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Abyssomicin 2 Reactivates Latent HIV-1 by a PKC- and HDAC-Independent Mechanism

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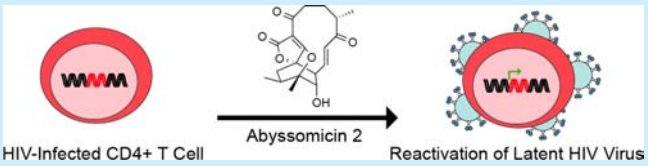
Supporting Information

ABSTRACT: Screening of a marine natural products library afforded three new analogues of the tetronic acid containing polyketide abyssomicin family and identified abyssomicin 2 as a selective reactivator of latent HIV virus. Examination of the mode of action of this new latent HIV reactivating agent demonstrated that it functions via a distinct mechanism compared to that of existing reactivating agents and is effective at reactivating latent virus in a subset of primary patient cell lines.



Highly active antiretroviral therapy (HAART), a combination of drugs that halts viral proliferation by mechanistic inhibition at different stages of the viral lifecycle, is the leading treatment for patients infected with human immunodeficiency virus (HIV). While a major accomplishment in managing the progression of the disease, HAART is not a curative therapy since it does not address the persistence of genomically integrated latent viral reservoirs, therefore requiring life-long patient treatment. There are concerns over increased incidence of heart disease, diabetes, and bone density reduction in patients on long-term HAART resulting from either low-level viremia or due to the specific treatment regime.[1] A promising strategy to achieve a cure for HIV is to reactivate the latent provirus in combination with HAART.[2] An example of this strategy is highlighted in prostratin (Supporting Information, Figure S2), a known latent HIV reactivator,[3] that is currently under consideration for investigative new drug (IND) status, with the goal of producing a treatment to cure HIV-infected patients.

While a number of potential pathways exist for the reactivation of latent HIV,[2] published reactivating agents have been predominantly limited to histone deacetylase (HDAC) inhibitors,[4] agonists of transcription elongation factors,[5] and protein kinase C (PKC) agonists (Supporting Information, Figure S2).[3,6] Of these known HIV reactivators, most have dose-limiting safety issues and/or unproven clinical efficacy.[7] The limited number of pathways that have been targeted highlight the importance of finding new latent-HIV reactivating agents, as the discovery of new leads could identify novel targets and methods of reactivation and could lead to noncytotoxic, clinically effective treatments for HIV infections.

To identify new HIV reactivating agents, we screened a microbially derived prefractionated natural products library using a model of *in vitro* HIV latency established in primary human CD4$^+$ T cells.[8,9] Examination of a number of prefractions from this screening campaign led to the identification of abyssomicin 2 (**2**) as a novel activator of latent HIV and to the discovery of three new abyssomicin analogs, abyssomicin 3−5 (**3** - **5**), from a marine-derived actinobacterium *Streptomyces* sp. (Figure 1). To orthogonally confirm reactivation activity, abyssomicin 2 activity was verified by RT-qPCR analysis of HIV RNA, which validated the reactivation activity originally identified via the primary screen. The mechanism by which compound **2** reactivates latent HIV

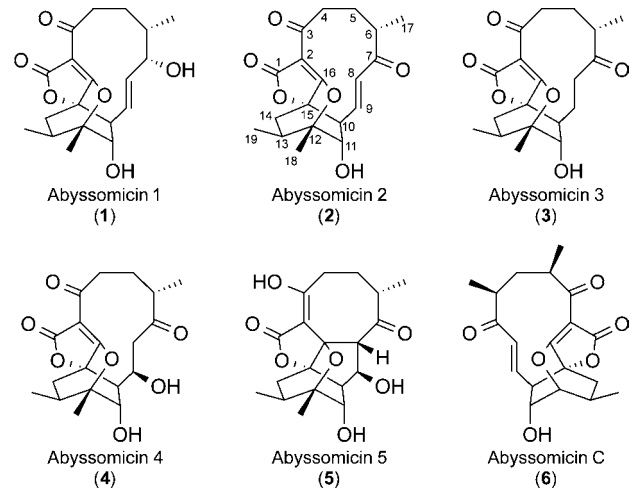


Figure 1. Abyssomicin analogues: abyssomicins **2**−**5** isolated as part of this study.

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was investigated through preliminary mechanistic assays involving chromatin remodeling (HDAC) and PKC activity. These experiments indicated that abyssomicin 2 possessed no activity in either mechanistic assay, suggesting a unique mode of action for reactivation of latent HIV. *Ex vivo* studies using $CD4^+$ cells isolated from HIV patients under HAART treatment demonstrated that abyssomicin 2 is capable of partial viral reactivation against natively infected cells, with two of the five donor T cell isolates showing reactivation of latent virus. These results offer encouragement for the application of small molecule-based approaches for the clearance of persistent HIV infections. The abyssomicins represent a novel structural class of reactivating agent with *ex vivo* activity through an HDAC- and PKC-independent mechanism, making them intriguing from a mechanistic perspective.

The abyssomicin class of natural products contains two related families: abyssomicins B−L, derived from microorganisms of the genus *Verrucosispora*, and abyssomicins 2−5 derived from microorganisms of the genus *Streptomyces*. Abyssomicins B−D were initially reported in 2004 as part of a screening campaign to identify *p*-aminobenzoic acid biosynthesis inhibitors, and their structures and absolute configurations were determined via a combination of NMR, X-ray, and chemical derivatization studies.[10] Subsequently, additional analogues were reported from biosynthetic studies as well as through bacterial screening campaigns.[11,12] Abyssomicin I and a synthetic analogue were discovered in 2010 as part of a program to identify novel metabolites from terrestrial Actinobacteria, and their structures were determined via a combination of NMR and chemical derivatization studies.[13] Since their discovery, the abyssomicin class has been explored through biosynthetic evaluation,[14] total synthesis,[15] and pharmacological studies surrounding abyssomicin C's promising antibacterial activity.[11,16] Here, we report the first identification of latent HIV reactivating activity for the abyssomicins.

Briefly, the natural product screening workflow consisted of three screening stages. First, a microbially derived prefractionated natural product library (5000 prefractions) was screened in triplicate at seven different concentrations in an *in vitro* model for HIV reactivation to identify primary hits capable of reactivating latent HIV. After identifying natural product prefractions that possessed reactivating activity, prefractions were then subjected to "peak library" LC−MS fractionation into deep-well 96-well plates. In this process, constituents in the prefraction were separated into individual wells, with simultaneous acquisition of mass spectrometric and UV absorbance data on each constituent. Peak library plates were then subjected to rescreening in the primary assay, and compounds that recapitulated the original activity were isolated and identified using standard structure elucidation methods. Through this screening workflow, we identified prefraction RLUS1487A as possessing reproducible reactivating activity in the primary assay. Peak library analysis indicated that four chemical constituents, sharing similar UV profiles, accounted for the observed biological activity. The potencies of the purified compounds were reassessed under original assay conditions, and from these data, compound **2** was prioritized for follow-up studies based on its robust reactivating activity.

On the basis of HRESITOF-MS and ^{1}H NMR data, the isolated material for compound **2** initially appeared to be identical to a previously published synthetic derivative of abyssomicin I.[13] Detailed analysis of 1D and 2D NMR data (see the Supporting Information) confirmed that compound **2** and the published structure shared the same chemical connectivities and were constitutionally identical. However, single-crystal X-ray analysis using a Cu irradiation source revealed that compound **2** was enantiomeric to the reported structure (Figure 2A). To confirm this assignment, the (*R*)- and (*S*)-

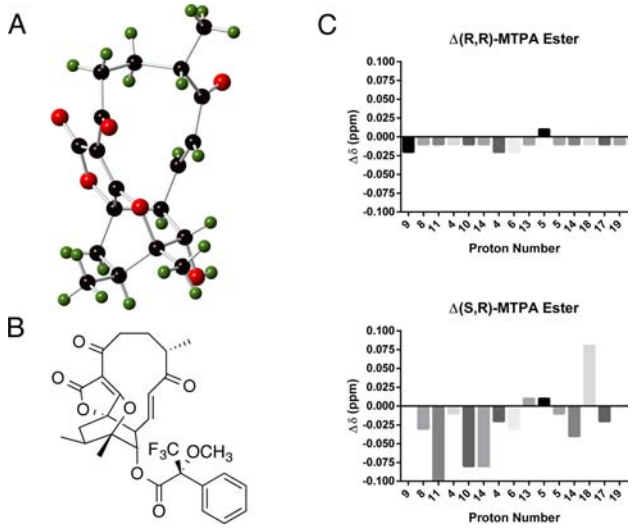

Figure 2. (A) X-ray crystal structure diagram for **2**; (B) structure of (*R*)-MTPA ester of **2**; (C) plots of differences in ^{1}H chemical shifts between published (*R*)-MTPA ester of the synthetic analogue of abyssomicin I and the (*R*) and (*S*)-MTPA ester of **2**.

MTPA esters of **2** (Figure 2B) were prepared and the ^{1}H NMR chemical shift values compared to the reported values for the (*R*)-MTPA ester from the original isolation (Figure 2C). The data from our (*R*)-MTPA ester matched those for the literature compound, confirming that the two Mosher's derivatives were identical. We therefore assigned the absolute configuration for abyssomicin 2 as 6*S*,10*S*,11*S*,12*S*,13*S*,15*S*. Additional review of the original isolation data revealed that while the C7 alcohol (abyssomicin I) was correctly assigned as *S*, the core scaffold was originally proposed with the incorrect absolute configuration. We therefore also reassign this structure from abyssomicin I to abyssomicin 1 (for full discussion of configurational reassignments, see the Supporting Information, Figure S3).

On the basis of HRESITOF-MS and ^{1}H NMR data, compound **3** appeared to be a novel analogue of abyssomicin 2, with a molecular formula of $C_{19}H_{24}O_6$ (a gain of 2H with respect to compound **2**). To determine the structure of compound **3**, 1D and 2D NMR experiments were acquired. Analysis of these data (COSY, TOCSY, and HSQC) revealed that compound **3** had four matching spin systems to **2** (see the Supporting Information). However, the α,β-unsaturated ketone-containing spin system was substituted by a hydrogenated derivative. The replacement in carbon and proton chemical shifts at positions 8 and 9 from sp^2- to sp^3-hybridized carbons corroborate the reduction across the α,β-unsaturated ketone (Supporting Information, Table S1). The absolute configuration of **3** was inferred by biosynthetic arguments, given the likely common biosynthetic origin for compounds **2** and **3**.

Compound **4** possessed an HRESITOF-MS adduct consistent with a molecular formula of $C_{19}H_{24}O_7$, differing from

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compound **2** by a gain of H_2O, indicating that compound **4** was also a novel abyssomicin analogue. ^{1}H NMR of compound **4** (Supporting Information, Table S1) was similar to that for compound **2**, suggesting the presence of a closely related analogue. Notably, a replacement of the vinylic protons at C8 and C9 was observed, with a corresponding gain of both an oxygenated methine and a diastereotopic methylene. COSY and TOCSY data revealed that these new protons were part of the spin system corresponding to carbons 8−11, with the oxygenation on C9. Relative configuration at the C9 hydroxyl was determined using the coupling constants $^3J_{H8-H9}$ = 8.2, $^3J_{H8'-H9}$ = 1.4 Hz, indicative of a dihedral angle for H_8-H_9 $|\phi|$ > 140° and $H_{8'}-H_9$ $|\phi| \approx 90°$. Furthermore, NOE correlations between H6 to H8 and H8 to H11 support these protons being on the same face. Another NOE correlation between H9 and H10 confirms these protons are on the opposite face, therefore suggesting H8 is syn to the hydroxyl group at C9. Through biosynthetic stereospecificity arguments, we can infer the absolute stereochemistry of **4** to be identical to **2**, therefore assigning the absolute configuration at C9 as *R*.

Finally, compound **5** possessed the same molecular formula as abyssomicin 4 ($C_{19}H_{24}O_7$) but differed in the UV profile by the absence of an absorbance at 215 nm, indicative of modification of the *β*-keto ester motif. Examination of the 1D and 2D NMR spectra revealed a change in the chemical shift of the carbon at C16, rearrangement of the olefin at C2−C16 to C2−C3, and conversion of the diastereotopic methylene at C8 to a corresponding methine. Key HMBC correlations from H8 to C7, C9, and C16 were supportive of an intramolecular Michael addition. In an analogous fashion to compound **4**, NOE correlations between H6 to H8 and H8 to H11 were observed. Together with detailed evaluation of COSY, HSQC, and HMBC data (see the Supporting Information), these results suggested that compound **5** was the cyclized analogue of compound **4**, a structure that has prior precedent in the abyssomicin B−L series.[11]

Compound **2** possessed the highest *in vitro* reactivation activity among the abyssomicin analogues tested, with a maximum reactivation of 56% (normalized to SAHA total activity) and an EC_{50} of 13.9 *μ*M (Figure 3A). Compounds **3** and **4** displayed only marginal activities (data not shown), indicating that the Michael acceptor at C8/9 is a critical structural feature for HIV reactivation activity. Since the primary screen is based on a reporter gene assay, false positives could arise from the compound-induced luminescence signal. To verify activity of compound **2** on reactivating latent HIV we performed RT-qPCR analysis of viral RNA (Figure 3B). Viral RNA concentration in $CD4^+$ cells latently infected with HIV increased in a dose-dependent manner when treated with compound **2**, with an EC_{50} value of 10.5 *μ*M, equivalent to its potency in the reporter gene assay. These data indicate that HIV reactivation is a direct result of treatment with compound **2** and that such treatment can induce the upregulation of HIV expression under the established *in vitro* conditions.

It has been almost two decades since the introduction of HAART, yet HIV is still a global pandemic requiring life-long drug treatment for patients. Reactivation of latent HIV is seen as an important component for developing a cure for HIV; however, only a few compounds have been validated as reactivating agents in *ex vivo* studies from HIV-positive patients. Currently, the best validated *ex vivo* HIV reactivating agents with therapeutic potential in the clinic are modulators of HDAC or PKC.[9,17,18] To explore the mode of action of

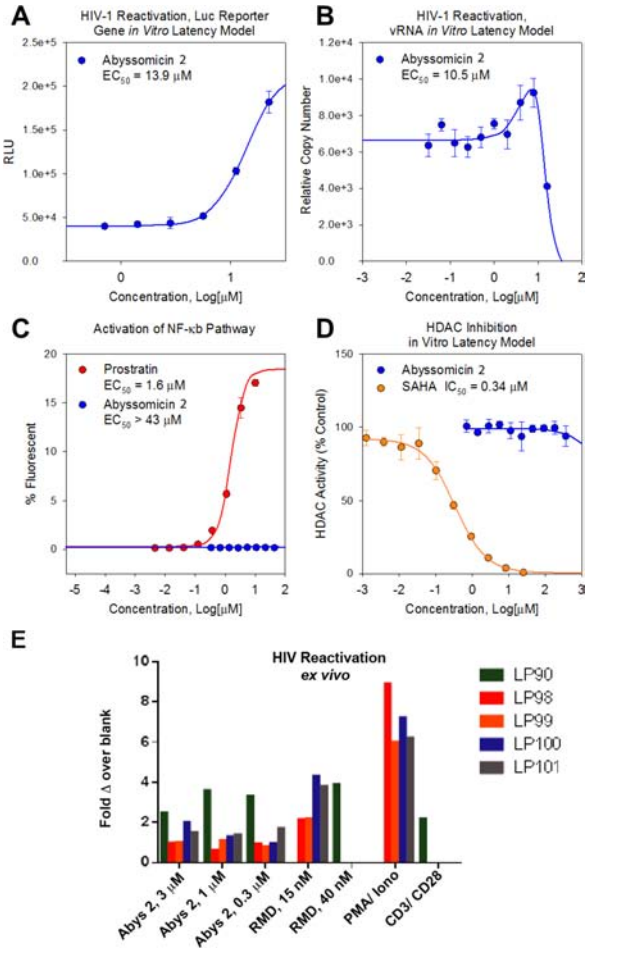

Figure 3. Reactivation of latent HIV by abyssomicin 2 (**2**). HIV reactivation an *in vitro* latency model measured by (A) luciferase reported gene activity and (B) RT-qPCR of HIV vRNA following 48 h of incubation with compound **2**. (C) NF*κ*B activation assay. GFP-based NF*κ*B reporter gene activity was quantified by FACS analysis in cells following 48 h incubation with compounds as indicated. (D) HDACi dose response curves. (E) Viral RNA levels in HIV positive patient cells treated with compound(s) *ex vivo* (experiment 1 with donor LP90 and romidepsin (RMD) and anti-CD3/CD28 antibodies positive controls, experiment 2 with donors LP98 to LP101 and PMA (50 ng/mL)/ionomycin (500 ng/mL) positive control).

abyssomicin 2, we tested compound **2** in both PKC activation and HDAC inhibition assays (Figures 3C,D). These results indicate that compound **2** has no activity in either assay up to the highest concentration tested, suggesting that the mechanism of action is independent of both PKC activation and HDAC inhibition.

Although a number of compounds have previously been reported as reactivators of latent HIV using *in vitro* latency models, only a few classes retain this activity *ex vivo* in cells isolated from HIV-infected patients with fully suppressed viral replication. To examine whether abyssomicin 2 was effective under these more relevant *ex vivo* conditions, we employed a reactivation assay that uses primary cells from HIV-positive donors under HAART treatment. In two separate experiments, treatment of patient cells, followed by quantification of vRNA levels in both cells and culture medium, revealed that compound **2** induced greater than 2-fold cell-associated vRNA increase in the cells of two of the five donors tested (Figure 3E). Although this narrow spectrum of activity limits

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the potential clinical utility of the native natural product, further characterization of the biological activity of the abyssomicins has the potential to reveal a novel mechanism for latent HIV reactivation. This partial reactivation activity has also been identified as a property of JQ1, a latent HIV reactivator by inhibition of BRD4,[5] suggesting that future development may yield insights into HIV latency mechanisms.

In conclusion, we report the identification of four abyssomicin analogues, including three novel compounds, as noncanonical reactivators of latent HIV, and present the reassignment of the absolute configuration of this compound class. Using a viral reactivation assay employing primary cells from HIV-positive donors under HAART treatment, we demonstrate that the most potent of these, abyssomicin 2, reactivated latent virus in some patient cells. The mechanism by which abyssomicins reactivate latent HIV remains to be elucidated and may lead to new target identification as well as better understanding of HIV latency maintenance.

■ ASSOCIATED CONTENT

Supporting Information

Materials and methods, comprehensive HIV screening information, and structural elucidation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

§These authors contributed equally.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Florescu, D.; Kotler, D. P. *Antiviral Ther.* **2007**, *12*, 149−162. (b) Weber, R.; Sabin, C. A.; Friis-Møller, N.; Reiss, P.; El-Sadr, W. M.; Kirk, O.; Dabis, F.; Law, M. G.; Pradier, C.; De Wit, S.; Akerlund, B.; Calvo, G.; Monforte, A. D.; Rickenbach, M.; Ledergerber, B.; Phillips, A. N.; Lundgren, J. D. *Arch. Int. Med.* **2014**, *166*, 1632−1641. (c) Bedimo, R. *Curr. HIV/AIDS Rep.* **2008**, *5*, 140−149. (d) Mondy, K.; Tebas, P. *Annu. Rev. Med.* **2007**, *58*, 141−155.

(2) Richman, D. D.; Margolis, D. M.; Delaney, M.; Greene, W. C.; Hazuda, D.; Pomerantz, R. J. *Science* **2009**, *323*, 1304−1307.

(3) Kulkosky, J.; Culnan, D. M.; Roman, J.; Dornadula, G.; Schnell, M.; Boyd, M. R.; Pomerantz, R. J. *Blood* **2001**, *98*, 3006−3015.

(4) Contreras, X.; Schweneker, M.; Chen, C.-S.; McCune, J. M.; Deeks, S. G.; Martin, J.; Peterlin, B. M. *J. Biol. Chem.* **2009**, *284*, 6782−6789.

(5) Zhu, J.; Gaiha, G. D.; John, S. P.; Pertel, T.; Chin, C. R.; Gao, G.; Qu, H.; Walker, B. D.; Elledge, S. J.; Brass, A. L. *Cell Rep.* **2012**, *2*, 807−816.

(6) DeChristopher, B. A.; Loy, B. A.; Marsden, M. D.; Schrier, A. J.; Zack, J. A.; Wender, P. A. *Nat. Chem.* **2012**, *4*, 705−710.

(7) Bullen, C. K.; Laird, G. M.; Durand, C. M.; Siliciano, J. D.; Siliciano, R. F. *Nat. Med.* **2014**, *20*, 425−429.

(8) Bosque, A.; Planelles, V. *Methods* **2011**, *53*, 54−61.

(9) Wei, D. G.; Chiang, V.; Fyne, E.; Balakrishnan, M.; Barnes, T.; Graupe, M.; Hesselgesser, J.; Irrinki, A.; Murry, J. P.; Stepan, G.; Stray, K. M.; Tsai, A.; Yu, H.; Spindler, J.; Kearney, M.; Spina, C. A.; McMahon, D.; Lalezari, J.; Sloan, D.; Mellors, J.; Geleziunas, R.; Cihlar, T. *PLoS Pathog.* **2014**, *10*, e1004071.

(10) Bister, B.; Bischoff, D.; Ströbele, M.; Riedlinger, J.; Reicke, A.; Wolter, F.; Bull, A. T.; Zähner, H.; Fiedler, H.-P.; Süssmuth, R. D. *Angew. Chem., Int. Ed.* **2004**, *43*, 2574−2576.

(11) Wang, Q.; Song, F.; Xiao, X.; Huang, P.; Li, L.; Monte, A.; Abdel-Mageed, W. M.; Wang, J.; Guo, H.; He, W.; Xie, F.; Dai, H.; Liu, M.; Chen, C.; Xu, H.; Liu, M.; Piggott, A. M.; Liu, X.; Capon, R. J.; Zhang, L. *Angew. Chem., Int. Ed.* **2013**, *52*, 1231−1234.

(12) Keller, S.; Nicholson, G.; Drahl, C.; Sorensen, E. J.; Fiedler, H.; Süssmuth, R. D. *J. Antibiot.* **2007**, *60*, 391−394.

(13) Igarashi, Y.; Yu, L.; Miyanaga, S.; Fukuda, T.; Saitoh, N.; Sakurai, H.; Saiki, I.; Alonso-Vega, P.; Trujillo, M. E. *J. Nat. Prod.* **2010**, *73*, 1943−1946.

(14) Gottardi, E. M.; Krawczyk, J. M.; von Suchodoletz, H.; Schadt, S.; Mühlenweg, A.; Uguru, G. C.; Pelzer, S.; Fiedler, H.-P.; Bibb, M. J.; Stach, J. E. M.; Süssmuth, R. D. *ChemBioChem* **2011**, *12*, 1401−1410.

(15) (a) Nicolaou, K. C.; Harrison, S. T. *J. Am. Chem. Soc.* **2007**, *129*, 429−440. (b) Bihelovic, F.; Karadzic, I.; Matovic, R.; Saicic, R. N. *Org. Biomol. Chem.* **2013**, *11*, 5413−5424. (c) Snider, B. B.; Zou, Y. *Org. Lett.* **2005**, *7*, 4939−4941. (d) Couladouros, E. A.; Bouzas, E. A.; Magos, A. D. *Tetrahedron* **2006**, *62*, 5272−5279. (e) Nicolaou, K. C.; Harrison, S. T.; Chen, J. S. *Synthesis (Stuttgart)* **2009**, 33−42. (f) Nicolaou, K. C.; Harrison, S. T. *Angew. Chem., Int. Ed.* **2006**, *45*, 3256−3260. (g) Zapf, C. W.; Harrison, B. A.; Drahl, C.; Sorensen, E. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 6533−6537.

(16) Keller, S.; Schadt, H. S.; Ortel, I.; Süssmuth, R. D. *Angew. Chem., Int. Ed.*. **2007**, *46*, 8284−8286.

(17) Lehrman, G.; Hogue, I. B.; Palmer, S.; Jennings, C.; Spina, C. A.; Wiegand, A.; Landay, A. L.; Coombs, R. W.; Richman, D. D.; Mellors, J. W.; Coffin, J. M.; Bosch, R. J.; Margolis, D. M. *Lancet* **2005**, *366*, 549−555.

(18) Beans, E. J.; Fournogerakis, D.; Gauntlett, C.; Heumann, L. V.; Kramer, R.; Marsden, M. D.; Murray, D.; Chun, T.-W.; Zack, J. A.; Wender, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *29*, 11698−11703.

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