Comparative effect of Various HDAC-inhibitors in-vitro on T-Cell Lymphoma cell lines alone and in combination with conventional anti-cancer drugs

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Introduction.

T-cell lymphomas are an uncommon and heterogeneous group of non-Hodgkin lymphomas.

Historically therapies for these diseases have been borrowed from treatments for other lymphomas.

More recently, efforts have be made to identify novel agents for their activity specifically in T-cell lymphomas.

A primary example of new agents with specific activity in T-cell lymphomas is the novel class of drug, histone deacetylase inhibitors.

Vorinostat and romidepsin are currently approved and are in clinical use for the treatment of cutaneous T-cell lymphomas.
Histones are core structural components of chromatin; DNA is wound around histones, and histones further associate to become and form chromatin.

Histone deacetylation inhibitors (HDAC) inhibitors induce accumulation of acetylated histones which leads to relaxation of chromatin structure and promotes access to transcriptional machinery and RNA polymerase.

HDACi also modify other cancer related proteins.
Chromatin Structure Regulates Transcriptional Activity

Histone Deacetylase Inhibitors (HDAC Inhibitors)

- Cause increased histone acetylation resulting in...
  - Uncoiling of chromatin and transcriptional activation of tumor suppressor genes leading to cell cycle arrest and/or apoptosis

Currently only Vorinostat is licensed for use in cutaneous T cell lymphoma (CTCL)
Genetic Variations and Epigenetic Changes Can Both Contribute to Oncogenesis

**GENETIC**
- DNA
  - Replication errors
  - Mutations/translocations
    - DNA sequence altered
    - Altered DNA/mRNA/proteins
    - Transformed cells
      - Oncogenesis

**EPIGENETIC**
- Chromatin
  - Enzyme modification errors
  - Open/closed chromatin
    - DNA sequence not altered
    - Altered mRNA/proteins
    - Transformed cells
      - Oncogenesis

Can be caused by:
- Abnormal modifications to histone proteins
- Abnormal DNA methylation
Deacetylation of Histones by HDAC Can Prevent Gene Expression

Acetylation by histone acetyltransferases (HATs) allows transcription and gene expression

Deacetylation by histone deacetylases (HDACs) can prevent transcription and gene expression

HAT

Transcription factors

Condensed chromatin

histones and other proteins

DNA strand

Deacetylated Histone
Closed chromatin
Transcription factors cannot access DNA

HISTONE ACETYLATION

Expanded chromatin

Acetylated Histone
Open chromatin
Transcription factors can access DNA

HISTONE DEACETYLATION

Ac: acetyl group
HDAC depicts a class I deacetylase
In Tumor Cells, Imbalanced HAT and HDAC Activity Can Result in Deregulated Gene Expression

Increased HDAC Activity

Decreased Tumor Suppressor Gene Activity (p21, p27)

Unchecked Cell Growth and Survival

Decreased HAT Activity

Tumor
HDAC Inhibition Restores Gene Expression in Tumor Cells

DAC Inhibition Increases Acetylation of Histones

Increased Tumor Suppressor Gene Activity (p21, p27)

Cell-Cycle Arrest and Differentiation

Normalized Cell

Growth arrest

TF: transcription factors

Ac: acetyl group

HDAC depicts a class I deacetylase
Deacetylase (DAC) Activity on Proteins is Associated with Downstream Effects that Promote Oncogenesis

Proteins modulated by DACs

Histone, p53, α-tubulin, HIF-1α, HSP90

Downstream effects

Tumor suppressor gene activity, Loss of tumor suppressor function, Microtubule depolymerization/aggresome formation, VEGF

Tumor effects

Cell-cycle arrest, Apoptosis, Cell motility and Invasion, Cell proliferation and survival, Angiogenesis, Oncoproteins
Pan-DAC Inhibition Interferes with the Multiple Hallmarks of Cancer

Proteins modulated by DACs

Histone
- Tumor suppressor gene activity
- Cell-cycle arrest
- Apoptosis

p53
- Loss of tumor suppressor function
- Cell motility and Invasion

α-tubulin
- Microtubule depolymerization/aggresome formation

HIF-1α
- VEGF
- Cell proliferation and survival

HSP90
- Oncoproteins

Downstream effects

Cell-cycle arrest
Apoptosis
Cell motility and Invasion
Angiogenesis

Tumor effects
Pan-DAC Inhibition May Have Potential in Several Cancers

Hematologic & Solid Tumors

Histone

DAC Inhibitor

α-tubulin

DACs

HIF-1α

p53

HSP90

Breast, Multiple Myeloma

RCC, Melanoma

CML, Breast, Prostate, NSCLC

50% of Cancers
Cells used

Loucy cell line: Loucy, was established from the peripheral blood of a patient with T-cell acute lymphoblastic leukemia.

HH Cell line: Cutaneous T- cell lymphoma.

SUP-T1: T-cell acute lymphoblastic leukemia
HDACi

Entinostat

Vorinostat Currently approved CTCL

LBH589

Doses: 100, 10, 1, 0.1, 0.01 and 0.001 in MM
Other Materials

RPMI
96 well plates.
Centrifuge tubes.
Cell counting chamber.
High power Microscope.
Colorimeter to measure fluorescence.
Alamar blue cell viability reagents.
Multi-channel micro- pipettes
Method.
Incubate each cell line with increasing dose of various HDACi
Continued.

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METHOD…..

100 Microliter of T-cell lines in each well.
100 microliter of HDAC inhibitor of various concentration or placebo was added to each well.
Incubate for 48 hour.
100 Microliter of Alamar blue was added after 48 hours of Incubation.
METHOD...

Plates were incubated for 4-6 hours with Alamar blue. Cell viability was measured by measuring fluorescence in each well using colorimeter. Fluorescence in each drugged well was compared with the placebo and the number was plotted for various concentration using SSPS software. SSPS software was used for analysis. Student’s t-test used for statistical analysis.
RESULTS
HH

P<0.05
LOUCY

![Graph showing % Viability vs. HDI Dose (uM)](image)

- Lines show Mean

P<0.05
SUP-T1

P<0.05
Combination of panobinostat with:

- Bortezomib
- Doxorubicin
- Cisplatin.
- Gemcitabine
The graphs show the effect of Panobinostat on the viability of cells in different conditions:

- **HH** and **Loucy** cell lines are tested at 0.00, 0.01, and 0.02 concentrations.
- **Supt 1** cell line is tested at 0.1 μM, 1 μM, and 10 μM concentrations.

Error bars display the mean ± 1.0 SE, and bars represent the means. The graphs indicate the percentage of viable cells for each condition.
Doxorubicin

HH

Loucy

Panobinostat

- 0.00
- 0.01
- 0.02

Error Bars show Mean +/- 1.0 SE

Bars show Means

% Viable Cells

% Viable Cells

RPMI

(0.16 μM)

(16 μM)

(16 μM)

chboro

Doxorubicin
Flow (Cell cycle studies)

Treated each cell line with three different HDACi for 24, 48 and 74 hours.
Supt1

![Cell cycle bar charts showing the effect of different drugs on cell cycle at 24, 48, and 72 hours.](chart.png)

Bars show Mean

**drug**
- control
- vorinostat
- entinostat
- panbinostat

**cell cycle**

- G1
- G2
- S
RESULTS:

All the three HDACi Exhibited potent killing effect on T-cell lymphoma cells lines in vitro.

Panobinostat is most potent of the HADCi studied and difference in activity was highly significant.

Panobinostat demonstrated additive killing effect in combination with Bortezomab and Doxirubacin. The additive effect is most likely due to different MOA.
Conclusion

The newer HDACi Panobinostat exhibited potent killing effect as compared to Vorinostat which is currently approved in the treatment T-cell lymphoma.

Combination with other anti-cancer drugs produced additive effect and holds promise for future.

The results would need to be validated by different method of assessing the killing effect and eventually testing on patient samples.
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