

11<sup>TH</sup> EDITION

DECEMBER 10-13, 2024

# HIV PERSISTENCE DURING THERAPY

Reservoirs & Eradication Strategies Workshop



## Persistence of Clonally Expanded Proviruses

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*Johns Hopkins Medicine*

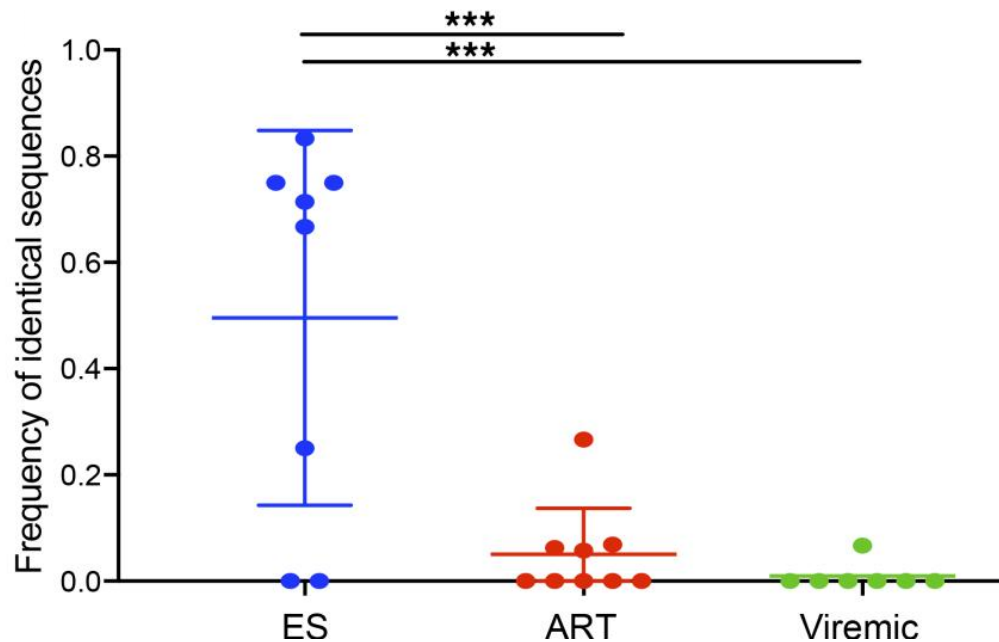
[www.hiv-persistence.com](http://www.hiv-persistence.com)

# CONFLICTS OF INTEREST

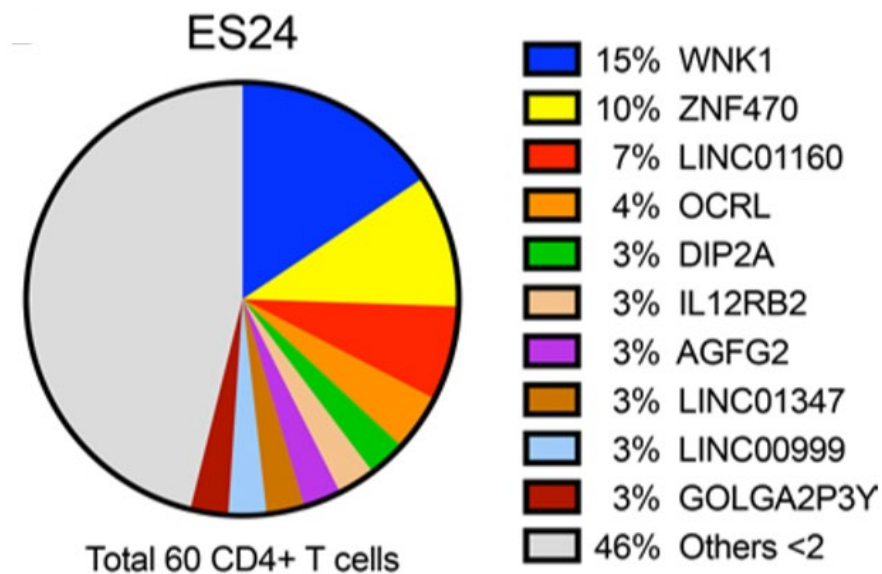
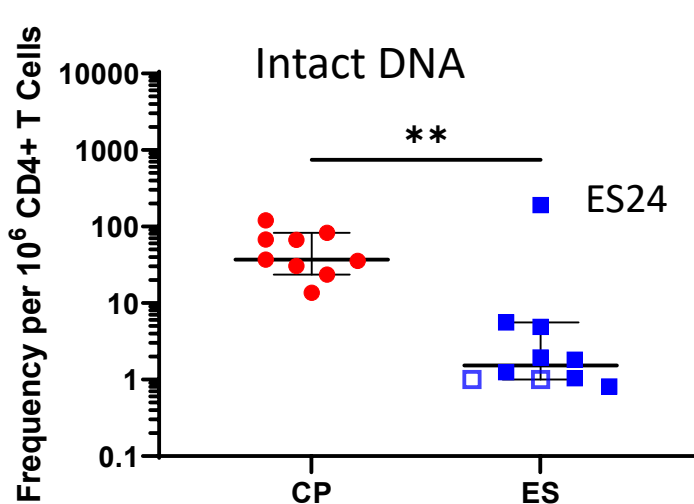
No conflicts of interest

# Elite suppressors (ES) have more clonally expanded virus than PLWH on ART

Elite suppressors/controllers control viral replication without ART and are a model for a functional cure



# ES24 has a large viral reservoir and a high degree of clonal expansion

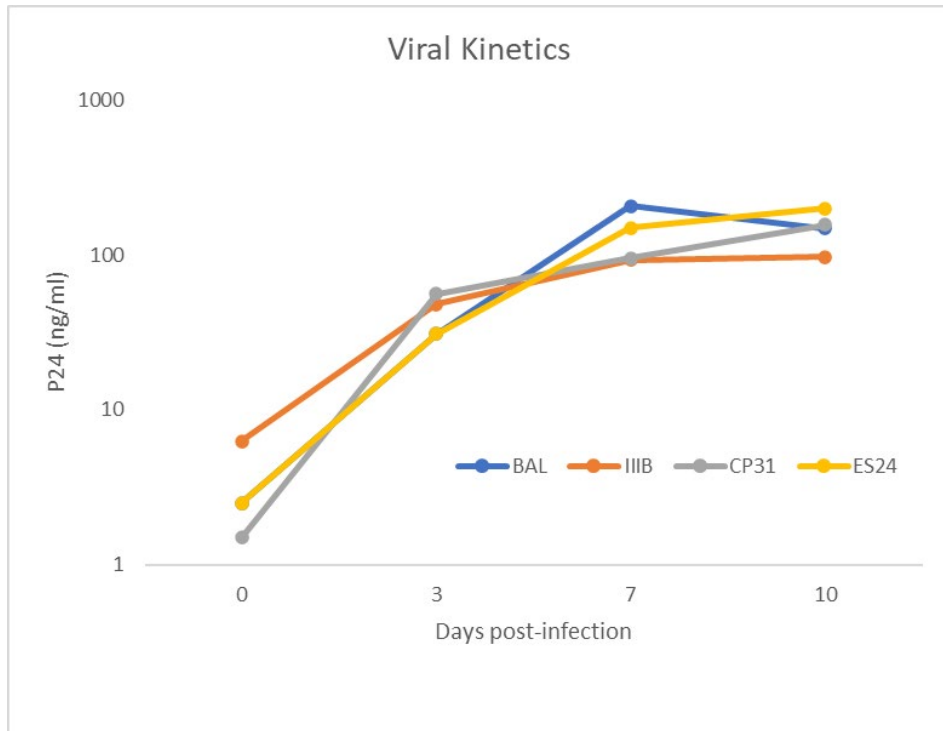


Rick Bushman PhD,  
U Penn

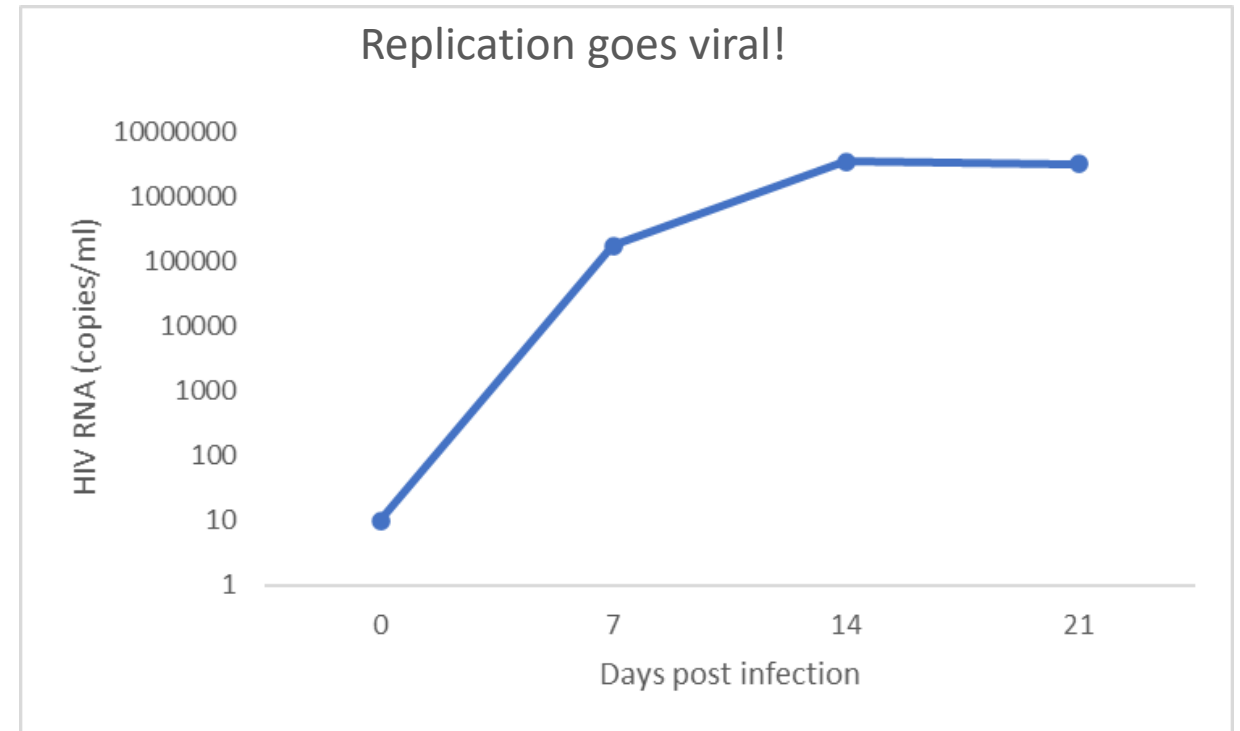
Grey slices represent unique integration sites, color represents clonally expanded integration sites

There were 10 genes where virus had integrated at the same exact site suggesting clonal expansion

# ES24 clonally expanded virus replicates vigorously in humanized mice

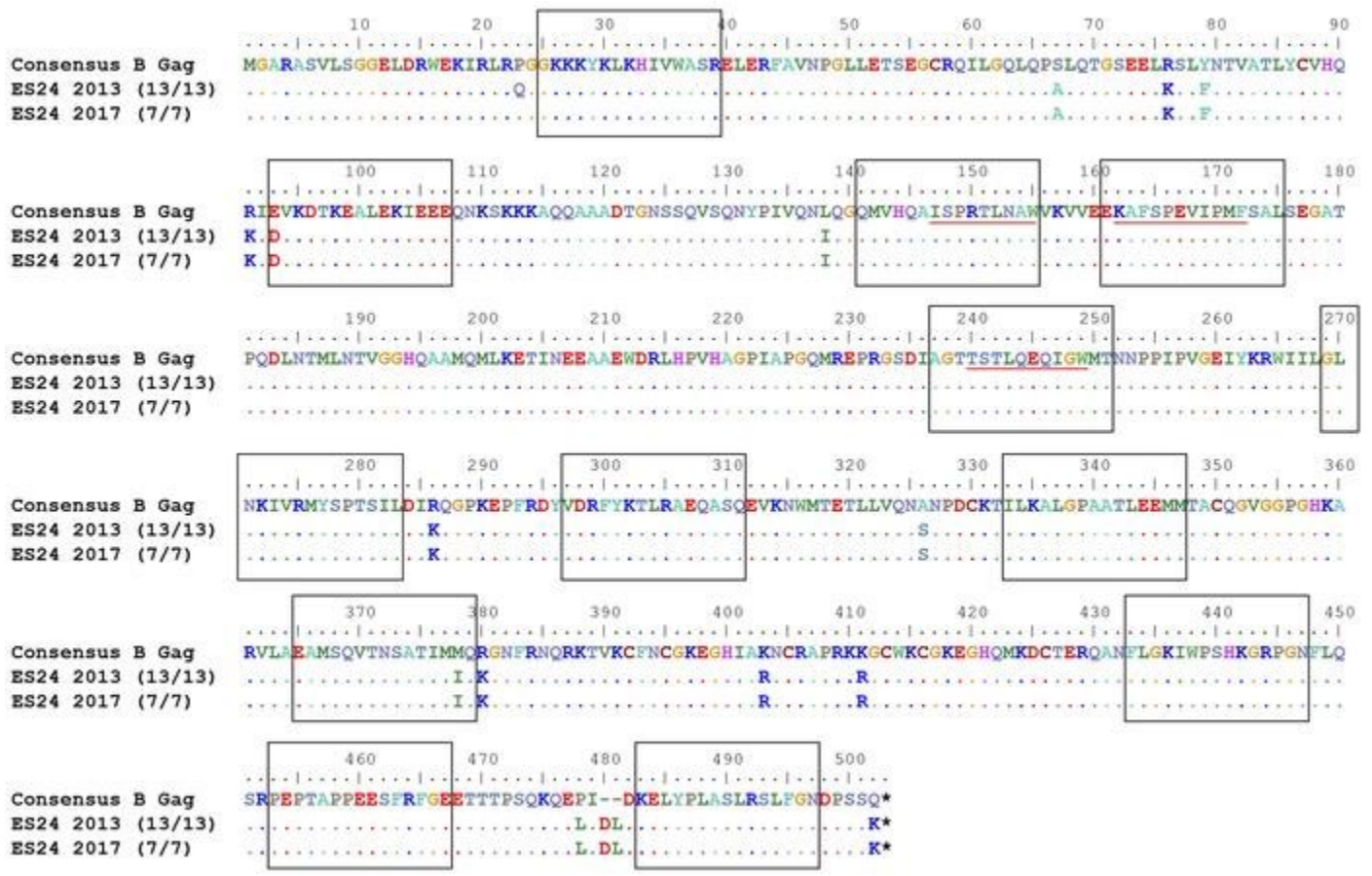


In vitro

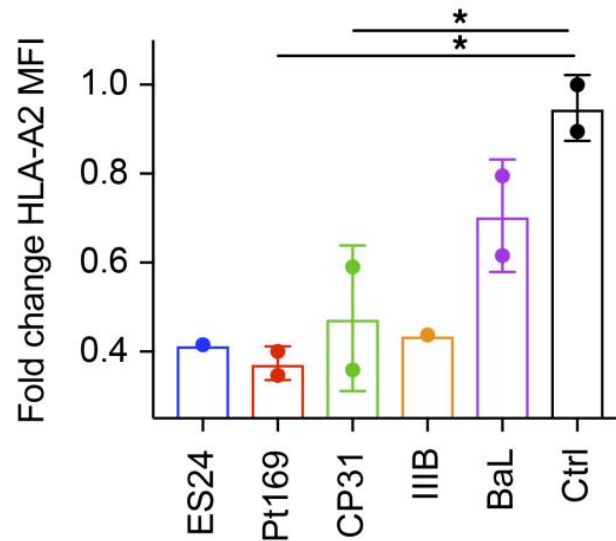
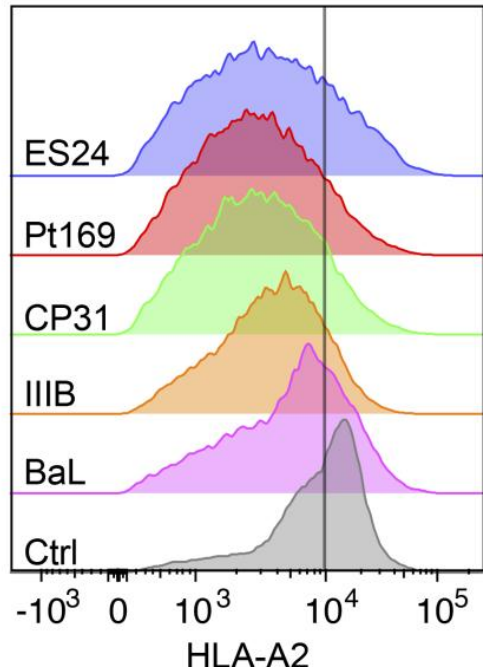


In a humanized mouse

# ES24 clonally expanded virus does not harbor escape mutations in Gag or Nef

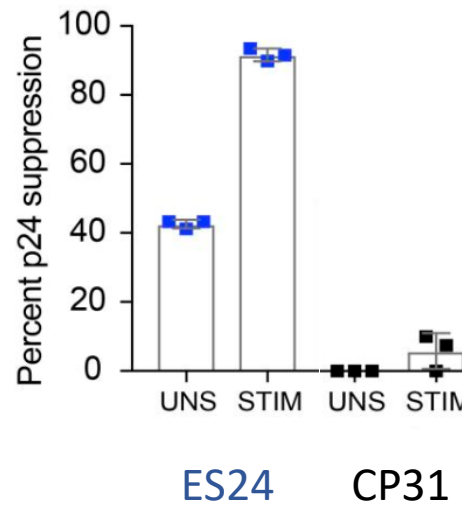
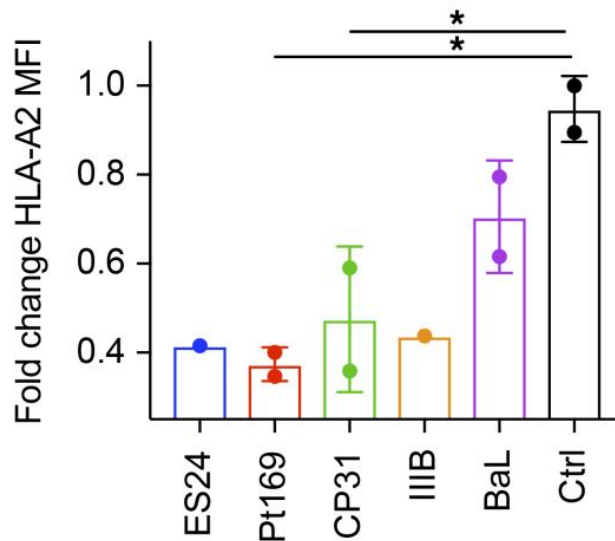
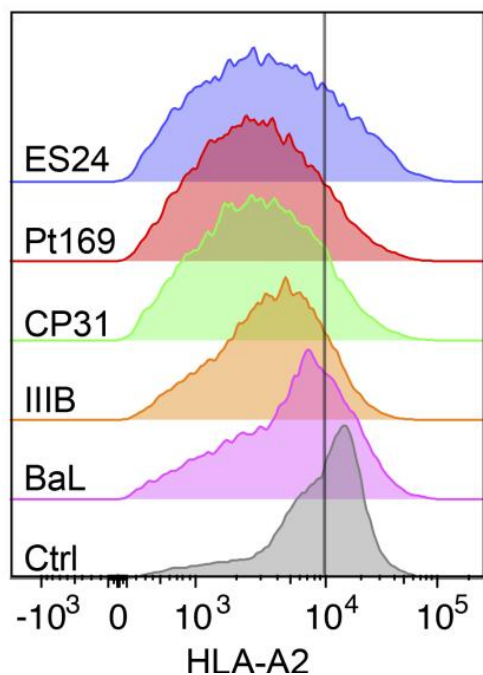


# Clonally expanded ES24 virus efficiently downregulates class I MHC



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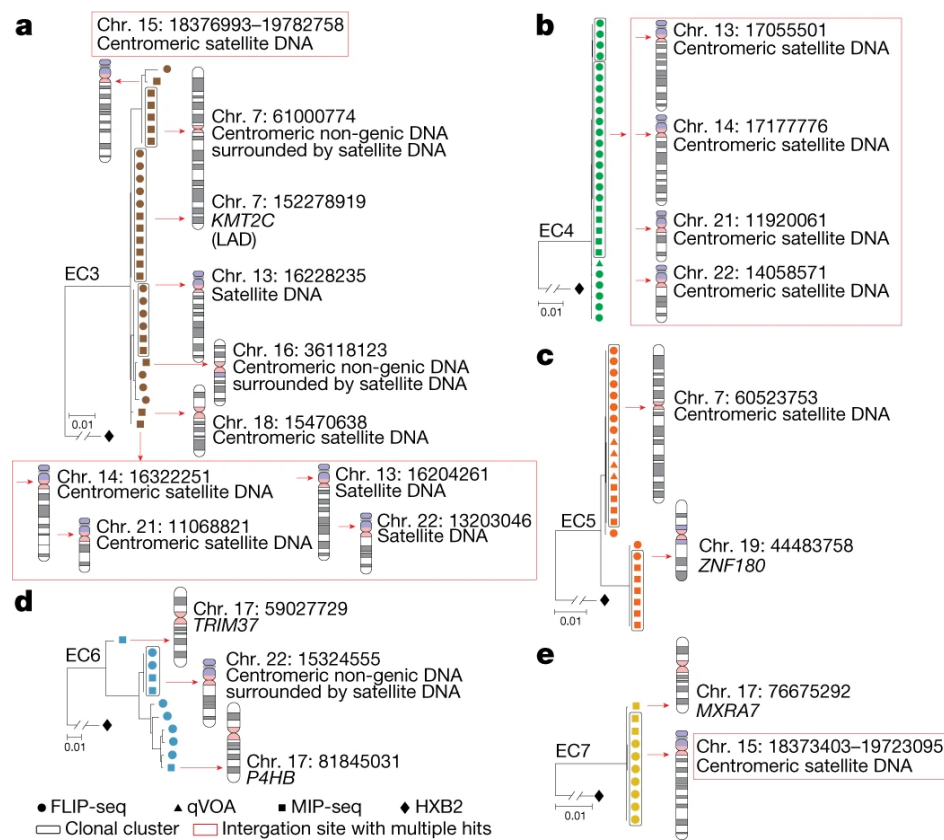
## ES24 CD8+ T cells suppress replication of autologous clonally expanded virus



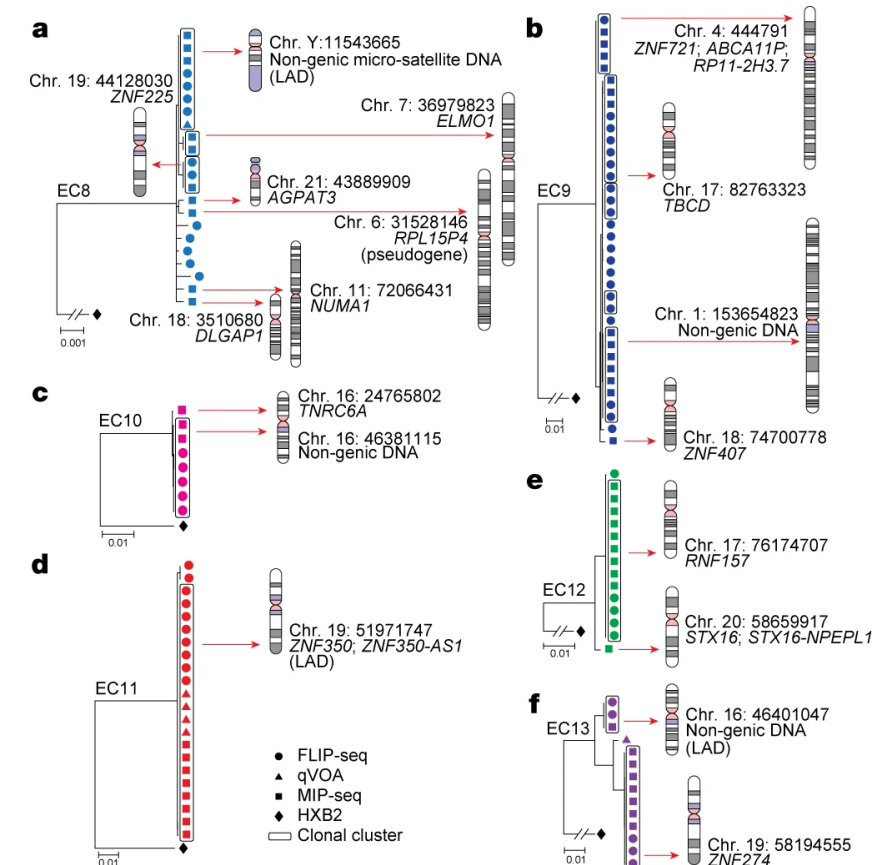


# Distinct integration sites in elite controllers: Cause versus consequence?

Increased frequency of genome-intact proviral sequences integrated in centromeric satellite DNA in elite controllers



Preferential location of genome-intact proviral sequences from elite controllers in genes that encode KRAB-ZNF proteins



# Proviruses are integrated into transcriptionally active and inactive sites

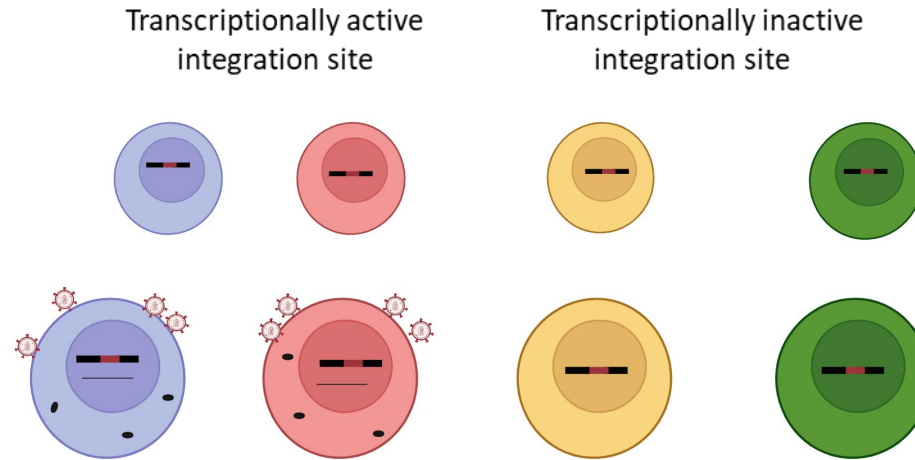
Transcriptionally active  
integration site



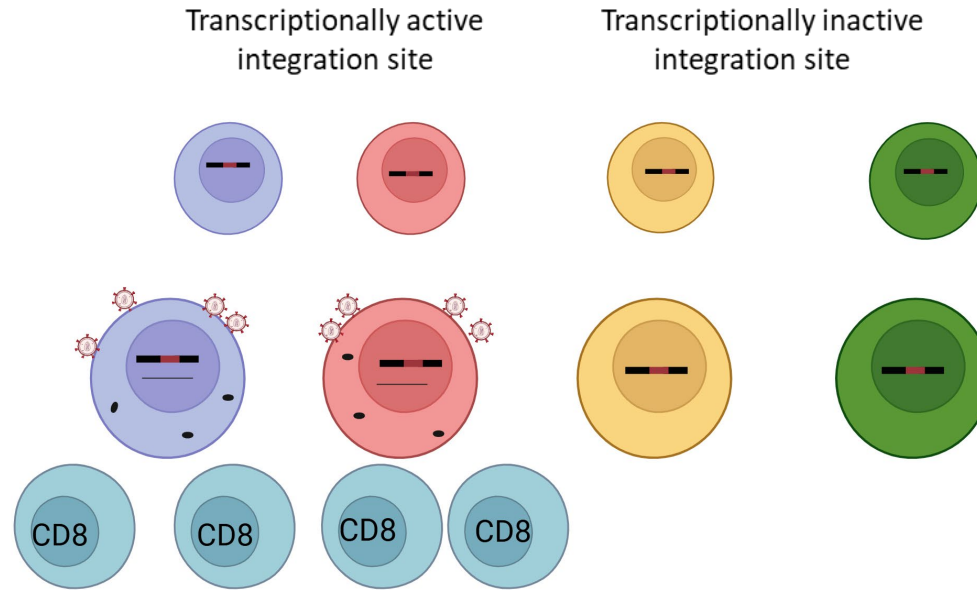
Transcriptionally inactive  
integration site



# Only proviruses in transcriptionally active sites will be induced following T cell activation



# CD8+ T cells will only recognize infected cells that produce viral proteins

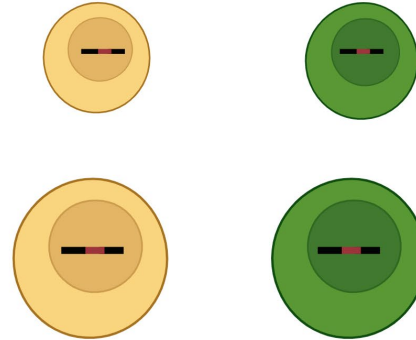


# Cells with inducible proviruses will be selectively killed by CD8+ T cells

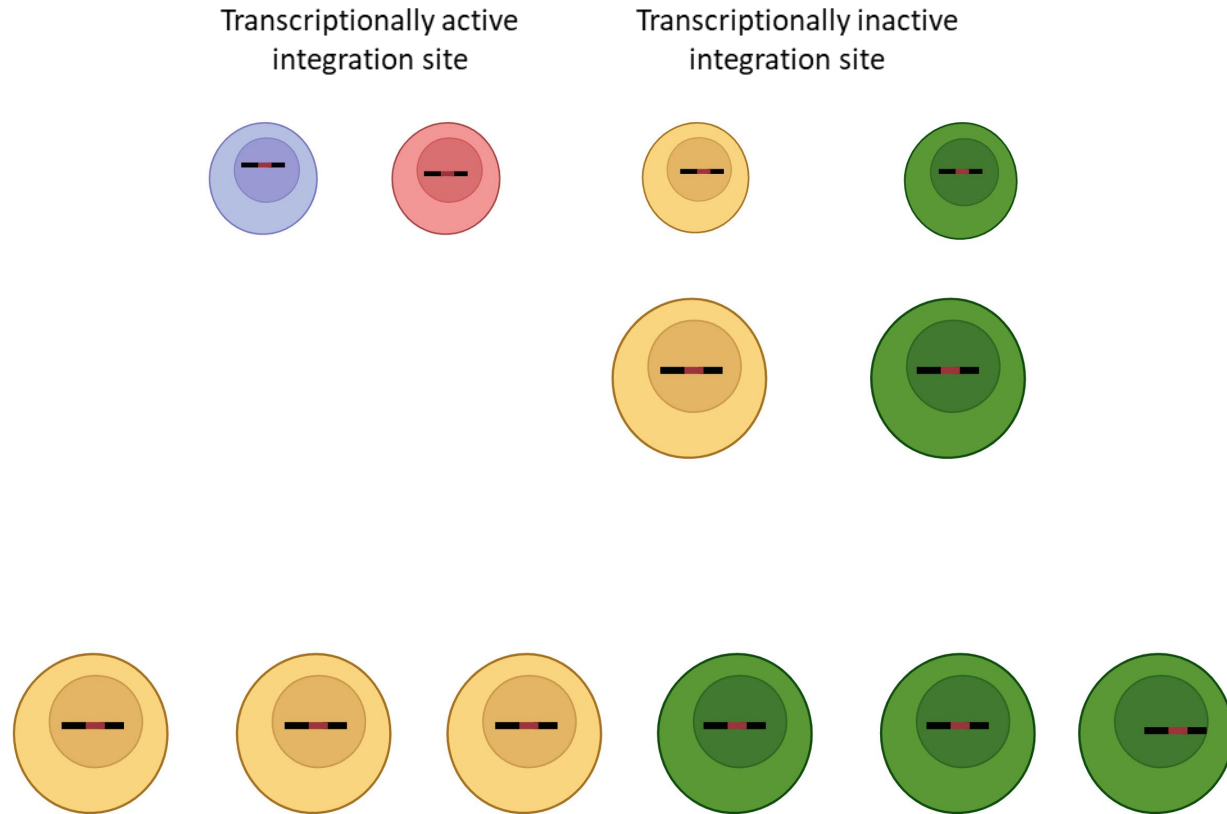
Transcriptionally active  
integration site



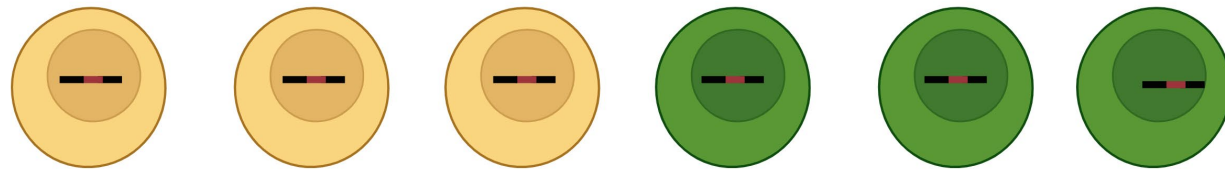
Transcriptionally inactive  
integration site



# Cells with non-inducible provirus will proliferate and expand



# After many years, clonally expanded cells with virus integrated into transcriptionally inactive sites will dominate the proviral landscape

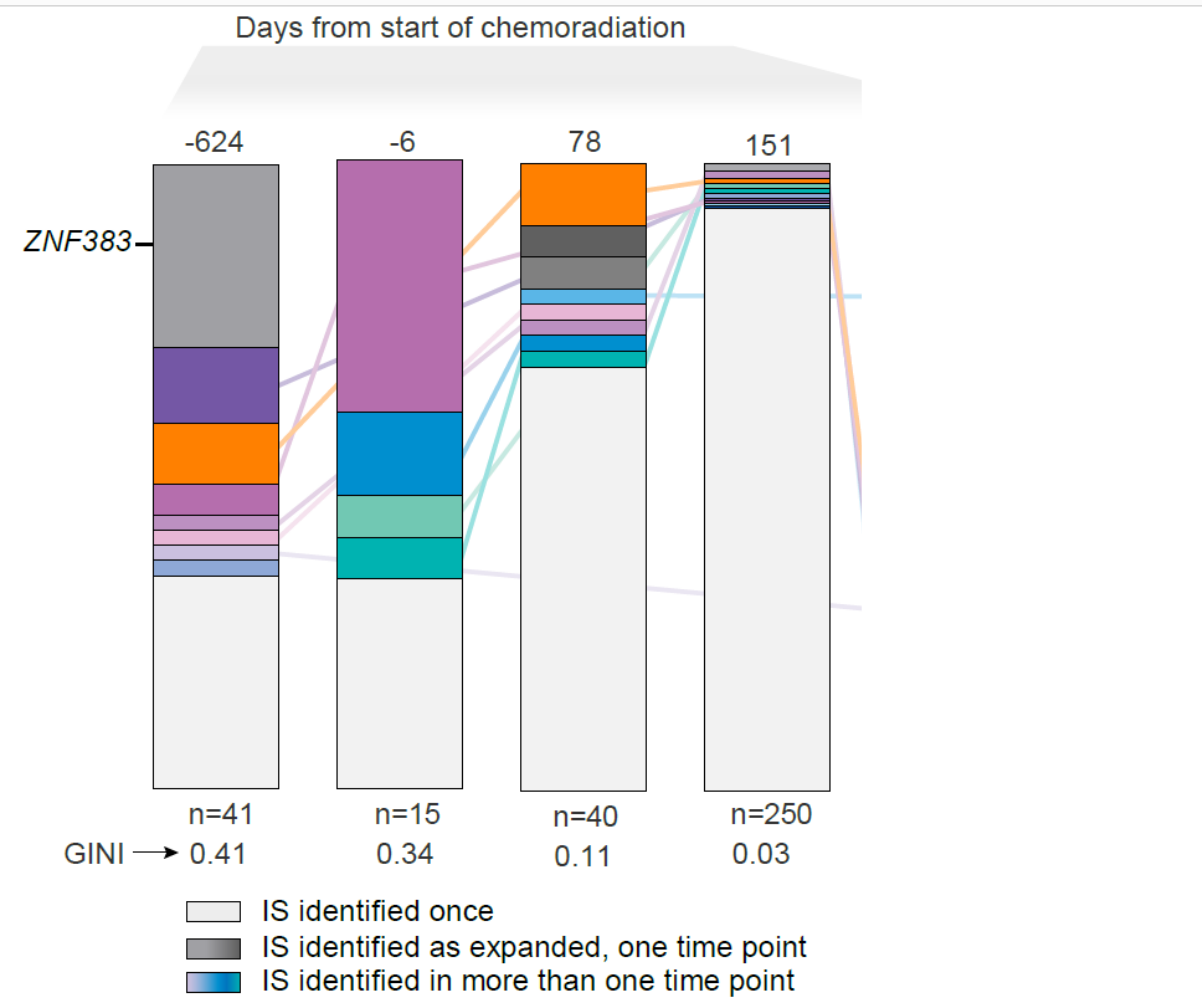


- ES24 was diagnosed with metastatic lung cancer in 2019
- He had a partial lobectomy but had residual disease
- He was put on ART
- He received chemotherapy with carboplatin and taxol and radiation therapy (CRT)
- Received a year of immunotherapy



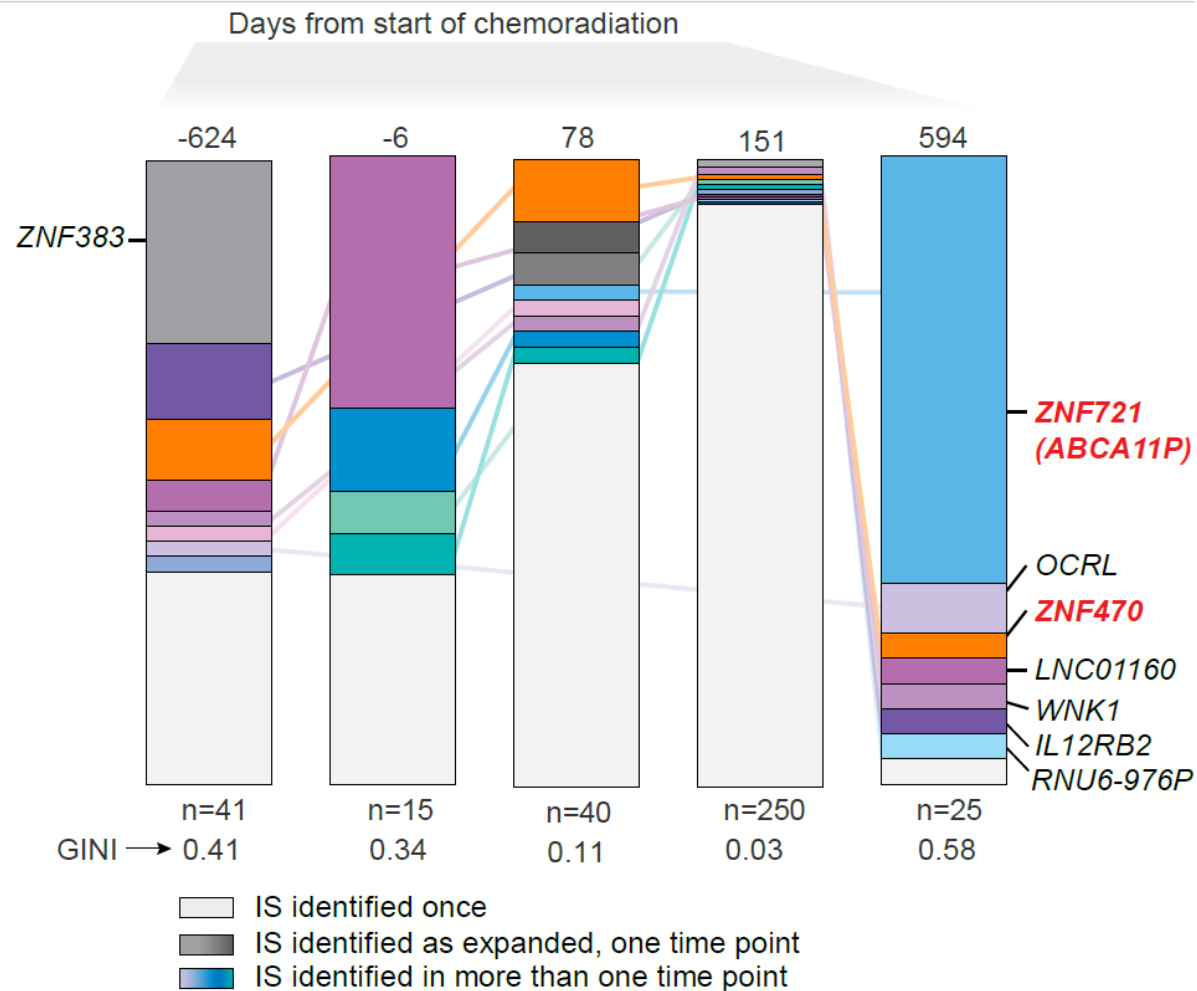
# ES24 had a high degree of clonal expansion 2 years prior to CRT Steady decline in clonal expansion observed during CRT

Light grey slices represent unique integration sites, color represents clonally expanded integration sites

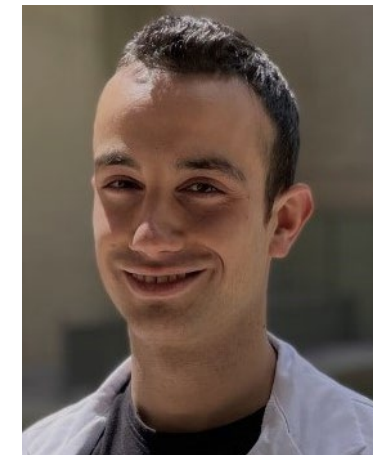
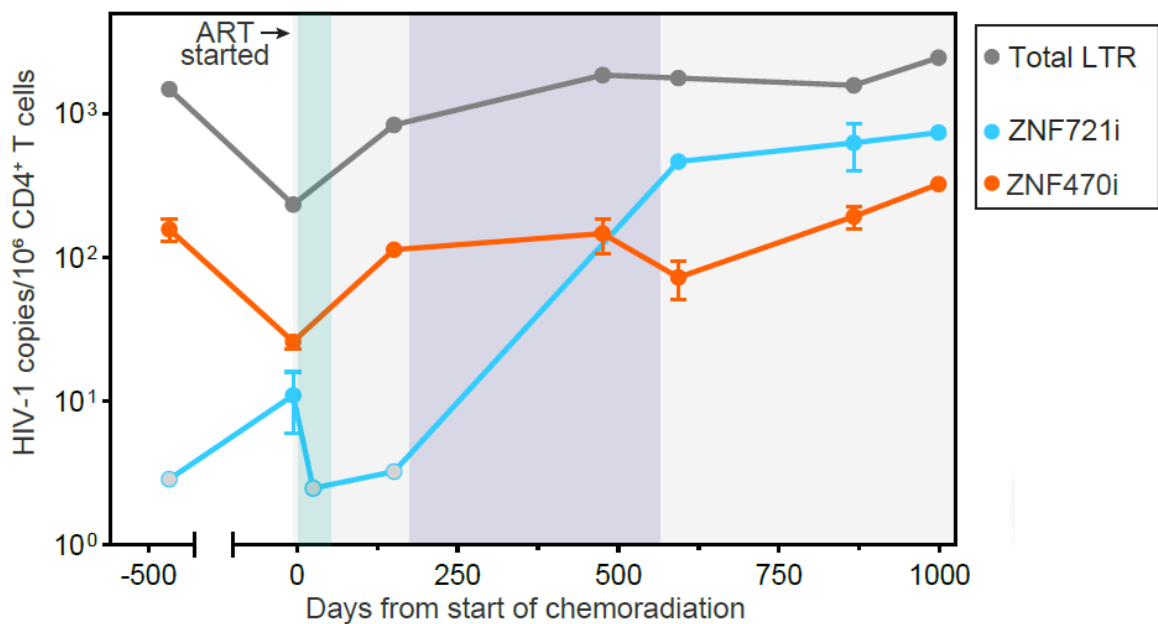


# There was a rebound in clonal expansion after a year of immunotherapy

Light grey slices represent unique integration sites, color represents clonally expanded integration sites



# Two clones with replication-competent virus integrated into ZNF genes dominated the landscape after chemoradiation and immunotherapy

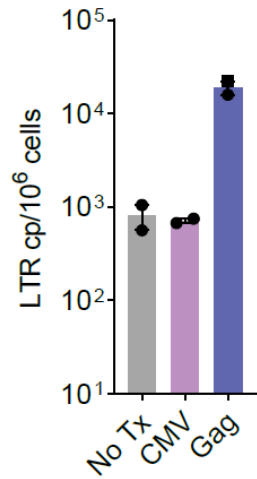


Filippo Dragoni  
PhD

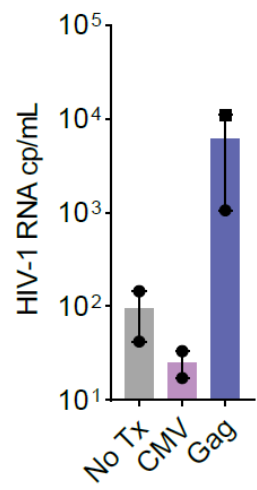
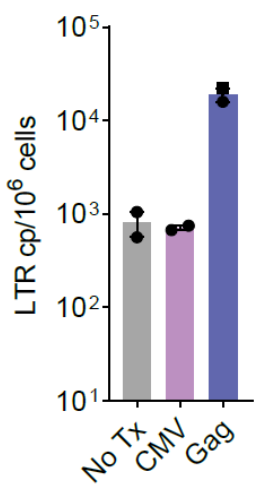


Francesco Simonetti  
MD, PhD

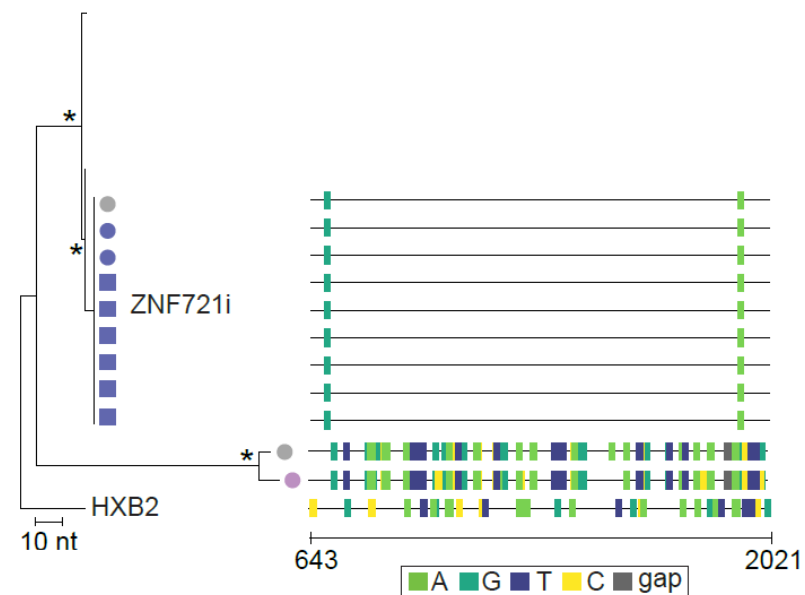
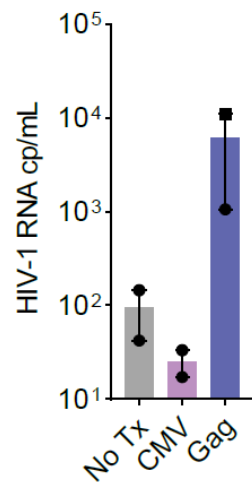
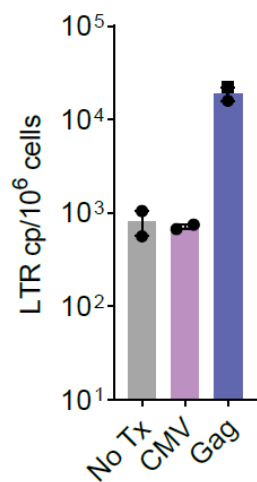
# Stimulation of ES24 CD4+ T cells with Gag peptides induces clonal expansion



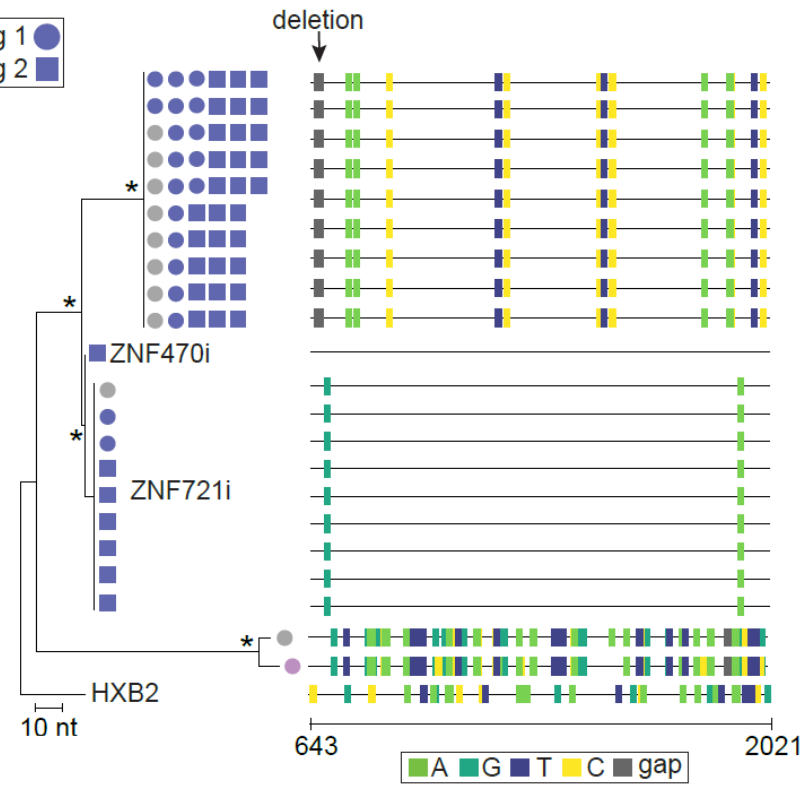
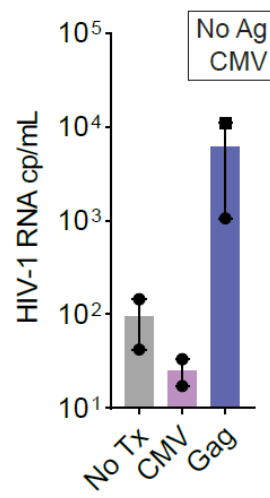
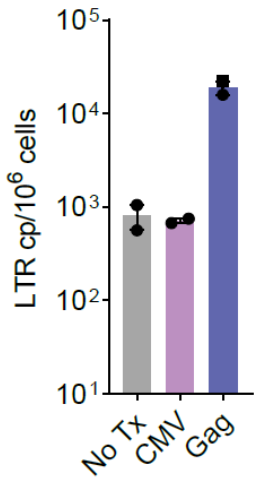
# Stimulation of ES24 CD4+ T cells with Gag peptides induces clonal expansion and viral transcription



# Sequence analysis reveals transcription of 2 clones, ZNF 721 and a clone with PBS deletion



# Sequence analysis reveals transcription of 2 clones, ZNF 721 and a clone with PBS deletion





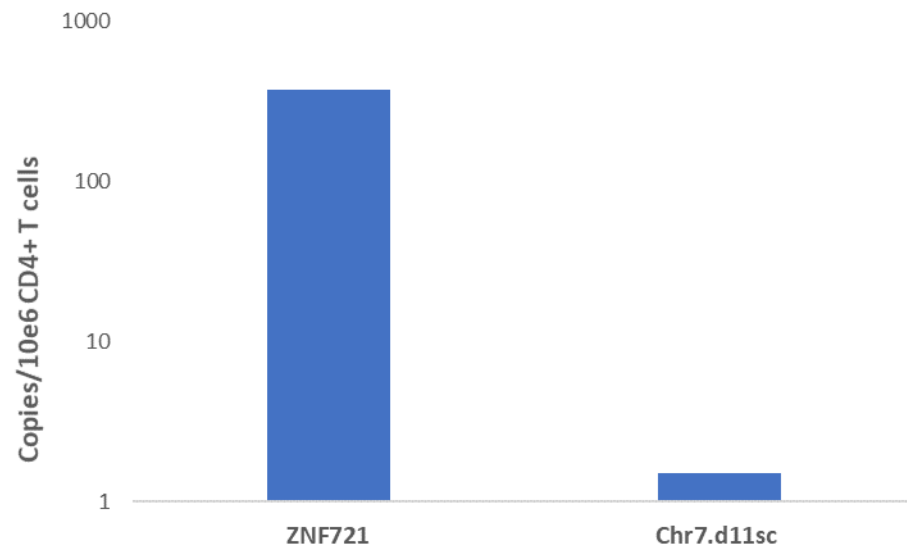


# A Tale of Two Clones

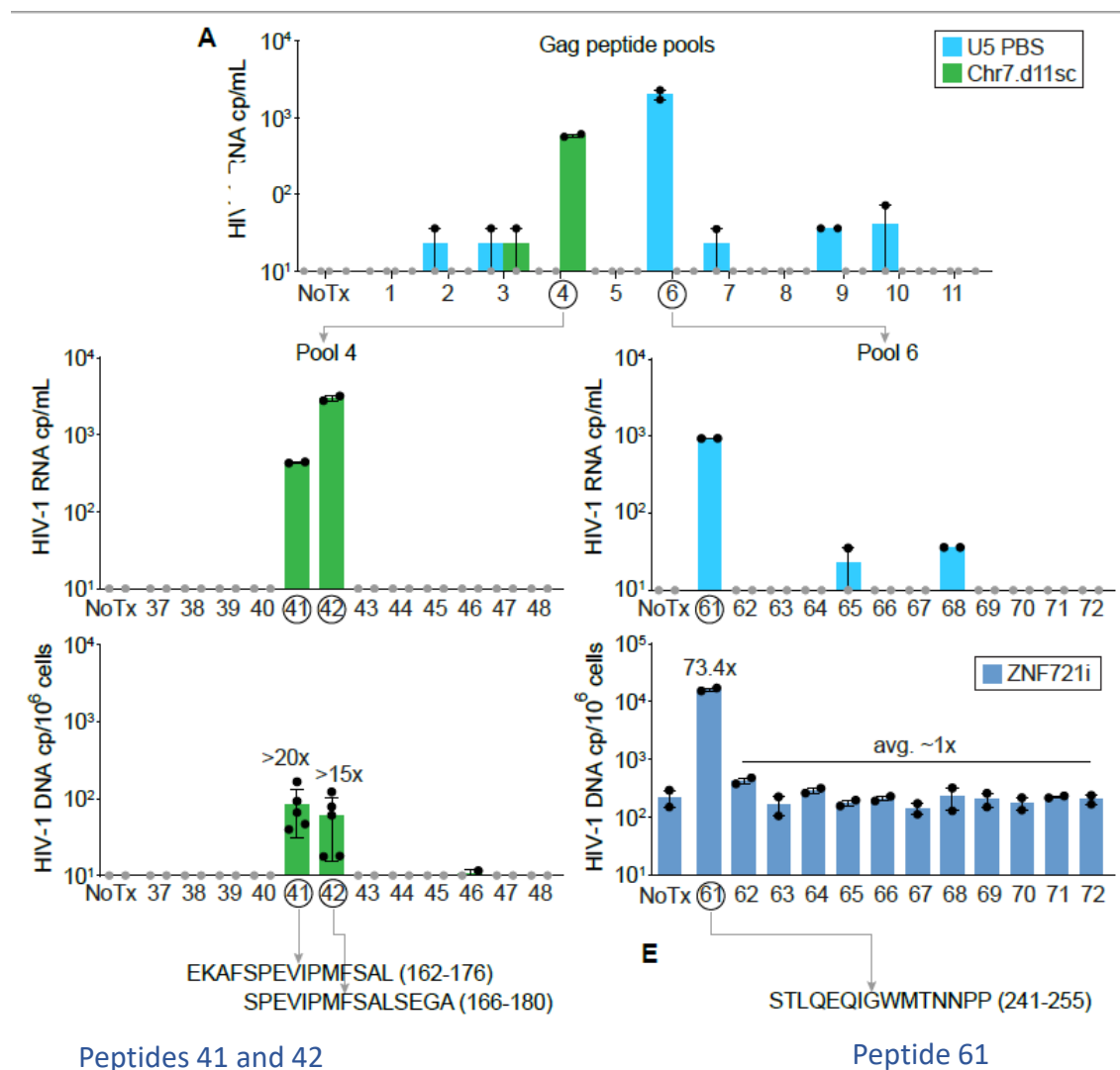
ZNF-721-high frequency

Chr7.d11sc- very low frequency

They both respond to Gag so why the difference?

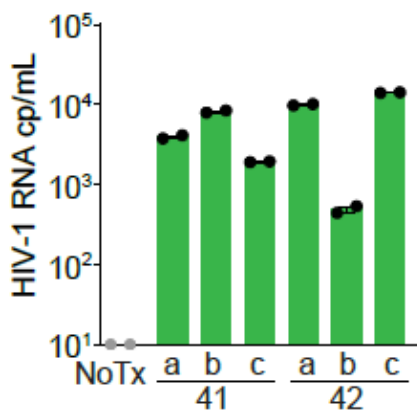


# We identified the exact Gag epitopes that were targeted by the ZNF-721 and Chr7.d11sc clones

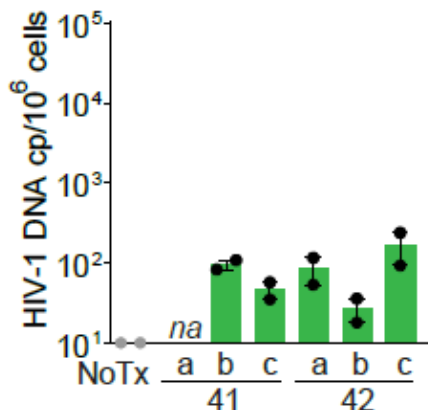


# Chr7.d11sc clone is much more transcriptionally active than the ZNF-721 clone

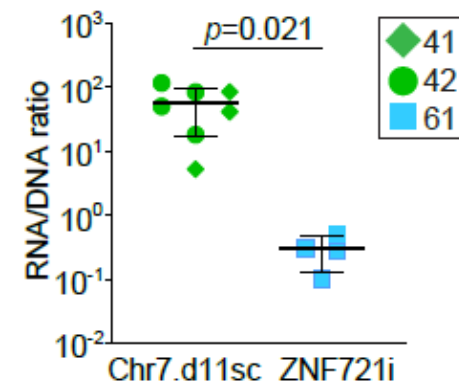
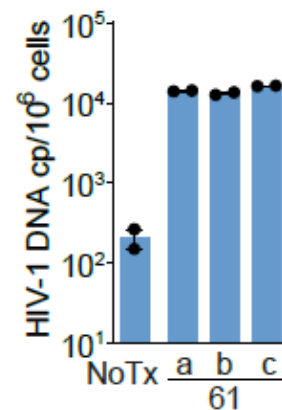
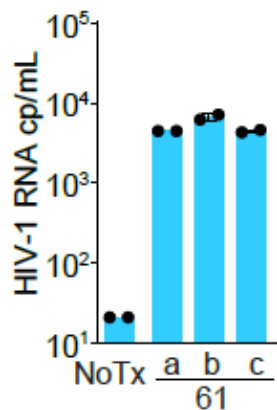
What are the consequences of this difference in transcriptional activity?



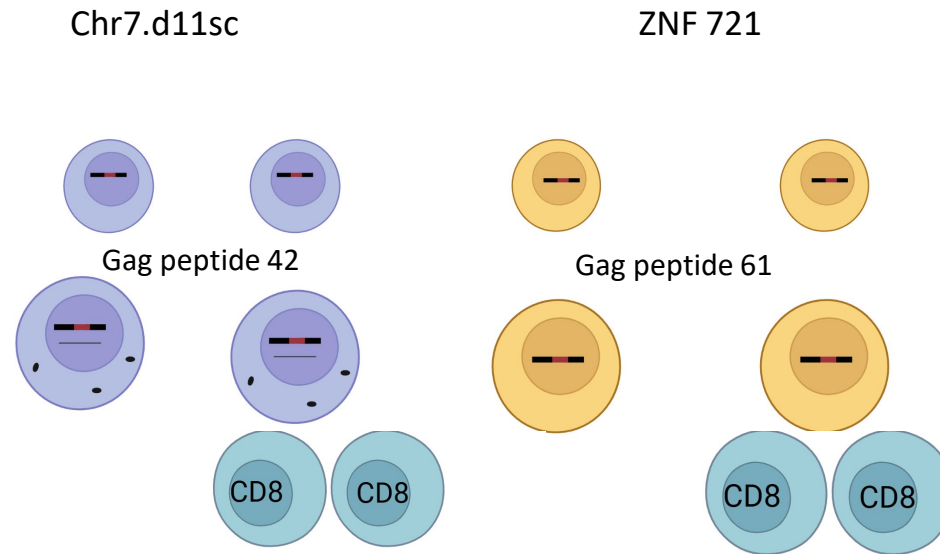
Chr7.d11sc



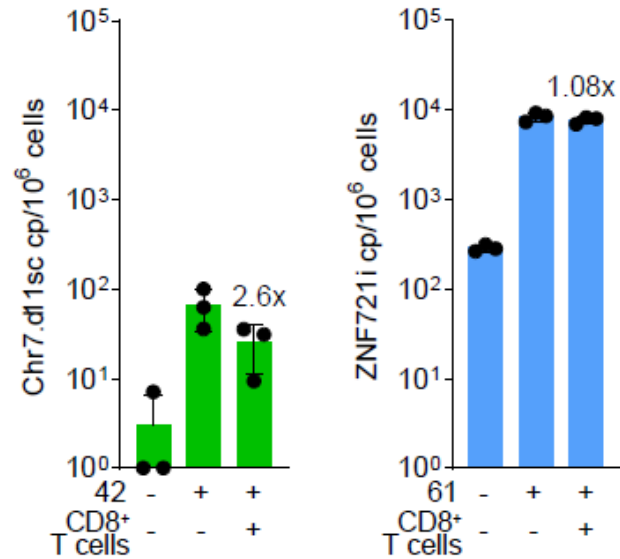
ZNF-721



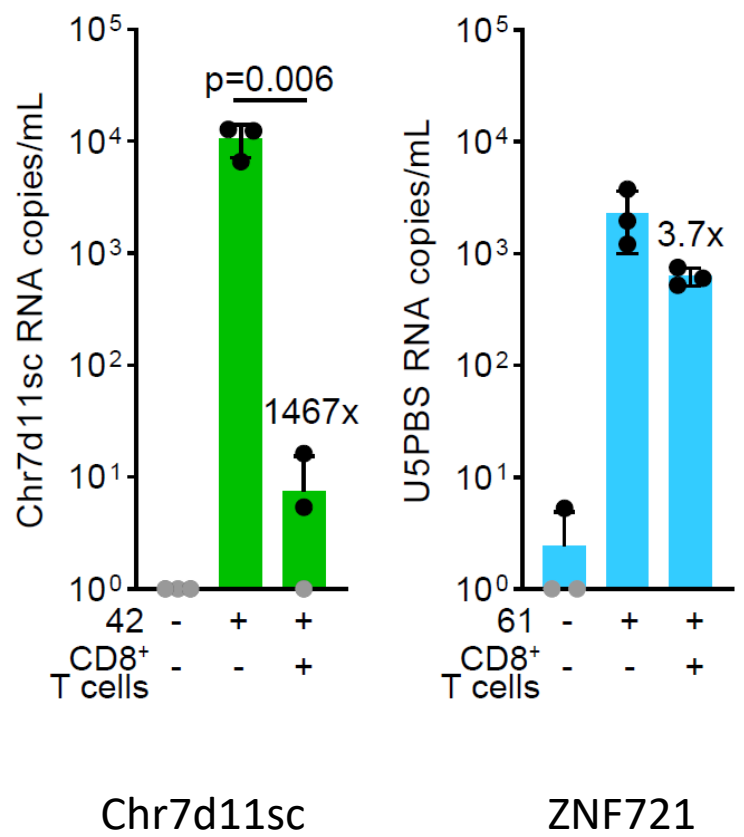
# After activation, cells were cultured in the presence and absence of HIV-specific CD8+ T cells



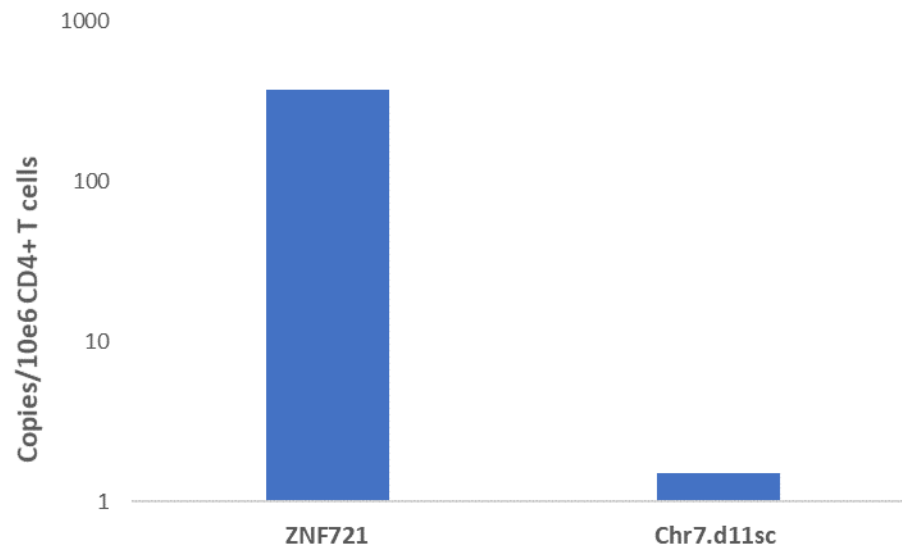
# CD8+ T cells have no effect on clonal expansion of ZNF721 but reduce the frequency of the Chr7.d11sc clone by 60%



# CD8+ T cells greatly impact virus production by the transcriptionally active Chr7d11sc clone



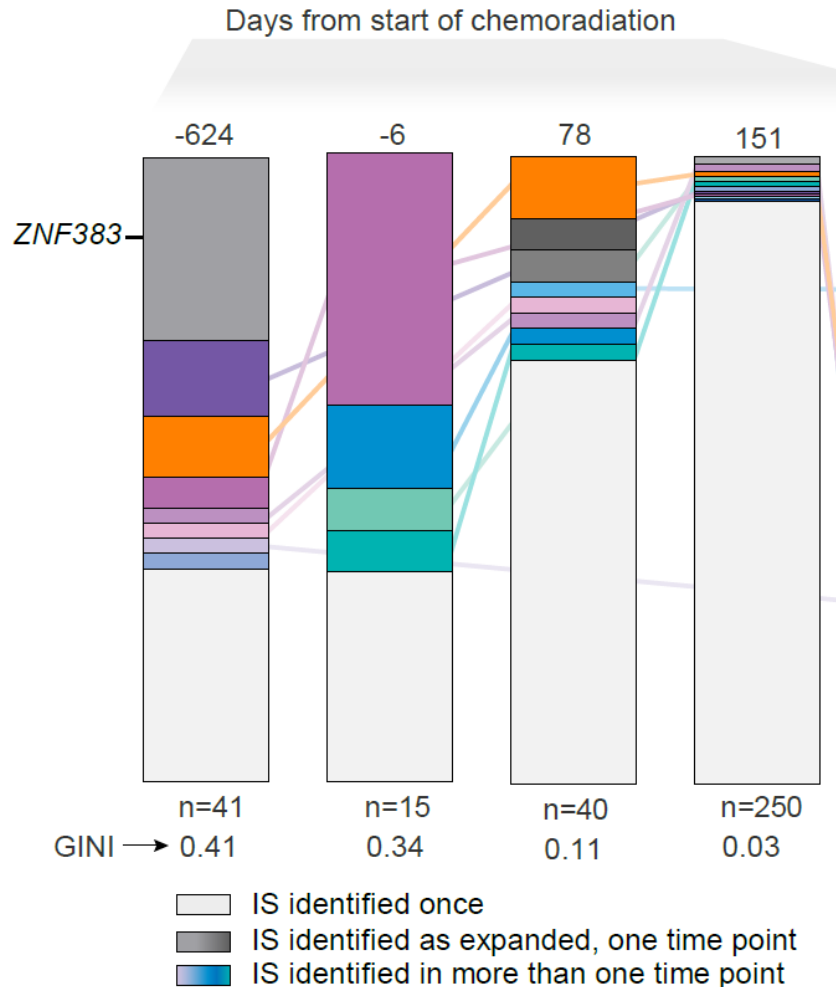
# Differential impact of HIV-specific CD8+ T cells probably explains the marked difference in the frequency of the clones in vivo



Transcriptional activity:	+	+++
Susceptibility to CD8+ cells	-	++

# Steady decline in clonal expansion during CRT

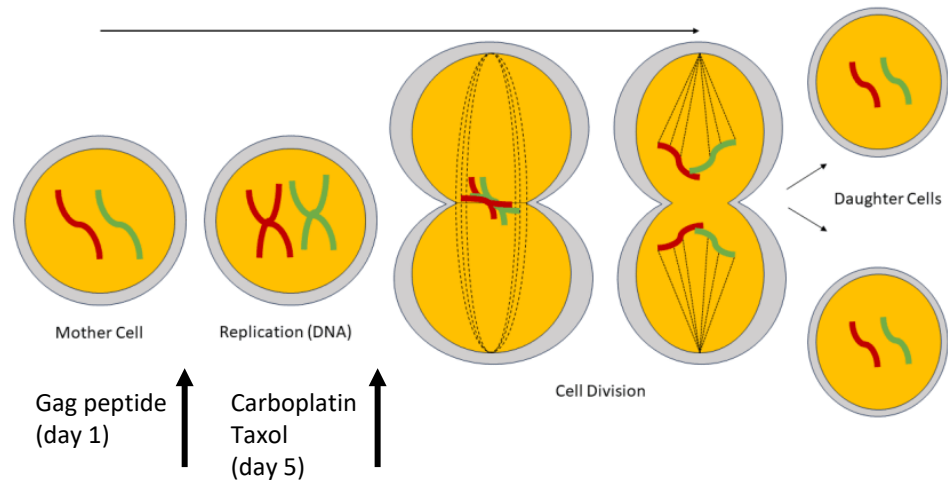
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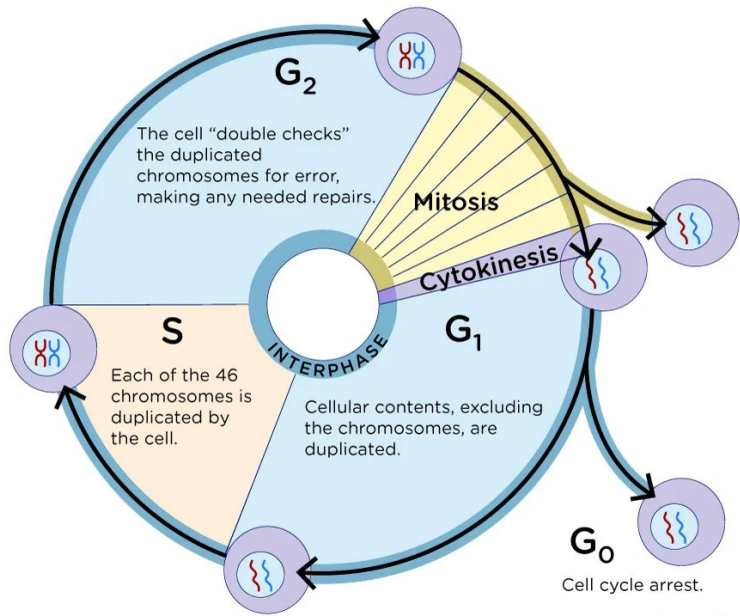
Hypothesis: Clones that were actively replicating were more susceptible to the chemotherapeutic drugs and were preferentially eliminated



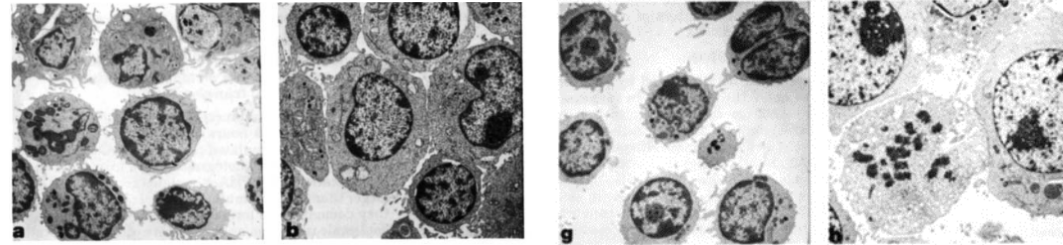
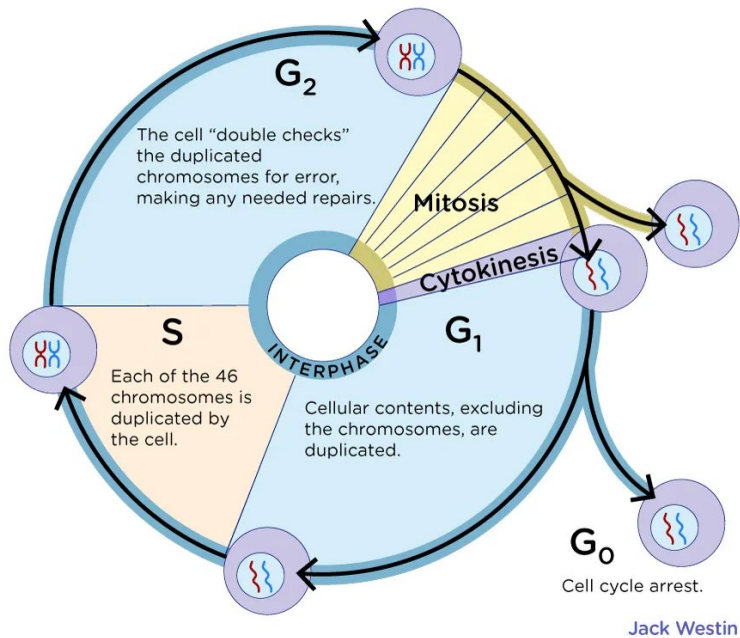
# Stimulate ZNF721 CD4+ T cell clone with cognate peptide Kill proliferating cells with chemo drugs



# Taxol inhibits microtubules resulting in spindle damage during mitosis



# Taxol does not prevent activation, but activated cells have increase in mitotic figures and will not survive another round of proliferation



Media

PHA

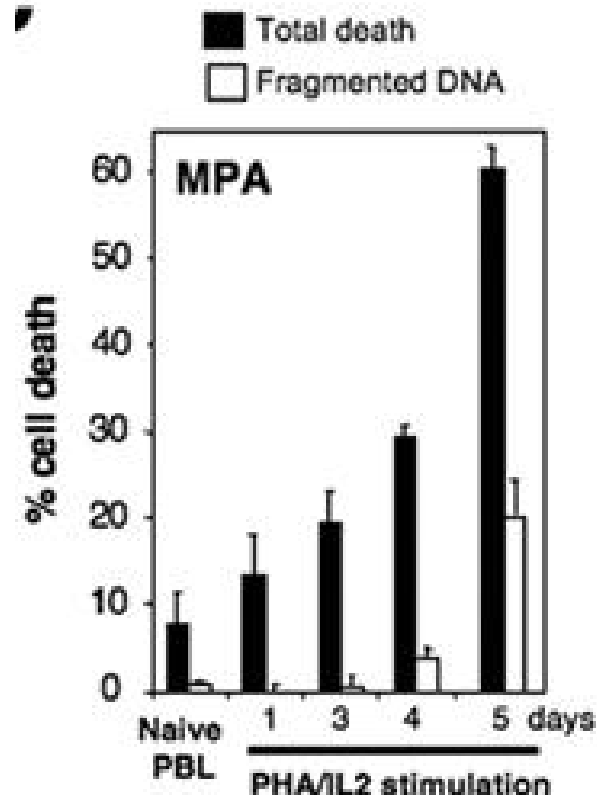
Taxol

PHA + Taxol

Cuthbert and Shay: J Cell Physiol 1983

# Mycophenolate mofetil (MMF) is a commonly used anti-proliferative drug

- MPA prevents resting cells from proliferating but kills activated cells



# Conclusions

- ES24 clonally expanded virus is fully susceptible to CD8+ T cells
- ES24 CD8+ T cells have shaped his proviral landscape
  - ZNF721 clone can expand in the presence of CD8+ T cells whereas proliferation of Chr7.d11 clone is impacted by CD8+ T cells
- Stimulation of clones with cognate peptide followed by treatment with an anti-proliferative agent may be an effective way to eliminate some clones
  - This strategy does not depend on latency reversal but you need to know the peptide the clone recognizes

# Acknowledgements



**BEAT-HIV**  
DELANEY COLLABORATORY

ES24!!!