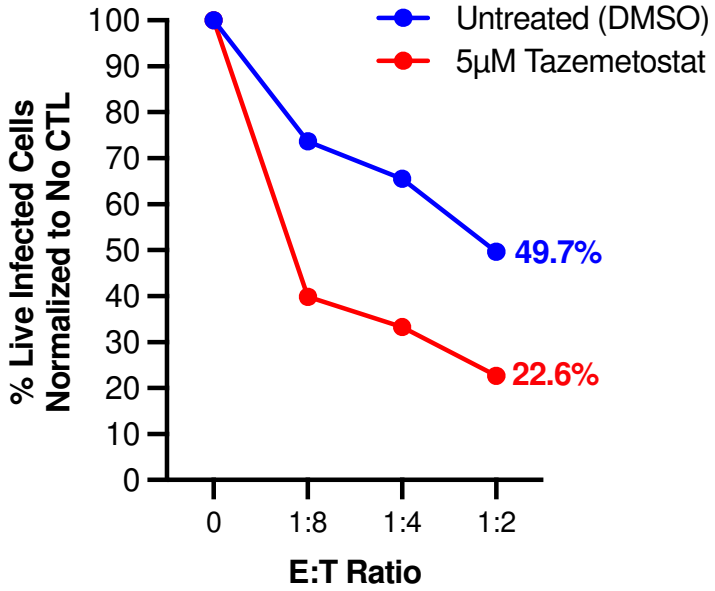


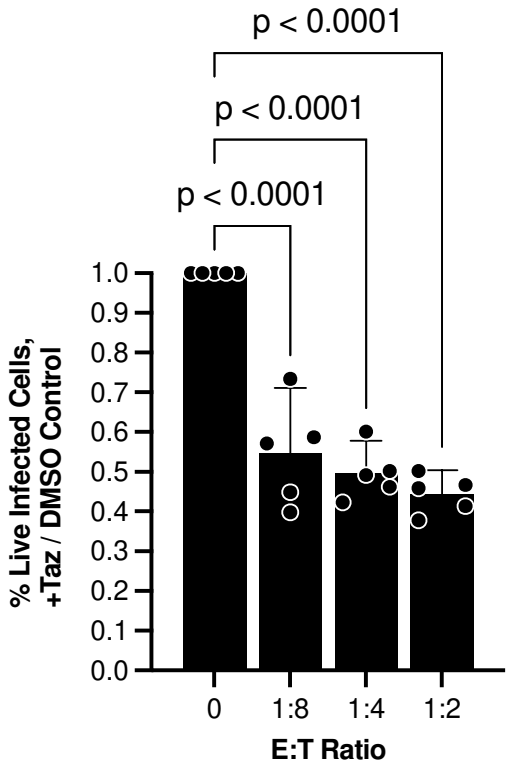
CTL-infected cell priming ⟶ Killing of infected cell



Tazemetostat Enhances Killing of Infected CD4+ T Cells In Vitro





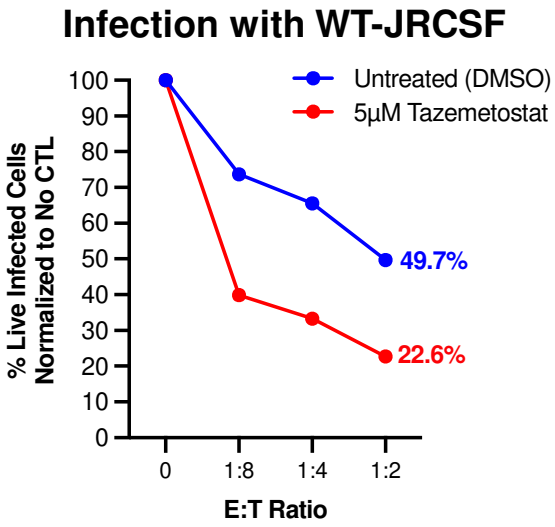


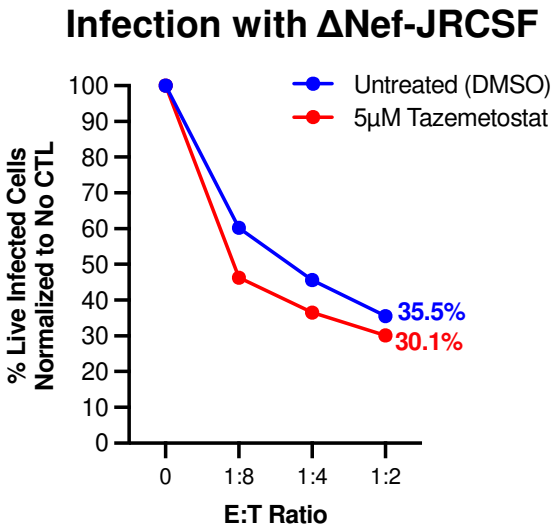
n=5 independent experiments. Statistical significance determined by 1-way ANOVA. Error bars represent 95% C.I.

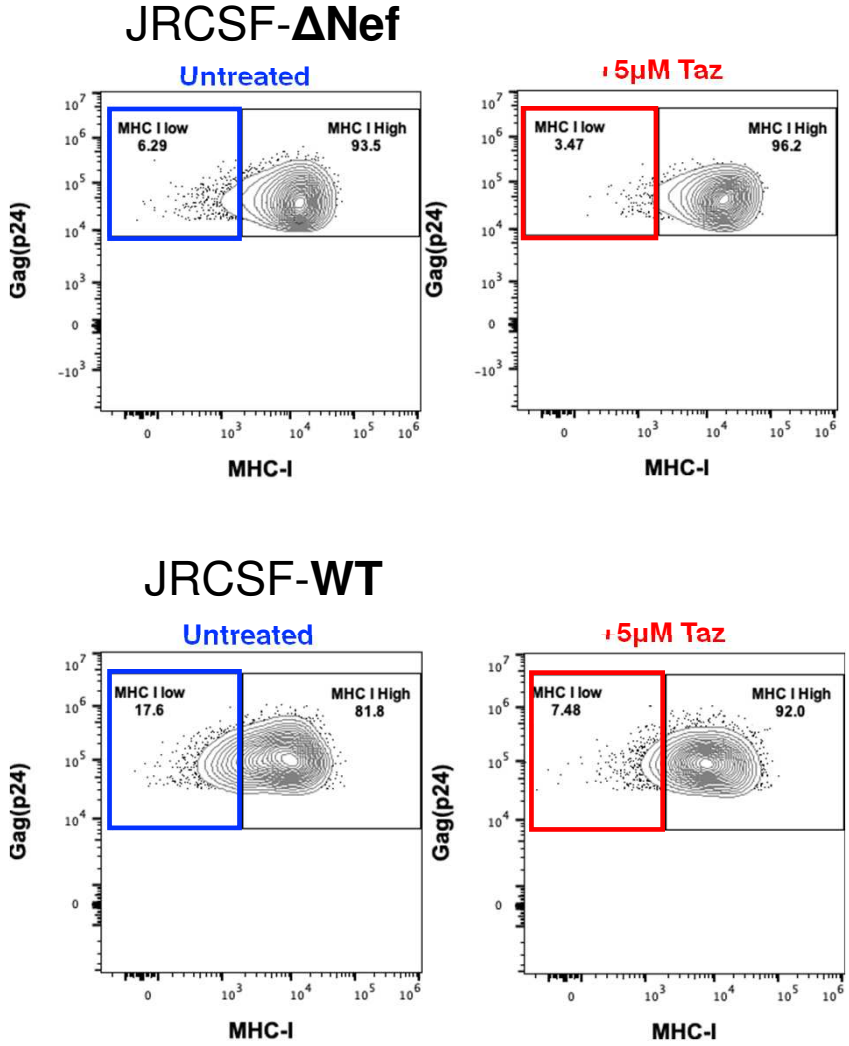
Tazemetostat Enhances CTL-mediated Killing Of Infected Cells Through Increased MHC-I Surface Expression

Does Tazemetostat Have The Same Sensitization Effect On Cells Infected With A Nef-deficient Virus?

Inhibition Of EZH2 In Cells Infected With A Nef-deficient (ΔNef) Virus Does Not Result In The Same Sensitization Effect

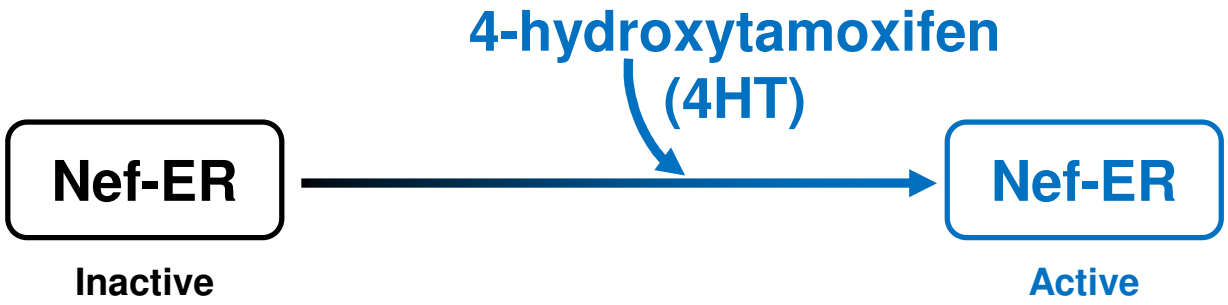






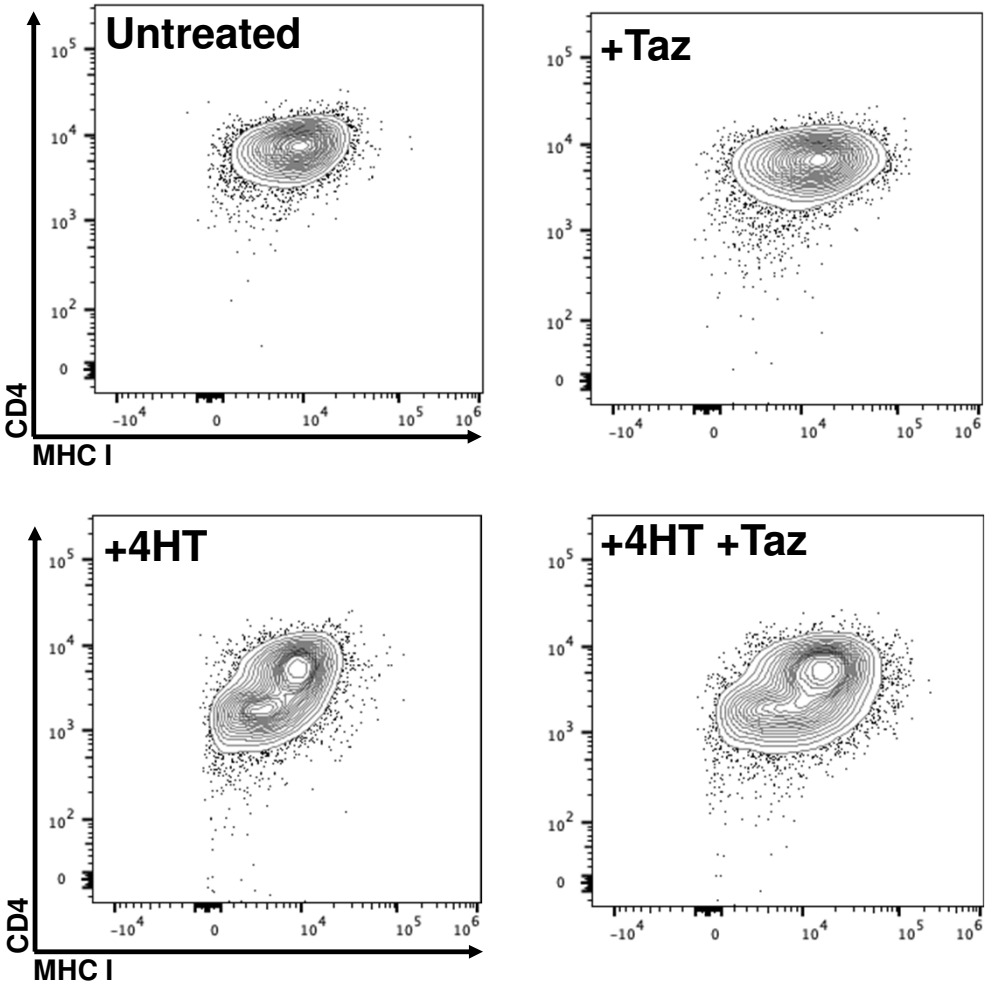
A Sup-T1 T-cell Line Expressing Chimeric Nef-estrogen Receptor

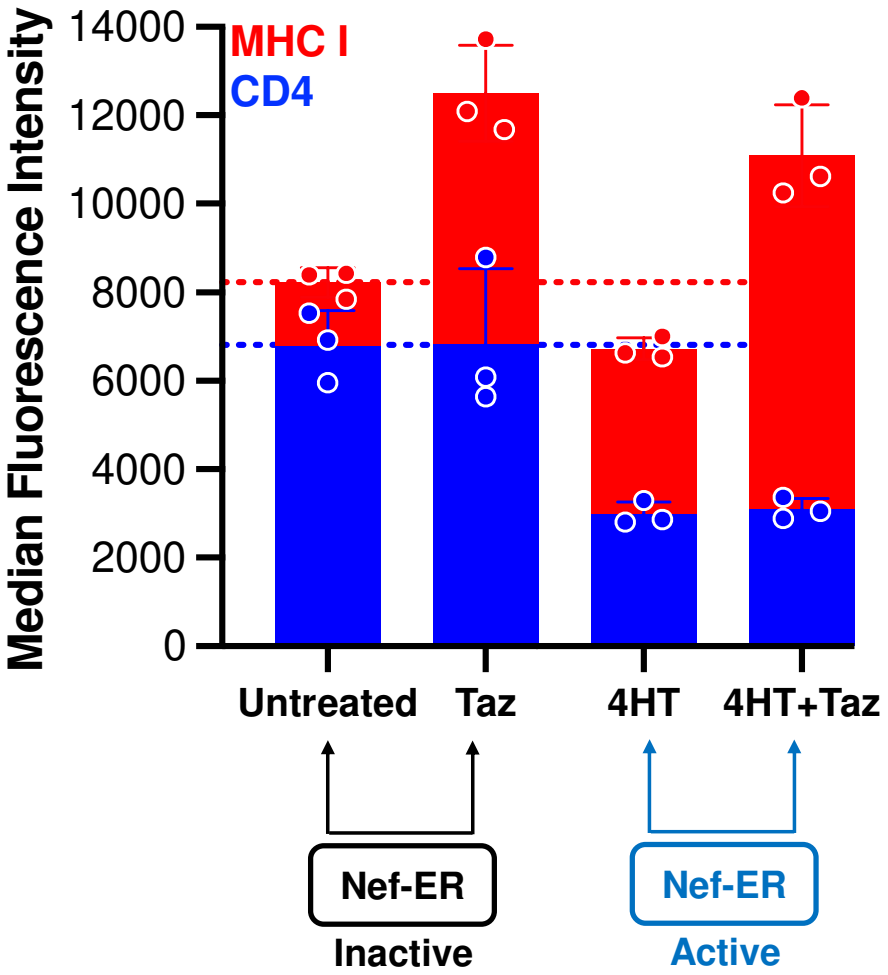
(AIDS Reagent Program: Nef-ER Expressing Sup-T1 Cells (Clone 31), ARP-6453,
contributed by Drs. **Scott Walk**, **Kodi Ravichandran** and **David Rekosh**)



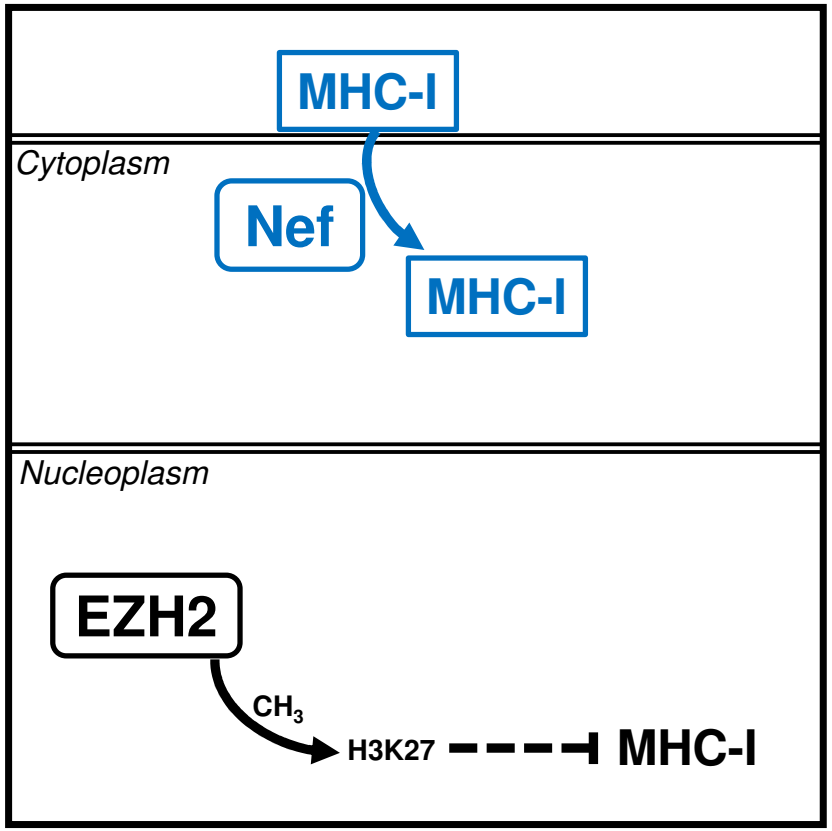
- This cell line expresses a fusion between full-length Nef and the estrogen receptor (ER) hormone-binding domain (Nef-ER)
- Nef-ER is kept in an inactive state due to steric hindrance
- Addition of the membrane-permeable drug 4-hydroxytamoxifen (4HT), which binds to the ER domain, leads to inducible activation of Nef-ER within cells

Tazemetostat Increases Surface MHC-I In The Presence Of 4HT

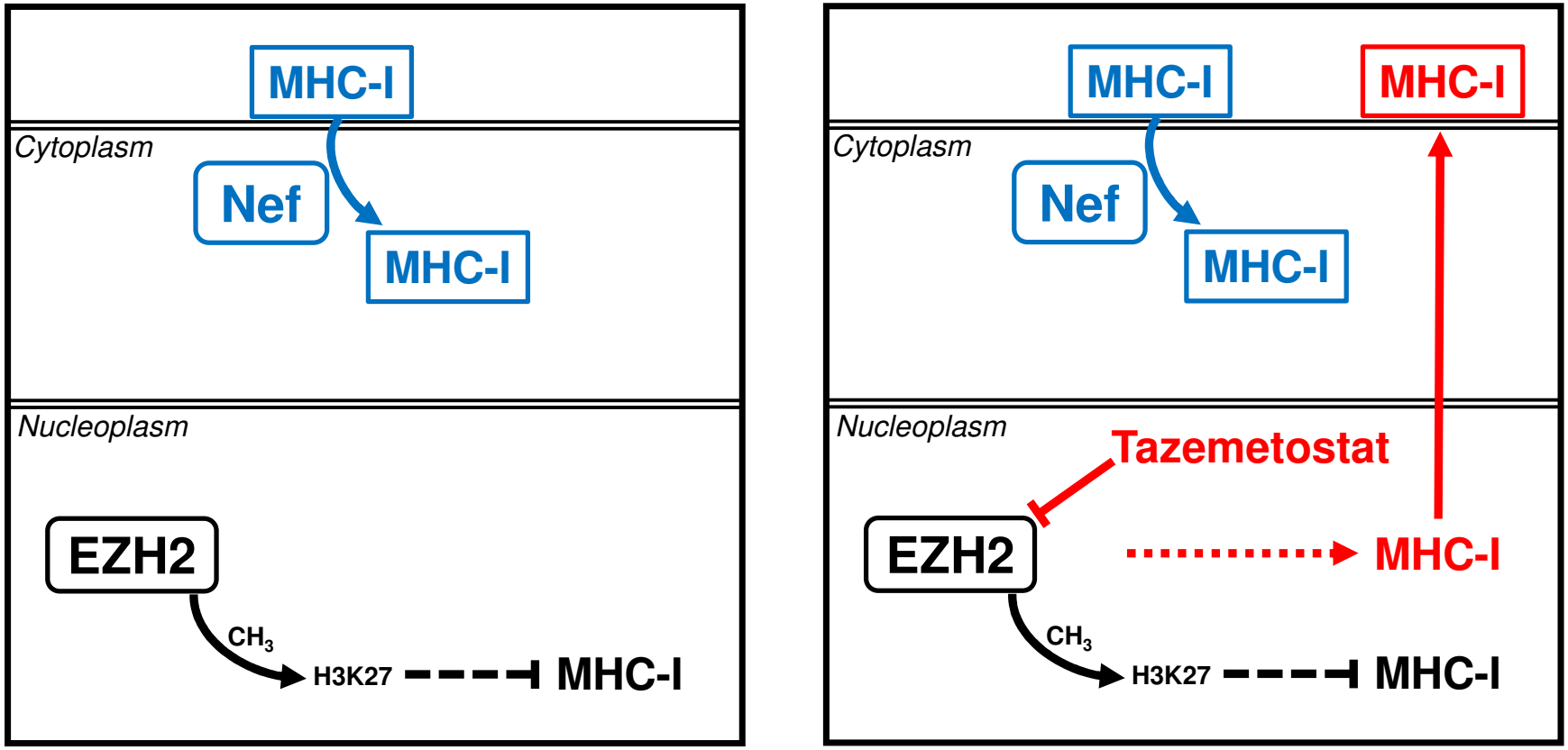




Tazemetostat Counteracts Nef-mediated Downregulation Of MHC-I By Increasing MHC-I Basal Transcription



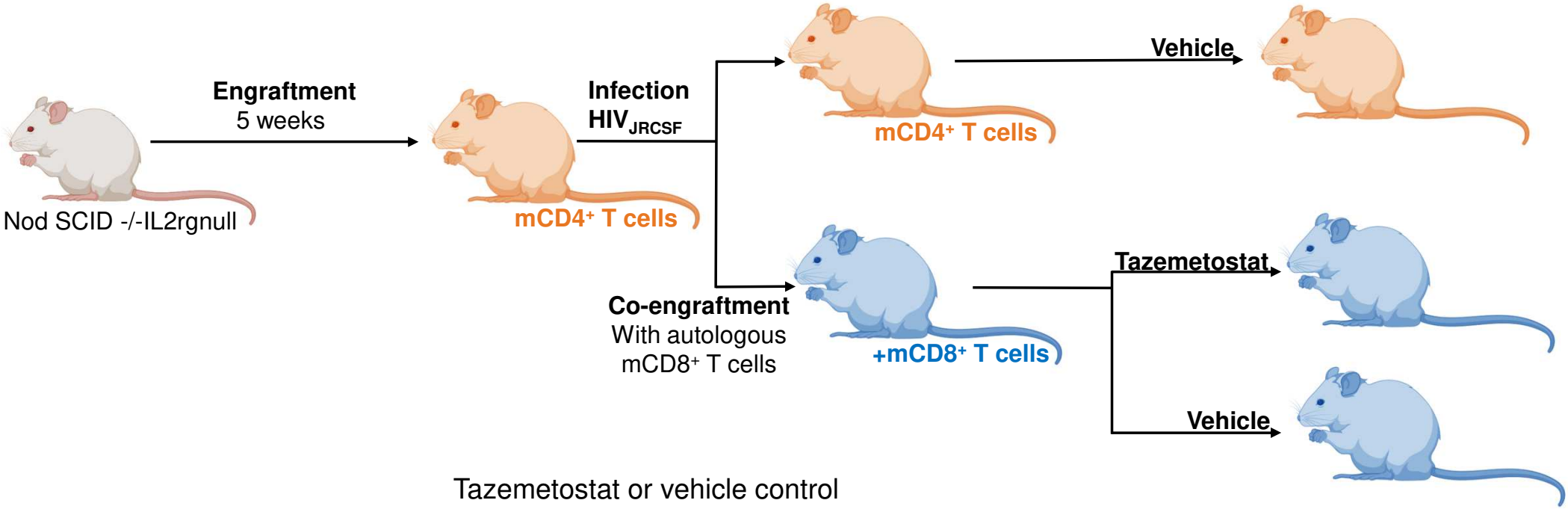
Tazemetostat Counteracts Nef-mediated Downregulation Of MHC-I By Increasing MHC-I Basal Transcription



Can Tazemetostat Increase MHC-I And Reduce Viral Loads In Vivo?

In vivo Treatment With Tazemetostat

Humanized HIV-infected mouse model from: ***CD. McCann, RB Jones et al***. *J Exp Med* (2021)

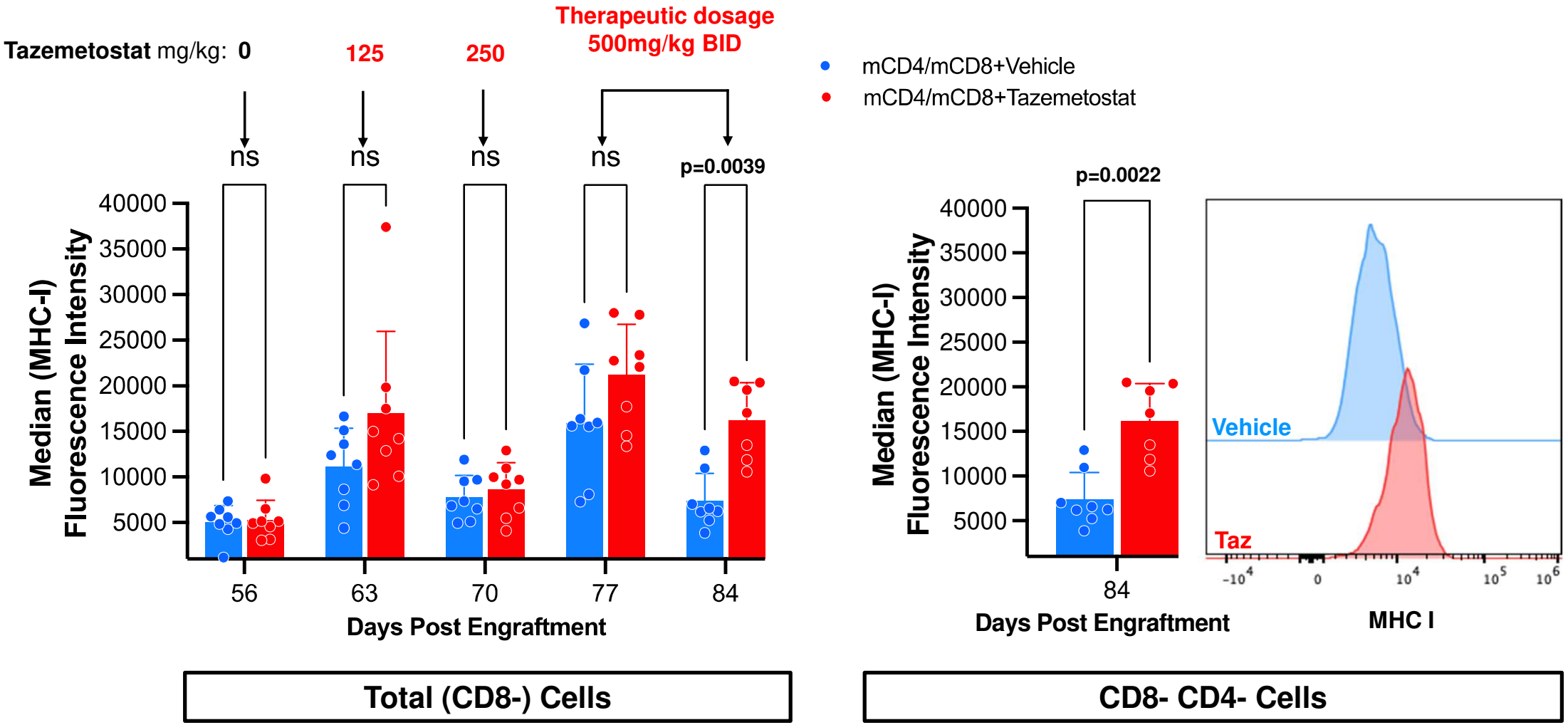


Tazemetostat or vehicle control
were administered orally, through oral gavage

125mg/kg (Week 1) → **250mg/kg** (Week 2) → **500mg/kg (twice/day)** (Week 3, 4)

Therapeutic dosage
(As reported in previous mouse study in cancer)

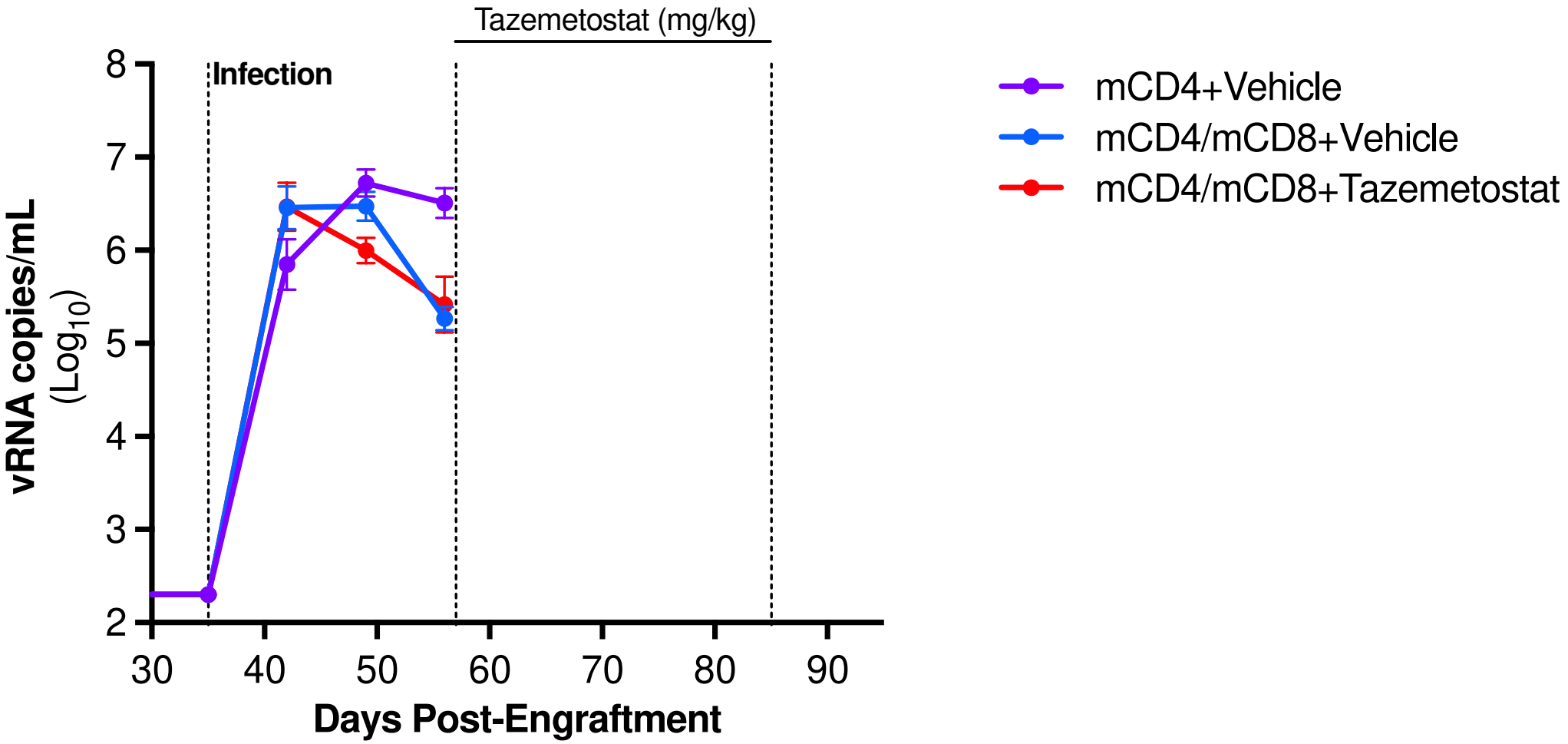
Tazemetostat Induces A Significant Increase In Surface MHC-I On Infected Cells In Vivo



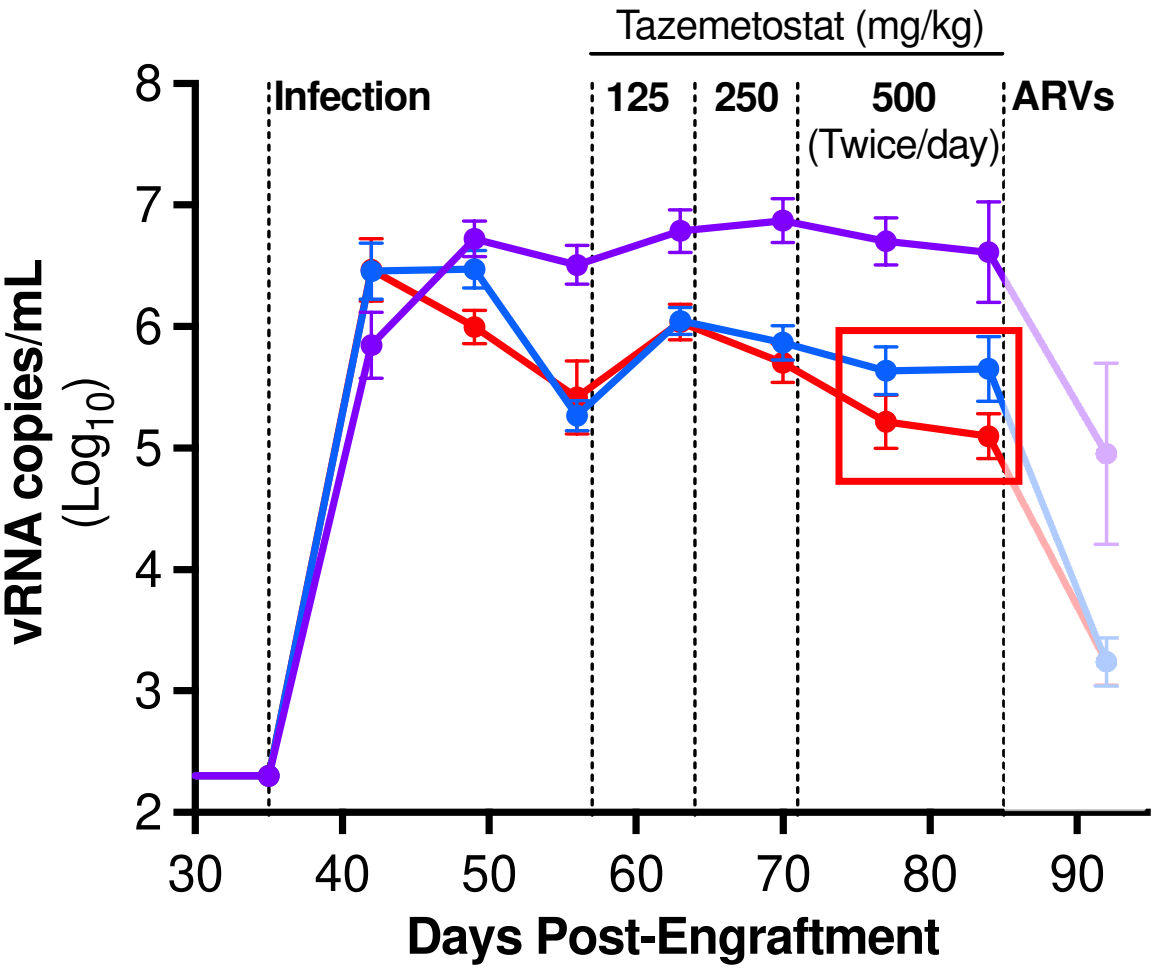
Total (CD8-) Cells

CD8- CD4- Cells

Viral Load Is Significantly Reduced In +CD8 Versus CD4only Mice



Treatment With Tazemetostat Results In Decrease Of Viral Load, Relative To Vehicle Control



mCD4+Vehicle
mCD4/mCD8+Vehicle
mCD4/mCD8+Tazemetostat

0.56-log decrease in VL (mean value)

Summary and Conclusions

Key question being asked:

Can we target intrinsic resistance mechanisms in infected cells to enhance their elimination by CTLs?

Key findings:

1. The FDA-approved drug Tazemetostat increases basal MHC-I expression on $CD4^+$ T cells
2. Increased MHC-I expression counterbalances HIV-Nef-mediated immunoevasion, resulting in enhanced infected-cell elimination *in vitro* and decreased viral loads *in vivo*

What are the next steps?

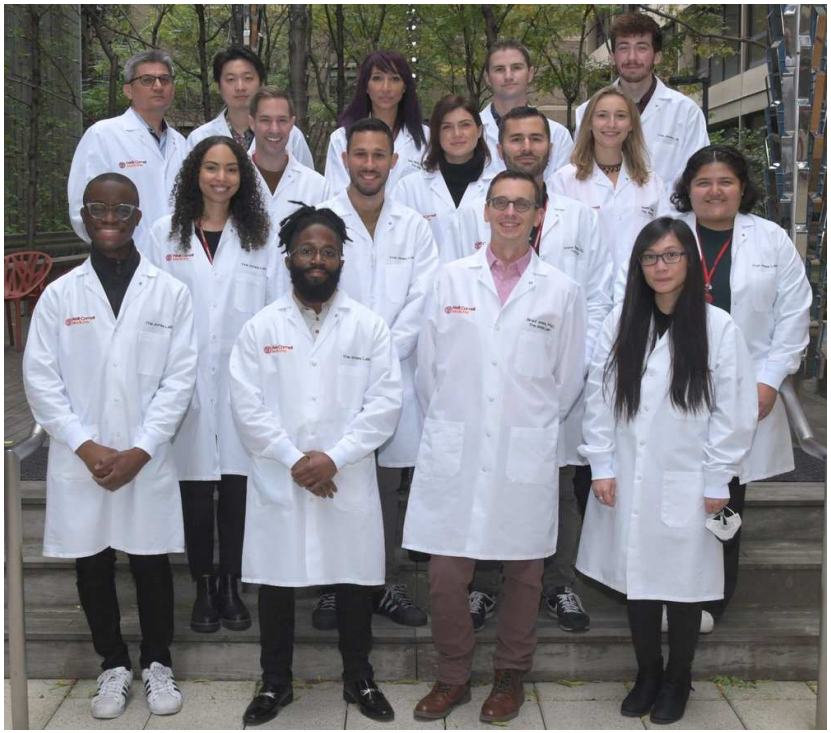
1. Repeat/confirm *in vivo* results with therapeutic dosage of Tazemetostat
2. Study whether treatment of viremic mice with Taz, prior to ART initiation, will induce CTL-mediated reduction of the pool of infected cells and will delay rebound of viral load when ART is interrupted

Acknowledgements

Weill Cornell Medicine

The Jones Lab

- Brad Jones
- Farzana Khan
- Ali Danesh
- Jared Weiler
- Itzyana Miller
- Dennis Copertino
- Adam Ward
- Uche Chukwukere
- Louise Leyre
- Talia Mota
- Sandra Terry



Martin Delaney Collaboratories for HIV Cure Research

REACH

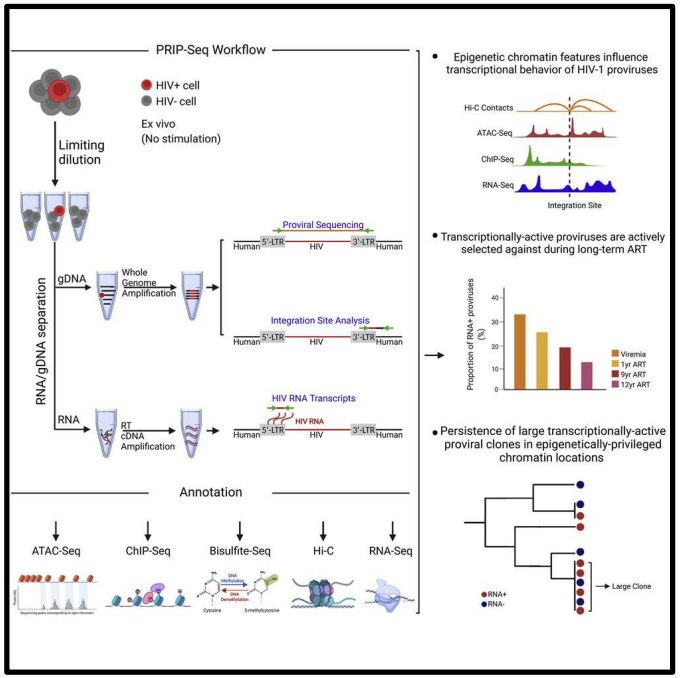
For the Cure

Research Enterprise to Advance a Cure for HIV

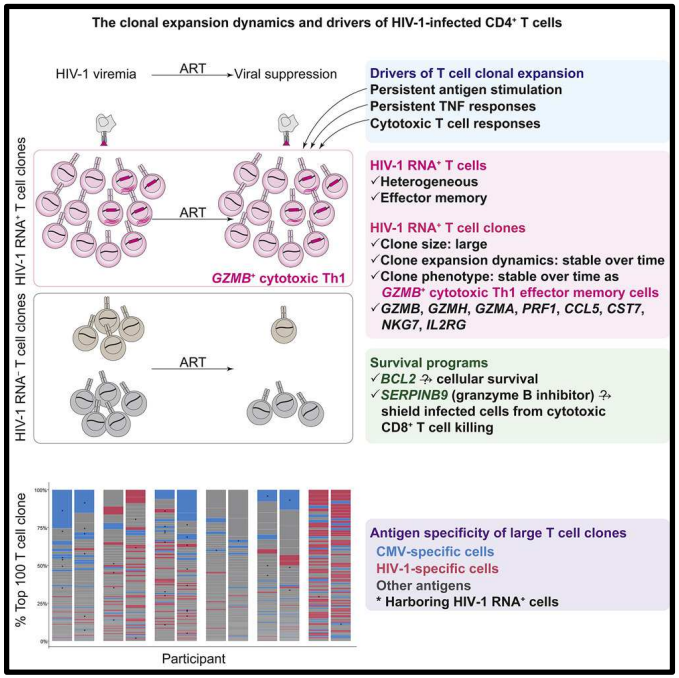
Evolution Of The HIV Reservoir In ART-treated People Supports The Hypothesis Of Resistance To CTL-mediated Elimination

Some infected cell clones are highly transcriptionally active and display a "survival program"

Einkauf KB, Lichterfeld M et al. Cell 2022

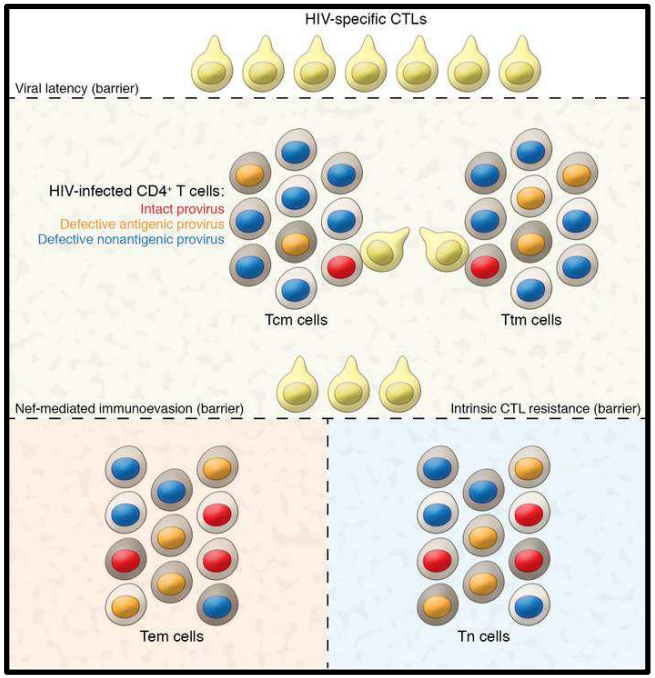


Collara J A, Ho Y et al., Immunity 2022



Evidence for on going CTL selection over years on ART

Duette G, Palmer S et al., JCI. 2022



EZH2 Inhibitors In HIV and Cancer Research

CLINICAL CANCER RESEARCH | TRANSLATIONAL CANCER MECHANISMS AND THERAPY

Targeting EZH2 Enhances Antigen Presentation, Antitumor Immunity, and Circumvents Anti-PD-1 Resistance in Head and Neck Cancer

Liye Zhou[1], Tenny Mudianto[1], Xiaojing Ma[1,2], Rachel Riley[1], and Ravindra Uppaluri[1,3]

Cell Host & Microbe
Article

SMYD2-Mediated Histone Methylation Contributes to HIV-1 Latency

Daniela Boehm,[1,2] Mark Jeng,[1,2] Gregory Camus,[1,2] Andrea Gramatica,[1,2,3] Roland Schwarzer,[1,2,3] Jeffrey R. Johnson,[1,] Philip A. Hull,[1,2] Mauricio Montano,[1,2,3] Naoki Sakane,[1,5] Sara Pagans,[1,2] Robert Godin,[6] Steven G. Deeks,[2] Nevan J. Krogan,[1,4] Warner C. Greene,[1,2,3] and Melanie Ott[1,2,7,*]

LETTER
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EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations

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H3K27 Demethylation at the Proviral Promoter Sensitizes Latent HIV to the Effects of Vorinostat in *Ex Vivo* Cultures of Resting CD4$^+$ T Cells

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Dramatic In Vivo Efficacy of the EZH2-Inhibitor Tazemetostat in *PBRM1*-Mutated Human Chordoma Xenograft

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Epigenetic Silencing of HIV-1 by the Histone H3 Lysine 27 Methyltransferase Enhancer of Zeste 2$^\nabla$

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