Title

Long-term use of valproic acid in US veterans is associated with a reduced risk of smoking-related cases of head and neck cancer

Journal

Cancer, 120(9)

ISSN

0008-543X

Authors

Kang, Hyunseok
Gillespie, Theresa W
Goodman, Michael
et al.

Publication Date

2014-05-01

DOI

10.1002/cncr.28479

Peer reviewed

Published in final edited form as:
Cancer. 2014 May 1; 120(9): 1394–1400. doi:10.1002/cncr.28479.

Long-term use of valproic acid in United States Veterans associates with reduced risk of smoking related head-and neck cancer

Hyunseok Kang, MD, MPH[6], **Theresa W Gillespie, PhD**[1,2,4], **Michael Goodman, MD MPH**[5], **Seth A Brodie, PhD**[1,2,3], **Mina Brandes, MD**[6], **Maria Ribeiro, MD**[1,2,3], **Suresh S Ramalingam, MD**[2,3], **Dong M Shin, MD**[2,3], **Fadlo R Khuri, MD**[2,3], and **Johann Christoph Brandes, MD, PhD**[1,2,3]

[1]Atlanta Veterans Affairs Medical Center

[2]Winship Cancer Institute, Emory University

[3]Department of Hematology and Medical Oncology, Emory University

[4]Department of Surgery, Emory University

[5]Rollins School of Public Health, Emory University

[6]Johns Hopkins University

Abstract

Purpose—Epigenetic events play a major role in the carcinogenesis of tobacco-related cancers. We conducted a retrospective cohort study to evaluate the effects of exposure to the anti-convulsant agent valproic acid (VPA), a HDAC inhibitor, on the risk for cancers of the lung, head and neck, prostate, bladder and colon.

Patients and Methods—The study was based on the 2002–2008 National Veterans Affairs (VA) medical SAS dataset linked to the VA Central Cancer Registry. The cohort was defined as subjects over 40 years of age who were followed in the VA system for at least one year for one of four diagnoses for which a VPA indication exists (bipolar disorder, PTSD, migraines and seizures). Multivariable Cox proportional hazard models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) reflecting the association between VPA use and cancer incidence.

Results—VPA use was associated with a significant reduction in the risk for cancers of the head and neck (HR 0.66, 95% CI (0.48–0.92)) Additional associations were seen with duration of treatment and median VPA drug levels. No significant differences in cancer incidence was observed for lung-(HR 1.00; 95%CI 0.84–1.19), bladder- (HR 0.86; 95% CI 0.64–1.15), colon-(HR 0.95; 95% CI 0.74–1.22) and prostate cancers (HR 0.96 95% CI 0.88–1.12)

Corresponding author: Johann C Brandes, M.D., PhD, Atlanta Veterans Affairs Medical Center, Winship Cancer Institute, Emory University, Atlanta, GA, 30322, johann.brandes@emory.edu.

Conflicts of interest
None

The contents of this publication do not reflect views of the Department of Veterans Affairs or the United States Government

Conclusion—VPA use is associated with a lower risk of head and neck cancers.

Introduction

Epigenetic changes, involving either DNA methylation or changes in chromatin structure[1, 2], are early carcinogenic events in many cancer sites including lung[3–5], prostate[6, 7], colon[8, 9], bladder[10, 11], and head and neck[12–14]. DNA methyltransferases (DNMTs) and histone-deacetylases (HDACs) are major epigenetic mediators for which pharmacologic inhibitors are available. In animal models, inhibition of DNMTs and HDACs has been shown to prevent the development of both lung[15] and prostate cancers[16]. In addition, our own data show that HDAC1,2 and 3 not only are associated with increased DNMT1 protein levels in lung cancers compared to normal controls, but that they are directly responsible for stabilizing DNMT1 expression[17]. Valproic acid (VPA), which has been widely used for psychiatric or neurologic disorders as a mood stabilizer or anti-epileptic drug, has recently been described to act as class I HDAC inhibitor[18] HDAC inhibition is observed at VPA concentrations as low as 30 ug/ ml[17]. . Epigenetic therapies such as the DNMT inhibitor azacytidine and the HDAC inhibitor vorinostat have been proven effective against several hematologic malignancies such as myelodysplastic syndrome[19–21]and cutaneous T-cell lymphomas[22]. A recent phase II study showed promise for the combination of azacytidine with the HDAC inhibitor entinostat for the treatment of lung cancer[23]. However, no clinical evidence exists so far on the association between use of HDAC inhibitors and cancer risk.

Given the importance of epigenetic mechanisms in early carcinogenesis[24], and the preclinical evidence supporting the anti-carcinogenic effects of VPA[25], we conducted a retrospective cohort study evaluating the risk of various malignancies in relation to VPA use.

Materials and Methods

Data sources

We searched the National Veterans Affairs (VA) Medical SAS datasets in conjunction with the VA Decision Support Systems (DSS) data from the VA Corporate Data Warehouse (CDW). The project was approved by the Institutional Review Board (IRB) at Emory University and by the Research and Development Committee at the Atlanta VA Medical Center (VAMC). Data were extracted by the VA Informatics and Computing Infrastructure (VINCI). The data elements obtained on each study subject included scrambled social security number, gender, age, first date of encounter, last day of encounter, first filled prescription of VPA, last filled prescription of VPA, serum drug levels for VPA where available, International Classification of Disease, 9th edition (ICD-9) codes of associated psychiatric (bipolar disorder, PTSD, depression, anxiety, schizophrenia, substance- and alcohol abuse) or neurologic (migraines and seizures) diagnoses and smoking related co-morbidities such as coronary artery disease and COPD. The smoking status of study subjects was determined from health-flags which are recorded by clinical providers at the end of a clinical visit and which serve as a quality measure for medical care delivered in the VA system. Smoking-related health flags characterize patients as "never-smoker, " "non-smoker, " “nonsmoker for more than × number of years”, "past smoker, " "current smoker" and also

include information on smoking cessation counseling. Cancer cases were ascertained by linking the data from the CDW with the VA Central Cancer Registry (VACCR) in Washington, DC using scrambled social security numbers as unique identifiers. The VACCR has been reported to capture at least 90% of cancer cases treated in the VA system.[26]

Study population

The inclusion criteria for the main cohort were as the following: 1) the presence of at least one diagnosis of psychiatric or neurologic diseases for which long term VPA has an accepted clinical indication (bipolar disorder, PTSD, migraines or seizures), 2) clinical follow up for a duration of at least one year 3) current smoker or former smoker, and 4) age at least 40 years at the inclusion of the cohort. We excluded patients without evidence of a smoking history because the preponderance of the preclinical evidence points towards a particular role of HDAC and DNMT1 mediated epigenetic changes in the etiology of smoking-related cancers. Subjects who had a cancer diagnosis within a year after their first visit to the VA were excluded to account for possible asymptomatic prevalent cases. Since all five types of cancer studied in this analysis are very rare in young patients, we furthermore excluded patients who were less than 40 years old. This enrichment of patients at risk for smoking related cancers based on age and smoking status is supported by numerous studies evaluating interventions for the risk reduction of such cancers[27–29]. Given a concern that the VACCR may have underreported cancer cases in its early years since inception in 1999, we defined the beginning of the observation period as the later date of either January 1, 2002 or the first visit to the VA for one of the four inclusion diagnoses. At the time of analysis VACCR data were available until December 31, 2008, which was defined as the end of the study period. . Since short-term drug exposure is unlikely to influence cancer risk, we excluded patients with less than 1 year of VPA use[30]. The start of the observation period was defined as the first visit for one of the 4 inclusion diagnoses for non-VPA users and the date of the first filled prescription for VPA users. The end of the observation period was either the date of a cancer diagnosis, the last visit date for one of the inclusion diagnoses or the end of the study period.

Statistical analysis

All statistical analyses were carried out using SAS9.2 software (SAS Institute Inc, Cary, NC). All the tests were two-sided with statistical significance set at $p < 0.05$. The characteristics of each cohort were compared using the chi-square test and student t-test. Cancer incidence over the follow-up period was calculated as a rate of new occurrence of cancer over the total number person-years and further explored using Kaplan-Meier method. Univariate analyses of cancer incidence and cancer-deaths were performed using log rank test. Multivariate adjusted hazard ratios (HR) for cancer incidence were determined using Cox-proportional hazard model. Covariates considered in the multivariate analyses were age, gender, race, smoking status, psychiatric disease (bipolar disorder, PTSD, schizophrenia, depression, anxiety), neurologic disease (seizures and migraines), COPD and evidence of alcohol and substance abuse. Formal tests were conducted to confirm the assumption of proportionality. To assess on the impact of VPA exposure duration and VPA serum drug levels, similar multivariate analyses were performed for different intervals of

VPA use and levels. For propensity score analyses, propensity scores for VPA use were calculated from logistic regression using age at study entry, gender, race, smoking status, alcohol and substance abuse, diagnosis of each individual psychiatric and/or neurologic disorder (by ICD9 code) and COPD as independent variables. Propensity scores were used in a multivariate model as an independent variable and in subgroup analyses divided by quintiles of propensity score[31]. Tests for interactions between VPA use, alcohol use and smoking were conducted.

Propensity scores for VPA use were calculated from the logistic regression model that used age at study entry, gender, smoking status, substance abuse, diagnosis of each individual psychiatric and/or neurologic disorder, COPD and CAD as independent variables. Subgroup analysis by propensity score quintile or matching by propensity score are well established methods to balance cofactors within a groups, thus reducing biases which could arise from vastly different distributions of these cofactors in a Cox proportional hazard model[31] Propensity scores were used in a multivariate model as an independent variable and in subclass analyses stratified by propensity score quintiles[31].

Results

Cohort characteristics

A total of 636,051 individuals over age 40 were initially identified. After excluding never-smokers and subjects with unknown smoking status and patients who had a cancer diagnosis within the first year after their first VA visit, the final cohort consisted of 439,628 patients of whom 26,911 had filled prescriptions for VPA for more than one year. The mean follow-up duration was 4.40 years (SD 2.29) for non-VPA users and 4.42 years (SD 2.05) for VPA users (p=0.11). VPA users on average were slightly younger than non-users (median age 59 years vs.60 years), and tended to have a higher incidence of bipolar disorder, schizophrenia, seizure d/o, migraines and alcohol and substance abuse. The prevalence of PTSD, depression and anxiety was lower in VPA users. No significant differences between VPA users and non-users were observed in terms of proportions of former (51%) and current smokers (49%) as well as prevalence of COPD (18%) at baseline (Table 1). Also, despite the risk for weight gain with VPA, body mass indices (BMI) were not significantly different between the two groups.

Cancer incidence

Crude and adjusted hazard ratios (HRs) for lung, head and neck, prostate, colorectal and bladder cancer comparing VPA users to non-users are shown in Table 2. In the multivariate analysis VPA use was associated with significant reductions in risk for head and neck cancers (HR 0.66, 95% CI **0.48–0.92**). No association between VPA use and risk reduction was observed for lung, colorectal, prostate or bladder cancers. No interaction was found between VPA use, smoking and alcohol use.

Relationship of VPA use duration and VPA blood levels to head and neck cancer risk

The relationship between VPA use duration and head and neck cancer risk is shown in Figure 1. Compared to VPA non-users, those who used VPA for 1 to 3 years had no

significant reduction in head and neck cancer risk (HR 0.96 (95% CI 0.58–1.60) while subjects with exposure of three years and more enjoyed a significant risk reduction (0.57 (0.39–0.85) (Figure 1). Next we analyzed the association between median VPA drug levels and the reduction of head and neck cancer risk by dose using the previously defined cutoff of 40 ug/ml for effective HDAC inhibition[17]. A statistically significant risk reduction for head and neck cancer was only observed in the group that had median VPA levels in the therapeutic range for HDAC inhibition (HR 0.59 (95%CI 0.35–0.99) (Figure 1). Both time and concentration-dependence of the VPA effect on head and neck cancer risk reduction support a causal relationship.

Impact of VPA use on clinical cancer characteristics

Squamous cell carcinomas of the head and neck have two very different etiologies. In addition to classic risk factors like tobacco and alcohol, infections with certain serotypes of human papilloma viruses (HPV), most notably HPV16 have emerged as a cause for the rapidly rising incidence of oropharyngeal carcinomas[32–34]. A distinction between oropharyngeal and non-oropharyngeal cancers is important since HDAC inhibitors have been described of being capable in vitro to induce apoptosis in HPV infected cells, suggesting that a preferential effect against HPV positive cancers may exist[35].

Our analysis revealed a nearly equal reduction in the risk for oropharyngeal (HR 0.67, 95%CI 0.32–1.37) and non-oropharyngeal cancers by VPA (HR 0.67, 95%CI 0.47–0.95) (Figure 1). Since it is possible that exclusion of non-smokers from the cohort disproportionally eliminated patients with HPV-related head and neck cancers, we also analyzed the risks for oropharyngeal (HR 1.02 (95% CI 0.63–1.66) and non-oropharyngeal cancers (HR 0.75 (95% CI0.57–0.98) in the entire cohort including never smokers. These findings argue against a preferential antiviral mechanism of action for VPA and suggest that VPA may be particularly effective against smoking induced squamous cell carcinomas.

Discussion

In this retrospective cohort study of US veterans, VPA use is associated with a significantly decreased incidence of head and neck squamous cell carcinomas. The observed relationship between long-term exposure to an HDAC inhibitor (in this case VPA) and reduced cancer risk in this VA population-based study complements preclinical data on the role of early epigenetic events in carcinogenesis[1] and confirms for the first time clinically that inhibitors of epigenetic repressors may serve as promising cancer prevention agents. Our data demonstrated that risk reduction for head and neck cancers was most pronounced after three years of VPA use. The optimal exposure duration could not be determined in this study, since the number of events in patients with longer exposures was limited. A longer follow-up of our cohort or a larger validation cohort will be necessary to address this question.

While differences in the baseline clinical characteristics of VPA-users vs. non-users in this cohort were adjusted for by multivariate and propensity score analysis, potential sources of bias remain. The most important variable in determining the cancer risk of the aerodigestive system is the cumulative lifetime smoking history. The health-flags collected in the VA databases give detailed information about the current smoking status, but do not provide the

cumulative amount of cigarettes smoked. Since the psychiatric co-morbidities on which our cohort selection is based is strongly associated with increased tobacco abuse, tobacco related morbidity and mortality[36, 37] and since these psychiatric co-morbidities are not entirely evenly distributed between VPA users and nonusers, it is possible that the life time exposure of cigarettes could have been imbalanced between the groups. However, since psychiatric co-morbidities were more common in the VPA group, this is likely to be weighted heavier in the VPA users and is thus unlikely to explain the observed results of decreased incidence of head and neck cancer. Given that the risk for COPD, another closely linked smoking related disease was identical between VPA users and non-users (Table 1), the cumulative smoking histories were most likely comparable.

Non-compliance with a medical regimen could have been a source of bias. Compliance is always a concern, particularly in patients with mental illness. Our data demonstrated that the head and neck cancer risk reduction in patients with a sub-therapeutic median VPA level was less evident. Thus it is possible that inclusion of patients with poor medication compliance in the VPA exposed group may have made the association less prominent.

Finally, evidence is emerging that the risk for smoking related cancers may be dependent on certain genotypes of nicotine and carcinogen metabolizing enzymes such as cytochrome P (CYP)-2A6 and others[38, 39]. The nature of our study does not grant us detailed knowledge of these genotypes. However, given the fact that the risk for other smoking related malignancies such as lung- and bladder cancer is unaffected by VPA exposure it is unlikely that significant imbalances in the genotypes of these metabolizing enzymes are responsible for the observed risk reduction in head and neck cancer.

A mechanistic explanation for why squamous cell carcinomas of head and neck are more susceptible to prevention by VPA is not straightforward, but recent whole genome sequencing, genotyping, methylation and gene expression projects have provided a wealth of information implicating epigenetic events, particularly in aerodigestive SCCs. Whole genome sequencing in head and neck and lung squamous cell carcinomas identified frequent mutations and amplifications of the histone methyltransferases PRDM9 and EZH2 in both malignancies in up to 20% of the cases[40, 41]. Recent whole genome approaches have revealed defects in four driver pathways in head and neck cancer: mitogenic signaling, cell cycle control, p53 signaling and NOTCH signaling[42]. All of these pathways are potentially affected by epigenetic changes: p16 is not expressed in the vast majority of non-HPV related head and neck cancers. In more than 50% of the cases p16 is silenced epigenetically by promoter methylation[43]. p53 is mutated in more than 80% of head an neck cancers, however even those tumors without mutation frequently show loss of expression. One potential mechanism for this is epigenetic silencing of p14 which shares a bi-directional promoter with p16 and controls the activity of the ubiquitin-ligase MDM2, which targets p53 for proteasomal degradation[44]. Notch signaling in head and neck cancer has a predominantly tumor- suppressive effect. It is possible that the upregulation of NOTCH1 after treatment with HDAC inhibitors can be an additional tumor suppressive mechanism[45, 46]. Finally, a recent study analyzing the DNA methylation pattern of head and neck cancers identified a subset of tumors with a hypermethylator phenotype, similar to the previously described CIMP phenotype in colon cancer. It will need to be explored if this subset of head and neck

cancers is particularly sensitive to the effects of epigenetic therapies[42]. While the precise underlying molecular events for these observations need to be unraveled, our data provide epidemiologic support for the susceptibility of head and neck cancers to the effects of HDAC inhibitors. While anticancer effects of HDAC inhibitors against HPV-related malignancies have been described in vitro[35], our data demonstrate that VPA use does not preferentially decrease the risk of cancers of the oropharynx, which account mostly for HPV-related cases[32–34], making an antiviral effect of VPA unlikely as the primary contributor to the observed decrease in head and neck cancer incidence at least in patients with smoking history.

In summary, long-term use of VPA is associated with a decreased risk for head and neck cancers in high risk veterans. The extensive preclinical and clinical evidence and the magnitude of the observed potential benefit warrant further investigation of VPA as a cancer chemoprevention agent, possibly in patients with premalignant lesions of the head and neck.

Acknowledgments

JCB had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Grant support:

This material is based upon work supported in part by the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development" (Biomedical Laboratory Research and Development)-7IK 2BX001283-02 to JCB. Other grant funding includes: NCI- 5 P50 CA128613-02 Career Development Project to JCB, NCI-P30CA138292 pilot grant to JCB, CHEST Foundation/Lungevity Foundation Clinical Lung Cancer Research award to JCB, Uniting against Lung Cancer /Lungevity Foundation research award to JCB, Suntrust Scholar Award to JCB.

References

1. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med. 2003; 349(21):2042–2054. [PubMed: 14627790]
2. McCabe MT, Brandes JC, Vertino PM. Cancer DNA methylation: molecular mechanisms and clinical implications. Clin Cancer Res. 2009; 15(12):3927–3937. [PubMed: 19509173]
3. Belinsky SA, Liechty KC, Gentry FD, et al. Promoter hypermethylation of multiple genes in sputum precedes lung cancer incidence in a high-risk cohort. Cancer research. 2006; 66(6):3338–3344. [PubMed: 16540689]
4. Belinsky SA, Palmisano WA, Gilliland FD, et al. Aberrant promoter methylation in bronchial epithelium and sputum from current and former smokers. Cancer research. 2002; 62(8):2370–2377. [PubMed: 11956099]
5. Palmisano WA, Divine KK, Saccomanno G, et al. Predicting lung cancer by detecting aberrant promoter methylation in sputum. Cancer research. 2000; 60(21):5954–5958. [PubMed: 11085511]
6. Alumkal JJ, Zhang Z, Humphreys EB, et al. Effect of DNA methylation on identification of aggressive prostate cancer. Urology. 2008; 72(6):1234–1239. [PubMed: 18387661]
7. Nelson WG, Yegnasubramanian S, Agoston AT, et al. Abnormal DNA methylation, epigenetics, and prostate cancer. Front Biosci. 2007; 12:4254–4266. [PubMed: 17485372]
8. Brandes JC, van Engeland M, Wouters KA, Weijenberg MP, Herman JG. CHFR promoter hypermethylation in colon cancer correlates with the microsatellite instability phenotype. Carcinogenesis. 2005; 26(6):1152–1156. [PubMed: 15760919]
9. Schuebel KE, Chen W, Cope L, et al. Comparing the DNA hypermethylome with gene mutations in human colorectal cancer. PLoS Genet. 2007; 3(9):1709–1723. [PubMed: 17892325]

10. Hoffman AM, Cairns P. Epigenetics of kidney cancer and bladder cancer. Epigenomics. 2011; 3(1):19–34. [PubMed: 22126150]
11. Kim YK, Kim WJ. Epigenetic markers as promising prognosticators for bladder cancer. Int J Urol. 2009; 16(1):17–22. [PubMed: 18721202]
12. Wong TS, Gao W, Li ZH, Chan JY, Ho WK. Epigenetic dysregulation in laryngeal squamous cell carcinoma. J Oncol. 2012:739461. [PubMed: 22645613]
13. Ha PK, Califano JA. Promoter methylation and inactivation of tumour-suppressor genes in oral squamous-cell carcinoma. Lancet Oncol. 2006; 7(1):77–82. [PubMed: 16389187]
14. Hoque MO, Kim MS, Ostrow KL, et al. Genome-wide promoter analysis uncovers portions of the cancer methylome. Cancer Res. 2008; 68(8):2661–2670. [PubMed: 18413733]
15. Belinsky SA, Klinge DM, Stidley CA, et al. Inhibition of DNA methylation and histone deacetylation prevents murine lung cancer. Cancer research. 2003; 63(21):7089–7093. [PubMed: 14612500]
16. McCabe MT, Low JA, Daignault S, Imperiale MJ, Wojno KJ, Day ML. Inhibition of DNA methyltransferase activity prevents tumorigenesis in a mouse model of prostate cancer. Cancer Res. 2006; 66(1):385–392. [PubMed: 16397253]
17. Brodie, SA.; Li, G.; Al-Kommos, A., et al. Class I HDACs are mediators of smoke-carcinogen induced stabilization of DNMT1 and serve as promising targets for chemoprevention of lung cancer. AACR special conference on chromatin and epigenetics; Atlanta. 2013.
18. Gottlicher M, Minucci S, Zhu P, et al. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. EMBO J. 2001; 20(24):6969–6978. [PubMed: 11742974]
19. Lubbert M, Suciu S, Baila L, et al. Low-dose decitabine versus best supportive care in elderly patients with intermediate- or high-risk myelodysplastic syndrome (MDS) ineligible for intensive chemotherapy: final results of the randomized phase III study of the European Organisation for Research and Treatment of Cancer Leukemia Group and the German MDS Study Group. J Clin Oncol. 2011; 29(15):1987–1996. [PubMed: 21483003]
20. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 2009; 10(3):223–232. [PubMed: 19230772]
21. Kantarjian H, Issa JP, Rosenfeld CS, et al. Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. Cancer. 2006; 106(8):1794–1803. [PubMed: 16532500]
22. Olsen EA, Kim YH, Kuzel TM, et al. Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. J Clin Oncol. 2007; 25(21):3109–3115. [PubMed: 17577020]
23. Juergens RA, Wrangle J, Vendetti FP, et al. Combination epigenetic therapy has efficacy in patients with refractory advanced non-small cell lung cancer. Cancer Discov. 2011; 1(7):598–607. [PubMed: 22586682]
24. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis. 2010; 31(1):27–36. [PubMed: 19752007]
25. Duenas-Gonzalez A, Candelaria M, Perez-Plascencia C, Perez-Cardenas E, de la Cruz-Hernandez E, Herrera LA. Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors. Cancer Treat Rev. 2008; 34(3):206–222. [PubMed: 18226465]
26. Zullig LL, Jackson GL, Dorn RA, et al. Cancer incidence among patients of the U.S. Veterans Affairs Health Care System. Mil Med. 2012; 177(6):693–701. [PubMed: 22730846]
27. Aberle DR, Adams AM, Berg CD, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med. 365(5):395–409. [PubMed: 21714641]
28. Henschke CI, Yankelevitz DF, et al. International Early Lung Cancer Action Program I. Survival of patients with stage I lung cancer detected on CT screening. The New England journal of medicine. 2006; 355(17):1763–1771. [PubMed: 17065637]

29. Hittelman WN, Liu DD, Kurie JM, et al. Proliferative changes in the bronchial epithelium of former smokers treated with retinoids. J Natl Cancer Inst. 2007; 99(21):1603–1612. [PubMed: 17971525]
30. Farwell WR, Scranton RE, Lawler EV, et al. The association between statins and cancer incidence in a veterans population. J Natl Cancer Inst. 2008; 100(2):134–139. [PubMed: 18182618]
31. Rubin DB. Estimating causal effects from large data sets using propensity scores. Ann Intern Med. 1997; 127(8 Pt 2):757–763. [PubMed: 9382394]
32. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 363(1):24–35. [PubMed: 20530316]
33. Gillison ML, D'Souza G, Westra W, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst. 2008; 100(6):407–420. [PubMed: 18334711]
34. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst. 2000; 92(9):709–720. [PubMed: 10793107]
35. Finzer P, Ventz R, Kuntzen C, Seibert N, Soto U, Rosl F. Growth arrest of HPV-positive cells after histone deacetylase inhibition is independent of E6/E7 oncogene expression. Virology. 2002; 304(2):265–273. [PubMed: 12504567]
36. Hurt RD, Offord KP, Croghan IT, et al. Mortality following inpatient addictions treatment. Role of tobacco use in a community-based cohort. JAMA. 1996; 275(14):1097–1103. [PubMed: 8601929]
37. Lasser K, Boyd JW, Woolhandler S, Himmelstein DU, McCormick D, Bor DH. Smoking and mental illness: A population-based prevalence study. JAMA. 2000; 284(20):2606–2610. [PubMed: 11086367]
38. Wassenaar CA, Dong Q, Wei Q, Amos CI, Spitz MR, Tyndale RF. Relationship between CYP2A6 and CHRNA5-CHRNA3-CHRNB4 variation and smoking behaviors and lung cancer risk. J Natl Cancer Inst. 2011; 103(17):1342–1346. [PubMed: 21747048]
39. Mwenifumbo JC, Tyndale RF. Genetic variability in CYP2A6 and the pharmacokinetics of nicotine. Pharmacogenomics. 2007; 8(10):1385–1402. [PubMed: 17979512]
40. Stransky N, Egloff AM, Tward AD, et al. The mutational landscape of head and neck squamous cell carcinoma. Science. 2011; 333(6046):1157–1160. [PubMed: 21798893]
41. Comprehensive genomic characterization of squamous cell lung cancers. Nature. 2012; 489(7417): 519–525. [PubMed: 22960745]
42. Pickering CR, Zhang J, Yoo SY, et al. Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. Cancer Discov. 3(7):770–781. [PubMed: 23619168]
43. Ohta S, Uemura H, Matsui Y, et al. Alterations of p16 and p14ARF genes and their 9p21 locus in oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009; 107(1): 81–91. [PubMed: 19101490]
44. Calmon MF, Colombo J, Carvalho F, et al. Methylation profile of genes CDKN2A (p14 and p16), DAPK1, CDH1, and ADAM23 in head and neck cancer. Cancer Genet Cytogenet. 2007; 173(1): 31–37. [PubMed: 17284367]
45. Adler JT, Hottinger DG, Kunnimalaiyaan M, Chen H. Histone deacetylase inhibitors upregulate Notch-1 and inhibit growth in pheochromocytoma cells. Surgery. 2008; 144(6):956–961. discussion 61-2. [PubMed: 19041003]
46. Platta CS, Greenblatt DY, Kunnimalaiyaan M, Chen H. Valproic acid induces Notch1 signaling in small cell lung cancer cells. J Surg Res. 2008; 148(1):31–37. [PubMed: 18570928]

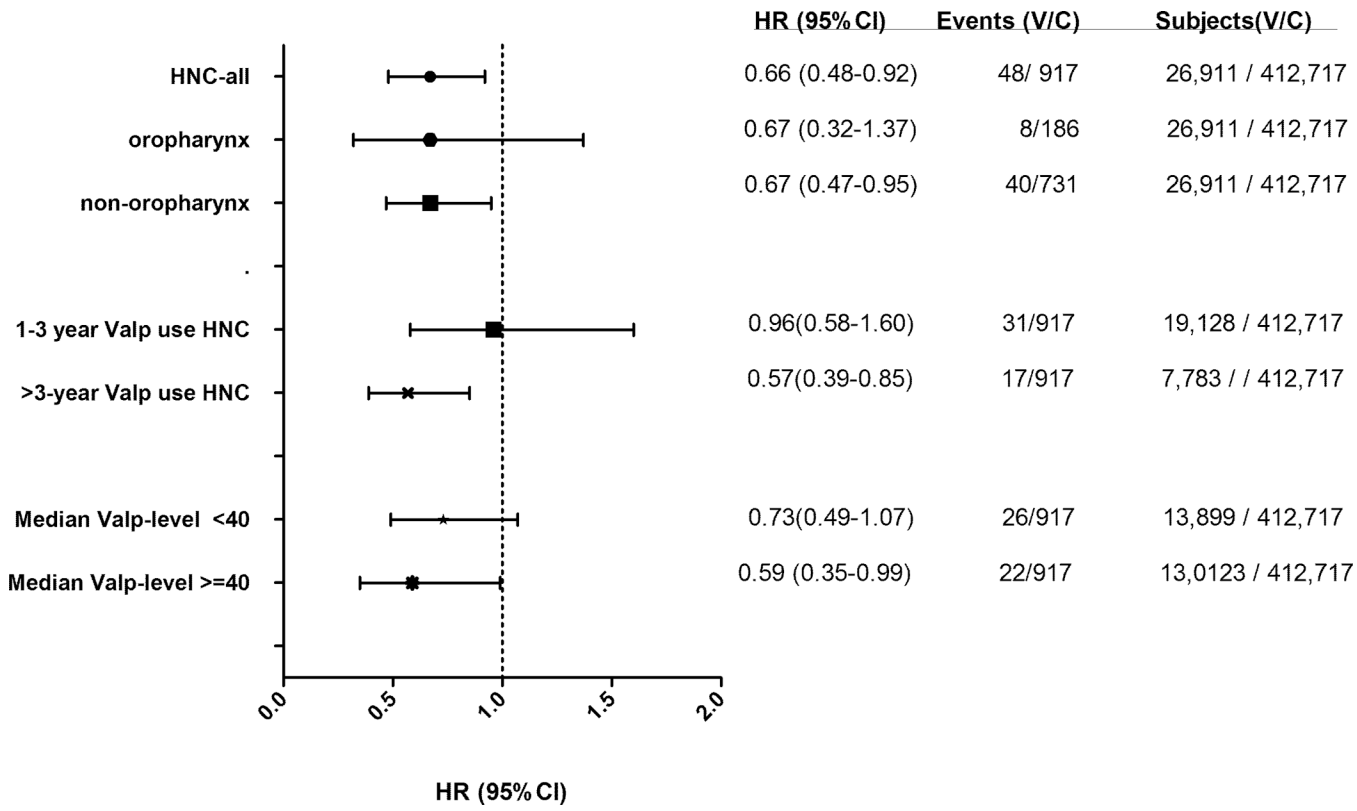

Figure 1.

Forrest Plot showing hazard ratio and 95% confidence intervals for the associations between VPA use and clinical scenarios. All analyses were conducted as Cox Proportional Hazard models and controlled for age, gender, race, smoking status, psychiatric disease (bipolar disorder, PTSD, schizophrenia, depression, anxiety), neurologic disease (seizures and migraines), and evidence of alcohol and substance abuse, COPD

Table 1

General characteristics

		Entire Cohort		
		VPA-use 26,911 (%)	**Non-VPA-use 412,717 (%)**	**p**
Age (years)	Mean	59.4	61.4	<0.001
	SD	9.2	9.8	
	Median	59	60	
Gender	Male	24,969 (92.78)	383,103 (92.82)	0.84
	Female	1,944 (7.22)	29,685 (7.18)	
Smoking	Past	13,660 (50.76)	208,964 (50.63)	0.68
	Current	13,251 (49.24)	203,753 (49.37)	
Race	Caucasian	19,753 (73.7)	294,391 (71.2)	<0.001
	African American	4,180 (15.6)	68,310 (16.5)	
	Hispanic	108 (0.40)	2,024 (0.49)	
	Asian	62 (0.23)	1,116 (0.27)	
	Hawaiian	263 (0.98)	3,835 (0.93)	
	Native American	175 (0.65)	3,390 (0.82)	
	unknown	2,265 (8.4)	39,886 (9.6)	
Bipolar		21,577 (80.18)	249,568 (46.47)	<0.001
PTSD		13,261 (49.28)	251,698 (61)	<0.001
Seizures		6,925 (25.73)	68,682 (16.74)	<0.001
Migraine		3,469(12.89)	46,661 (11.31)	<0.001
Anxiety		5,587 (20.76)	87,706 (21.25)	0.057
Depression		6,151 (22.86)	108,732 (26.35)	<0.001
Schizophrenia		3,377 (12.95)	16,128 (3.91)	<0.001
COPD		4,984 (18.52)	75,338 (18.25)	0.27
Alcohol and substance abuse		6,363 (26.64)	75,749 (18.35)	<0.001
BMI (mean)		29.68	29.5	0.41
Follow-up (mean) in years		4.42	4.40	0.11
Propensity score-mean		0.083	0.055	<0.001

Table 2

Cancer specific Hazard Ratios

Cancer site-specific analyses	Event counts	Incidence hazard ratios (95% confidence intervals)		
	Cases	Crude	Model 1¶	Model 2§
Lung				
VPA non-users*	2,151	1 (ref)	1 (ref)	1 (ref)
VPA users**	155	0.96 (0.81–1.14)	1.00 (0.84–1.19)	1.00(0.84–1.20)
Head and Neck				
VPA non-users*	916	1 (ref)	1 (ref)	1 (ref)
VPA users*	48	**0.68 (0.50–0.93)**	**0.66 (0.48–0.92)**	**0.67(0.48–0.92)**
Prostate				
VPA non-users***	4,334	1 (ref)	1 (ref)	1 (ref)
VPA users****	317	0.97 (0.86–1.10)	0.96(0.88–1.12)	0.96(0.85–1.09)
Colon and Rectum				
VPA non-users*	1,168	1 (ref)	1 (ref)	1 (ref)
VPA users*	83	0.90 (0.71–1.15)	0.95 (0.74–1.22)	0.95 (0.74–1.21)
Bladder				
VPA non-users*	1,388	1 (ref)	1 (ref)	1 (ref)
VPA users*	91	0.93 (0.71–1.21)	0.86 (0.64–1.15)	0.85 (0.63–1.14)

* 412,717 subjects (non-VPA-use),

** 26,911 subjects (VPA use)

*** 383,103 subjects (non-VPA-use),

**** 24,969 subjects (VPA use)

¶ Cox proportional hazard model adjusted for age, gender, race, smoking status, psychiatric disease (bipolar disorder, PTSD, schizophrenia, depression, anxiety), neurologic disease (seizures and migraines), and evidence of alcohol and substance abuse, COPD

§ Cox proportional hazard model adjusted for propensity score