The HIV-1 splicing inhibitor, SPL-464, compromises viral replication in vitro and induces a long lasting anti-viral effect in humanized mice infected with HIV-1

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Alternative splicing and HIV life cycle
Viral RNA

Viral entry

Integration

Reverse transcription

Viral DNA

ARN viral

Protease

Viral structural proteins

Splicing

Non splicing

Regulatory proteins (Tat and Rev)

Alternative splicing initiates HIV replication
Alternative splicing is a key process for HIV replication
Late transcripts

classe 4 kb

classe 9 kb

Early transcripts

classe 2 kb

Translation

Viral proteins
(Env/Vpu, Vif, Tat, Vpr)

Precursors

Nef
HIV-1 RNA has weak 3’ splice sites

Consensus

\[ Y(n)\quad C\quad U\quad AG\quad G \]

A1
\[ AAUUUUCGGUUUAUUACAG\quad G \]
A2
\[ UAUUACUUUGACUGUUUUUCA\quad A \]
A3
\[ ACACUGCUUUUAAUCCAUUCCAG\quad A \]
A4a
\[ AGUUUGUUUCACAAACAAAG\quad C \]
A4b
\[ AGUUUGUUUCACAAACAAAGCCUUAAG\quad G \]
A4c
\[ AAGUGUUGCUUAUUGCCCAAG\quad U \]
A5
\[ AGUUUGUUUCACAAACAAAGCCUUAAG\quad G \]
A7
\[ GGAAUUAUCAUUCUACGUUUCAG\quad A \]
HIV-1 RNA splicing depends on splicing regulators that target HIV-1 RNA sequences
Splicos has a proprietary library of small chemical compounds targeting the splicing machinery
Lead SPL-464 characterization
SPL-464 efficacy and cytotoxicity on PBMCs

Inhibition of HIV-1 p24 production (%) vs. Concentration (nM)

Viability (%) vs. Concentration (nM)
• SPL-464 induces a dose-dependent inhibition of HIV-1 replication in primary macrophages from different donors

• SPL-464 inhibits viral replication of different HIV-clades including B and C types

• SPL-464 inhibits viral replication of ART escape mutants

• SPL-464 inhibits viral replication of HIV-2

• After six months of *in vitro* treatment with SPL-464 no resistant viruses have emerged, whereas drug-resistant viruses are selected following three weeks of treatment with either 3TC or EFV
Deep sequencing of YU-2 virus after SPL-464 treatment did not reveal any selected mutation.
Profiling cellular alternative splicing events shifted by Splicos drugs

Robotic liquid handling

High throughput capillary electrophoresis

‘Percent Spliced In’, psi or Ψ

\[ \Psi = \frac{[\text{LONG}] \times 100\%}{[\text{LONG} + \text{SHORT}]} \]
SPL-464 did not induce global changes of alternative splicing in PBMCs.
Efficacy of SPL-464 in mouse models
SPL-464 HIV-1 inhibition on hu-PBL-SCID mouse after 40 mg/kg/day treatment by twice-daily per os administration started simultaneously to HIV-1 infection.
Treatment with SPL-464 rescues CD8/CD4 ratio in infected mice
SPL-464 (40mg/kg daily by gavage) reduces viral loads in engrafted humanized NSG mice infected by YU2 HIV-1 strain. Comparison with ART
Long lasting HIV-1 inhibitory effect of SPL-464 in infected humanized mice.
Summary

- SPL-464 is a novel anti-HIV agent active against different HIV-1 clades and mutants as well as HIV-2
- SPL-464 did not induce emergence of HIV-1 mutants
- SPL-464 has a new mode of action inhibiting HIV-1 splicing but not splicing of cellular genes
- SPL-464 induces a long lasting effect in humanized mice
- SPL-464 rescues CD8/CD4 ratio in infected humanized mice
Anti – HIV therapeutic strategies

- Entry Inhibiteur
- Integrase Inhibitors
- Reverse transcriptase inhibitors
- Protease Inhibitors
- Viral entry
- Intégration
- Splicing
- Non splicing
- ARN viral
- Protease
- Viral structural proteins
- Regulatory proteins (Tat and Rev)
- SPL-464
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