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RESEARCH ARTICLE

Pharmacoepidemiology evaluation of bumetanide as a potential candidate for drug repurposing for Alzheimer's disease

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Abstract

INTRODUCTION: Bumetanide, a loop diuretic, was identified as a candidate drug for repurposing for Alzheimer's disease (AD) based on its effects on transcriptomic apolipoprotein E signatures. Cross-sectional analyses of electronic health records suggest that bumetanide is associated with decreased prevalence of AD; however, temporality between bumetanide exposure and AD development has not been established.

METHODS: We evaluated Medicare claims data using Cox proportional hazards regression to evaluate the association between time-dependent use of bumetanide and time to first AD diagnosis while controlling for patient characteristics. Multiple sensitivity analyses were conducted to test the robustness of the findings.

RESULTS: We sampled 833,561 Medicare beneficiaries, 60.8% female, with mean (standard deviation) age of 70.4 (12). Bumetanide use was not significantly associated with AD risk (hazard ratio 1.05; 95% confidence interval, 0.99–1.10).

DISCUSSION: Using a nationwide dataset and a retrospective cohort study design, we were not able to identify a time-dependent effect of bumetanide lowering AD risk.

KEYWORDS
Alzheimer's disease, drug repurposing, loop diuretics, pharmacoepidemiology

Highlights

- Bumetanide was identified as a candidate for repurposing for Alzheimer's disease (AD).
- We evaluated the association between bumetanide use and risk of AD.
- We used Medicare data and accounted for duration of bumetanide use.
- Bumetanide use was not significantly associated with risk of AD.

1 | INTRODUCTION

Therapeutic development in Alzheimer's disease (AD) has faced very low rates of success with high costs of late-stage failures.[1,2] Recently, anti-amyloid monoclonal antibodies (AAMAs) including aducanumab, lecanemab, and donanemab have achieved phase 3 success[3–5] with aducanumab earning US Food and Drug Administration approval under the accelerated pathway and lecanemab full approval.[6] These treatments, however, are expensive,[7] and require significant infrastructure to deliver their infusions and to monitor their safety for amyloid-related neuroimaging abnormalities.[8] Effective small molecule treatments would help address these challenges and potentially provide wider and more equitable drug access.

Repurposing medications, particularly small molecules, represents an approach that can diversify treatment targets, shorten the time to initiate clinical trials, and provide new therapeutic candidates.[9] There are numerous potential advantages to repurposed medications, as they are known to be pharmacologically active in humans, have known safety and tolerability profiles, have preclinical packages that are complete (including toxicological studies), with dose ranges that have been established for their primary indication, as well as having their metabolic and clearance pathways and drug–drug interactions characterized. At the same time, to be fit for purpose in AD, their central nervous system bioavailability, plasma/cerebrospinal fluid ratio, and optimal dose for activity in AD still need to be ascertained.

"Powder for Pennies" (P4P) is a collaborative multi-institutional effort hosted by the Alzheimer's Disease Cooperative Study (ADCS) at University of California San Diego, to develop and test a pipeline of repurposed medications or natural products for early development studies for AD. This program is building its pipeline through (1) drug screening in 3D human neural triculture, (2) in silico predictive modelling, (3) systematic reviews and evaluations of candidates, (4) mechanistic studies using networked human induced pluripotent stem cells and neurotransmitter profiles, and (5) pharmacoepidemiological assessments.

Bumetanide is a loop diuretic approved for the treatment of fluid overload in congestive heart failure and other disorders. Its diuretic action occurs at the kidney through inhibition of the Na-K-2Cl- co-transporter isoform NKCC2.[10] Bumetanide has been recognized as a potential compound of interest for repurposing in AD through drug screening and computational analysis of gene expression patterns with prediction that it can reverse the transcriptomic brain aging patterns in apolipoprotein (Apo) E4 knock-in mice.[11] It has also been shown to improve neuronal hyperexcitability, long-term potentiation, plasticity, and spatial learning in this mouse model. Additionally, existing evidence from J20/ApoE4 knock-in mice suggests that bumetanide rescues functional deficits and reduces amyloid beta plaque load.[11]

Recent analyses of electronic health record data observed a 35% to 75% lower prevalence of AD among individuals exposed to bumetanide.[11,12] These studies, however, followed a cross-sectional design, which is not able to establish temporal precedence between bumetanide exposure and AD development. Additionally, existing studies failed to consider how the risk of AD differs with the duration of bumetanide exposure.

RESEARCH IN CONTEXT

1. **Systematic review**: Based on computational screening of drug effects on transcriptomic apolipoprotein E signatures, experimental data with apolipoprotein E4 knock-in and amyloid mouse models and cross-sectional analyses of electronic health records data, bumetanide has been proposed as a potential compound of interest for drug repurposing in Alzheimer's disease (AD).
2. **Interpretation**: Our study design, which measured bumetanide exposure prior to incidence of AD, failed to replicate prior findings that bumetanide use was associated with a decreased risk to AD.
3. **Future directions**: Evaluations of real-world data can inform the identification of candidate compounds for repurposing; however, careful study design is needed to reduce risk of bias and confounding.

Following this lead, P4P has undertaken a systematic review of bumetanide including its pharmaceutical properties, potential mechanism of brain action, and activity when tested in other neurological conditions including epilepsy, autism, and Parkinson's disease.[13] In this report, we present the findings of the pharmacoepidemiology evaluation of bumetanide, which incorporated a longitudinal study design that enabled us to establish temporality between bumetanide exposure and AD development, and account for duration of bumetanide use.

2 | METHODS

2.1 | Overview of study design

We conducted a retrospective cohort study of claims data from a 5% random sample of Medicare beneficiaries to overcome limitations of previous work in examining the association of bumetanide use with AD and all-cause dementia risk. Our analysis included bumetanide and other loop diuretics (furosemide, torsemide). Additionally, we included variables representing use of other antihypertensive agents to mitigate confounding. De-identified claims data were obtained under a Data User Agreement with the Centers for Medicare and Medicaid Analyses. The study was deemed exempt by the University of California, San Diego Institutional Review Board, as only de-identified data were used in analyses.

Figure 1 shows the study design, outlining the timing of measurement of drug use and study outcomes. We selected individuals free of AD or all-cause dementia who filled at least one prescription for any drug of interest (loop diuretics, non-loop diuretics, and non-diuretic

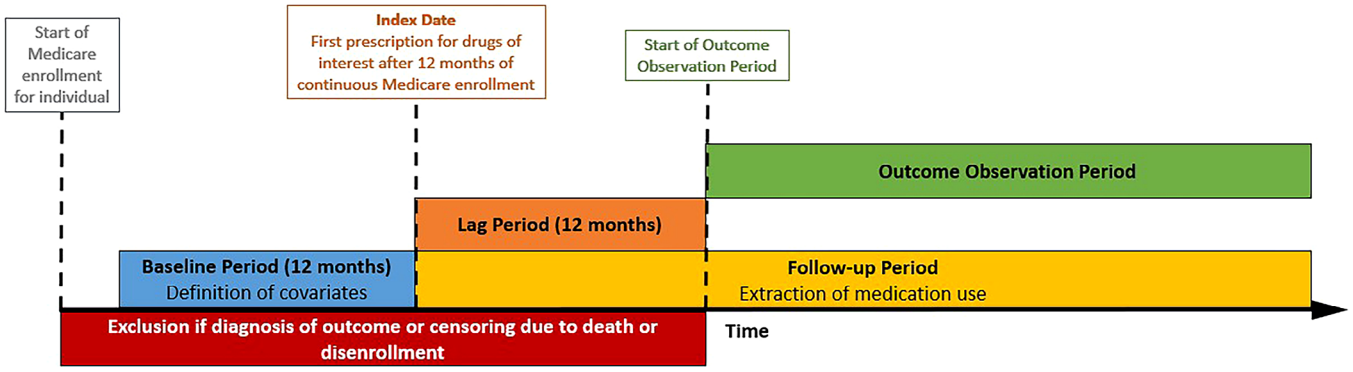

FIGURE 1 Study design. The figure summarizes the sample selection and study design. First, we selected individuals who were continuously enrolled in Medicare fee-for-service for at least 1 year. Index date was defined as the date of the first prescription for a drug of interest (loop diuretics, non-loop diuretics, and non-diuretic antihypertensives). The 360-day period preceding index date was the baseline period used for the definition of covariates. Medication use was assessed starting on index date and throughout all the follow-up period available for a patient. To enable sufficient time for medications to have a detectable effect on outcomes, we applied a 360-day lag period after index date, and only started to collect outcome events 360 days after index date (start of the outcome observation period). The start of the outcome observation period was used as time zero for survival analyses. Patients who had a diagnosis of the outcome or were censored before the start of the outcome observation period were excluded from analyses, as they would not have time at risk.

antihypertensives) after being enrolled in Medicare fee-for-service Parts A, B, and D for at least 1 year. The date of the first prescription for a drug of interest was defined as the index date. We measured outcome events (AD and all-cause dementia) starting 360 days after the index date. We applied this lag period because AD and all-cause dementia develop over time; therefore, we assumed a minimum exposure was needed for the drugs of interest to have a plausible association with the outcomes of interest, as previously done in the literature.[14] Outcome events were collected from the end of the lag period until the occurrence of an outcome event, death, disenrollment, or end of the study period.

2.2 | Data source and study participants

We used claims data collected from January 1, 2006, through December 31, 2020, and selected the sample of Medicare beneficiaries in eight steps (Figure S1 in supporting information). First, we identified beneficiaries who filled at least one pharmacy claim for a loop diuretic, non-loop diuretic, or non-diuretic antihypertensive medication between 2006 and 2018 after being continuously enrolled in Medicare fee-for-service for 365 days. The date of the first prescription for a drug of interest after this 360-day period was defined as the index date. By constraining the filling of prescriptions to before the end of 2018, we ensured that patients had a minimum follow-up of 2 years available, if alive and enrolled in Medicare-fee-for-service. Second, we excluded beneficiaries who were not continuously enrolled for 365 days after the index date. Third, we excluded beneficiaries with a diagnosis of mild cognitive impairment or AD at baseline or during the lag period. Finally, we excluded patients who died, disenrolled from Medicare fee for service, or were censored by the end of the 360-day lag period. This ensured that follow-up data were available for the totality of the sample selected. We followed the selected sample until the first of the following events: disenrollment from Medicare, death, or end of the study period (December 31, 2020).

2.3 | Exposure

To define the exposures of interest, we extracted all prescriptions for drugs of interest starting on the index date and throughout the follow-up period. The drugs of interest included the loop diuretics bumetanide, furosemide, and torsemide, which were assessed independently, and non-loop diuretics, and non-diuretic antihypertensives, which were assessed as a class. Non-loop diuretics included thiazides and potassium-sparing diuretics. Non-diuretic antihypertensives included angiotensin II receptor blockers (ARBs), angiotensin-converting enzyme (ACE) inhibitors, beta-blockers, and non-dihydropyridine calcium channel blockers (list of drugs in Table S1 in supporting information). Using the prescription fill date and the days of supply, we created a supply diary. The supply diary assessed whether beneficiaries had possession of bumetanide, furosemide, torsemide, non-loop diuretics, or non-diuretic antihypertensives each day of the exposure assessment window. If patients filled a prescription before the previous one ran out, it was assumed that they began using the new prescription after completing the previous one.

We defined time-dependent exposures using two different approaches used in peer-reviewed literature.[11] In the first set of analyses, for each 30-day interval, we evaluated whether an individual had possession of each of the drug groups of interest (bumetanide, furosemide, torsemide, non-loop diuretics, and non-diuretic antihypertensives). An individual was considered to have used a drug in a given 30-day interval if they had possession of the drug at least 1 day of the interval, regardless of the dose used. In this manner, we created time-dependent indicator variables denoting use of bumetanide, furosemide, torsemide, non-loop diuretics, and non-diuretic

antihypertensives for each 30-day interval of the study period. In the second set of analyses, we summed the number of 30-day intervals that a patient had used each drug group of interest, thus creating a variable that represented total months of possession of each of the drug groups up to that point. This approach enabled us to code time-dependent continuous *cumulative exposure* variables denoting the number of 30-day intervals that an individual had used these medications. We divided the resulting variables by 12 to express the *cumulative use* of medications in 1-year increments and improve interpretability of the findings.

2.4 | Outcomes

The primary outcome was time to first occurrence of AD, and the secondary outcome was time to first occurrence of all-cause dementia. Outcomes were defined following the Centers for Medicare & Medicaid Services (CMS) Chronic Conditions Data Warehouse definitions, which defines outcomes as having one inpatient or outpatient claim among specific International Classification of Diseases (ICD)-9 or ICD-10 codes.[15] Specifically, AD was defined as having one inpatient or outpatient claim with the ICD-9 code 331, or ICD-10 code G.30x. All-cause dementia was defined as having one inpatient or outpatient claim with ICD-9 codes 331, 331.x, 331.2, 290.4x, 331.7, 290.0, 290.1x, 290.2x, 290.3, 294.0, 294.1x, 294.2x, and 797, or ICD-10 codes G30.x, G31.0x, G31.1, F01.5x, G13.8, G31.2, G94, F02.8x, F03.9x, F04, F05, F06.1, F06.8, F41.81, and R54. Against data from the Consortium to Establish a Registry for Alzheimer's Disease, the sensitivity of AD and all-cause dementia was 79% and 87%, respectively, for patients continuously enrolled in Medicare fee-for-service.[16] As described above, we ascertained outcomes starting 360 days after the index date. We applied this lag period because AD and all-cause dementia develop over time; so, we assumed a minimum exposure was needed for the drugs of interest to have a plausible association with AD and all-cause dementia.[14]

2.5 | Covariates

Covariates were selected a priori based on their potential role as confounders (i.e., variables that are associated with antihypertensive medication use and AD outcomes) and were all defined as of the index date.[14] Sociodemographic characteristics included sex, race/ethnicity (Black, Hispanic, White, Asian, North American Native, other, or unknown race and ethnicity), and receipt of low-income subsidy or Medicaid status. Clinical factors measured at baseline included atrial fibrillation, chronic heart failure, chronic kidney disease, depression, diabetes, ischemic heart disease, hypertension, and stroke or transient ischemic attack, all were defined using CMS Chronic Conditions Data Warehouse definitions.[15] In sensitivity analyses, we further included as covariates a history of traumatic brain injury, defined as having one inpatient or outpatient claim with ICD-9 codes 800.0–801.9, 803.0–804.9, 850.0–854.1, 950.1–950.3, 995.55, and 959.01, or ICD-10-CM codes S02.0, S02.1, S02.8, S02.91, S04.02, S04.03, S04.04, S06, S07.1, and T74.4,[17,18] and the number of other CMS Chronic Conditions Data Warehouse priority conditions that were not included as separate indicator variables in the model.

2.6 | Statistical analysis

We compared baseline patient characteristics across individuals who ever used or never used bumetanide at any point during the follow-up period. We calculated the incidence rate of outcomes in each time-dependent treatment group per 100 person-years. In the first set of analyses, we constructed a cause-specific hazards regression analysis using Cox proportional hazard models and the five time-dependent indicator variables denoting *ever use* of each drug in any 30-day interval up to that time point. Death was a competing risk. Covariates included all variables listed above and were incorporated in the model as time-fixed variables with the exception of hypertension. Hypertension was not included due to concerns related to collinearity, as the exposure variables represent use of antihypertensive medications. Time zero was the end of the lag period and the time at risk was censored at disenrollment from the Medicare fee-for-service program, or the end of the study period (December 31, 2020), whichever came first. We used time since study entry as the time scale in time-to-event analyses. Time-dependent variables denoting drug use were lagged to the 30-day interval prior to ensure that the exposure was measured prior to the measure of the outcome. For example, the incidence of AD in the second 30-day interval was regressed against medication use in the first 30-day interval.

In the second set of analyses, we executed Cox proportional hazard models incorporating the time-dependent continuous variables denoting the *cumulative duration of use* of each drug group. As explained above, we transformed these variables into 1-year increments in duration of use of each treatment, to aid interpretation of the results.

All analyses were performed with SAS statistical software, version 9.4 (SAS Institute Inc.). All p values were from Cox proportional hazard models and results were deemed statistically significant at $p < 0.05$. Data extraction and statistical analyses were conducted from August 1, 2022, to June 30, 2023.

2.7 | Sensitivity analyses

We tested the robustness of our findings to several modifications in the sample selection criteria and list of covariates. First, to address confounding by indication, we constrained sampling to individuals who had a diagnosis of chronic heart failure at baseline, the leading indication for loop diuretic use. Chronic heart failure was defined using the CMS Chronic Conditions Data Warehouse definition.[15] Second, we constrained sampling to those < 70 years of age as of the index date to determine any age differences. Third, we restricted the sample to only individuals with an index date before January 2010, which allowed us to constrain sampling to participants with a longer follow-up period. Finally, we controlled for traumatic brain injury and number of CMS Chronic Condition Data Warehouse priority conditions that

are not independently specified as indicator variables,[15] that is, not already a part of the model, to account for potential differences in the underlying health status of study participants. These included a history of myocardial infarction, chronic obstructive pulmonary disease, cataract, glaucoma, hip or pelvic fracture, osteoporosis, rheumatoid arthritis/osteoarthritis, breast cancer, colorectal cancer, endometrial cancer, lung cancer, and prostate cancer.

2.8 | Negative control calibration

To evaluate the potential for residual confounding in analyses, we reproduced the base case analyses using two negative control outcomes, which are believed not to be associated with use of diuretics. These include cataract surgery, defined as having Current Procedural Terminology codes 66830, 66840, 66850, 66852, 66920, 66940, 66982, 66983, 66984, 66987, and 66988,[19] and non-melanoma skin cancer, defined as having ICD-9 code 173.X or ICD-10 code C44.[20] Both were measured in the outcomes observation period, and defined as time-to-event variables.

3 | RESULTS

3.1 | Baseline beneficiary characteristics

The final sample included 833,561 Medicare beneficiaries (mean [SD] age, 70.4 [12] years; 506,830 [60.8%] were women and 326,731 [39.2%] were men; 690,894 [82.9%] White, 85,360 [10.2%] Black, 16,861 [2%] Hispanic, and 40,446 [4.9%] individuals of other racial/ethnic groups). Table 1 compares baseline characteristics of individuals who used bumetanide at any point of the study period versus those who did not. Bumetanide users were older compared to non-bumetanide users (mean [SD] age 74.7 [12.1] vs. 70.3 [12]). At baseline, bumetanide users had higher prevalence of chronic conditions, including atrial fibrillation, chronic heart failure, chronic kidney disease, depression, diabetes, ischemic heart disease, hypertension, and stroke or transient ischemic attack. Other characteristics such as race, sex, and low-income subsidy and Medicaid status were similar between bumetanide users and non-bumetanide users.

3.2 | Mean follow-up time in time-dependent treatment groups

Individuals were followed for a median of 5.5 years (interquartile range [IQR], 3.42–8.25 years) in the first set of analyses examining AD and 5.17 years (IQR, 3.17–7.92 years) for the second set of analyses examining all-cause dementia. Measures of distribution of the follow-up period are shown in Table 2. In sensitivity analyses for individuals with an index date before January 2010, individuals were followed for a median of 6.75 years (IQR, 3.33–11.67 years) for the outcome AD and a median of 6.0 years (IQR, 3.0–11.08 years) for the outcome of all-cause dementia. Beneficiaries spent 28,179 person-years (0.9% of follow-up time) in the bumetanide group, 316,523 person-years in the furosemide group (15.2% of follow-up time), 28,503 person-years in the torsemide group (1.0% of follow-up time), 295,310 person-years in the non-loop diuretics group (15.2% of follow-up time), and 792,628 person-years in the non-diuretic antihypertensives group (74.6% of follow-up time; Table 3).

3.3 | Unadjusted rates of AD and all-cause dementia

The unadjusted incidence density rate of AD was 1.18 cases per 100 person-years for the overall sample, with a total of 50,865 study participants experiencing AD (Table 2). The unadjusted incidence density rate of all-cause dementia was 3.95 cases per 100 person-years, with 159,181 persons developing all-cause dementia. The incidence rate of both AD and all-cause dementia was higher for the subset of individuals with heart failure, lower for the subset of individuals < 70 years of age at baseline.

3.4 | Hazard ratios of AD

In fully adjusted models, *ever use* of bumetanide was associated with a slightly increased risk of AD, but this association was not significant (hazard ratio [HR], 1.05; 95% confidence interval [CI], 0.999–1.10; Figure 2, left). There were no significant associations between *ever use* of furosemide, torsemide, or non-diuretic antihypertensives and AD risk. *Ever use* of non-loop diuretics was associated with 11% lower risk of AD (HR, 0.89; 95% CI, 0.87–0.90).

There were no significant associations between the cumulative number of years of bumetanide use, furosemide use, or torsemide use and AD risk (Figure 3, left). A 1-year increase in the use of non-loop diuretics was associated with 3.6% decreased risk of AD (HR, 0.964 per 1-year increment; 95% CI, 0.959–0.969). The number of years of non-diuretic antihypertensive use was significantly associated with AD risk, but the magnitude of the association was small (HR, 1.009 per 1-year increment; 95% CI, 1.005–1.014).

3.5 | Hazard ratios of all-cause dementia

In fully adjusted models, *ever use* of bumetanide, torsemide, or furosemide was associated with 23% (HR, 1.23; 95% CI, 1.20–1.26), 27% (HR, 1.27; 95% CI, 1.25–1.28), and 21% (HR, 1.21; 95% CI, 1.18–1.24) higher risk of all-cause dementia, respectively (Figure 2, right). *Ever use* of non-loop diuretics was associated with 4% lower risk of all-cause dementia (HR, 0.96; 95% CI, 0.95–0.97). There was no significant association between use of non-diuretic antihypertensives and all-cause dementia.

A 1-year increase in the use of bumetanide was associated with 4.1% increase in the risk of all-cause dementia (HR, 1.041; 95% CI, 1.032–1.051; Figure 3, right); a similar association was found for furosemide and torsemide (HR, 1.036; 95% CI, 1.033–1.038 for furosemide; HR,

TABLE 1 Baseline sample characteristics.

	Overall sample (*n* = 833561)	Bumetanide users (*n* = 28179)	Non-bumetanide users (*n* = 805382)
Age, mean (SD), years	70.4 (12)	72 (12)	70.4 (12)
Aged < 65, no. (%)	157,958 (19)	5660 (20.1)	152,298 (18.9)
Aged 65–69, no. (%)	236,962 (28.4)	5662 (20.1)	231,300 (28.72)
Aged 70–74, no. (%)	154,845 (18.6)	4912 (17.4)	149,933 (18.6)
Aged 75–79, no. (%)	116,872 (14)	4587 (16.3)	112,285 (13.9)
Aged 80 +, no. (%)	166,924 (20)	7358 (26.11)	159,566 (19.8)
Sex, no. (%)			
Male	326,731 (39.2)	10,344 (36.7)	316,387 (39.3)
Female	506,830 (60.8)	17,835 (63.3)	488,995 (60.7)
Race, no. (%)			
White	690,894 (82.9)	23,988 (85.1)	666,906 (82.8)
Black	85,360 (10.2)	2988 (10.6)	82,372 (10.2)
Hispanic	16,861 (2)	452 (1.6)	16,409 (2)
Other[a]	40,446 (4.9)	751 (2.7)	39,695 (4.9)
Low income or Medicaid, no. (%)			
Medicaid	180,205 (21.6)	6693 (23.8)	173,512 (21.5)
Partial Medicaid	57,569 (6.9)	2212 (7.9)	55,357 (6.9)
No Medicaid, low-income subsidy	46,694 (5.6)	1914 (6.8)	44,780 (5.6)
No Medicaid, not low-income subsidy	549,093 (65.9)	17,360 (61.6)	531,733 (66)
Chronic condition at baseline, no. (%)			
Atrial fibrillation	112,216 (13.5)	7836 (27.8)	104,380 (13)
Chronic heart failure	222,230 (26.7)	16,529 (58.7)	205,701 (25.5)
Chronic kidney disease	170,028 (20.4)	10,979 (39)	159,049 (19.8)
Depression	235,234 (28.2)	9521 (33.8)	225,713 (28)
Diabetes	311,233 (37.3)	15,758 (55.9)	295,475 (36.7)
Ischemic heart disease	382,621 (45.9)	19,477 (69.1)	363,144 (45)
Hypertension	693,125 (83.2)	24,378 (86.5)	668,747 (83)
Stroke or transient ischemic attack	92,882 (11.1)	4415 (15.7)	88,467 (11)

Abbreviation: SD, standard deviation.
[a]Other race/ethnicity include individuals identified as Asian or North American Native, and those categorized as other or unknown race/ethnicity in the Medicare data.

1.041; 95% CI, 1.032–1.050 for torsemide). A 1-year increase in the use of non-loop diuretics was associated with 2.5% decreased risk of all-cause dementia (HR, 0.975; 95% CI, 0.972–0.978). A 1-year increase in the use of non-diuretic antihypertensives was associated with 2.9% decreased risk of all-cause dementia (HR, 0.971; 95% CI, 0.969–0.974).

3.6 | Sensitivity analyses

Findings of sensitivity analyses were generally consistent with the primary results. In brief, after constraining sampling to individuals with heart failure, the association between bumetanide use and AD remained non-significant, regardless of whether bumetanide use was measured as *ever use* (Table S2 in supporting information) or as number of years of use (Table S3 in supporting information). *Ever use* of bumetanide, however, remained associated with increased risk of all-cause dementia (Table S4 in supporting information).

When constraining the sample to individuals ≤ 70 years of age at baseline, *ever use* of bumetanide was associated with a slightly increased risk of AD; however, the number of years of bumetanide use was not significantly associated with AD (Tables S2 and S3). Bumetanide use, like furosemide and torsemide use, was associated with increased risk of all-cause dementia, regardless of the functional form of the variable (Tables S4 and S5 in supporting information).

When constraining the sample to individuals with an index date before January 2010, which extended the average follow-up

TABLE 2 Unadjusted incidence rate of outcomes.

AD analysis	*n*	Total person-years (P - Y)	Minimum to maximum follow-up (years)	Average follow-up (years)	Median (25th percentile, 75th percentile) follow-up (years)	No. of AD events	AD rate per 100 P-Y
Overall sample	833,561	4,293,688	(0.08, 13.25)	5.15	5.5 (3.42, 8.25)	50,865	1.18
Sensitivity analysis I: heart failure cohort	222,230	961,310	(0.08, 13.25)	4.33	4.58 (2.75, 7.08)	18,025	1.88
Sensitivity analysis II: overall sample < 70 years	394,920	2,169,362	(0.08, 13.25)	5.49	6.0 (3.75, 8.83)	9185	0.42
Sensitivity analysis III: index date before January 2010	333,487	2,121,750	(0.08, 13.25)	6.36	6.75 (3.33, 11.67)	32,893	1.55
All-cause dementia analysis	***n***	**Total P-Y**	**Minimum to maximum follow-up (years)**	**Average follow-up (years)**	**Median (25th percentile, 75th percentile) follow-up (years)**	**No. of All-cause dementia events**	**All-cause dementia rate per 100 P - Y**
Overall sample	833,488	4,034,865	(0.08, 13.25)	4.84	5.17 (3.17, 7.92)	159,181	3.95
Sensitivity analysis I: heart failure cohort	222,197	869,809	(0.08, 13.25)	3.91	4.08 (2.42, 6.67)	61,377	7.06
Sensitivity analysis II: overall sample < 70 years	394,912	2,092,406	(0.08, 13.25)	5.30	5.75 (3.58, 8.50)	37,683	1.80
Sensitivity analysis III: index date before January 2010	333,449	1,961,048	(0.08, 13.25)	5.88	6.0 (3.0, 11.08)	90,591	4.62

Note: Samples for the AD and all-cause dementia analyses are different as patients are assigned to treatment groups on a time-dependent manner, and time at risk differs between two outcomes, as patients are only followed until first diagnosis (of AD or all-cause dementia). Results for Sensitivity Analysis IV are not shown in the table as it was performed on the overall sample and results were unchanged.
Abbreviations: AD, Alzheimer's disease; P -Y, person-years.

TABLE 3 Proportion of time at risk, by treatment group.

	Bumetanide	Furosemide	Torsemide	Non-loop diuretics	Non-diuretic antihypertensives
n	28,179	316,523	28,503	295,310	792,628
Person-years	39,418	654,403	44,213	653,243	3,204,123
% follow-up time	0.9%	15.2%	1.0%	15.2%	74.6%

Note: Proportion of time at risk by treatment group is shown based on analysis for the Alzheimer's disease outcome; proportion of time at risk may differ for the all-cause dementia outcome as patients may have been censored at earlier times. Non-loop diuretics included diuretic drugs that are not loop diuretics (thiazides and potassium-sparing diuretics). Non-diuretic antihypertensives include ACE inhibitors, ARBs, calcium channel blockers, and beta-blockers.
Abbreviations: ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blockers.

period available in the sample, the association between bumetanide use and AD remained non-significant, regardless of whether bumetanide use was measured as *ever use* (Table S2) or as number of years of use (Table S3). Use of bumetanide, furosemide, or torsemide was associated with increased risk of all-cause dementia (Tables S4 and S5).

When reproducing the base case analyses on the overall sample with the addition of a history of traumatic brain injury and the number of other CMS chronic conditions as covariates, the association between bumetanide use and AD remained non-significant, regardless of whether bumetanide use was measured as *ever use* (Table S2) or as number of years of use (Table S3). Use of bumetanide, furosemide, or torsemide was associated with increased risk of all-cause dementia (Tables S4 and S5).

3.7 | Negative control calibration

Ever use of bumetanide was not significantly associated with cataract surgery or non-melanoma skin cancer; however, *ever use* of furosemide was associated with a modest increase in the risk of cataract surgery (HR, 1.08; 95% CI, 1.06–1.09) and non-melanoma skin cancer (HR, 1.03; 95% CI, 1.01–1.04; Table S6 in supporting information). When measured as number of years of use, neither bumetanide nor

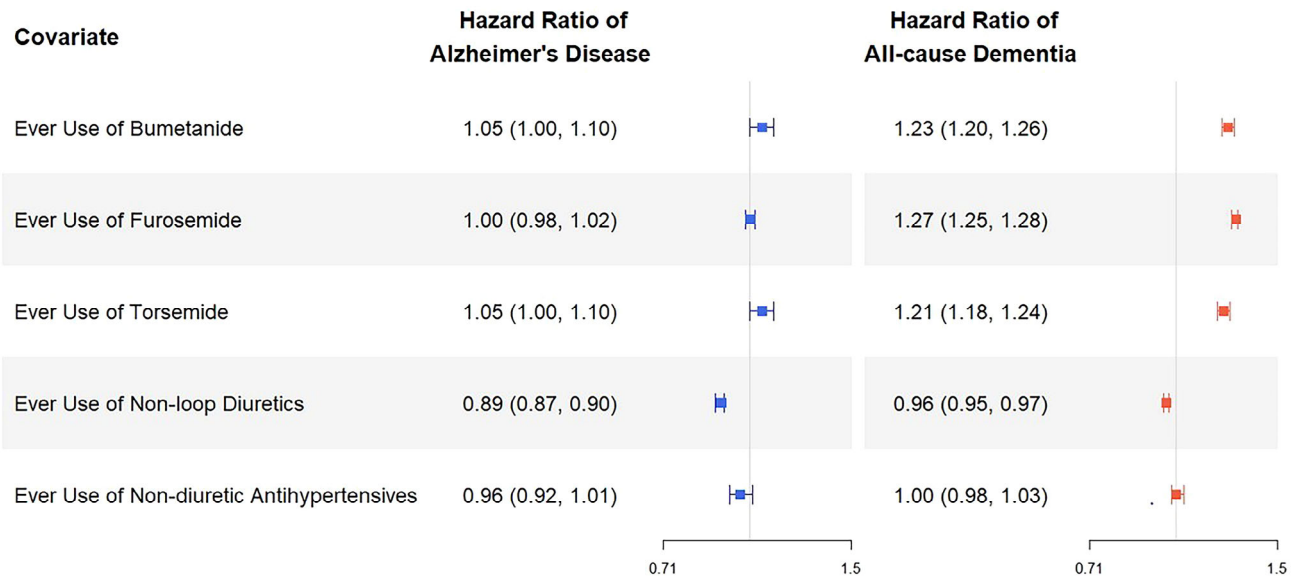

FIGURE 2 Adjusted hazard ratios of Alzheimer's disease and all-cause dementia for *ever use* of medications of interest. *Ever use* of of medications was defined with time-varying indicator variables, which denoted whether an individual had used a drug of interest at any point of time prior to the period of assessment. In other words, once an individual used a drug, the indicator variable for *ever used* remained 1 throughout follow-up. Non-loop diuretics included diuretic drugs that are not loop diuretics (thiazides and potassium-sparing diuretics). Non-diuretic antihypertensives include ACE inhibitors, ARBs, calcium channel blockers, and beta-blockers. The model was adjusted for age, sex, race, low-income subsidy, Medicaid eligibility, and all chronic conditions listed in Table 1 with the exception of hypertension. ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blockers.

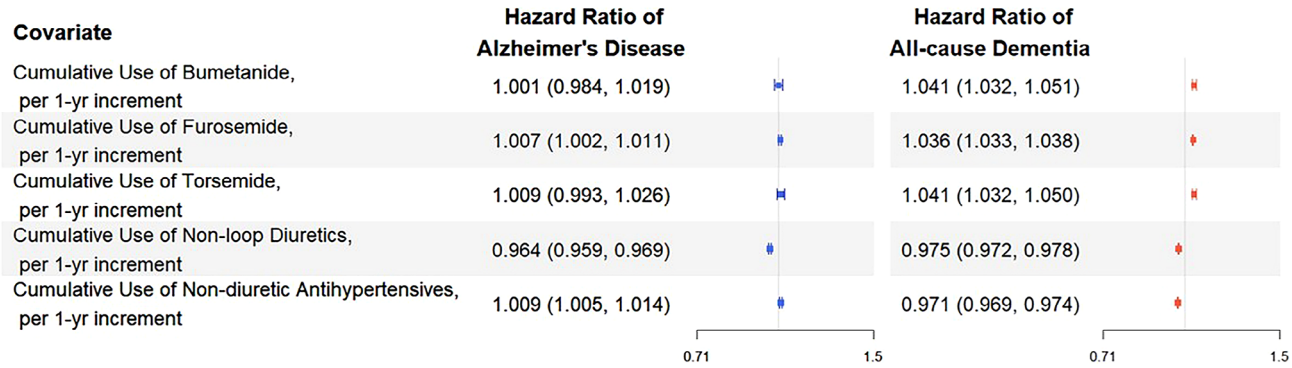

FIGURE 3 Adjusted hazard ratios of AD and all-cause dementia, per 1-year increment in drug use. *Cumulative use* of medications was defined with continous time-dependent variables, and was measured at each interval as the cumulative number of 30-day intervals that each subject had used a specific medication. To improve interpretability, the variable was expressed per 1-year increments. For example, the hazard ratio of AD associated with bumetanide can be interpreted as follows: For each 1-year increment in the use of bumetanide, the hazards of AD increased by 0.1% (95% CI, −1.6% to 1.9%). Non-loop diuretics included diuretic drugs that are not loop diuretics (thiazides and potassium-sparing diuretics). Non-diuretic antihypertensives include ACE inhibitors, ARBs, calcium channel blockers, and beta-blockers. The model was adjusted for age, sex, race, low-income subsidy, Medicaid eligibility, and all chronic conditions listed in Table 1 with the exception of hypertension. AD, Alzheimer's disease; ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blockers; CI, confidence interval.

furosemide were significantly associated with the negative control outcomes (Table S7 in supporting information).

4 | DISCUSSION

Based on computational screening of drug effects on transcriptomic apolipoprotein E (*APOE*) signatures, experimental data with ApoE4 knock-in and amyloid mouse models and cross-sectional analyses of electronic health records data, bumetanide has been proposed as an important candidate drug for repurposing for AD. In this study, we overcame the limitations of existing pharmacoepidemiologic assessments to further evaluate its potential clinical utility. Specifically, we applied a longitudinal study design to claims data from a nationally representative sample of Medicare beneficiaries and measured the association between bumetanide exposure and incidence of AD and

all-cause dementia. Our study design was unable to replicate prior findings associating bumetanide with decreased risk of AD. Use of bumetanide or other loop diuretics, such as furosemide and torsemide, was not significantly associated with AD, regardless of whether the exposure was measured as ever use of the drug or as the number of years of drug use. Loop diuretic use was, however, associated with increased risk of all-cause dementia. This association may represent confounding by indication, as loop diuretic users are more likely to have underlying cardiovascular disease associated with increased risk of dementia of vascular etiology.[21,22]

The results of the current analysis are an important contribution to the existing literature exploring the potential of bumetanide as a candidate for drug repurposing for AD. Previous studies based on electronic health record data have associated bumetanide exposure with a 35% to 75% reduced prevalence of AD.[11,12] These studies, however, are limited by their cross-sectional designs, which prevents establishment of temporality. That is, the existing cross-sectional studies could not determine whether bumetanide use preceded the development of AD. Bumetanide users are likely to have unfavorable outcomes earlier in life due to the underlying cardiovascular pathology for which bumetanide was prescribed; this can lead to survival bias and lower prevalence of AD compared to individuals who were never exposed to bumetanide, who are likely to live longer.

To overcome the limitations of prior work, we used a retrospective cohort design, which ensured that drug use was measured prior to the development of dementia. We incorporated time-dependent variables to account for changes in medication use over time as well as duration of drug use. In sensitivity analyses, we further controlled for the number of CMS Chronic Condition Data Warehouse priority conditions, which enabled us to control for potential differences in the underlying health status of patients. To minimize the risk of confounding by indication, we performed a sensitivity analysis restricting the sample to individuals with chronic heart failure, the leading indication for bumetanide. Then, to rule out the possibility that bumetanide may be protective of AD when individuals are exposed earlier in life, we performed sensitivity analyses constraining sampling to individuals who were $\leq$ 70 years of age at baseline. None of these analyses yielded findings suggestive of an association between bumetanide and decreased AD risk. We further evaluated the association between bumetanide use and two negative control outcomes (cataract surgery and non-melanoma skin cancer) to evaluate the potential for residual confounding. Bumetanide use was not significantly associated with either of the negative control outcomes; however, there was a minor increase in the hazards of cataract surgery and non-melanoma skin cancer associated with furosemide, which may be indicative of residual confounding in those analyses.

Despite our adjustment for multiple clinical characteristics and the execution of several sensitivity analyses, the results presented herein may have been vulnerable to inadequate control of confounding by indication, as patients receiving bumetanide may represent particularly severe cases of heart failure or other serious illnesses who cannot be clinically managed with other diuretics commonly prescribed as first-line.[23] Confounding by indication may explain why we found that use of bumetanide was associated with increased risk of all-cause dementia—the underlying cardiovascular pathology for which bumetanide was prescribed was likely a risk factor for the development of dementia of vascular etiology. This is one of the reasons why our failure to find an association between bumetanide exposure with decreased risk of AD does not necessarily mean that there is not a mechanism through which bumetanide may present with AD protective benefits. It should be noted, however, that our null findings are consistent with an investigation of the AD protective effect of medications prescribed in the United States, which identified five therapeutic classes as candidates for drug repurposing, none of which were diuretics.[24] It is also possible that, while bumetanide may be pharmacologically active in improving impairment in ApoE4 knock-in mice, the brain bioavailable levels of bumetanide in humans may be insufficient to reach a therapeutic effect.[10] These results illustrate some of the discordance that can rise across the different types of evidence generated for drug repurposing across pre-clinical to pharmacoepidemiology assessments.

We observed that use of non-loop diuretics and of non-diuretic antihypertensives was associated with decreased risk of all-cause dementia. This is consistent with previous evidence on the association between use of diuretic antihypertensives and blood pressure control with development of dementia.[25] However, use of non-diuretic antihypertensives was not associated with risk of AD; this difference may be explained by the more prominent role of hypertension in the development of vascular etiology rather than AD.[22,26]

This study has several limitations. First, due to unavailability of medical claims for individuals enrolled in Medicare Advantage, we constrained sampling to Medicare fee-for-service beneficiaries, which could limit the generalizability of the findings. While the racial/ethnic composition of the sample was consistent to that of prior studies based on Medicare fee-for-service beneficiaries,[14,27–29] it was overly representative of White individuals and was not able to capture the current racial/ethnic diversity of the US population. Second, claims data do not contain information on genetic (*APOE* genotype) or lifestyle (smoking patterns and diet) risk factors for AD; thus, findings may be vulnerable to residual confounding. Further, claims data do not report vital signs or results of laboratory and diagnostic tests, so we were not able to control for body mass index, blood pressure, or cholesterol levels. Although we were unable to control for education level, we adjusted for eligibility for Medicaid coverage or low-income subsidy as a proxy for socioeconomic status. Third, we measured AD with ICD-9 or ICD-10 codes, as claims data do not contain information on cognitive function or results of diagnostic testing. It is possible that some AD cases were misclassified as unspecified dementia if they were diagnosed in clinical practice without the diagnostic tests that would identify AD specifically. Fourth, as individuals become eligible for Medicare coverage after the age of 65 years, we were not able to test whether exposure to bumetanide in mid-life is associated with AD or all-cause dementia risk. Fifth, our analyses using time-dependent continuous variables capturing cumulative duration of drug use assume that each 1-year increment in drug use had a comparable effect. That is, these analyses assumed that the difference in hazards of AD between individuals who used bumetanide

for 1 year and those who never used bumetanide was the same as the difference in hazards between individuals who used bumetanide for 2 years versus 1 year. Sixth, we did not consider differences in drug use dose in our analysis.

5 | CONCLUSIONS

Our longitudinal analysis of Medicare claims data was unable to replicate prior findings from cross-sectional analyses associating bumetanide with decreased risk of AD, regardless of whether the exposure was measured as *ever use* of the drug or as the number of years of drug use. Use of bumetanide as well as of other loop diuretics was associated with increased risk of all-cause dementia, which may be suggestive of confounding by indication. Pharmacoepidemiology evaluations of real-world data may contribute to the identification of candidate compounds for repurposing; however, rigorous study designs are needed to account for survivor bias and confounding.

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CONFLICT OF INTEREST STATEMENT

Dr. Feldman reports grant funding from Annovis (QR Pharma), Vivoryon (Probiodrug), Biohaven Pharmaceuticals, AC Immune, and LuMind; service agreements for consulting activities with LuMind, Genentech (DSMB), Roche/Banner (DMC), Tau Consortium (SAB), Biosplice Therapeutics, Axon Neuroscience, Janssen Research & Development LLC, and Arrowhead Pharmaceuticals with no personal funds received and all payments to UCSD. Dr. LaCroix reports grant funding from Biohaven Pharmaceuticals with no personal funds received and all payments to UCSD. Dr. Hernandez reports consulting fees from Pfizer and BMS. The remaining co-authors have no conflicts of interest. Author disclosures are available in the supporting information.

CONSENT STATEMENT

Informed consent was not necessary as only de-identified data were used in analysis and the institutional review board approved the research as exempt.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX

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