Both Immune Activation and Viral Load are Reduced Within 28 Days by VS411, the First AntiViral-HyperActivation Limiting Therapeutic (AV-HALT). A Phase II Study

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Background

Excessive immune activation drives HIV disease progression and HIV-associated pathologies even with successful, maximally suppressive HAART. Traditional antiretrovirals inhibit viral replication but do not directly address excessive immune activation.

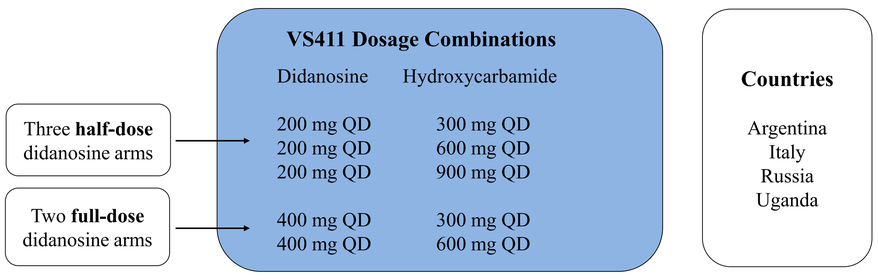
A new antiretroviral class, AV-HALTs (AntiViral-HyperActivation Limiting Therapeutics), has been proposed to reduce both viral load and excessive immune activation. VS411, the first-in-class NRTI-based AV-HALT, combines two approved medications - low-dose, slow-release 2',3'-dideoxyinosine (didanosine, ddI) and low-dose hydroxycarbamide (HC) – into a single once-daily capsule to accomplish both objectives with a favorable toxicity profile.

To test the Proof-of-Concept that an AV-HALT can both inhibit viral replication and directly reduce markers of excessive immune system activation, a 28-day Phase IIa study was fielded that not only followed traditional safety and efficacy parameters, but also incorporated measurements of four accepted markers of immune system activation – PD-1 (exhaustion), Ki-67 (proliferation), CD38 (activation), and HLA-DR (activation).

The results obtained in this study confirm the Proof-of-Concept that AV-HALTs have the potential to become a valuable tool in the management of HIV infection and encourage the testing of new compounds with similar properties and improved druggability.

Methods

This was a multinational, double-blind, 28-day Phase IIa study of 60 antiretroviral therapy-naïve HIV-1-infected adults randomized into five VS411 dose arms. Subjects were randomized in a 1:1:1:1:1 ratio into one of the following five dose pairs of didanosine and hydroxycarbamide as VS411 daily for 28 days.



Traditional parameters of safety and efficacy were followed. Post-study follow-up continued for an additional 14 days to gather data on viral rebound and further safety data. Fifty-eight evaluable subjects were analyzed for safety, viral load reduction, changes in $CD4^+$ cell counts, activation/proliferation markers, and HIV-specific T cell responses by flow cytometry and ELISPOT analysis.

Results were analyzed using ANOVA, Kruskal-Wallis, ANCOVA, and two-tailed paired *t* tests.

Results

A total of 58 evaluable subjects* were enrolled and analyzed (ITT-exposed).

Baseline Characteristics

Median $CD4^+$: 406.5 cells/mm^3 Median Viral Load: 4.48 $\log_{10}$ copies/mL (30,200 copies/mL)

50% Female 50% Male 50% Black 43.1% Caucasian 6.9% Hispanic

Mean Age: 34.2 yrs Mean Weight: 72.5 kg

* One subject discontinued after first dose due to severe dog bite;
One subject withdrew consent upon electing to breastfeed her newborn

VS411 was safe and well-tolerated

There were no serious adverse events (SAEs) or discontinuations due to study drug, nor were there major differences between arms in terms of clinical or laboratory safety. A total of 83 Adverse Events (AEs) were experienced by 36 of the 58 evaluable subjects. Interestingly, the total number of AEs was similar between the 25 subjects receiving full-dose didanosine and 33 subjects receiving half-dose didanosine, that is, 72% of subjects receiving full-dose didanosine versus 54% of subjects receiving half-dose didanosine experienced an AE.

Number of AEs reported per study arm (% of subjects within arm)

ddI/HC 200mg/300mg	ddI/HC 200mg/600mg	ddI/HC 200mg/900mg	TOTAL AEs
8 (66.7%)	6 (54.5%)	4 (40.0%)	18 (54.5%)
ddI/HC 400mg/300mg	**ddI/HC 400mg/600mg**	**TOTAL AEs**	
8 (66.7%)	10 (76.9%)	18 (72.0%)	

There were only two severe Adverse Events:

-One subject *(ddI 400 mg/HC 600 mg)* experienced grade 3 *neutropenia* at Visit 5 (Day 8); however, the subject reduced to grade 1 at Visit 6 (Day 15) with no change in therapy.
The subject was experiencing grade 2 neutropenia at baseline, prior to receiving VS411.

-One subject *(ddI 400 mg/HC 300 mg*) severely *bitten by a dog* discontinued from the trial.

There were no significant signals in laboratory chemistry or hematological parameters.

VS411 did not select for nucleoside resistance in any study arm

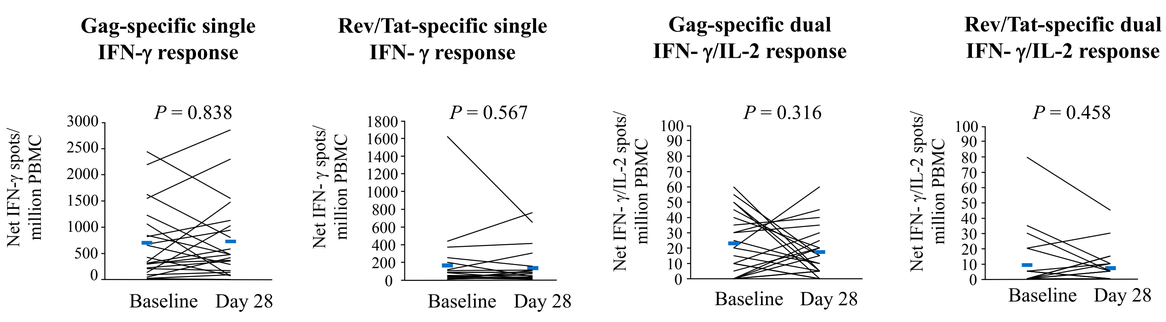
No changes in nucleoside analogue sensitivity were detected utilizing genotypic testing performed before and following 28 days of therapy with VS411.

This result was expected as, unlike traditional antiretrovirals, hydroxycarbamide targets a highly conserved ***human*** cellular enzyme (ribonucleotide reductase) with little, if any, potential for resistance.[1]

Moreover, viruses genotypically resistant to didanosine regain phenotypic sensitivity in the presence of hydroxycarbamide.[2, 3]

VS411 had no immunosuppressive effect on HIV-specific immune responses

There were no significant changes in the subjects' ability to respond against the HIV antigens Gag, Rev and Tat.



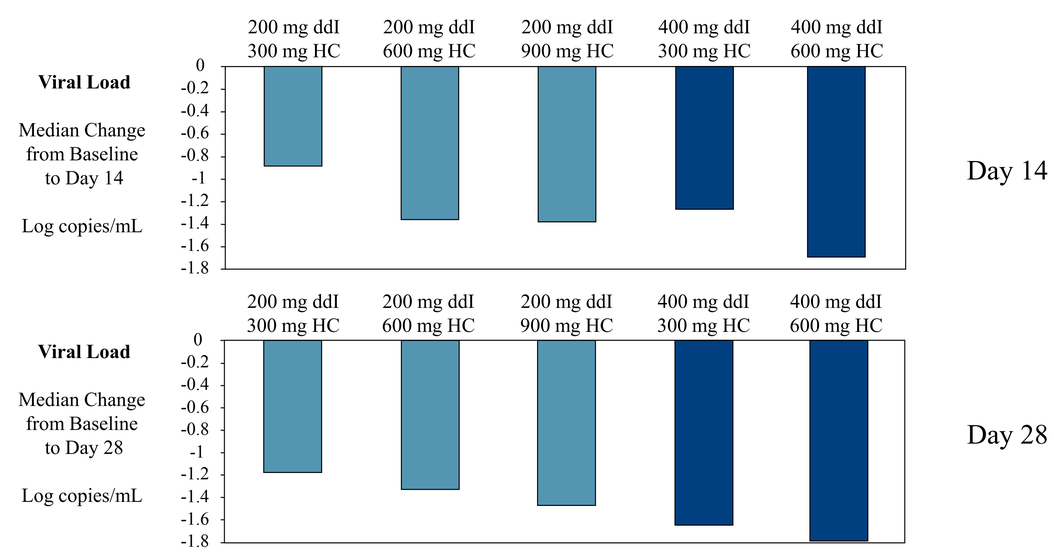
Blue bars indicate mean values

VS411 produced significant (P < .001) viral load reductions after 14 and 28 days

Viral load reductions were seen in each of the five VS411 dose arms. The magnitude of the reduction increased from Day 14 to Day 28. There was a trend toward greater viral load reductions with increasing doses of both didanosine and hydroxycarbamide.

Only 2 of 58 evaluable subjects (3 %) achieved viral load reductions of < 50 copies/mL by Day 28.

Median viral load reduction across all study arms at Day 28 was -1.47 $\log_{10}$

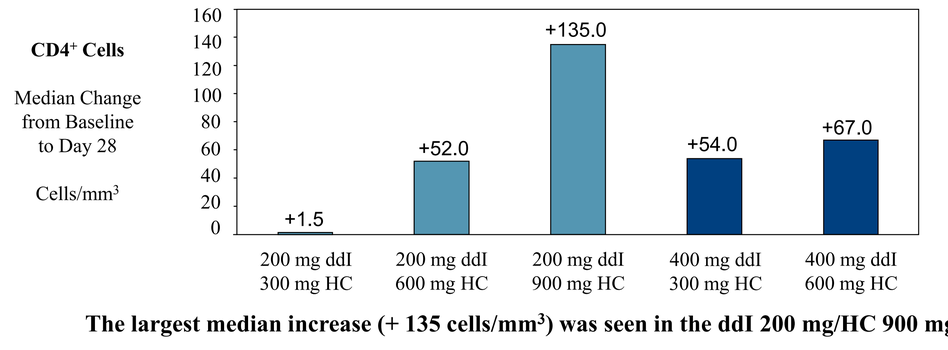


VS411 produced significant (P <.002) $CD4^+$ cell count increases

Reducing the doses of hydroxycarbamide contained in VS411 from those traditionally administered allowed the agent to act as a cytostatic, rather than cytotoxic, drug.

As a result, the "blunting" of $CD4^+$ cell increases historically seen with the didanosine/hydroxycarbamide combination was not seen with VS411.

Median CD4+ cell increase across all study arms was +53.0 cell/mm^3

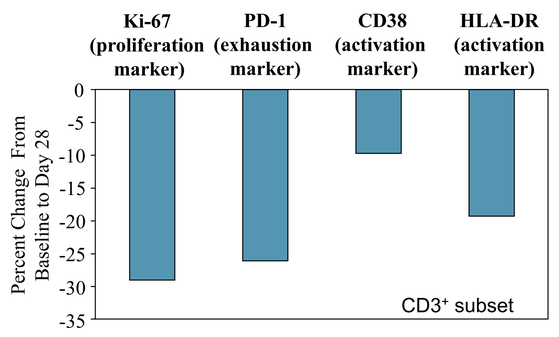


The largest median increase (+ 135 cells/mm^3) was seen in the ddI 200 mg/HC 900 mg arm

VS411 produced rapid and significant immune activation marker reductions

In a sub-study of 32 subjects, rapid and significant reductions in the four markers of excessive immune system activation were seen at Day 28.

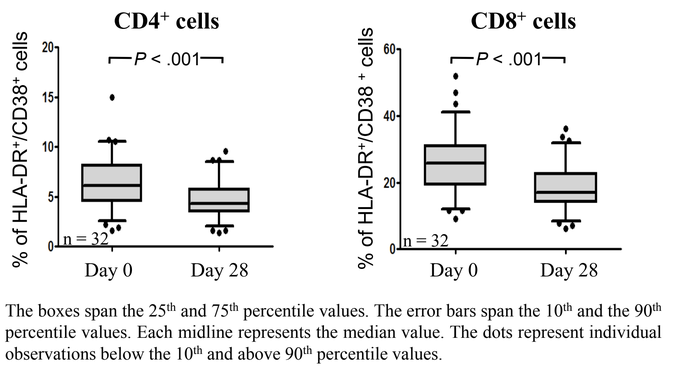
$CD3^+$ Subset (n=32)	Baseline (mean)	Day 28 (mean)	*P*
Ki-67%	3.1 ± 1.7	2.2 ± 0.9 **-29.0%**	<0.005
PD-1 %	15.3 ± 6.1	11.3 ± 5.0 **-26.1%**	<0.005
CD38 %	58.5 ± 14.8	52.8 ± 14.2 **-9.7%**	<0.005
HLA-DR %	27.5 ± 10.1	22.2 ± 7.5 **-19.3%**	<0.005



These reductions were obtained despite the fact that VS411 did not attain maximal viral load suppression over this time, suggesting that the effect was due to the HALT component of this AV-HALT combination.

VS411 produced rapid reductions in $CD4^+$ / $CD8^+$ cells co-expressing CD38/HLA-DR

In a sub-study of 32 subjects receiving VS411 for 28 days, rapid and statistically significant reductions were seen in the number of $CD4^+$ and $CD8^+$ cells co-expressing the two markers of cellular activation, CD38 and HLA-DR. At Day 28, the percentage of $CD4^+$ and $CD8^+$ cells co-expressing the two markers decreased by 28.9 percent and 34.4 percent, respectively.



$HLA\text{-}DR^+/CD38^+$ cells Median percentage

	Baseline	Day 28
$CD4^+$	6.09 %	4.33 %
$CD8^+$	25.87 %	16.98 %

$CD4^+$ cell population:
A 28.9% decrease at Day 28

$CD8^+$ cell population:
A 34.4% decrease at Day 28

The boxes span the 25th and 75th percentile values. The error bars span the 10th and the 90th percentile values. Each midline represents the median value. The dots represent individual observations below the 10th and above 90th percentile values.

These reductions were obtained despite the fact that VS411 did not attain maximal viral load suppression, suggesting the effect was due to the HALT component of this AV-HALT combination.

Conclusions

VS411 represents the first entry in a new class, AV-HALTs, combining antiviral efficacy with novel, potentially beneficial reductions in the excessive immune system activation associated with HIV disease progression.

In this study, VS411 achieved Proof-of-Concept for the novel AV-HALT class. VS411 was well-tolerated, increased $CD4^+$ counts and reduced viral load (antiviral effects), and reduced T cell activation and proliferating (Ki-67$^+$) $CD4^+$ T cells (hyperactivation limiting effects) without suppressing HIV-specific immune responses. Specifically,

- **VS411 safely lowered HIV replication over 28 days by 1.5 logs without inducing resistance**
- **VS411 significantly increased $CD4^+$ T cell counts over 28 days, up to +135 cells/mm^3**
- **VS411 rapidly and significantly reduced markers of excessive immune activation over 28 days**
  This was achieved despite incomplete viral load suppression and without suppressing HIV-specific immune responses
- **The didanosine 200 mg/hydroxycarbamide 900 mg QD formulation (doses lower than traditionally investigated) demonstrated the greatest $CD4^+$ cell increase (+135 cells/mm^3), fewest Adverse Events, and a viral load reduction of 1.47 $\log_{10}$ at Day 28**
- **These results were achieved in a population mirroring the global pandemic: 50% female and 50% black from both the northern and southern hemispheres**

Based upon this successful Proof-of Concept study, additional work is underway to identify and develop new agents that combine both attributes of AV-HALTs in a single molecule.

Literature cited

1. Donehower RC. 1992. An overview of the clinical experience with hydroxyurea. *Seminars in Oncology* 19(3 Suppl 9): 11-9
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