ORIGINAL RESEARCH ARTICLE

Open Access

A Pilot Study of Raltegravir Plus Combination Antiretroviral Therapy in Early Human Immunodeficiency Virus Infection: Challenges and Lessons Learned

Ann C. Collier,^{1,*} Tae-Wook Chun,² Janine Maenza,¹ Robert W. Coombs,^{1,3} Kenneth Tapia,⁴ Ming Chang,³ Claire E. Stevens,⁵ J. Shawn Justement,² Danielle Murray,² Joanne D. Stekler,¹ James I Mullins,^{1,3,6} and Sarah E. Holte⁷

Abstract

Availability of integrase strand transfer inhibitors created interest in determining whether their use would decrease persistently infected cell numbers. This study hypothesized that adding raltegravir (RAL) to standard antiretroviral therapy (ART) would decrease human immunodeficiency virus (HIV)-infected CD4⁺ T cells more than standard combination ART. This was a pilot, randomized study comparing open-label standard triple ART to standard triple ART plus RAL over 96 weeks in ART-naïve adults with early HIV infection. The primary objective was to compare quantity and trajectory of HIV DNA. Eighty-two persons were referred. A diverse set of reasons precluded the enrollment of all but 10. Those who enrolled and completed the study had an estimated median duration of HIV infection of 74 days at ART start. The groups had similar baseline characteristics. The RAL group had more rapid first phase plasma HIV RNA decay (0.67 log₁₀ copies/mL/day) than with combination ART (0.34 log₁₀ copies/mL/day), $p=0.037$. Second phase HIV RNA decay, residual viremia, cell-associated RNA, HIV DNA, CD4⁺ T-cells with replication-competent virus, and 2LTR circle levels did not differ between groups. Among those with entry plasma HIV RNA levels above the median, 2LTR circles were significantly lower over time than in those with lower entry HIV RNA levels ($p=0.02$). Our results suggest homogeneity of responses in cell-associated RNA, HIV DNA, CD4⁺ T-cells with replication-competent virus, and 2LTR circles with early HIV in both ART groups. The kinetics of 2LTR DNA did not reflect the kinetics of plasma HIV RNA decline following ART initiation.

Key words: antiretroviral therapy; integrase inhibitor; primary HIV; reservoir

Introduction

Availability of the first integrase strand transfer inhibitor created interest in determining whether its use would decrease the number of cells persistently infected with human immunodeficiency virus (HIV) type one. Raltegravir (RAL, Isentress®) added to standard combination antiretroviral therapy (ART) in persons with chronic HIV decreased latently infected,

resting CD4⁺ T cells and had favorable effects on ileal cell-associated unspliced HIV RNA in CD4⁺ T cells^{1,2}; other studies showed no virologic impact of RAL intensification.^{3–6} No difference in HIV RNA or DNA was seen in randomized studies of 5-drug RAL-containing ART versus three-drug ART in recent HIV infection.^{7,8} Persons who start ART earlier versus later have lower HIV DNA burden.^{9–11} We performed a pilot study to

¹Division of Infectious Diseases, Department of Medicine, University of Washington, Seattle, Washington.

²Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

³Department of Laboratory Medicine, University of Washington, Seattle, Washington.

⁴Department of Global Health, University of Washington, Seattle, Washington.

⁵Department of Neurology, University of Washington, Seattle, Washington.

⁶Department of Microbiology, University of Washington, Seattle, Washington.

⁷Program in Biostatistics and Biomathematics, Division of Fred Hutch, Department of Biostatistics, University of Washington, Seattle, Washington.

*Address correspondence to: Ann C. Collier, Department of Medicine (Infectious Diseases), University of Washington, Harborview Medical Center, Box 359929; 325 9th Avenue, Seattle, WA 98104, E-mail: acollier@uw.edu

evaluate impact of RAL in addition to standard three-drug ART in persons with early HIV infection on virologic measures and describe the challenges and lessons learned.

Materials and Methods

Study participants

Participants were ≥ 18 years old, ART-naïve, had HIV RNA ≥ 500 copies/mL within 14 days before entry and had early HIV infection defined as follows: A current positive HIV EIA and western blot with either a negative HIV EIA within the past 6 months or a negative point-of-care HIV test or a nonreactive less-sensitive (LS) HIV EIA within the past month. Exclusion criteria included pregnancy, breastfeeding, and prior HIV vaccines. Complete entry criteria are listed on ClinicalTrials.gov number NCT00781287. This study was approved by University of Washington (UW)'s Human Subjects Review Committee. Participants provided written informed consent.

Study design and procedures

This was a pilot randomized study comparing open label standard triple ART to standard triple ART plus RAL (hereafter called RAL group). We hypothesized that adding RAL to standard ART would decrease HIV-infected CD4⁺ T cell number more than standard ART. Standard ART, prescribed by the subject's primary care provider, was two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-NRTI or a protease inhibitor. The study provided RAL 400 mg twice daily for up to 96 weeks. The study statistician performed the 1:1 randomization.

Participants underwent evaluations at entry, day 3, weekly for 4 weeks, at weeks 8, 12, 16, 24, and every 12 weeks until week 96. Consenting subjects underwent leukapheresis at entry, weeks 48 and 96 for assessment of infectious HIV in resting CD4⁺ T cells. Assays were performed by laboratory personnel blinded to treatment. Plasma HIV RNA levels were determined in Seattle until viral suppression using the Abbott m2000sp/rt HIV-1 RealTime assay (Abbott Molecular) and subsequently, in Bethesda using the COBAS[®] Ampliprep/COBAS Taqman HIV-1 Test, version 2.0 (Roche Diagnostics) done in triplicate as previously described¹² with a limit of detection of 20–48 copies/mL. Residual plasma viremia (<20 HIV RNA copies/mL) (by low copy assay) was determined by averaging Ct values obtained in triplicate from plasma specimens. Frequency of CD4⁺ T cells carrying HIV proviral DNA was determined by

real-time polymerase chain reaction (PCR).¹² For detection of cell-associated unspliced HIV-1 RNA, 500 ng of total cellular RNA was subjected to real-time PCR using TaqMan RNA-to-C_T 1-Step Kit (Applied Biosystems) and HIV-specific primers and probe.¹³ Frequency of CD4⁺ T cells producing infectious (replication competent) HIV was measured by quantitative coculture assays.¹⁴ Extra-chromosomal 2LTR circular DNA was assessed on stored peripheral blood mononuclear cells using a modified assay procedure.^{4,15} The quantity of 2LTR circles was reported as log₁₀copies/10⁶ CD4⁺ T cells. CD4⁺ T cells and routine toxicity assessments were performed using standard methods by UW Department of Laboratory Medicine clinical laboratories. Toxicities were graded by standard tables.¹⁶

Statistical considerations

The primary objective was to compare quantity and trajectory of HIV-infected CD4⁺ T cells, assessed by HIV DNA. Secondary endpoints included levels and trajectory of plasma HIV RNA, cell-associated RNA, quantity and half-lives of CD4⁺ T-cells with infectious virus, and clinical and laboratory toxicities. Residual plasma HIV viremia and 2LTR circles were added as secondary endpoints when these assays became available.

The study originally had a sample size of 10 per arm and 88% power to detect a 0.7 log₁₀ difference in HIV DNA at 96 weeks, assuming a standard deviation (SD) of 0.5 log₁₀ and using a two-sided *t*-test with alpha of 0.05. However, due to slow enrollment, the study was terminated prematurely. With five subjects per arm and an SD of about 0.2727, we would have 88% power to detect a difference of 0.54 log₁₀copies/mL. We used Wilcoxon rank-sum and Fisher's exact tests to compare continuous and categorical baseline characteristics. To evaluate differences in endpoints at single time points and changes between time points, two sample *t*-tests were used. To evaluate longitudinal levels, linear mixed effects models were used to account for repeated measures. All analyses used SAS v9.3 (SAS Institute, Inc.). Estimates of first and second nonlinear viral decay rates used the model developed by Perelson et al.¹⁷ and nonlinear random effects to account for repeated measures.

Results

Enrollment, participant characteristics, and follow-up

Subjects enrolled from March 2009 to 2011. Follow-up ended in February 2012. Figure 1 describes the disposition of individuals referred for participation and enrollees.

FIG. 1. Disposition of individuals who screened for and participated in the study. ART, antiretroviral therapy; HIV, human immunodeficiency type 1; LS-EIA, low-sensitivity enzyme immunoassay.

Table 1 describes baseline characteristics of enrollees. Participants had symptoms consistent with primary HIV infection; nine sought medical attention. Each had early HIV infection with an estimated median HIV infection duration of 74 days at ART start. Each group's characteristics were similar except for a

trend toward higher HIV RNA values at screening in the RAL group ($p=0.047$). No participants were lost to follow-up. Eight subjects completed 96 weeks. Two had follow-up truncated when the study ended; one completed 72 weeks and the other 48 weeks. All but one subject were adherent to treatment; one in

Table 1. Baseline Characteristics of Participants

Characteristics	Combination ART (n = 5)	Combination ART + raltegravir (n = 5)	Total (n = 10)
Age, years, median (range)	26 (21–32)	31 (22–43)	27 (21–43)
Male, No.	5 (100)	5 (100)	10 (100)
Race, No.			
Nonhispanic white	3	5	8
Hispanic or nonwhite	2	0	2
HIV risk factor, No.			
MSM	5	5	10
Symptoms with 1° HIV, No.			
Mild (no medical attention)	1	0	1
Mild (sought medical attention)	0	2	2
Severe	4	3	7
Fiebig stage at screening, No.			
V ^a	2	4	6
VI	3	0	3
Not available	0	1	1
CCR5 delta-32 mutation status, No.			
Wild-type	5	3	8
Heterozygote	0	2	2
Baseline CD4 ⁺ cells/mm ³ , median (range)	432 (412–722)	690 (432–980)	511 (412–980)
Baseline CD8 ⁺ cells/mm ³ , median (range)	996 (572–1,499)	849 (471–1,829)	923 (471–1,829)
Baseline CD4:CD8, median (range)	0.55 (0.37–0.76)	0.92 (0.24–1.15)	0.67 (0.24–1.15)
Screening HIV RNA, log ₁₀ copies/mL, median (range)	4.26 (2.97–5.06)	5.25 (4.42–5.89)	4.78 (2.97–5.89)
Baseline HIV RNA, log ₁₀ copies/mL, median (range)	4.26 (2.20–4.92)	5.21 (3.90–6.00)	4.28 (2.20–6.00)
Baseline HIV RNA (low copy assay)log ₁₀ copies/mL, median (range)	4.43 (3.20–5.09)	4.86 (4.05–5.89)	4.48 (3.20–5.89)
Baseline HIV cell-associated RNA, log ₁₀ copies/mL, median (range) ^b	3.19 (2.65–3.40)	3.21 (2.83–4.48) ³	3.19 (2.65–4.48)
Baseline HIV DNA, log ₁₀ copies/10 ⁶ CD4 ⁺ T-cells, median (range)	3.37 (3.01–3.65)	3.57 (3.12–4.05)	3.47 (3.01–4.05)
Baseline infectious virus, log ₁₀ infectious units/10 ⁶ PBMCs, median (range) ^c	61 (8–421)	157 (41–323)	82 (8–421)
2-LTR circles log ₁₀ copies/10 ⁶ CD4 ⁺ T-cells, median (range)	77 (3–224)	25 (0–146)	25 (0–224)
Days from estimated date of infection to ART start, median (range)	69 (44–98)	78 (56–83)	74 (44–98)
First ART regimen, No.			
NNRTI-based	2	2	4
PI-based	3	3	6

^aTwo subjects had Fiebig I/II at diagnosis.

^bOne subject is missing in the raltegravir-containing group.

^cOne subject is missing data in each group.

ART, antiretroviral therapy; CCR5, C-C chemokine receptor 5; HIV, human immunodeficiency virus; LTR, long terminal repeat; mL, milliliter; mm, millimeter; NNRTI, non-nucleoside reverse transcriptase inhibitor; No., number; PBMCs, peripheral blood mononuclear cells; 1°, primary HIV; PI, protease inhibitor; RNA, ribonucleic acid.

the RAL group stopped ART 5 days before week 96's leukapheresis.

Virologic, immunologic, and clinical outcomes

The RAL group had more rapid first phase plasma HIV RNA decay (0.67 log₁₀copies/mL/day) than the other group (0.34 log₁₀copies/mL/day), $p=0.037$ (Fig. 2, part A). Rates of second phase decay were not significantly different in the groups. Eight subjects achieved plasma HIV RNA <50 copies/mL by week 12 of ART (part B). No differences were seen between groups in cell-associated RNA, HIV DNA (primary endpoint with a difference in means of 0.083 log₁₀copies/mL), or CD4⁺ T-cells with infectious virus at 48 or 96 weeks or in change from baseline to either 48 or 96 weeks (parts D–F). Similarly, levels of residual viremia (based upon the low copy assay) (part C) from samples collected 20

or more weeks post-infection or of 2LTR circles (part G) did not differ between groups.

Given a trend toward differences between groups in screening plasma HIV RNA levels, we reanalyzed virological parameters by dividing subjects by median entry plasma HIV RNA level of 4.48 log₁₀copies/mL. Among those with higher entry plasma RNA, 2LTR circle levels were significantly lower over time than in those with lower entry RNA ($p=0.03$) (Table 2). There were no differences between high and low baseline RNA groups in cell-associated HIV RNA, HIV DNA, or numbers of CD4⁺ T-cells with infectious virus.

There were no differences between randomized groups in CD4⁺ or CD8⁺ T cell counts or CD4:CD8 ratios. No subjects developed AIDS-defining or HIV-associated illnesses. One subject had transient, self-limited grade 3 neutropenia unrelated to ART. All

FIG. 2. Virologic parameters by treatment group over time. **(A)** Plasma HIV RNA ($\log_{10}$ copies/mL) with two phase decay to undetectable model. **(B)** Plasma HIV RNA, $\log_{10}$ copies/mL; values below the limit of detection were set to half the limit of detection. **(C)** Residual viremia, $\log_{10}$ RNA copies/mL. **(D)** HIV Cell-associated RNA, $\log_{10}$ copies/mL. **(E)** HIV DNA, $\log_{10}$ copies/ 10^6 $CD4^+$ T cells. **(F)** $CD4^+$ T cells with replication-competent HIV, $\log_{10}$ infectious units/ 10^6 peripheral blood mononuclear cells. **(G)** 2LTR circles, $\log_{10}$ copies/ 10^6 $CD4^+$ T cells.

other signs, symptoms, and laboratory values were grade 2 or less and typical of the ART used.

Table 2. Comparison of Longitudinal Virologic Levels by High Versus Low Baseline HIV RNA

Virologic parameter	Low HIV RNA, N=7	High HIV RNA, N=3	p
HIV DNA, $\log_{10}$ copies/ 10^6 $CD4^+$ T-cells	2.92	2.77	0.46
Infectious virus, $\log_{10}$ infectious units/ 10^6 PBMCs	1.15	0.77	0.39
HIV cell-associated HIV RNA, $\log_{10}$ copies/mL	1.84	1.57	0.62
2LTR circles, $\log_{10}$ copies/ 10^6 $CD4^+$ T-cells	2.05	0.85	0.03

DNA, deoxyribonucleic acid; HIV, human immunodeficiency virus; PBMCs, peripheral blood mononuclear cells; RNA, ribonucleic acid.

Discussion

When ART was started during early HIV, our results suggest homogeneity of responses in residual viremia, cell-associated RNA, HIV DNA, and $CD4^+$ T cells with infectious virus. First phase decay of HIV RNA was faster in subjects receiving RAL in addition to standard triple ART.

It was of interest that levels of 2LTR circles were lower over time among subjects with higher compared with lower pre-ART HIV RNA levels. We hypothesize that the difference in 2LTR kinetics represents differences in infected $CD4^+$ cell clearance and different

rates of replenishment between these two groups of initially viremic subjects. Some studies have suggested that 2LTR circles may increase in persons with chronic HIV when they are treated with RAL-containing regimens but others, including the present study in persons with early HIV, have not seen such changes.^{11,18,19} Following ART initiation, kinetics of 2LTR DNA did not reflect the kinetics of plasma HIV RNA decline.

Enrolling our study was unexpectedly challenging. Our group has enrolled 394 subjects with acute or early HIV since 2002 in an observational study that does not require or exclude ART; the proportion of immediate ART start has varied over time, likely reflecting evolving views of ART's potential benefits and risks. When the current study was conducted, U.S. treatment guidelines were not supportive of early ART.²⁰ Other issues included the lack of sensitive, readily available assays during the study to detect early HIV; eight potential participants with recent sexual HIV exposure and documented past negative HIV tests (as recently as 7 months previously), suggesting recent HIV infection, had positive LS-EIA assays so were excluded from participation. A wide variety of other reasons prevented potentially eligible persons from enrolling.

Limitations of this pilot study include being underpowered for treatment comparisons, selection bias toward participants with symptomatic primary infection, and lack of subject diversity (although reflective of the epidemic in the Pacific Northwest). Nonetheless, with increased interest in early ART and understanding relationships among different virologic parameters, the lessons learned from this study may increase knowledge about effects of ART in early HIV and help the design and implementation of future studies.

Conclusions

A diverse set of reasons adversely impacted enrollment in this pilot study. The RAL group had more rapid first phase decay than combination ART without RAL. Our results suggest homogeneity of responses in cell-associated RNA, HIV DNA, CD4⁺ T-cells with replication competent virus, and 2LTR circles to early ART in both treatment groups. Following ART initiation, the kinetics of 2LTR DNA did not reflect the kinetics of plasma HIV RNA decline.

Acknowledgments

This study was supported in part by a research grant from the Investigator-initiated Studies Program of Merck & Co., Inc., by a grant for supplies from Roche Molecular

Systems, the University of Washington/Fred Hutch Center for AIDS Research (P30 AI027757), and NIH grant AI57005. We wish to thank the study participants, Helen Horton and her laboratory for separation and shipment of peripheral blood mononuclear cells, Audrey Wong and the UW Clinical Retrovirology Laboratory for performing the 2LTR assays, and the Puget Sound Blood Center for performing leukaphereses. We also acknowledge Terri Smith and Bess Sorensen for their assistance with data management.

The opinions expressed in this article are those of the authors and do not necessarily represent those of Merck & Co., Inc.

Author Disclosure Statement

A.C.C.: Membership on a Data and Safety Monitoring Board for Merck-sponsored studies and previous stock ownership in Abbott Laboratories, Bristol-Myers-Squibb, Johnson & Johnson, and Pfizer.

M.C., T.-W.C., R.W.C., D.M., S.E.H., J.S.J., J.M., J.I.M., K.T., J.D.S., and C.E.S.: No competing financial interests exist.

References

1. Yukl SA, Shergill AK, McQuaid K, et al. Effect of raltegravir-containing intensification on HIV burden and T-cell activation in multiple gut sites of HIV-positive adults on suppressive antiretroviral therapy. *AIDS*. 2010;24:2451–2460.
2. Vallejo A, Gutierrez C, Hernandez-Novoa B, et al. The effect of intensification with raltegravir on the HIV-1 reservoir of latently infected memory CD4 T cells in suppressed patients. *AIDS*. 2012;26:1885–1894.
3. Gandhi RT, Zheng L, Bosch RJ, et al. The effect of raltegravir intensification on low-level residual viremia in HIV-infected patients on antiretroviral therapy: a randomized controlled trial. *PLoS Med*. 2010;7:Pii:1000321.
4. Gandhi RT, Coombs RW, Chan ES, et al. No effect of raltegravir intensification on viral replication markers in the blood of HIV-1-infected patients receiving antiretroviral therapy. *JAIDS*. 2012;59:229–235.
5. Hatano H, Hayes TL, Dahl V, et al. A randomized, controlled trial of raltegravir intensification in antiretroviral-treated, HIV-infected patients with suboptimal CD4⁺ T cell response. *J Infect Dis*. 2011;203:960–968.
6. Besson G, McMahon D, Maldarelli F, et al. Short-course raltegravir intensification does not increase 2 long terminal repeat episomal HIV-1 DNA in patients on effective antiretroviral therapy. *Clin Infect Dis*. 2012;54:451–453.
7. Markowitz M, Evering TH, Garmon D, et al. A randomized open-label study of 3- versus 5-drug combination antiretroviral therapy in newly HIV-1-infected individuals. *JAIDS*. 2014;66:140–147.
8. Cheret A, Nembot G, Melard A, et al. Intensive five-drug antiretroviral therapy regimen versus standard triple-drug therapy during primary HIV-1 infection (OPTIPRIM-ARNS 147): a randomized, open-label, phase 3 trial. *Lancet Infect Dis*. 2015;15:387–396.
9. Jain V, Hartogensis W, Bacchetti P, et al. Antiretroviral therapy initiated within 6 months of HIV infection is associated with lower T-cell activation and smaller HIV reservoir size. *J Infect Dis*. 2013;208:1202–1211.
10. Laanai M, Ghosn J, Essat A, et al. Impact of the timing of initiation of antiretroviral therapy during primary HIV-1 infection on the decay of cell-associated HIV-DNA. *Clin Infect Dis*. 2015;60:1715–1721.
11. Koelsch KK, Boesecke C, McBride K, et al. Impact of treatment with raltegravir during primary or chronic HIV infection on RNA decay characteristics and the HIV viral reservoir. *AIDS*. 2011;25:2069–2078.

12. Chun TW, Murray D, Justement JS, et al. Relationship between residual plasma viremia and the size of HIV proviral DNA reservoirs in infected individuals receiving effective antiretroviral therapy. *J Infect Dis.* 2011;204:135–138.
13. Chun TW, Justement JS, Lempicki RA, et al. Gene expression and viral production in latently infected, resting CD4⁺ T cells in viremic versus aviremic HIV-infected individuals. *Proc Natl Acad Sci U S A.* 2003;100:1908–1913.
14. Chun TW, Engel D, Mizell SB, et al. Effect of interleukin-2 on the pool of latently infected, resting CD4⁺ T cells in HIV-1-infected patients receiving highly active anti-retroviral therapy. *Nat Med.* 1999;5:651–655.
15. Pauk J, Huang ML, Brodie SJ, et al. Mucosal shedding of human herpes-virus 8 in men. *N Engl J Med.* 2000;343:1369–1377.
16. Division of AIDS Table for Grading Adverse Events, Version 1.0. Available at http://rsc.tech-res.com/safetyandpharmacovigilance/grading_tables.aspx Accessed April 14, 2015.
17. Perelson AS, Essunger P, Cao Y, et al. Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature.* 1997;387:188–191.
18. Hatano H, Strain MC, Scherzer RS, et al. Increase in 2-long terminal repeat circles and decrease in D-dimer after raltegravir intensification in patients with treated HIV infection: a randomized, placebo-controlled trial. *J Infect Dis.* 2013;208:1436–1442.
19. Stephan C, Baldauf HM, Barry J, et al. Impact of raltegravir on HIV-1 RNA and DNA forms following initiation of antiretroviral therapy in treatment-naïve patients. *J Antimicrob Chemother.* 2014;69:2809–2818.
20. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. December 1, 2009; 1–161, pp. 84–87. Available at <http://aidsinfo.nih.gov/guidelines/archive/adult-and-adolescent-guidelines>. Accessed January 20, 2015.

Abbreviations Used

1° = primary HIV
 ART = antiretroviral therapy
 CCR5 = C-C chemokine receptor 5
 HIV = human immunodeficiency virus
 LS = less-sensitive
 LTR = long terminal repeat
 mL = milliliter
 mm = millimeter
 NNRTI = non-nucleoside reverse transcriptase inhibitor
 No. = number
 NRTIs = nucleoside reverse transcriptase inhibitors
 PBMCs = peripheral blood mononuclear cells
 PCR = polymerase chain reaction
 PI = protease inhibitor
 RAL = raltegravir
 RNA = ribonucleic acid
 SD = standard deviation

Cite this article as: Collier AC, Chun T-W, Maenza J, Coombs RW, Tapia K, Chang M, Stevens CE, Justement JS, Murray D, Stekler JD, Mullins JI, Holte SE (2016) A pilot study of raltegravir plus combination antiretroviral therapy in early human immunodeficiency virus infection: challenges and lessons learned, *BioResearch Open Access* 5:1, 15–21, DOI: 10.1089/biores.2015.0038.

Publish in BioResearch Open Access

- Broad coverage of biomedical research
- Immediate, unrestricted online access
- Rigorous peer review
- Compliance with open access mandates
- Authors retain copyright
- Highly indexed
- Targeted email marketing

liebertpub.com/biores