



Research Advocacy Training

TALK NERDY TO ME

Nurturing Engagement
in Research and
Development with You

Fairmont Dallas Hotel
1717 N. Akard Street
Dallas, TX 75201
November 19, 2019

Akin Gump Strauss Hauer & Feld LLP
2300 N. Field Street, Suite 1800
Dallas, TX 75201
November 20-21, 2019

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November 19, 2019

Dear Talk NERDY to Me Network Advocates, Advisors, and Guests,

Welcome and thank you for joining us!

We are very excited and grateful to have you here for the training of the Talk N.E.R.D.Y. to Me Network.

As you know, the purpose of this program is to develop an older adult patient and family caregiver-led nationwide group of advocates with the following:

- Basic understanding of patient-centered outcomes research (PCOR)
- Ability to develop research questions that are important to older adult patients and their family caregivers and will ultimately help inform research design, encourage broader participation, and produce meaningful health outcomes
- Willingness to provide the patient and family caregiver perspective by participating in PCOR opportunities at the national or local level.

You each bring a unique perspective to this training, and that has already helped us to think about this curriculum in ways we would not have considered otherwise.

Some of you have advanced degrees and many years of professional experience. Some of you have less formal education, but extensive personal experience living with a chronic condition or caring for someone who has that condition. Some of you may have variations of one or both.

To set a level from the beginning, let me say this: everyone's experience is valid and important to this process. My request is for all of us to approach this training with an openness and willingness to both teach and learn from each other. If someone you are training with is struggling to understand something, be patient and help them along.

If you have a question, be willing to ask—chances are, you are not the only one who has that question.

Please use this opportunity to learn, teach, and connect with each other. The psychologist, Dr. Joanne Cacciatore, states it well: "There is no pharmacy that can fill the need for compassionate interaction with others. There is no panacea. The answer to human suffering is both within us and between us."

Best,

A handwritten signature in cursive script that reads "Susan Peschin".

Sue Peschin



TALK NERDY TO ME NETWORK ADVOCACY TRAINING
***NURTURING ENGAGEMENT IN RESEARCH AND
DEVELOPMENT WITH YOU***
NOVEMBER 19-21, 2019

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Talk NERDY to Me Network Advocacy Training
Nurturing Engagement in
Research and Development with You (NERDY)
November 19-21, 2019

AGENDA

Tuesday, November 19

Location: Fairmont Dallas Hotel

(1717 North Akard Street, Dallas, TX 75201)

- 6:00 pm Registration and Dinner
- 6:30 pm Welcome and Introductions: Susan Peschin, MHS (Alliance for Aging Research (AAR))
- 7:15 pm Guest Speakers: Lia Hotchkiss, MPH (Patient-Centered Outcomes Research Institute) and Mellanie True Hills, CSP (StopAfib.org)
- 8:00 pm Wrap-up

Wednesday, November 20

Location: Akin Gump Strauss Hauer & Feld, LLP

(2300 North Field Street, Suite 1800, Dallas, TX 75201)

- 8:00 am Breakfast
- 8:30 am Program begins
- 8:40 am **Session One: Clinical Trials**
George Perry, PhD (University of Texas at San Antonio), Maria Langas, PharmD (Janssen Pharmaceuticals), Srinu Potluri, MD (The Heart Hospital, Baylor), Carolyn Carman, OD, FAAO, and Patrick Dougherty, PhD (The University of

Texas MD Anderson Cancer Center and The University of Texas Health Science Center) Participants will learn about clinical trial design from researchers in the field.

- 10:00 am Break
- 10:15 am **Session One, Continued**
Participants will practice extracting key information from a scientific journal article.
- 11:30 am Break and then Lunch
- 12:15 pm Lunch
- 1:15 pm **Session One, Continued**
Participants will explore current research.
- 2:40 pm **Session Two: Patient-Centered Outcomes Research**
Susan Peschin, MHS (AAR) and Sara Collina, JD (Blueberry Hill Strategies)
Participants will evaluate research and identify ways to make it more patient-centered.
- 4:00 pm Wrap-up

Dinner on your own; we encourage you to join your colleagues and will provide restaurant recommendations.

Thursday, November 21

**Location: Akin Gump Strauss Hauer & Feld, LLP
(2300 North Field Street, Suite 1800, Dallas, TX 75201)**

- 8:30 am Breakfast
- 9:00 am **Session Three: Advocate to Advocate and Action Plans**
Penney Cowan (American Chronic Pain Association), Susan Strong (Heart Valve Voice, US), and Jeff Todd (Prevent Blindness)

Participants will engage with experienced research advocates to better understand how to find and create advocacy opportunities, then work on personalized action plans.

- 11:30 am *Participants will share their action plans with the larger group.*
- 12:30 pm *Wrap-up*

Boxed lunches available to go.

A Clinical Study of Lupron Depot in the Treatment of Women with Alzheimer's Disease: Preservation of Cognitive Function in Patients Taking an Acetylcholinesterase Inhibitor and Treated with High Dose Lupron Over 48 Weeks

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Handling Editor: Massimo Tabaton

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Abstract. To test the efficacy and safety of leuprolide acetate (Lupron Depot[®]) in the treatment of Alzheimer's disease (AD), we conducted a 48-week, double-blind, placebo-controlled, dose-ranging study in women aged 65 years or older with mild to moderate AD. A total of 109 women with mild to moderate AD and a Mini-Mental State Examination score between 12 and 24 inclusive were randomized to low dose Lupron Depot[®] (11.25 mg leuprolide acetate), high dose Lupron Depot[®] (22.5 mg leuprolide acetate), or placebo injections every 12 weeks. There were no statistically significant differences in primary efficacy parameters (ADAS-Cog and ADCS-CGIC), although there was a non-statistically significant trend in favor of the high dose Lupron group on the ADAS-Cog. There were no statistically significant differences in secondary efficacy parameters (NPI, ADCS-ADL, BI, and ADCS-Severity Rating). However, in the *a priori* designated subgroup analysis of patients taking an

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acetylcholinesterase inhibitor (AChEI), there was a statistically significant benefit in the high dose group compared to both the low dose and placebo groups as determined by ADAS-Cog (mean decline: 0.18, 4.21, and 3.30), ADCS-CGIC (% subjects experiencing decline: 38, 82, and 63), and ADCS-ADL (mean decline: -0.54 , -8.00 , and -6.85), respectively. No differences between treatment groups were seen on the NPI, ADCS-CGI Severity Rating, or the BI in the subgroup analysis. These data indicate that cognitive function is preserved in patients treated with high dose Lupron who were already using AChEIs. The positive interaction between Lupron and AChEIs warrants further investigation for the treatment of AD.

Keywords: 17 β -estradiol, acetylcholinesterase inhibitor, Alzheimer's disease, apolipoprotein E, clinical trial, cognitive testing, gonadotropin-releasing hormone, Lupron, luteinizing hormone, women

INTRODUCTION

Age-related changes in hormones of the hypothalamic-pituitary-gonadal axis have been suggested as a major etiological factor in Alzheimer's disease (AD) [1–4]. In addition to the age-related decline in circulating sex steroids, there is evidence to suggest that simultaneous elevations in the circulating concentrations of gonadotropins and gonadotropin-releasing hormone (GnRH) at this time play a role in AD [5–8]. Evidence for suppressing GnRH and gonadotropin signaling in the treatment of AD comes from a growing number of epidemiological, preclinical and biological studies.

Compelling epidemiological data suggest that Lupron Depot[®] (otherwise referred to as Lupron) treatment decreases the risk for AD in men. The most frequent use of Lupron is for the treatment of prostate cancer. A study, utilizing the Medicare inpatient database, of men who underwent prostatectomy for prostate cancer ($n = 115,789$) found that the incidence of dementia within 5 years of the procedure date was ~ 34 – 55% that of age-matched men undergoing a similar surgical procedure ($n = 433,736$) ([9] and Beard, Bowen, Perry, Atwood et al., unpublished data). That GnRH agonist treatment was the cause of this dramatic decrease in AD incidence was verified by D'Amico and colleagues [10] who demonstrated a significant 55% reduction in the risk of death from AD in men with prostate cancer treated with a GnRH agonist compared with untreated patients.

Preclinical evidence for the use of Lupron in the treatment of AD comes from studies of both normal and amyloid- β protein precursor (A β PP)-transgenic models of AD. Suppression of gonadotropins with Lupron improves cognitive performance in aged A β PP-transgenic mice [2] while increases in luteinizing hormone (LH)/human chorionic gonadotropin (hCG) have been attributed to cognitive decline in ovariectomized rats [11], LH β -transgenic mice [12], and ovariectomized C57/Bl6 mice [13]. Moreover, Lupron

treatment has been shown to decrease amyloid- β (A β) production in C57/Bl6 mice [8] and A β load in aged A β PP-transgenic mice [2]. The role of LH in mediating A β PP processing was confirmed in a bigenic mouse model that expresses A β PP sw^+ in the background of a LH receptor (*Lhr*) knockout (A β PP sw^+ /*Lhr*^{-/-}; [14]). Despite the ~ 10 -fold elevation in A β PP/A β production by A β PP sw^+ mice [15], genetic ablation of *Lhr* significantly reduced amyloid load and the total number of A β plaques in the hippocampus and cerebral cortex of male and female mice. Genetic ablation of *Lhr* in A β PP sw^+ mice also decreases tau phosphorylation by $\sim 50\%$ that induced by A β PP overexpression in these mice [14].

Pathological and biochemical studies support the role of gonadotropins in amyloidosis and neurofibrillary tangle formation. LH/hCG promotes the processing of A β PP toward the amyloidogenic pathway *in vitro* [16]. LH induced an increase in the generation and secretion of A β , coupled with decreased secretion of A β PP and increased A β PPCT100 production in human neuroblastoma cells [8].

This clinical study was conducted as a dose-ranging study designed to investigate the efficacy and safety of Lupron in the treatment of individuals with AD. In order to minimize any effects due to the loss of sex steroids, it was decided to make this a woman only study since women in this age group are postmenopausal and have little if any endogenous sex steroid production. The study design, patient selection criteria, and outcome measures were guided by regulatory standards in clinical studies. We find that Lupron treatment in combination with acetylcholinesterase inhibitor (AChEI) halts or slows the progression of cognitive decline in women with mild-moderate AD.

METHODS

The study was conducted from April 16, 2003 through December 16, 2004. Participants were

recruited from five U.S. sites. The institutional review board at each site (Baumel-Eisner Neuromedical Institute – three sites; Sun Health Research Institute; Meridien Research) reviewed and approved the study protocol. 109 patients were enrolled who met all of the following criteria: had given their consent by signing the Informed Consent Form and the responsible caregiver also had signed the consent form; or, if the patient was judged by the investigator to be unable to give consent, the legally authorized representative gave consent by signing the consent form and the patient gave assent, in accord with local regulations; were female; were 65 years of age or older; had a diagnosis of probable AD according to the National Institute of Neurological and Communicative Disorders-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria, and the investigator ascertained that the condition had been present at least 6 months prior to screening; were either presently taking a AChEI, and had begun taking it at least 90 days prior to baseline and, in the investigator's opinion, the dosage would likely remain stable throughout the study or they had never taken AChEIs or stopped taking such medication at least 90 days prior to baseline and would likely remain off AChEIs throughout the study; if they were taking other drugs or substances that have purported cognition enhancing properties such as *Ginkgo biloba* and vitamin E, they had begun taking it at least 60 days prior to baseline and, in the investigator's opinion, the dosage would remain stable throughout the study; had scored no lower than 12 or higher than 24 on the Mini-Mental State Examination (MMSE) administered at the screening visit; had a brain imaging study (CT scan, MRI, or PET) performed at the time of their initial diagnosis of AD or after that time, and the findings had been consistent with a diagnosis of probable AD (if a brain imaging study had not been performed, one was performed during the screening process); had a Rosen Modified Hachinski score of 4 or lower at the screening visit, supporting the investigator's clinical judgment that the patient's dementia was "probable AD" and not a dementia of vascular origin; were fluent in English or Spanish and had completed at least 6 years of education; lived at home or in a congregate living facility for requirements other than skilled nursing care, and had a caregiver who saw the patient at least three times a week for a total of at least 10 hours and could sign the consent form, provide information pertinent to the patient's cognitive status, accompany the patient on clinic visits, and participate in the evaluations; hormone replacement therapy, if any, had been stable for at least 60 days prior to baseline, and was not expected

to change during the course of the study; scored less than 15 on the Hamilton Depression Scale (17-item version) administered as part of the screening evaluation; values on their screening laboratory tests did not indicate significant medical conditions that would have interfered with their participation in, and completion of, the study.

Exclusion criteria were: The presence of a significant neurological disease affecting the brain, or psychiatric disease other than AD, such as major depression, schizophrenia, epilepsy, Parkinson's disease, or stroke; current significant systemic illness or symptoms of organ failure; a screening electrocardiogram (ECG) that showed evidence of a serious and/or unstable condition or a recent (within 6 months) myocardial infarction; a history of cancer within the last 5 years, except for basal cell or squamous cell cancer, or cervical carcinoma *in situ*; receiving Coumadin or anti-Parkinsonian medications; receiving other investigational drugs within 30 days or 5 half-lives prior to randomization, whichever was longer; taking other medications known to affect serum gonadotropin concentrations, such as gosorelin or danazol, except for estrogen and/or progesterone; had a history of bone fracture secondary to low bone mineral density; had a history of osteoporosis/osteopenia, unless they were receiving therapy for osteoporosis/osteopenia for at least 3 weeks prior to baseline, and the treatment regimen was expected to remain stable; abuse or dependence on alcohol or other substances satisfied criteria for DSM-IV categories 303.9 or 305; had donated blood within 30 days of baseline or were likely to do so during the course of the study.

Intervention

The study was a 48-week, double-blind, placebo-controlled, stratified, parallel-group study conducted in a group of women aged 65 years or older with mild to moderate AD at five sites in the United States. Those whose screening assessments showed that they were eligible to enter the study were assigned to receive either: An 11.25 mg formulation (marketed by TAP Pharmaceuticals Inc. of Lake Forest, Illinois, as Lupron Depot[®]-3 Month 11.25 mg) given as intramuscular injections; a 22.5 mg formulation (marketed by TAP Pharmaceuticals Inc. of Lake Forest, Illinois, as Lupron Depot[®]-3 Month 22.5 mg) given as intramuscular injections; or a placebo (physiologic saline) injection. Patients received intramuscular injections of study drug at Day 0 (baseline visit), week 12 (visit 5), week 24 (visit 7), and week 36 (visit 10) (see Table 1

Table 1
Schedule of assessments

Visit Number	Screening	Baseline		Post-baseline Visits								
	1	2	3 ¹	4	5	6 ¹	7	8	9 ¹	10	11	12
Weeks Post-baseline	≤ -6	0	1	4	12	18	24	26	30	36	42	48
Informed Consent	X											
Medical & Social History	X											
Diagnosis of Probable AD ^{2,3}	X											
MMSE	X											
Rosen Modified HIS	X											
ECG	X											
Ham-D (17 item version)	X	X						X				X
Review Inclusion/Exclusion Criteria	X	X										
AE Assessment		X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination	X											
Height	X											
Hematology & Chemistry	X	X		X	X		X	X		X	X	X
Urinalysis	X											
DEXA scan	X											X
APOE Assay		X										
TSH	X											
FSH, LH, Estradiol Assays ⁴		X		X	X		X	X		X	X	X
Weight & Vital Signs	X	X		X	X		X	X		X	X	X
Randomization		X										
Administer Study Drug		X			X		X			X		
Phone Contact			X			X			X			
ADAS-Cog		X		X	X		X	X		X	X	X
ADCS-CGIC				X	X		X	X		X	X	X
NPI		X						X				X
BI		X		X	X		X	X		X	X	X
ADCS-ADL		X		X	X		X	X		X	X	X
ADCS-CGI Severity Rating		X										X

¹Patients and caregivers were contacted by phone for assessments of safety and concomitant medications. ²Defined in the NINCDS-ADRDA, including neuro imaging, history or cognitive and memory loss, and examinations to exclude other causes of dementia. ³Brain imaging was obtained during screening period if not previously obtained after onset of symptoms of AD. ⁴Optional blood samples were to be collected only if patients had consented to them in the Informed Consent Form. AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; HIS, Hachinski Ischemic Score; ECG, electrocardiography; Ham-D, Hamilton Depression Rating Scale; AE, adverse event; DEXA, dual-energy x-ray absorptiometry; APOE, apolipoprotein E; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; ADAS-Cog, Alzheimer's Disease Assessment Scale - Cognitive Subscale; ADCS-CGIC, Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change; NPI, neuropsychiatric inventory; BI, burden interview; ADCS-ADL, Alzheimer's Disease Cooperative Study-Activities of Daily Living Inventory.

for Schedule of Assessment). The study randomization was stratified so that the number of patients with and without evidence of osteoporosis or osteopenia, based on dual-energy x-ray absorptiometry (DEXA) scan findings, was balanced among the three treatment groups. Lupron Depot[®] is composed of leuprolide acetate, an analogue of the endogenous decapeptide GnRH. It has a substitution of a D-amino acid for glycine at position 6 and deletion of glycine at position 10 with the insertion of ethylamide, causing it to have a longer half-life and much higher affinity for the GnRH receptor than endogenous GnRH [17]. Once administered, it elicits an initial surge in LH and subsequently sex steroids, but within 2 weeks, GnRH receptors are

down regulated resulting in very low levels of LH and follicle-stimulating hormone (FSH) [18] (Table 2).

Outcome measures

Outcome and safety measures were evaluated at baseline and weeks 4, 12, 24, 26, 36, 42, and 48. Additional telephone assessments were performed at weeks 1, 18, and 30. The primary efficacy parameters were the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) and the Alzheimer's Disease Cooperative Study Clinical Global Impression of Change (ADCS-CGIC). Secondary efficacy parameters were the Neuropsychiatric Inventory (NPI),

Table 2

Serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations in per protocol patients

Serum LH in patients treated with placebo or lupron (mean \pm SD; mIU/mL)			
Study week	11.25 mg <i>n</i> = 24	22.5 mg <i>n</i> = 21	Placebo <i>n</i> = 24
Baseline	27.7 \pm 11.2*	30.9 \pm 19.1*	24.1 \pm 14.5
Week 4	2.3 \pm 0.9	2.6 \pm 1.8	25.7 \pm 14.7
Week 24	0.7 \pm 0.3	0.3 \pm 0.2	26.5 \pm 15.4
Week 26	0.4 \pm 0.2	0.3 \pm 0.2	25.8 \pm 15.1
Week 48	0.8 \pm 0.4	0.4 \pm 0.2	25.9 \pm 15.3
Serum FSH in patients treated with placebo or lupron (mean \pm SD; mIU/mL)			
Study week	11.25 mg <i>n</i> = 24	22.5 mg <i>n</i> = 21	Placebo <i>n</i> = 24
Baseline	52.3 \pm 20.9*	48.5 \pm 31.6*	46.3 \pm 19.9
Week 4	5.0 \pm 1.9	3.8 \pm 2.4	47.4 \pm 20.1
Week 24	7.3 \pm 2.6	5.1 \pm 3.3	50.8 \pm 20.7
Week 26	4.9 \pm 1.9	4.7 \pm 3.1	50.5 \pm 20.9
Week 48	6.8 \pm 2.9	5.3 \pm 3.5	50.1 \pm 20.8

*Statistical difference ($p < 0.001$) between Baseline and Weeks 4, 24, 26, and 48 for low and high dose Lupron for serum LH and FSH.

Alzheimer's Disease Cooperative Study-Activities of Daily Living Inventory (ADCS-ADL), Burden Interview (BI), and ADCS-Severity Rating.

Safety was assessed by reviews of treatment-emergent adverse events and post-baseline changes in vital signs, physical examinations, clinical laboratory measures, and bone mineral density.

Bone mineral density

Bone mineral density was measured by means of DEXA scans of the lumbar vertebrae and a hip (including femoral neck). A DEXA scan was performed at screening and the end of study (week 48). The final DEXA scan was performed within 2 weeks before or after the final visit.

APOE genotyping

Direct sequencing of APOE genotype was performed by the Michigan State University DNA Diagnostic Program, East Lansing, MI.

Hormonal analyses

Serum LH, FSH and 17 β -estradiol concentrations were measured at Quest Diagnostics, Miramar, FL.

Statistical analyses

All groups were analyzed for primary and secondary efficacy endpoints. In addition, pre-defined subgroup analyses included AChEI use and APOE status.

Primary efficacy analyses

The primary efficacy analyses were defined as comparisons between treatment groups for scores on the ADAS-Cog and ADCS-CGIC and were performed on the Intent-to-Treat population. The Intent-to-Treat population was defined as patients who received at least one dose of randomized drug and who had at least one post-baseline assessment of at least one primary efficacy variable.

ADAS-Cog: The efficacy analysis of the ADAS-Cog score for both treatment groups (low and high doses of Lupron Depot[®]) and the placebo group were analyzed by the method of analysis of variance and analysis of covariance. The primary analysis was the two-way analysis of variance model containing the main effects for both the treatment groups and the study sites along with their possible interaction. The final analysis was carried out on the 48-week endpoint by using the change in ADAS-Cog score from baseline.

ADCS-CGIC: The primary efficacy comparisons of the ADCS-CGIC score for both active treatment groups and the placebo group were analyzed by the Cochran-Mantel-Haenszel test which treats study sites as strata. In order to adjust for the other covariate effects, similar tests were based on the strata according to the levels of covariates. These covariates included the baseline osteoporosis/osteopenia status, the APOE genotype status, and the education level. If there was a significant association between the treatment groups and the ADCS-CGIC score, the common odds ratios were estimated by the Mantel-Haenszel estimator and the corresponding confidence interval determined across strata that ADCS-CGIC improved (or at least stabilized) over time between each active treatment group and the placebo group. The final analysis was carried out on the 48-week endpoint for ADCS-CGIC score.

Secondary efficacy analyses

The secondary efficacy analyses were the comparisons between treatment groups in scores on the ADCS-ADL, NPI (degree of behavioral disturbances associated with AD), BI (the impact of the patient's illness on the caregiver), and ADCS-CGI Severity Rating. Methods of statistical analysis similar to those

used for ADAS-Cog score were used to analyze the change from baseline for the ADCS-ADL, NPI, and BI. The change from baseline in ADCS-ADL, NPI, and BI were analyzed by ANOVA and ANCOVA with the incorporation of important covariates such as the baseline age, the baseline osteoporosis/osteopenia status, the *APOE* status, and the education level. The effects of treatment on the change in ADCS-ADL, NPI, and BI were assessed using the appropriate hypotheses tests and confidence interval estimations.

In addition, the ADCS-CGI severity rating was summarized descriptively using frequency and percentage for each level of the rating at baseline, and using continuous statistics in the change from baseline at week 48.

In the use of all of these techniques in efficacy analysis, a variety of technical assumptions were required for each type of analysis. In order to assure that the reported results were not simply artifacts of the particular method of analysis, different analyses with a variety of analytic techniques that have slightly differing theoretical assumptions were carried out and compared. In order to control the Type I error rate for the final analysis, Bonferroni's method was used to adjust for the multiple comparisons made between each of the two active treatment groups and the placebo group. However, no adjustment was made for multiple analyses in the *a priori* subgroup analysis of patients taking AChEIs.

RESULTS

Demographic and clinical characteristics

The demographics and baseline characteristics of each treatment group are listed in Table 3. Each group was comparable for all demographic and clinical characteristics ($p > 0.05$) which included: age, race, height, weight, education level, *APOE* genotype, AChEI usage, MMSE score, Rosen Modified Hachinski Ischemic Score, Hamilton Psychiatric Rating Scale for Depression, abnormal physical exam findings at screening, abnormal ECG findings at screening, and 17β -estradiol, LH and FSH concentrations.

Of the 109 patients who entered the study, 37 were assigned to low dose, 36 to high dose, and 36 to placebo. There was no significant difference in completion rates between the groups: 72 patients (66%) completed the study; 25 (68%) in the low dose group, 22 (61%) in the high dose group, and 25 (69%) in the placebo group (Supplementary Table 1).

Primary outcomes

In the primary analysis there was a trend, although not statistically significant, in favor of the high dose Lupron group on the ADAS-Cog. The mean decline in the ADAS-Cog scores after 48 weeks of treatment was 1.7 points in the high dose group compared to 2.4 points in the placebo group and 4.9 points in the low dose group (Fig. 1A). A similar, although not as pronounced trend, was observed for ADCS-CGIC scores with 39% of patients in the high dose group exhibiting decline compared to 54% in the placebo group and 72% in the low dose group (Fig. 1B).

However, in the *a priori* designated subgroup analysis of patients taking AChEIs, there was a statistically significant benefit to subjects as determined by the ADAS-Cog and the ADCS-CGIC in the high dose Lupron group compared to both the placebo and low dose groups (Fig. 2). The mean decline in the ADAS-Cog scores after 48 weeks of treatment was 0.18 points in the high dose group compared to 3.30 points in the placebo group and 4.21 points in the low dose groups (Fig. 2A). Similarly, 9% of patients in the high dose group exhibited decline on ADCS-CGIC scores after 48 weeks of treatment compared to 63% in the placebo group and 82% in the low dose group (Fig. 2B). In patients not taking AChEIs, there was no significant difference by the ADAS-Cog and the ADCS-CGIC between individuals in the high dose Lupron, low dose Lupron, or placebo groups (see Supplementary Figure 1).

Secondary outcomes

In the primary analysis, there was no statistically significant difference on any of the secondary outcome measures, which included the ADCS-ADL (Fig. 3), the NPI, the ADCS-CGI Severity Rating, and the BI.

However, in the *a priori* subgroup analysis, patients taking high dose Lupron showed a statistically significant benefit seen on the ADCS-ADL. The mean decline in the high dose group was 0.54 points compared to 6.9 points in the placebo group and 8.0 points in the low dose group (Fig. 3). No differences between treatment groups were seen on the NPI, ADCS-CGI Severity Rating, or the BI in the subgroup analysis. In patients not taking AChEIs, there was no significant difference by the ADCS-ADL between individuals in the high dose Lupron, low dose Lupron, or placebo groups (see Supplementary Material).

It is known that patients who are homozygous for *APOE* $\epsilon 4$ allele have an increased risk of AD.

Table 3
Demographics and baseline characteristics of each treatment group

Characteristic	Category	Lupron 11.25 mg (n = 36)	Lupron 22.5 mg (n = 36)	Placebo (n = 36)	p-value
Age (years)	Mean ± Std	78.75 ± 6.25	78.25 ± 6.01	76.97 ± 5.54	0.461 ¹
	Median	80.0	80.0	77.5	
	Interquartile Range	73.5 – 83.0	73.5 – 83.0	74.0 – 80.0	
	Min-Max	67–93	67–88	65–88	
Race	Caucasian	30 (83.3%)	26 (72.2%)	27 (75%)	0.514 ²
	African-American	1 (2.8%)	2 (5.6%)	0	
	Hispanic	5 (13.9%)	8 (22.2%)	9 (25.0%)	
Height (inches)	Mean ± Std	60.95 ± 1.94	60.97 ± 2.88	61.58 ± 2.53	0.508 ¹
	Median	61	61.5	61.3	
	Interquartile Range	59.0 – 62.0	59.0 – 62.8	60.0 – 64.0	
	Min-Max	57.0 – 64.5	55.0 – 67.0	56.0 – 67.0	
Weight (pounds)	Mean ± Std	131.9 ± 27.3	139.4 ± 20.9	140.4 ± 25.1	0.373 ¹
	Median	132.0	134.8	136.5	
	Interquartile Range	113.0 – 147.0	127.5 – 146.0	123.0 – 152.3	
	Min-Max	92.0 – 220.0	108.0 – 193.0	95.0 – 223.0	
Education	Grade 6	6 (16.7%)	6 (16.7%)	6 (16.7%)	0.817 ²
	High school Grad	20 (55.6%)	21 (58.3%)	23 (63.9%)	
	Some College	5 (13.9%)	4 (11.1%)	3 (8.3%)	
	College Grad	5 (13.9%)	4 (11.1%)	2 (5.6%)	
	Post-Grad	0	1 (2.8%)	2 (5.6%)	
APOE Genotype	2/3	2 (5.6%)	2 (5.6%)	0	0.484 ²
	2/4	0	3 (8.8%)	1 (2.8%)	
	3/3	15 (41.7%)	16 (44.4%)	12 (33.3%)	
	3/4	16 (44.4%)	12 (33.3%)	18 (50.0%)	
	4/4	1 (2.8%)	2 (5.6%)	2 (5.6%)	
AChEI	Yes	28 (77.8%)	23 (63.9%)	26 (72.2%)	0.462 ²
	No	8 (22.2%)	13 (36.1%)	10 (27.8%)	
Estrogen supplementation	Yes	4	1	3	
Serum 17β-estradiol (pg/mL)	Mean±Std	73.8 ± 214.9 (n = 36)	21.1 ± 25.0 (n = 35)	32.3 ± 48.3 (n = 35)	>0.05 ³
	Median	15.5	15.0	17.0	
	Interquartile Range	11.0 – 25.0	11.0 – 20.0	10.0 – 22.0	
	Min-Max	10.0 – 1170.0	10.0 – 155.0	10.0 – 214.0	
FSH (mIU/mL)	Mean±Std	48.2 ± 22.2	52.4 ± 28.8	48.8 ± 21.8	>0.05 ³
	Median	45.1	48.0	43.8	
	Interquartile Range	32.5 – 63.7	30.9 – 70.8	32.7 – 65.4	
	Min-Max	7.4 – 105.0	13.3 – 145.0	15.7 – 106.0	
LH (mIU/mL)	Mean±Std	27.7 ± 15.0	33.6 ± 22.6	27.6 ± 14.8	>0.05 ³
	Median	26.3	30.5	23.5	
	Interquartile Range	22.0 – 32.3	18.9 – 40.9	17.6 – 36.1	
	Min-Max	4.3 – 84.5	3.9 – 130.1	3.3 – 71.9	
ADAS-Cog	Overall	19.73 ± 6.41	20.14 ± 9.36	21.90 ± 9.50	>0.29 ³
	Sub-group analysis (AChEI users)	20.73 ± 5.94	20.31 ± 9.03	24.29 ± 9.93	
ADCS-ADL	Sub-group analysis (AChEI users)	59.2 ± 7.8	55.8 ± 12.7	55.6 ± 13.9	>0.06 ⁴
NPI	Overall	8.9 ± 11.8	8.8 ± 9.6	9.1 ± 8.5	1.00 ⁵
MMSE	Overall	18.2 ± 3.3	18.6 ± 3.5	17.9 ± 3.3	0.33 ⁶
Rosen Modified HIS	Overall	0.72 ± 0.74	0.50 ± 0.56	0.72 ± 0.88	0.82 ⁶
Ham-D	Overall	3.3 ± 3.0	4.1 ± 3.6	4.6 ± 3.2	0.12 ⁶
Abnormal physical findings	Overall	29 (81%)	32 (89%)	26 (72)	0.13 ⁷
Abnormal ECG findings	Overall	20 (56%)	30 (83%)	27 (75)	0.008 ⁷

¹p-values for treatment comparisons using a two-way analysis of variance test with factors of treatment and site (if the assumptions of ANOVA are satisfied) or using Friedman's test if these assumptions are not satisfied. ²p-values for treatment comparisons using the Cochran-Mantel-Haenszel test for general association, adjusted for site. ³p-values for baseline serum hormone concentrations. ⁴p-value for placebo versus high dose group. ⁵p-values and confidence intervals for treatment comparisons from analysis of variance with treatment and site as factors. ⁶p-values for treatment comparisons using Friedman's test with factors of treatment and site. ⁷p-values for treatment comparisons using Cochran-Mantel-Haenszel test for general association, adjusted for site. MMSE, Mini-Mental State Examination; HIS, Hachinski Ischemic Score; ECG, electrocardiography; Ham-D, Hamilton Depression Rating Scale; APOE, apolipoprotein E; FSH, follicle-stimulating hormone; LH, luteinizing hormone; ADAS-Cog, Alzheimer's Disease Assessment Scale - Cognitive Subscale; NPI, neuropsychiatric inventory; ADCS-ADL, Alzheimer's Disease Cooperative Study-Activities of Daily Living Inventory; AChEI, acetylcholinesterase inhibitor.

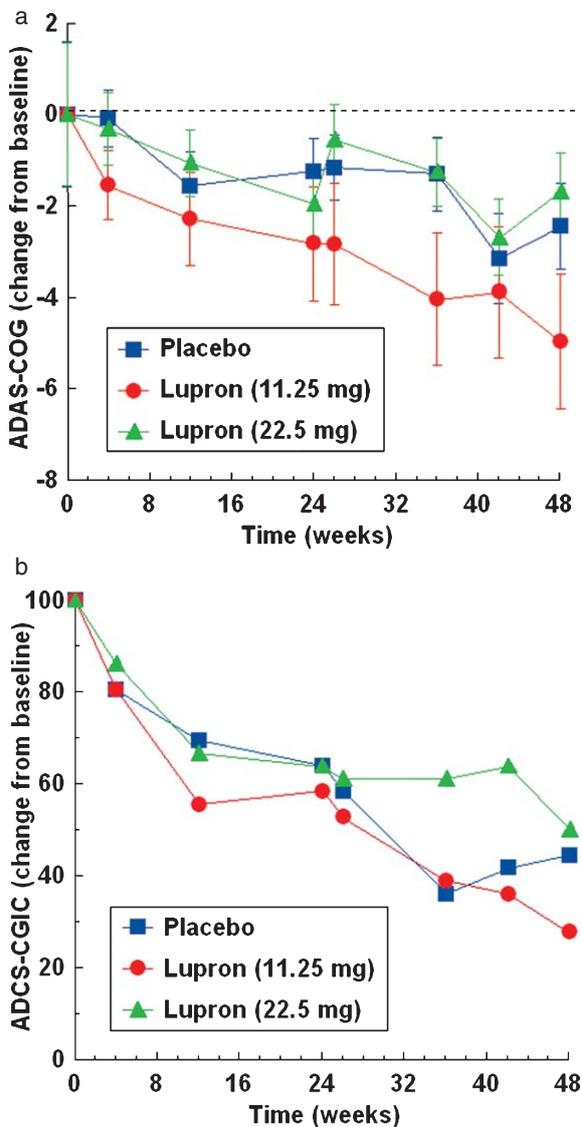


Fig. 1. Changes in cognitive performance over 48 weeks in individuals treated with placebo, low dose Lupron, or high dose Lupron as determined with (A) ADAS-Cog and (B) ADCS-CGIC ($n=36$ in each group). For ADAS-Cog and ADCS-CGIC, there were no significant differences for placebo versus high dose, placebo versus low dose, and low dose versus high dose at 26 and 48 weeks.

Sub-analyses were performed for efficacy endpoints based upon patients' *APOE* status. No statistical differences were found.

Safety

The safety profile of Lupron at doses similar to those used in this study has been established in other indications such as the treatment of advanced prostate cancer,

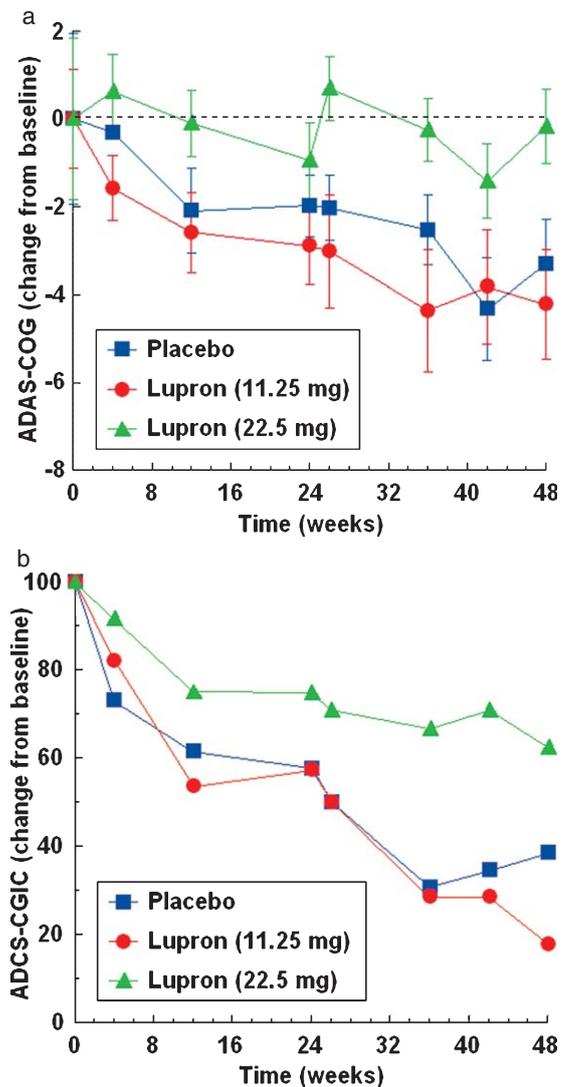


Fig. 2. Changes in cognitive performance over 48 weeks in individuals taking AChEIs and treated with placebo ($n=26$), low dose ($n=28$), or high dose ($n=24$) Lupron as determined with (A) ADAS-Cog and (B) ADCS-CGIC. A) Statistical differences, ADAS-Cog - Adjusted for multiple comparisons: p -value and 95% confidence interval for the high dose group versus placebo with treatment and site as factors using ANOVA = 0.037 ($-5.78, -0.18$) and with treatment and site as factors and baseline ADAS-Cog score as covariate using ANCOVA = 0.057 ($-5.67, 0.08$) at week 26. p -value and 95% confidence interval for the high dose group versus placebo with treatment and site as factors using ANOVA = 0.042 ($-6.14, -0.11$) and with treatment and site as factors and baseline ADAS-Cog score as covariate using ANCOVA = 0.060 ($-6.08, 0.12$) at week 48. ADAS-Cog - Unadjusted for multiple comparisons: The p values, unadjusted for multiple analyses for high dose and placebo were = 0.0009 and 0.026 at weeks 26 and 48, respectively. B) Statistical differences, ADCS-CGIC - Adjusted for multiple comparisons: There were no statistical differences between any treatment groups. ADCS-CGIC - Unadjusted for multiple comparisons: The p -values, unadjusted for multiple analyses for high dose and placebo, were 0.223 and 0.031 at weeks 26 and 48, respectively.

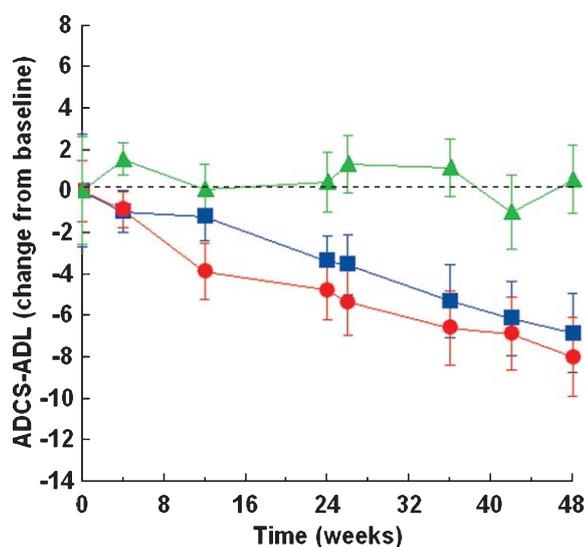


Fig. 3. Changes in cognitive performance as determined by ADCS-ADLs over 48-weeks in individuals treated with placebo ($n=26$), low dose ($n=28$), or high dose ($n=24$) with AChEIs. The p -values, unadjusted for multiple analyses for high dose and placebo, were = 0.016 and 0.015 at weeks 26 and 48, respectively.

children with central precocious puberty, endometriosis, and uterine fibroids. However, the safety of Lupron treatment in patients with AD has not previously been described. The majority of patients (77 of 109 patients or 71%) experienced at least one adverse event (AE) (Table 4 and Supplementary Table 2). These were mostly mild or moderate in severity and the ADCS safety monitoring committee regarded these as mainly unrelated to study drug. The most common AEs reported were consistent with the known safety profile of Lupron (Supplementary Table 3). There were 8 patient discontinuations due to AEs.

Twenty serious AEs were reported in 18 patients including two deaths. One death was attributed to respiratory failure in the high dose group, and one death was attributed to cerebral hemorrhage in the placebo group. The ADCS safety monitoring committee categorized three AEs as possibly related to study drug: Two cases (upper gastrointestinal hemorrhage and a syncopal episode) occurred in the low dose group and one case (deep vein thrombosis) occurred in the placebo group. Therefore, based on this data, there were no unexpected safety concerns in this patient population. This is consistent with the well-established safety profile of Lupron in the treatment of other conditions.

The extended use of Lupron is known to cause the loss of bone mineral density in men [19–22] and

pre-menopausal women [23, 24]. Whether any similar effect occurs in postmenopausal women has not been studied. In our study, there were no significant differences in bone mineral density between any of the treatment groups over 48 weeks of treatment.

DISCUSSION

This Phase II dose-ranging study demonstrated that high dose Lupron in combination with AChEIs halted the progression of cognitive decline in women with mild to moderate AD over a 48-week period (Figs. 2 and 3). A similar effect was not observed in the low dose Lupron group taking AChEIs or in the placebo group taking AChEIs (Figs. 2 and 3). This combination treatment was safe and well tolerated at both dose levels (Table 4) and AEs were consistent with the current product labels for other indications.

In the primary analysis there was a trend, although not statistically significant, in favor of the high dose Lupron group on the ADAS-Cog (Fig. 1). In the *a priori* designated subgroup analysis of patients already taking AChEIs, there was a clear statistically significant benefit demonstrated on the ADAS-cog, ADCS-CGIC, and the ADCS-ADL in the high dose Lupron group compared to the low dose and placebo groups (Figs. 2 and 3).

All drugs currently approved for the treatment of AD confer an initial improvement in cognitive function followed by a decline whose rate is similar to placebo [25]. In contrast to these treatments, there was no initial improvement in cognitive function following initiation of Lupron treatment but most importantly, there was no decline in cognitive performance in the high dose/AChEI group. These findings together with biological and epidemiological evidence suggest that the effects seen with high dose Lupron are one of potential disease modification rather than symptomatic improvement [1–9, 11–14, 26].

The mechanism by which Lupron acts with AChEI to improve cognitive performance is unclear. It is known that the AChEI rivastigmine can reduce the lipopolysaccharide-induced decreases in GnRH and LH, and perhaps stimulate GnRH/LH secretion [27]. In this connection, the modulation of GnRH release has been suggested to be mediated via cholinergic (and GABAergic) neurotransmission [28]. Thus, one possible additive mechanism of action might involve the further downregulation of GnRH receptor signaling and LH expression/signaling. Alternatively, since GnRH mediates neurotransmission itself [29, 30], Lupron might act directly to improve cognitive per-

Table 4
Summary of adverse events (AEs)

	Treatment			<i>p-value</i> ¹		
	Leuprolide 11.25 mg (<i>n</i> = 37)	Leuprolide 22.5 mg (<i>n</i> = 36)	Placebo (<i>n</i> = 36)	11.25 versus placebo	22.5 versus placebo	11.25 versus 22.5
Patients with at least 1 AE	27 (72.9%)	27 (75.0%)	23 (63.9%)	0.40	0.31	0.84
Not related	13 (35.1%)	12 (33.3%)	8 (22.2%)			
Probably not related	6 (16.2%)	10 (27.7%)	6 (16.7%)			
Possibly related	7 (18.9%)	3 (8.3%)	8 (22.2%)			
Probably related	1 (2.7%)	2 (5.5%)	1 (2.8%)			
Related	0	0	0			
Patients with serious AE	10 (27.0%)	4 (11.1%)	5 (13.8%)	0.17	1.0	0.08
Not related	7 (18.9%)	1 (2.8%)	2 (5.6%)			
Probably not related	1 (2.7%)	3 (8.3%)	2 (5.6%)			
Possibly related	2 (5.4%)	0	1 (2.8%)			
Probably related	0	0	0			
Related	0	0	0			
Patients with AEs that led to discontinuation	4 (10.8%)	2 (5.5%)	0	0.12	0.49	0.67
Patients with AEs resulting in death	0	1 (2.8%)	1 (2.8%)	0.49	1.0	0.49

At each level of summarization each patient is only counted once. ¹*p-values* for treatment comparisons from Pearson's Chi-square test or Fisher's exact test if appropriate.

formance. Another possibility is that Lupron acts to halt any further neurodegeneration thereby allowing AChEIs to act on remaining neurons to maintain cholinergic function.

The dose effect seen in this study suggests that Lupron's action is not solely due to its suppression of peripheral circulating concentrations of gonadotropins, which were similarly suppressed in low dose and high dose groups (Table 2). Therefore, Lupron's actions might also be due to direct effect on GnRH receptor signaling within the brain [31]. GnRH receptors are expressed throughout the brain and their expression correlates to those areas with AD neuropathology [31]. In this connection, we recently identified the existence of autocrine/paracrine feedback loops within the brain, in essence a feedback loop similar to the hypothalamic-pituitary-gonadal axis that regulates neurohormone production [32]. Since GnRH receptor mediates neuronal LH expression and LH receptor signaling, high doses of Lupron might suppress the neuroautocrine production of LH, which we have previously demonstrated is elevated in expression and colocalizes with AD neuropathology [33], while low doses might stimulate LH production.

This dose effect might also explain some conflicting preclinical results. Most researchers have found that lowering LH signaling with the GnRH agonist Lupron decreases A β levels and improves cognitive performance in wild-type mice [8, 34] and A β PP-transgenic mice [2]. However, a decrease in brain A β and improvement in cognition following leuprolide

acetate treatment was not observed in the overexpressing A β PP(Swt), PS1(M146 V), and tau(P301L) (triple) transgenic mice [35]. Whether this is a dose effect (or an artifact of the 3xTg mice) is not clear since multiple doses have not been evaluated. Future animal studies are warranted to help understand the dose effect and the synergism with AChEIs.

In conclusion, our data demonstrate that cognitive function was preserved in patients treated with high dose Lupron who were already using AChEIs. Caution should be used in the interpretation of the results due to: The small sample size, which did not allow determination of whether this treatment is best suited to early or later phases of the disease; the fact that baseline demographics were not compared for the subgroup; and non-adjustment for multiple analyses. The results of this study should however encourage further investigation of GnRH agonist therapy for the treatment of AD. Future clinical studies should be conducted with Lupron at doses providing systemic exposure at least equivalent to those provided by Lupron 22.5 mg every 12 weeks. Such studies could be expanded to include the use of GnRH antagonists.

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Authors' disclosures available online (<http://www.jalz.com/disclosures/view.php?id=2545>).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: <http://dx.doi.org/10.3233/JAD141626>.

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The antibody aducanumab reduces A β plaques in Alzheimer's disease

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Alzheimer's disease (AD) is characterized by deposition of amyloid- β (A β) plaques and neurofibrillary tangles in the brain, accompanied by synaptic dysfunction and neurodegeneration. Antibody-based immunotherapy against A β to trigger its clearance or mitigate its neurotoxicity has so far been unsuccessful. Here we report the generation of aducanumab, a human monoclonal antibody that selectively targets aggregated A β . In a transgenic mouse model of AD, aducanumab is shown to enter the brain, bind parenchymal A β , and reduce soluble and insoluble A β in a dose-dependent manner. In patients with prodromal or mild AD, one year of monthly intravenous infusions of aducanumab reduces brain A β in a dose- and time-dependent manner. This is accompanied by a slowing of clinical decline measured by Clinical Dementia Rating—Sum of Boxes and Mini Mental State Examination scores. The main safety and tolerability findings are amyloid-related imaging abnormalities. These results justify further development of aducanumab for the treatment of AD. Should the slowing of clinical decline be confirmed in ongoing phase 3 clinical trials, it would provide compelling support for the amyloid hypothesis.

The amyloid hypothesis posits that A β -related toxicity is the primary cause of synaptic dysfunction and subsequent neurodegeneration that underlies the progression characteristic of AD^{1,2}. Genetic, neuropathological, and cell biological evidence strongly suggest that targeting A β could be beneficial for patients with AD^{3,4}. So far, attempts at therapeutically targeting A β have not been successful^{5–7}, casting doubt on the validity of the amyloid hypothesis. However, the lack of success may have been due to the inability of the antibodies to adequately engage their target or the proper target in the brain, or selecting the wrong patient population.

We describe the development of an antibody-based immunotherapeutic approach by selecting human B-cell clones triggered by neo-epitopes present in pathological A β aggregates. The screening of libraries of human memory B cells for reactivity against aggregated A β led to molecular cloning, sequencing, and recombinant expression of aducanumab (BIIB037), a human monoclonal antibody that selectively reacts with A β aggregates, including soluble oligomers and insoluble fibrils. In preclinical studies, we show that an analogue of aducanumab is capable of crossing the blood-brain barrier, engaging its target, and clearing A β from plaque-bearing transgenic mouse brains. These results prompted the start of clinical trials⁸.

We report here interim results from a double-blind, placebo-controlled phase 1b randomized trial (PRIME; ClinicalTrials.gov identifier NCT01677572) designed to investigate the safety, tolerability, pharmacokinetics, and pharmacodynamics of monthly infusions of aducanumab in patients with prodromal or mild AD with brain A β pathology confirmed by molecular positron emission tomography (PET) imaging. Together, our data support further development of aducanumab as an A β -removing, disease-modifying therapy for AD.

Removal of brain A β plaques in patients with AD

In the PRIME study, 165 patients were randomized and treated between October 2012 and January 2014 at 33 sites in the United States. Patients with a clinical diagnosis of prodromal or mild AD and visually positive A β PET scan⁹ were given monthly intravenous infusions of placebo or aducanumab at doses of 1, 3, 6 or 10 mg kg⁻¹ for one year. Of these patients, 125 completed and 40 discontinued treatment, most commonly due to adverse events (20 patients) and withdrawal of consent (14 patients): 25% of the placebo group discontinued compared with 23%, 19%, 17%, and 38% of the 1, 3, 6 and 10 mg kg⁻¹ aducanumab dose groups, respectively (Extended Data Fig. 1). Baseline characteristics, including cognitive measures, were generally well-balanced across the groups, although the 1 mg kg⁻¹ dose group included a higher proportion of patients with mild AD, and the aducanumab treatment groups tended to have a higher Clinical Dementia Rating—Sum of Boxes (CDR-SB) score (Table 1).

Treatment with aducanumab reduced brain A β plaques as measured by florbetapir PET imaging in a dose- and time-dependent fashion (Fig. 1, 2a). The mean PET standard uptake value ratio (SUVR) composite score at baseline was 1.44. After 54 weeks of treatment, this had decreased significantly ($P < 0.001$) in the 3, 6 and 10 mg kg⁻¹ dose groups; whereas change for the placebo group was minimal (Fig. 2a, Extended Data Table 1). In the 10 mg kg⁻¹ dose group, the SUVR composite score was 1.16 after 54 weeks of treatment, a value near the purported quantitative cut-point of 1.10 that discriminates between positive and negative scans (Fig. 2b)¹⁰. The adjusted mean changes in SUVR composite scores in the 6 and 10 mg kg⁻¹ groups treated for 26 weeks were similar in magnitude to the dose group below (3 and 6 mg kg⁻¹, respectively) treated for 54 weeks (Fig. 2a). Reductions in amyloid PET SUVR composite score in aducanumab-treated patients

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Table 1 | Baseline characteristics

Characteristic		Aducanumab					Total (n = 165)*
		Placebo (n = 40)	1 mg kg ⁻¹ (n = 31)	3 mg kg ⁻¹ (n = 32)	6 mg kg ⁻¹ (n = 30)	10 mg kg ⁻¹ (n = 32)	
Years of age (mean ± s.d.)		72.8 ± 7.2	72.6 ± 7.8	70.5 ± 8.2	73.3 ± 9.3	73.7 ± 8.3	72.6 ± 8.1
Female sex (n (%))		23 (58)	13 (42)	17 (53)	15 (50)	15 (47)	83 (50)
ApoE ε4 (n (%))	Carriers	26 (65)	19 (61)	21 (66)	21 (70)	20 (63)	107 (65)
	Non-carriers	14 (35)	12 (39)	11 (34)	9 (30)	12 (38)	58 (35)
Clinical stage (n (%))	Prodromal	19 (48)	10 (32)	14 (44)	12 (40)	13 (41)	68 (41)
	Mild	21 (53)	21 (68)	18 (56)	18 (60)	19 (59)	97 (59)
MMSE (mean ± s.d.)		24.7 ± 3.6	23.6 ± 3.3	23.2 ± 4.2	24.4 ± 2.9	24.8 ± 3.1	24.2 ± 3.5
Global CDR (n (%))	0.5	34 (85)	22 (71)	22 (69)	25 (83)	24 (75)	127 (77)
	1	6 (15)	9 (29)	10 (31)	5 (17)	8 (25)	38 (23)
CDR-SB (mean ± s.d.)		2.66 ± 1.50	3.40 ± 1.76	3.50 ± 2.06	3.32 ± 1.54	3.14 ± 1.71	3.18 ± 1.72
FCSRT sum of free recall score (mean ± s.d.)		15.2 ± 8.5	13.2 ± 9.0	13.8 ± 8.0	14.4 ± 8.3	14.6 ± 8.3	14.3 ± 8.3
PET SUVR composite score (mean ± s.d.)		1.44 ± 0.17	1.44 ± 0.15	1.46 ± 0.15	1.43 ± 0.20	1.44 ± 0.19	1.44 ± 0.17
AD medications use† (n (%))		24 (60)	19 (61)	28 (88)	20 (67)	17 (53)	108 (65)

Percentages are rounded to the nearest integer. AD, Alzheimer's disease; ApoE ε4, apolipoprotein E ε4 allele; CDR, Clinical Dementia Rating; CDR-SB, Clinical Dementia Rating—Sum of Boxes; FCSRT, Free and Cued Selective Reminding Test; MMSE, Mini-Mental State Examination; PET, positron emission tomography; SD, standard deviation; SUVR, standard uptake value ratio.

*Number of patients dosed.

†Cholinesterase inhibitors and/or memantine.

were similar in patients with mild and prodromal AD, and apolipoprotein E (ApoE) ε4 carriers and non-carriers (Extended Data Fig. 2a, b). Pre-specified regional analyses of SUVR changes demonstrated statistically significant dose-dependent reductions in all brain regions, except for the pons and sub-cortical white matter, two areas in which Aβ plaques are not expected to accumulate (Extended Data Fig. 3).

Effect on clinical measures

Clinical assessments were exploratory as the study was not powered to detect clinical change. The test of dose response was the pre-specified primary analysis for the clinical assessments. Analysis of change from baseline on the CDR-SB (adjusted for baseline CDR-SB and ApoE ε4 status) demonstrated dose-dependent slowing of clinical progression with aducanumab treatment at one year (dose-response, $P < 0.05$), with the greatest slowing for 10 mg kg⁻¹ ($P < 0.05$ versus placebo) (Fig. 3a, Extended Data Table 1). Sensitivity analysis using a mixed model for repeated measures (MMRM) also showed a trend for slowing of decline on the CDR-SB at one year ($P = 0.07$ with 10 mg kg⁻¹ aducanumab versus placebo). A dose-dependent slowing of clinical progression on the Mini Mental State Examination (MMSE) with aducanumab treatment was also observed at one year (dose-response, $P < 0.05$), with the greatest effects at 3 and 10 mg kg⁻¹ aducanumab ($P < 0.05$ versus placebo) (Fig. 3b, Extended Data Table 1). On sensitivity analysis using MMRM, the greatest difference was retained for 10 mg kg⁻¹ aducanumab ($P < 0.05$ versus placebo), with a smaller difference at 3 mg kg⁻¹ ($P = 0.10$ versus placebo). No changes from baseline after one year were found on the composite neuropsychological test battery (NTB) or the Free and Cued Selective Reminding Test (FCSRT) free recall (Extended Data Table 1), but skewed non-normal (floor) effects at baseline were observed. The floor effects on the NTB were seen in the individual tests; specifically, in the two most clinically relevant components given the stage of the population enrolled: Wechsler Memory Scale-Fourth Edition Verbal Paired Associates II (WMS-IV VPA II) and Rey Auditory Verbal Learning Test (RAVLT) delayed recall of the NTB memory domain.

Safety and tolerability

The most common adverse effects were amyloid-related imaging abnormalities (ARIA), headache, urinary tract infection, and upper respiratory tract infection (Table 2). Using the most specific descrip-

tion of ARIA by magnetic resonance imaging (MRI), ARIA-vasogenic oedema (ARIA-E) abnormalities occurred in no patients receiving placebo compared with 1 (3%), 2 (6%), 11 (37%), and 13 (41%) patients receiving 1, 3, 6 and 10 mg kg⁻¹ aducanumab, respectively (Extended Data Table 2). ARIA-E was generally observed early in the course of treatment, MRI findings typically resolved within 4–12 weeks, and of the 27 patients who developed ARIA-E, 15 (56%) continued treatment (Supplementary Information). All cases of symptomatic ARIA were

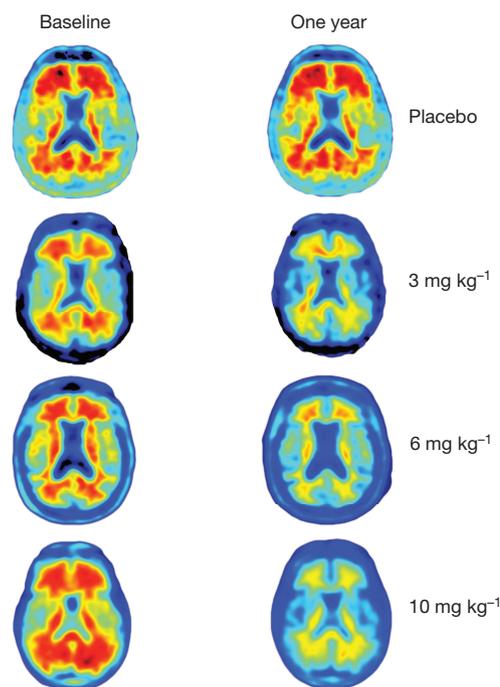


Figure 1 | Amyloid plaque reduction with aducanumab: example amyloid PET images at baseline and week 54. Individuals were chosen based on visual impression and SUVR change relative to average one-year response for each treatment group ($n = 40, 32, 30$ and 32 , respectively). Axial slice shows anatomical regions in posterior brain putatively related to AD pathology. SUVR, standard uptake value ratio.

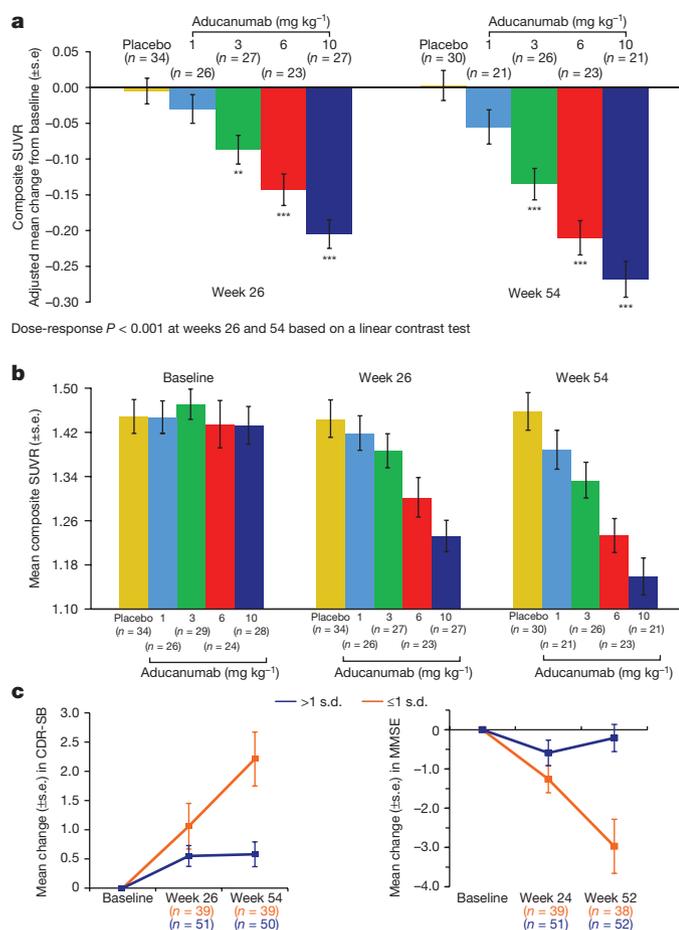


Figure 2 | Amyloid plaque reduction with aducanumab. **a–c**, Change from baseline (**a**, analyses using ANCOVA), SUVR values (**b**), and categorization of change in amyloid PET (**c**) at week 54 and associated change from baseline CDR-SB and MMSE in aducanumab-treated patients (post hoc analysis). Categorization of amyloid PET at week 54 based on s.d. of change from baseline in placebo-treated patients. ** $P < 0.01$; *** $P < 0.001$ versus placebo; two-sided tests with no adjustments for multiple comparisons. Mean \pm s.e. ANCOVA, analysis of covariance; CDR-SB, Clinical Dementia Rating—Sum of Boxes; MMSE, Mini Mental State Examination; SUVR, standard uptake value ratio.

required to be reported as medically important serious adverse effects. No patients were hospitalised for ARIA. The only serious adverse effects (by preferred term) that occurred in more than one patient in any treatment group were ARIA (0, 1 (3%), 1 (3%), 4 (13%), and 5 (16%) of patients receiving placebo, and 1, 3, 6 and 10 mg kg⁻¹ aducanumab, respectively) and superficial siderosis of the central nervous system (0, 1 (3%), 0, 2 (7%), and 3 (9%) of patients receiving placebo and 1, 3, 6 and 10 mg kg⁻¹ aducanumab, respectively). Owing to the requirement for repeated MRI assessments of those patients who developed ARIA, these individuals were partially unblinded to treatment. Other adverse effects and serious adverse effects were consistent with the patient population. There were no drug-related deaths (Supplementary Information).

Pharmacokinetics

The pharmacokinetics of aducanumab (maximum concentration (C_{max}) and cumulative area under the concentration curve (AUC)) were linear across the dose range in patients who received all 14 planned doses (Extended Data Table 3). The median plasma half-life was 21 days. In total, 3 of 118 evaluable patients (3%) in the combined aducanumab groups developed treatment-emergent anti-aducanumab antibodies within the first year of treatment. Antibody responses were

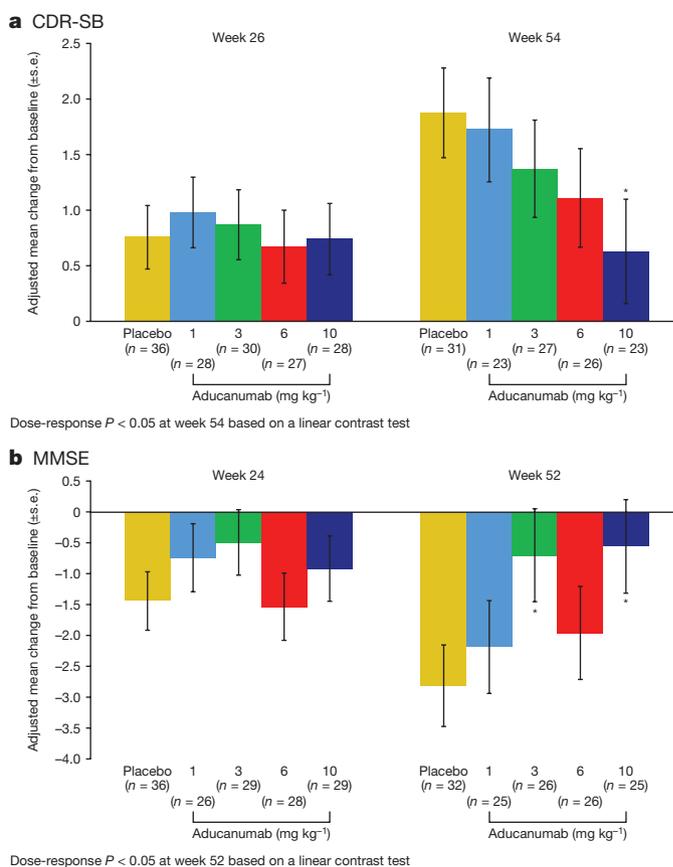


Figure 3 | Aducanumab effect (change from baseline) on CDR-SB and MMSE. **a, b**, Aducanumab effect on CDR-SB (**a**) and MMSE (**b**).

* $P < 0.05$ versus placebo; two-sided tests with no adjustments for multiple comparisons. CDR-SB and MMSE were exploratory endpoints. Adjusted mean \pm s.e. Analyses using ANCOVA. CDR-SB, Clinical Dementia Rating—Sum of Boxes; MMSE, Mini Mental State Examination.

transient, with minimal titres, and had no apparent effect on aducanumab pharmacokinetics or safety.

Brain penetration and binding to A β plaques

In the preclinical studies which preceded PRIME, systemically administered aducanumab (single dose, 30 mg kg⁻¹ intraperitoneally (i.p.)) bound to diffuse and compact A β plaques in the brains of 22-month-old female Tg2576 transgenic mice ('Target engagement study'; Extended Data Fig. 4a–d). C_{max} in plasma was 181 μ g ml⁻¹, with a terminal elimination half-life ($t_{1/2}$) of 2.5 days. The C_{max} in brain was 1,062 ng g⁻¹ of tissue, and approximately 400–500 ng g⁻¹ of drug was measured 3 weeks after dosing, suggesting long-term retention. Consequently, the brain:plasma AUC ratio of 1.3% was higher than the 0.1% frequently reported for systemically administered antibodies^{11,12}.

Administration of a single dose of aducanumab did not affect plasma (Extended Data Fig. 4b) or brain (data not shown) A β concentrations, consistent with the observation that aducanumab does not bind to soluble A β monomers. In contrast, the murine bapineuzumab precursor antibody 3D6, which binds to A β monomers, triggered a transient plasma A β spike (Extended Data Fig. 4b). Similarly, plasma A β concentrations were stable after repeated dosing with aducanumab in the PRIME study (data not shown). Within 24 h of dosing, aducanumab bound to parenchymal brain A β with a spatial pattern essentially superimposable with *ex vivo* pan-A β antibody staining, confirming that aducanumab binds all morphological types of brain A β plaques *in vivo*, including diffuse A β deposits and compact A β plaques (Extended Data Fig. 4c, d). Aducanumab binding to A β deposited in cerebral amyloid angiopathy (CAA) lesions within brain blood vessel walls was less

Table 2 | Summary of adverse events and most common adverse events

Adverse event (n (%))	Placebo (n=40)	Aducanumab			
		1 mg kg ⁻¹ (n=31)	3 mg kg ⁻¹ (n=32)	6 mg kg ⁻¹ (n=30)	10 mg kg ⁻¹ (n=32)
Any adverse event	39 (98)	28 (90)	27 (84)	28 (93)	29 (91)
Serious event	15 (38)	3 (10)	4 (13)	4 (13)	12 (38)
Discontinuing treatment due to an adverse event	4 (10)	3 (10)	2 (6)	3 (10)	10 (31)
Common adverse events					
ARIA	2 (5)	2 (6)	4 (13)	11 (37)	15 (47)
Headache	2 (5)	5 (16)	4 (13)	8 (27)	8 (25)
Urinary tract infection	4 (10)	3 (10)	2 (6)	4 (13)	5 (16)
Upper respiratory tract infection	6 (15)	2 (6)	2 (6)	2 (7)	6 (19)
Diarrhoea	3 (8)	0	6 (19)	1 (3)	3 (9)
Arthralgia	2 (5)	0	6 (19)	2 (7)	1 (3)
Fall	8 (20)	3 (10)	2 (6)	2 (7)	2 (6)
Superficial siderosis of CNS	0	2 (6)	1 (3)	2 (7)	4 (13)
Constipation	0	3 (10)	1 (3)	1 (3)	3 (9)
Nausea	2 (5)	2 (6)	5 (16)	0	1 (3)
Anxiety	4 (10)	4 (13)	1 (3)	1 (3)	1 (3)
Nasopharyngitis	0	1 (3)	5 (16)	0	1 (3)
Cough	2 (5)	3 (10)	1 (3)	0	1 (3)
Alanine aminotransferase increased	0	3 (10)	0	1 (3)	0
Aspartate aminotransferase increased	0	3 (10)	0	0	1 (3)

Common adverse events are those with an incidence of $\geq 10\%$ in any aducanumab treatment group. Incidence of ARIA based on adverse event reporting. Adverse events of ARIA-E (oedema) and ARIA-H (micro-haemorrhage) are both coded to the MedDRA preferred term of amyloid-related imaging abnormalities, and ARIA-H (superficial siderosis) codes to the MedDRA preferred term of superficial siderosis of the CNS. ARIA, amyloid-related imaging abnormalities; CNS, central nervous system; MedDRA, Medical Dictionary for Regulatory Activities.

prominent than parenchymal A β binding, when compared with the total amount of A β (Extended Data Fig. 4c, d).

Reduction of brain A β in transgenic mice

Exposure in plasma and brain correlated linearly with dose after chronic dosing in plaque-bearing transgenic mice (Extended Data Fig. 5) (Supplementary Information). ^{ch}aducanumab, a murine IgG2a/ κ chimaeric analogue, dose-dependently reduced A β measured in brain homogenates by up to 50% relative to the vehicle control in the diethylamine (DEA) fraction that extracted soluble monomeric and oligomeric forms of A β_{40} and A β_{42} , and in the guanidine hydrochloride (GuHCl) fraction that extracted insoluble A β fibrils (Fig. 4a, b).

Quantitative 6E10 immunohistochemistry showed significant reductions in all forms of A β deposits by up to 70% (Fig. 4c, d). Thioflavin S (ThioS) staining of compact A β plaques showed dose-dependent and statistically significant reductions in the cortex and hippocampus by up to 63% (Fig. 4c, d). Quantitative histology indicated that ^{ch}aducanumab significantly reduced the number of plaques of all sizes, including plaques $>500\ \mu\text{m}^2$ and plaques $<125\ \mu\text{m}^2$ (Extended Data Fig. 6a–c). Quantification of ThioS-positive vascular and parenchymal A β plaques separately showed that ^{ch}aducanumab did not affect vascular A β in either cortex or hippocampus (Fig. 4e–h).

To identify the mechanism of A β clearance, we analysed the involvement of microglia which are known to display enhanced phagocytic activities through binding to the Fc region of an antibody^{13,14}. ^{ch}aducanumab significantly increased recruitment of Iba-1-positive microglia to A β plaques, suggesting Fc γ R-mediated phagocytosis of antibody–A β complexes as a possible clearance mechanism (Extended Data Fig. 7a–c and Supplementary Information).

Biochemical characterization

The apparent affinities of aducanumab and ^{ch}aducanumab for aggregated A β_{42} , with half maximal effective concentration (EC₅₀) values of 0.1 nM, were comparable to 3D6 (ref. 13) (Fig. 5a). Neither aducanumab nor ^{ch}aducanumab bound monomeric soluble A β_{40} at concentrations

up to 1 μM , indicating $>10,000$ -fold selectivity for aggregated A β over monomer, whereas 3D6 bound soluble A β_{40} with an EC₅₀ of 1 nM (Fig. 5b). In contrast to 3D6, which immunoprecipitated both monomeric and aggregated A β , ^{ch}aducanumab bound soluble A β_{42} oligomers and insoluble A β_{42} fibrils prepared *in vitro*, but not A β_{42} monomers (Fig. 5c). Histological staining of autopsy tissue from patients with AD or aged amyloid precursor protein (APP) transgenic mice confirmed binding of aducanumab to bona fide human A β fibrils (Fig. 5d, e).

Discussion

The PRIME study shows that aducanumab penetrates the brain and decreases A β in patients with AD in a time- and dose-dependent manner. Within 54 weeks of treatment, 3, 6 and 10 mg kg⁻¹ doses of aducanumab significantly decreased the amyloid PET SUVR. Patients receiving placebo showed virtually no change in their mean PET SUVR composite scores over one year, indicating that A β pathology had already reached an asymptote of accumulation. Considering that it may have taken up to 20 years for A β to have accumulated to the levels in these patients at study entry¹⁵, the observed kinetics of A β removal within a 12-month time period appears encouraging for a disease-modifying treatment for patients with AD.

The cognitive results for CDR-SB and MMSE provide support for the clinical hypothesis that reduction of brain A β confers a clinical benefit. Post hoc analysis showed that those aducanumab-treated patients who had decreased SUVR scores >1 standard deviation unit relative to placebo-treated patients after one year of treatment experienced a stabilization of clinical decline on both CDR-SB and MMSE scores; whereas, those patients with a smaller or no decrease experienced clinical decline similar to placebo patients (Fig. 2c). The apparent clinical benefit observed in PRIME could also be explained by the binding of aducanumab to oligomeric forms of A β , which would not be directly detected by PET imaging. The reductions in SUVR scores may be surrogates for reductions in toxic soluble A β oligomers which may have had a more functionally relevant impact on cognition. Whereas significant A β reduction was detectable by 6 months, clinical effects were not

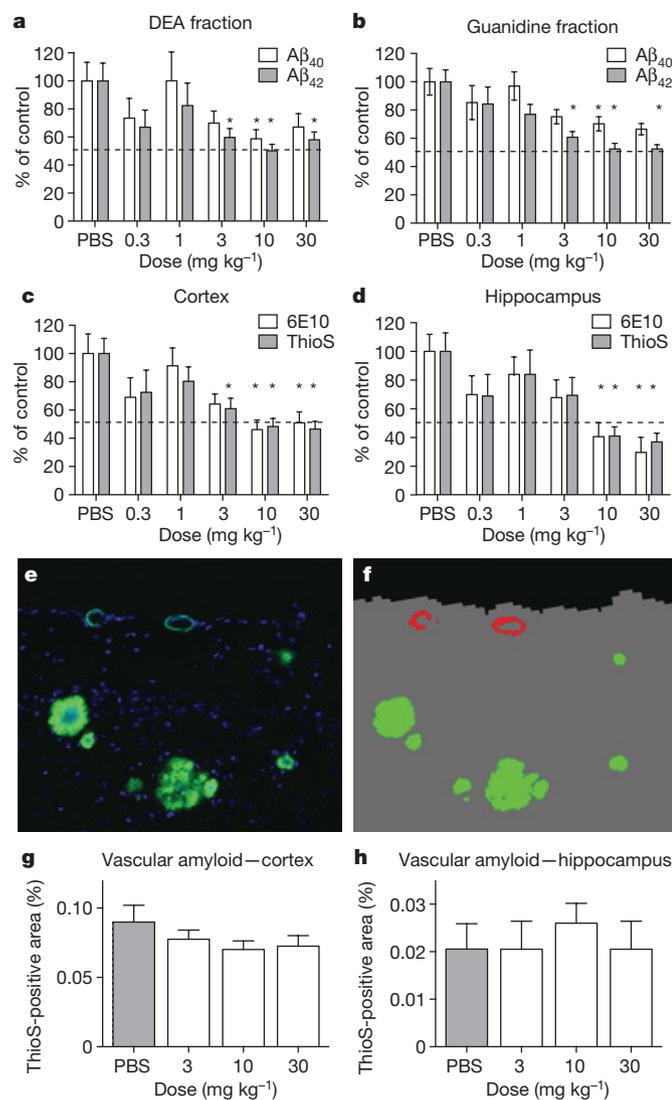


Figure 4 | Reduction of amyloid burden following weekly dosing with ^{ch}aducanumab in 9.5- to 15.5-month-old Tg2576 transgenic mice. **a, b**, Aβ₄₀ and Aβ₄₂ levels in soluble DEA (**a**) and insoluble GuHCl (**b**) brain fractions. **c, d**, Total brain Aβ (6E10) and compact amyloid plaques (ThioS) in cortex (**c**) and hippocampus (**d**) (mean ± s.e.; n = 20–55; dotted line 50% reduction; *P < 0.05 versus control). **e–h**, ThioS staining of amyloid deposits (**e**) and Visiopharm software (**f**) differentiated parenchymal deposits (green) from vascular deposits (red) (representative pictures 10× magnification), and quantified area of vascular amyloid (**g, h**; mean ± s.e.; n = 20–24).

apparent until one year. Given that clearance of Aβ could be followed by recovery of neuronal function, a lag between reduction of Aβ burden and slowing of disease progression is not altogether surprising.

The main safety finding, ARIA-E, was dose-dependent and more common in ApoE ε4 carriers, consistent with findings with other anti-Aβ monoclonal antibodies^{7,16,17}. Although the underlying cause of ARIA is not well understood, it is likely that the MRI signal of ARIA is due to increased extracellular fluid. This may be a result of underlying CAA, changes in perivascular clearance and vascular integrity, or local inflammatory processes associated with Aβ-targeting therapies^{17–20} (see Supplementary Information for further discussion).

Study limitations of the PRIME phase 1b study included staggered parallel-group design, small sample sizes, limited region (USA only), and possible partial unblinding due to ARIA-E. Measures were taken to maintain blinding to adverse effects: raters of given tests were not permitted to perform other clinical assessments, and were blinded to other assessments (for example, MMSE and CDR raters were required

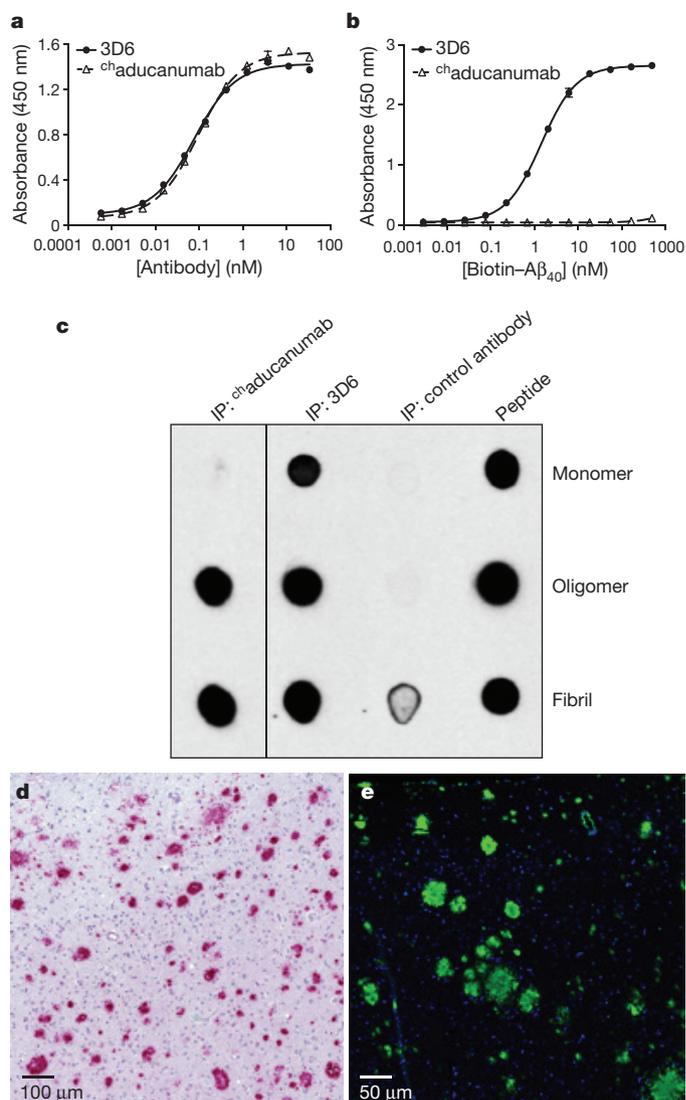


Figure 5 | Aducanumab binds selectively to insoluble fibrillar and soluble oligomeric Aβ aggregates. **a**, Binding of ^{ch}aducanumab or 3D6 to immobilized fibrillar Aβ₄₂. Mean ± s.d., in triplicate. **b**, Capture of soluble monomeric Aβ₄₀ with immobilized ^{ch}aducanumab or 3D6. Mean ± s.d., in triplicate. **c**, Dot blots of Aβ₄₂ monomer, soluble oligomers, or insoluble fibrils immunoprecipitated with ^{ch}aducanumab, 3D6, or irrelevant antibody control. Equivalent concentrations confirmed by direct dot blotting (Peptide). **d, e**, Immunostaining of Aβ in autopsy brain tissue from a patient with AD with ^{ch}aducanumab (0.2 μg ml⁻¹) (**d**) and 22-month-old Tg2576 transgenic mouse brain tissue with aducanumab (60 ng ml⁻¹) (**e**).

to be different and neither were permitted to perform other study assessments). Post hoc analyses of change from baseline PET SUVR composite score and cognition by presence/absence of ARIA suggested no apparent difference in treatment effect when comparing patients with and without ARIA-E (Extended Data Table 4). There was overlap in enrolment in Arms 1–3 (aducanumab 1 and 3 mg kg⁻¹, placebo) and Arms 4 and 5 (aducanumab 10 mg kg⁻¹, placebo) but Arms 6 and 7 (aducanumab 6 mg kg⁻¹, placebo) were initiated after enrolment in Arms 1–5 was complete. This was a small study designed for assessment of safety and tolerability, and for detecting a pharmacological effect on brain Aβ levels measured by PET imaging. The trial was not powered for the exploratory clinical endpoints, thus the clinical cognitive results should be interpreted with caution. Primary analyses were based on observed data with no imputation for missing values, nominal P values were presented with no adjustments for multiple comparisons, and they were supported by sensitivity analyses using a MMRM.

The initiation of the PRIME study and its results are supported by extensive preclinical data. Detection on parenchymal A β plaques following a single systemic administration confirmed that aducanumab penetrates the brain to a sufficient extent to allow accumulation on A β plaques. This is consistent with earlier findings showing that, in the presence of significant A β deposition, plaque-binding antibodies can be detected bound to the target over an extended period^{14,21}. The minimal effective dose upon repeated systemic administration of ^{ch}aducanumab in transgenic mice was 3 mg kg⁻¹ (corresponding to minimally effective concentrations of 13.8 \pm 1.9 μ g ml⁻¹ in plasma and 99.8 \pm 30.0 ng g⁻¹ in brain) with reductions of A β ₄₂ in soluble and insoluble brain fractions of approximately 50%, and reductions in A β plaque of approximately 40%. Since exposure at 3 mg kg⁻¹ in animals and humans is approximately equivalent, the observed dose-response in the model was consistent with the clinical doses that led to reductions in amyloid PET SUVR. ^{ch}aducanumab cleared plaques of all sizes, suggesting that aducanumab triggered clearance of pre-existing A β plaques and prevented formation of new plaques.

In transgenic mice, aducanumab preferentially bound to parenchymal A β over vascular A β deposits, consistent with the lack of effect on vascular A β following chronic dosing. The effect of anti-A β antibody therapies on the vascular A β compartment could be related to micro-haemorrhages or oedema in transgenic mice, and may relate to ARIA in clinical trials²². Nevertheless, the preferential binding of aducanumab to parenchymal versus vascular A β may have been critical in allowing the use of relatively high doses in the clinical study so as to achieve robust target engagement in the brains of patients with AD.

Several mechanisms may be involved in aducanumab's A β -lowering activity. The clearance of A β deposits was accompanied by enhanced recruitment of microglia. Together with the reduced potency of the aglycosylated form of ^{ch}aducanumab (data not shown), and the *ex vivo* phagocytosis data, this suggests that Fc γ R-mediated microglial recruitment and phagocytosis played an important role in A β clearance in these models. Activated microglia appeared to encapsulate the remaining central dense core of plaques in treated animals, possibly isolating them from the surrounding neuropil. It is commonly thought that soluble A β oligomers, rather than monomers or plaques, may be the primary toxic species^{23,24}. Considering that A β plaques might be a source of A β oligomers^{25–28}, this suggests that treatment with aducanumab might slow their release into the neuropil, thereby limiting their toxic effect on neurons²⁹. In fact, chronic dosing of 18-month-old Tg2576 transgenic mice with ^{ch}aducanumab led to normalization of neuritic calcium overload in the brain³⁰. Other studies have linked calcium dyshomeostasis in neurons and microglia to binding of A β oligomers to metabotropic receptors^{31–33}. Aducanumab binding to soluble A β oligomers may prevent their interaction with those receptors, thereby preventing the detrimental effect of membrane depolarization. Restoration of this functional endpoint suggests that aducanumab treatment may lead to beneficial effects on neuronal network function underlying cognitive deficits.

Together, the clinical and preclinical data support continued development of aducanumab as a disease-modifying treatment for AD. The clinical study results provide robust support to the biological hypothesis that treatment with aducanumab reduces brain A β plaques and, more importantly, to the clinical hypothesis that A β plaque reduction confers clinical benefit. This concurs with preclinical data demonstrating brain penetration, target engagement, and dose-dependent clearance of A β plaques in transgenic mice. The clinical effects of aducanumab need to be confirmed in larger studies. Both the long-term extension (LTE) phase of this study and phase 3 development are ongoing.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.S. (alfred.sandrock@biogen.com).

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METHODS

Clinical study subjects. Patients were screened for inclusion in three stages. First, patients were evaluated on demographic, and clinical and laboratory criteria, including being between 50–90 years of age, and meeting clinical criteria for either prodromal or mild AD, as determined by the investigator. The criteria for prodromal AD were: MMSE score between 24–30 (inclusive), a spontaneous memory complaint, objective memory loss defined as a free recall score of ≤ 27 on the FCSRT³⁴, a global CDR score of 0.5, absence of significant levels of impairment in other cognitive domains and essentially preserved activities of daily living, and an absence of dementia³⁵. The criteria for mild AD were: MMSE score between 20–26 (inclusive), a global CDR of 0.5 or 1.0, and meeting the National Institute on Aging–Alzheimer’s Association core clinical criteria for probable AD³⁶. Second, patients who remained eligible underwent MRI to exclude those with confounding pathology, including acute or sub-acute micro- or macro-haemorrhage, prior macro-haemorrhage, >4 micro-haemorrhages, superficial siderosis or any finding that might be a contributing cause of the patient’s dementia, pose a risk to the patient, or prevent a satisfactory MRI assessment for safety monitoring. Third, remaining eligible patients underwent a florbetapir PET scan, and those with a positive scan based on a visual assessment, as determined by a qualified reader, were eligible. The A β PET screening process has been described in a separate publication⁹. Stable use of most concomitant background medications was permitted and, in the case of cholinesterase inhibitors and/or memantine, patients were required to be on a stable dose for a minimum of 4 weeks before screening with no adjustment of dosing during the double-blind phase of the study. Patients were excluded if they had a medical condition that might be a contributing cause of cognitive impairment.

Clinical study design. This was a multicentre, randomized, 12-month, double-blind, placebo-controlled, multiple-dose study of aducanumab followed by a 42-month, dose-blinded LTE study in patients with either prodromal or mild AD who were A β PET-positive (ClinicalTrials.gov identifier NCT01677572). The primary objective was to evaluate the safety and tolerability of multiple doses of aducanumab in patients with prodromal AD or mild AD dementia. The secondary objectives were to: (i) assess the effect on cerebral A β plaque content as measured by ¹⁸F-florbetapir PET imaging at week 26; (ii) assess the multiple-dose serum concentrations of aducanumab; and (iii) evaluate the immunogenicity of aducanumab after multiple-dose administration. The key exploratory objectives were assessments of the effect of aducanumab on the following: the clinical progression of AD as measured by change from baseline on the CDR-SB, a NTB, and the FCSRT; disease-related biomarkers in blood, cerebral A β plaque content as measured by ¹⁸F-florbetapir PET imaging at week 54; and cerebral A β plaque content by ApoE $\epsilon 4$ carrier status (carrier/non-carrier). Other exploratory endpoints were change from baseline on the Neuropsychiatric Inventory Questionnaire, Cognitive Drug Research computerized test battery, volumetric MRI, and, in a subset of patients, glucose metabolism as measured by fluorodeoxyglucose PET, functional connectivity by task-free functional MRI, cerebral blood flow by arterial spin labelling MRI, and disease-related biomarkers in cerebrospinal fluid. MMSE was another exploratory assessment.

During the 12-month, double-blind, placebo-controlled phase, patients received aducanumab or placebo by IV infusion once every 4 weeks for 52 weeks. In a staggered, parallel-group design, the treatment arms were enrolled as follows: first Arms 1–3 (aducanumab 1 mg kg⁻¹ ($n = 30$); aducanumab 3 mg kg⁻¹ ($n = 30$); placebo ($n = 20$), respectively) in parallel. Once enrolment was open, Arms 4 and 5 (aducanumab up to 10 mg kg⁻¹ ($n = 30$) (actual dose 10 mg kg⁻¹); placebo ($n = 10$), respectively) were enrolled in parallel with Arms 1–3. Once enrolment in Arms 1–5 was complete, enrolment in Arms 6 and 7 (aducanumab up to 30 mg kg⁻¹ ($n = 30$) (actual dose 6 mg kg⁻¹); placebo ($n = 10$), respectively) began. The trial was initially designed to dose up to 30 mg kg⁻¹, but when ARIA were detected at 10 mg kg⁻¹ it was decided not to proceed to doses higher than 10 mg kg⁻¹ with repeated infusions. Dose escalation in Arms 4 and 5, and then Arms 6 and 7, was based on review of existing safety, tolerability, and pharmacokinetic data, and recommendation of the external Data Monitoring Committee. Patients were randomized (using a centralized interactive voice and web response System (IXRS)) to a treatment group within Arms 1–3, 4 and 5, or 6 and 7, stratified by ApoE $\epsilon 4$ status (carrier or non-carrier). Patient enrolment was monitored so that the ratio of ApoE $\epsilon 4$ carriers to non-carriers was no more than 2:1 and no less than 1:2. During the overlap in enrolment of Arms 1–3 and Arms 4 and 5, patients were randomized using a minimization algorithm. Patients who discontinued study treatment for any reason were encouraged to remain in the study and complete all assessments during the double-blind period. Patients completing the double-blind period and meeting certain eligibility criteria entered the LTE. After enrolment on Arms 6 and 7 were completed, the protocol was amended to include a titration arm and a corresponding placebo group—Arms 8 and 9. Both the LTE and Arms 8 and 9 are ongoing and were not part of this interim analysis.

Investigators, study site staff (except for a designated pharmacist/technician), and study patients were blinded to the patients’ randomized treatment assignment

for the placebo-controlled period. Only the designated pharmacist/technician at each site was aware of the assigned treatment for each patient. Aducanumab was supplied as a sterile clear-to-yellow solution for IV infusion at a dose of 200 mg in 4 ml. For patients randomized to receive aducanumab, undiluted aducanumab (required volume based on patient weight) was added to a 100 ml 0.9% saline bag to reach the assigned dose (an equivalent amount of saline was first withdrawn so that the final total volume of all IV bags was identical). All IV bags (active and placebo (100 ml 0.9% saline)) were covered with a sealed brown light-protective bag to maintain blinding with a label including protocol and patient randomization number.

Cases of ARIA were managed in accordance with protocol-defined rules using centrally read MRI findings coupled with clinical symptoms, if present. The rules were consistent with the guidelines published by the Alzheimer Association Research Roundtable Working Group¹⁸. Briefly, patients developing mild ARIA-E or ARIA-H (≤ 4 incident micro-haemorrhages) without clinical symptoms could continue at the same dose; patients developing moderate or severe ARIA-E without clinical symptoms, or those with ARIA-E accompanied by mild clinical symptoms, could suspend treatment and resume at the next lower dose level once ARIA (and symptoms, if any) resolved. Patients who developed ARIA-E or ARIA-H (≤ 4 incident micro-haemorrhages) accompanied by moderate, severe, or serious clinical symptoms, >4 incident micro-haemorrhages, any incident macro-haemorrhage, or >1 incident haemosiderosis at any time during the study were to permanently discontinue treatment.

The study was conducted in accordance with the Declaration of Helsinki, and the International Conference on Harmonisation and Good Clinical Practice guidelines, and had ethics committee approval at each participating site. All patients provided written informed consent.

Clinical study assessments. Amyloid plaque content, as measured by florbetapir PET imaging, was assessed at screening, and at weeks 26 and 54. Detailed PET scanning protocols have been described in a separate publication⁹. Briefly, for each florbetapir scan, a dose of 370 MBq was injected intravenously, with PET scanning starting around 50 min later and continuing for approximately 20 min.

Visual reads, the basis for meeting the inclusion criterion of a positive A β PET scan, were based upon PET image data, with the registered MRI and fused PET/MRI data providing supplementary anatomical information. Scans were independently interpreted by two board-certified neuroradiologists who, in accordance with the Amyvid Prescribing Information³⁷, had successfully completed a training programme (provided by the manufacturer using either an in-person tutorial or an electronic process). Images were designated as positive or negative, following guidelines described in the Amyvid Prescribing Information³⁷.

A composite cortical SUVR was computed using a volume-weighted average across six brain regions of interest (frontal, parietal, lateral temporal and sensorimotor, anterior, and posterior cingulate cortices), as previously described¹⁶, normalized to whole cerebellar activity^{10,38}.

Clinical tests including the CDR and an NTB (comprising RAVLT Immediate and Delayed Recall, Wechsler Memory Scale Verbal Pair Associate Learning Test Immediate and Delayed Recall, Delis–Kaplan Executive Function System Verbal Fluency Conditions 1 and 2, and the Wechsler Adult Intelligence Scale Fourth Edition Symbol Search and Coding Subsets) were performed during screening and at weeks 26 and 54. The FCSRT was performed at screening and at week 52. These clinical tests were administered by a trained, certified clinician or rater experienced in the assessment of patients with cognitive deficits. When possible, the same rater would administer a given test across all visits. In order to maintain blinding to adverse events, raters were not permitted to perform other clinical assessments, and were blinded to other clinical and safety assessments. The rater who conducted the CDR for a patient could not complete any other rating scales for that same patient, and was blinded to the results of all other cognitive scales.

The following safety assessments were performed at regular intervals: physical examination, neurological examination, vital signs, electrocardiogram, and laboratory safety assessments. During the placebo-controlled period, brain MRI was performed at screening and at weeks 6, 18, 30, 42, and 54, and end of study or termination. The MMSE was completed at screening, and at weeks 24, 52, and end of study or termination, and, in patients who developed ARIA, at every scheduled visit until ARIA resolved.

The concentrations of aducanumab in serum and presence of anti-aducanumab antibodies were determined using validated ELISA techniques (Supplementary Information).

Statistical analysis in the clinical study. This interim analysis included all patients randomized to a fixed-dose regimen and completing the double-blind period of the study (data cut-off February 2015). For all analyses, all patients assigned to placebo were treated as a single group. The safety population was defined as all patients who were randomized and received at least one dose of study treatment. Adverse events were coded using the Medical Dictionary for Regulatory Activities

classification. The pharmacodynamic and pharmacokinetic populations were defined as all patients who were randomized, received at least one dose of study treatment, and had at least one post-baseline assessment of the pharmacodynamic parameter or at least one measurable aducanumab concentration in serum, respectively.

The primary analysis of the pharmacodynamic and efficacy data was based on Analysis of Covariance (ANCOVA), adjusting for baseline and ApoE ϵ 4 status (carrier and non-carrier) using observed data. No imputation was performed for missing data. For each time point, adjusted means for each treatment, pairwise adjusted differences with placebo, 95% confidence intervals for the pairwise differences, and associated nominal *P* values for comparison were calculated. No adjustments were made for multiple comparisons/multiple interim analyses. Dose-response was tested using a linear contrast from the ANCOVA model. The linear contrast test is sensitive to a variety of positive dose-response shapes, including a linear dose-response relationship. This served as the primary analysis for the cognition analyses. To account for missing data, a MMRM was used as a sensitivity analysis for the longitudinal data change from baseline data, adjusting for baseline and ApoE ϵ 4 status (carrier and non-carrier). Visit and treatment group were treated as categorical variables in the model along with their interactions. An unstructured covariance matrix was assumed to model the within-patient variability. This model imposes no assumptions on mean trend and correlation structure, and is considered robust.

Subgroup analyses were performed for change from baseline A β PET and change from baseline for cognition measures (CDR-SB and MMSE) for baseline clinical stage and ApoE ϵ 4 status (carrier and non-carrier). The subgroup analysis of the pharmacodynamic and efficacy data was based on ANCOVA, adjusting for baseline and ApoE ϵ 4 status (carrier and non-carrier) (for baseline clinical stage only) using observed data.

Serum pharmacokinetics were determined by nonlinear mixed effects model (NONMEM) approach. Sparse samples in the multiple-ascending-dose study and intensive samples from an earlier single-ascending-dose study⁸ were combined to construct a population pharmacokinetic model. The model was built in NONMEM software using the first-order conditional estimation with interaction method. Cumulative AUC up to month 12 was estimated for each patient. The plasma terminal elimination half-life was estimated in the pharmacokinetic analysis population. The analysis population for the primary immunogenicity analysis was defined as all patients who were randomized, received study treatment, and had at least one post-dose immunogenicity sample evaluated for immunogenicity.

Interim analyses were specified in the protocol for the purpose of planning future studies; no changes were to be made for this study based on the interim analysis results.

A sample size of 30 patients per treatment group would provide more than 90% power to detect a treatment difference of 1 standard deviation with respect to the reduction of A β from baseline, based on comparison of each aducanumab group with placebo, at a two-sided significance level of 0.05, and assuming a dropout rate of 20%.

Transgenic mouse studies. Penetration of aducanumab into the brain and target engagement were assessed in 22-month-old female Tg2576 mice following a single dose of aducanumab at 30 mg kg⁻¹ administered i.p. ('Target engagement study'; *n* = 4–5 per time point). The ability of aducanumab to reduce A β burden was assessed following chronic treatment of 9-month-old male and female Tg2576 transgenic mice dosed weekly i.p. for 6 months with PBS or 0.3, 1, 3, 10, or 30 mg kg⁻¹ of the murine chimaeric variant ^{ch}aducanumab ('Chronic efficacy study'; *n* = 20–55 per treatment group). An additional dosing study ('Chronic efficacy study with Agly'; *n* = 12–14 per treatment group) comparing the plaque clearing ability of ^{ch}aducanumab to that of an effector function-impaired variant (^{ch}aducanumab-Agly) was conducted using a similar study design (chronic treatment of 9.5-month-old Tg2576 transgenic mice dosed weekly i.p. for 6 months with PBS or 3 mg kg⁻¹ of ^{ch}aducanumab or ^{ch}aducanumab-Agly).

Mice were killed following anaesthesia with ketamine/xylazine (100/10 mg kg⁻¹ i.p.). Blood was collected by cardiac puncture, and mice were perfused with ice-cold heparinized saline (0.9%) using a peristaltic pump. The brain was removed and halved along the medio-sagittal line. The right hemisphere was frozen on dry ice and stored at -80 °C for biochemical analysis. The left hemisphere was fixed by immersion in 10% neutral buffered formalin.

Size of the treatment groups was determined to take into account natural mortality (10–20%) and high inter-animal variability specific to the Tg2576 strain of mice. No animals were excluded from the analyses, unless the animal died prematurely. 'n' reported in the manuscript represents the number of animals in each group that were euthanized as scheduled at the end of the study. The allocation of animals to treatment groups took into account date of birth, gender, and weight at baseline. Each treatment group was balanced for mean age, gender, and mean weight. Dosing solutions were coded with letters so that all experimenters were

blinded to the treatment. The labelling of the samples collected did not reflect treatment group, so that experimenters processing and analysing the samples were still blinded. Codes were broken once all analyses were completed, including statistical analysis.

All in-life procedures were conducted in strict accordance with protocols approved by Biogen's Institutional Animal Care and Use Committee.

Biochemical measurements. Please see Supplementary Information.

Histological assessment. Please see Supplementary Information.

Preparation of different A β peptide conformations. Synthetic A β 1–42 (A β ₄₂) peptide (AnaSpec, Fremont, California, USA) was reconstituted in hexafluoroisopropanol at a concentration of 1 mg/ml, aliquoted, air-dried, and vacuum-concentrated to form a film, and dissolved in dimethyl sulfoxide (DMSO) at a concentration of 5 mg/ml. A β ₄₂ oligomers and A β ₄₂ fibrils were prepared by diluting DMSO-reconstituted monomeric into PBS at a concentration of 100 μ g/ml and incubating at 37 °C for at least 3 days and 1 week, respectively. The solution was centrifuged at 14,000g for 15 min at 4 °C, and oligomers were recovered from the supernatant following the shorter incubation, whereas fibrils were recovered from the pellet following the longer incubation. For details on the biophysical characterization of high molecular weight A β ₄₂ aggregates, please see Supplementary Information.

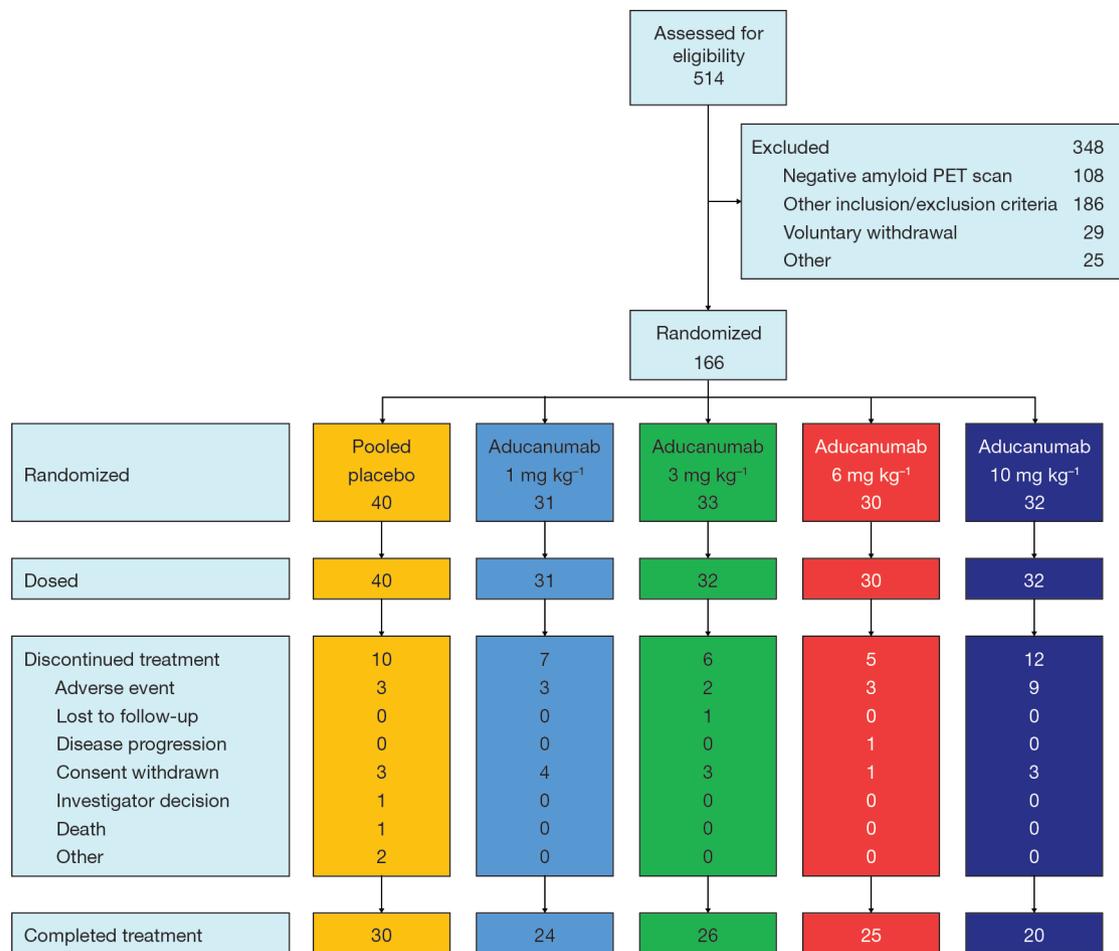
In immunoprecipitation experiments, samples of freshly prepared monomeric, soluble oligomeric, or insoluble fibrillar A β ₄₂ were immunoprecipitated with ^{ch}aducanumab, 3D6 or a murine IgG2a control antibody (P1.17), dot-blotted onto a nitrocellulose membrane, and detected with biotinylated pan-A β antibody 6E10. Similar results were observed for ^{ch}aducanumab when immunoblotted with 3D6.

ELISA. Please see Supplementary Information.

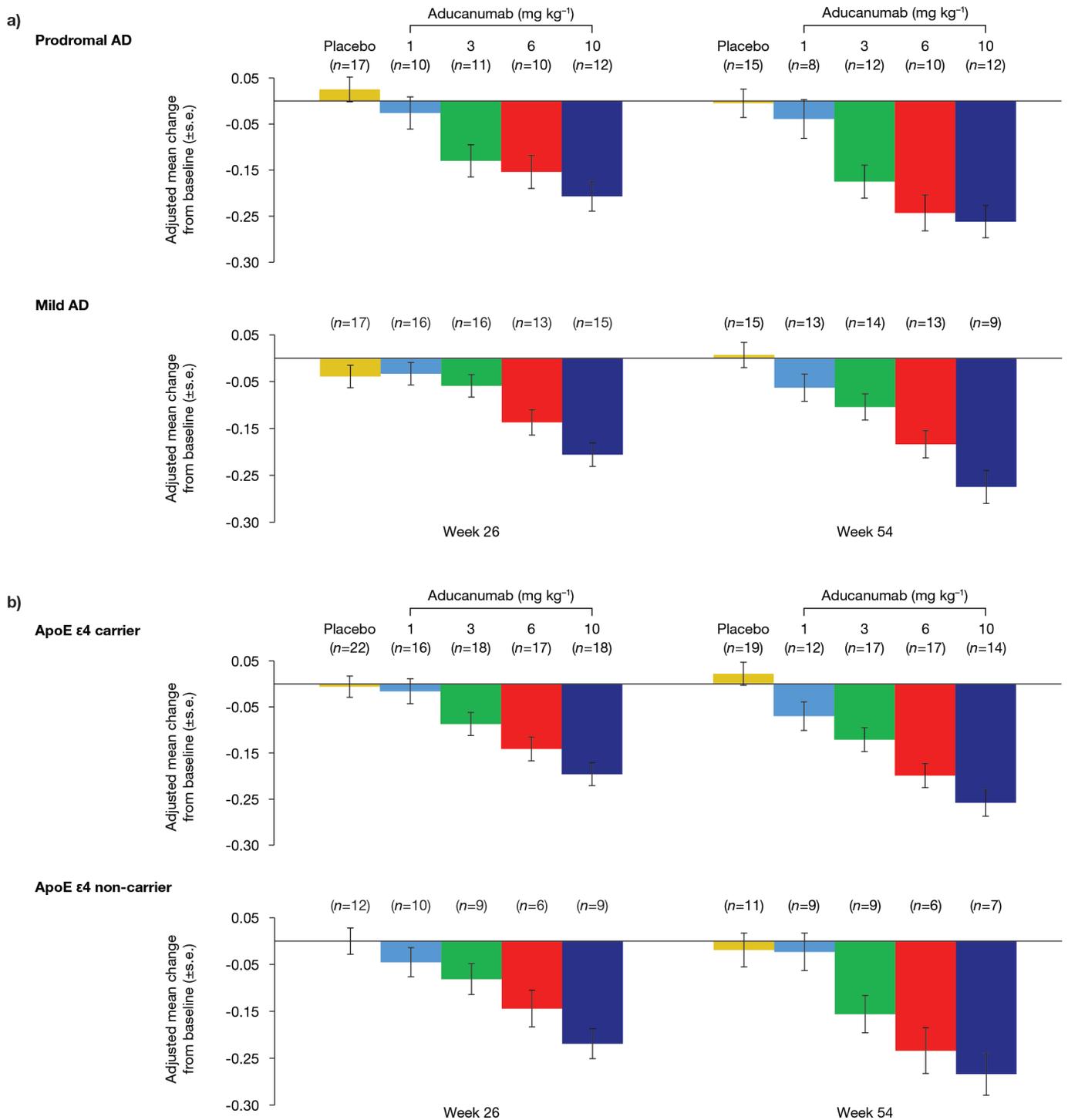
Antibody generation using reverse translational medicine. Aducanumab was derived from a de-identified blood lymphocyte library collected from healthy elderly subjects with no signs of cognitive impairment and cognitively impaired elderly subjects with unusually slow cognitive decline. Memory B cells, isolated from peripheral blood lymphocyte preparations by anti-CD22-mediated sorting were cultured on gamma-irradiated human peripheral blood mononuclear cell feeder layers. Supernatants from isolated B cells were screened for their ability to stain A β plaques on brain tissue sections, from either patients with AD or aged APP transgenic mice³⁹, and for their binding to aggregated forms of A β ₄₀ and A β ₄₂ *in vitro*. Positive hits meeting the above criteria were counter-screened to exclude clones cross-reacting with full-length APP expressed on stably transfected HEK293 cells (provided by U. Konietzko, University of Zurich, Switzerland; tested negative for mycoplasma contamination; not independently authenticated). Selected A β -reactive B-cell clones were subjected to cDNA cloning of IgG heavy and κ or λ light chain variable region sequences, and sub-cloned in expression constructs using Ig-framework specific primers for human variable heavy and light chain families in combination with human J-H segment-specific primers. Aducanumab was engineered to incorporate glycosylated human IgG1 heavy and human κ light chain constant domain sequences. A murine chimaeric IgG2a/ κ version of aducanumab (^{ch}aducanumab) was generated for use in chronic efficacy studies in APP transgenic mice. An aglycosylated variant of ^{ch}aducanumab (^{ch}aducanumab-Agly), incorporating a single point mutation (N297Q, using standard Kabat EU numbering) which eliminates N-glycosylation of the Fc region and severely reduces Fc γ R binding⁴⁰, was generated to test for Fc-related activities. The recombinant mouse IgG2b monoclonal antibody 3D6⁴¹ was used as a comparator in some studies.

Ex vivo phagocytosis assay. Please see Supplementary Information.

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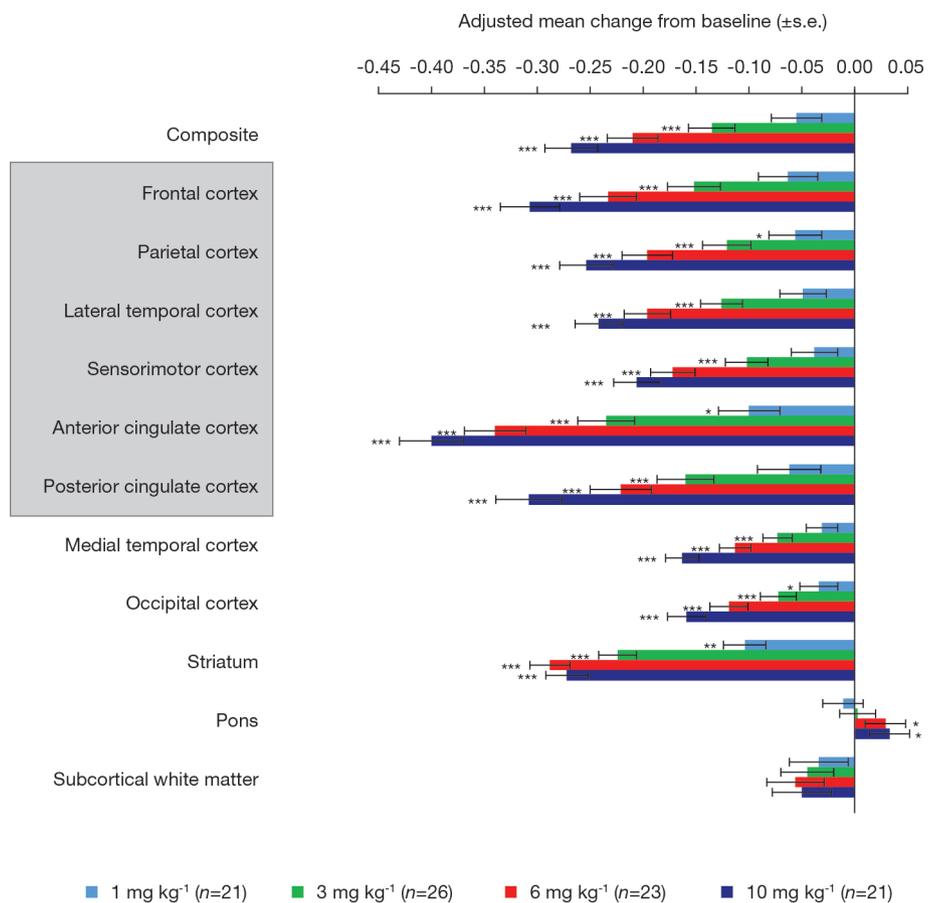


Extended Data Figure 1 | Participant accounting. PET, positron emission tomography.

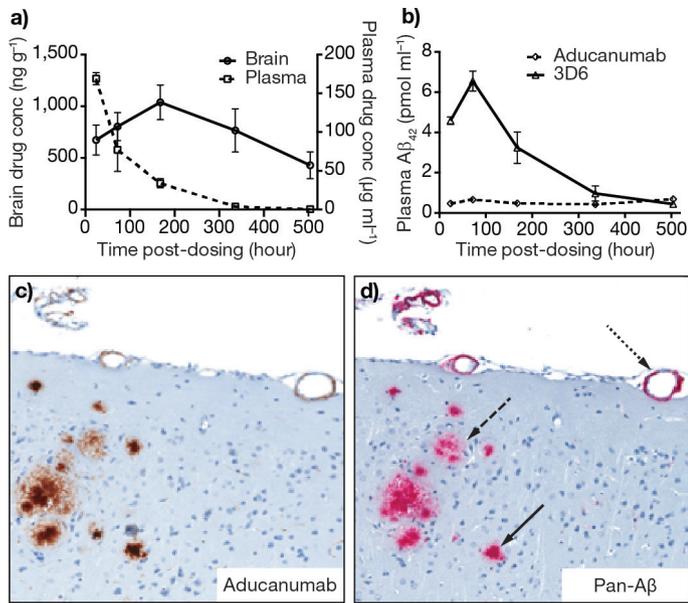


Extended Data Figure 2 | Amyloid plaque reduction with aducanumab by baseline clinical stage and baseline ApoE ε4 status. a, b, Analyses by baseline clinical stage were performed using ANCOVA for change from baseline with factors of: treatment, ApoE ε4 status (carrier and

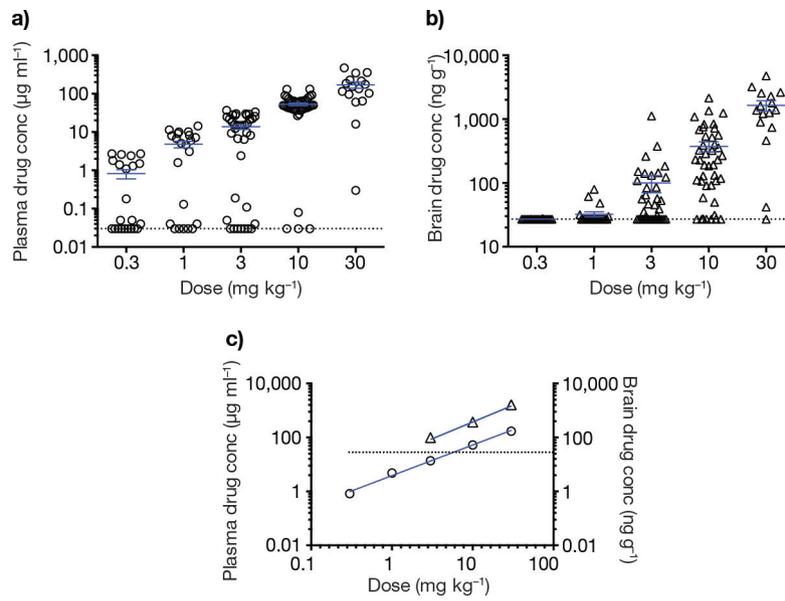
non-carrier) and baseline composite SUVR (a), and for analyses by ApoE ε4 status, using treatment and baseline composite SUVR (b). Adjusted mean ± s.e. ApoE ε4, apolipoprotein E ε4 allele; SUVR, standard uptake value ratio.



Extended Data Figure 3 | Amyloid plaque reduction: regional analysis SUVR at week 54. The boxed area indicates the six regions included in the composite score. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus placebo; two-sided tests with no adjustments for multiple comparisons. Adjusted mean \pm s.e. Analyses using ANCOVA. SUVR, standard uptake value ratio.



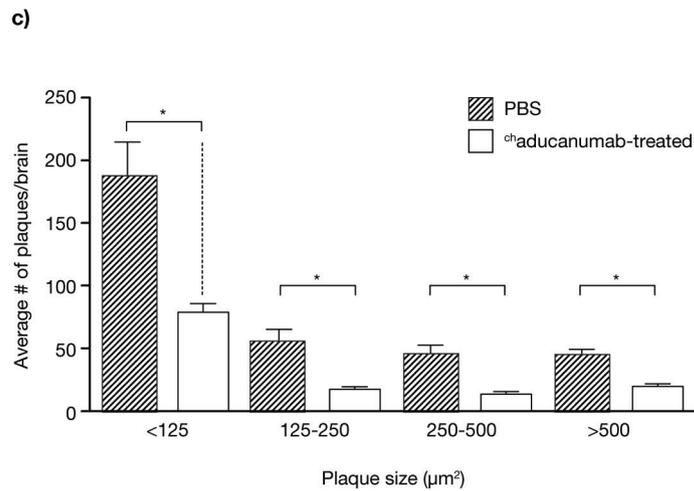
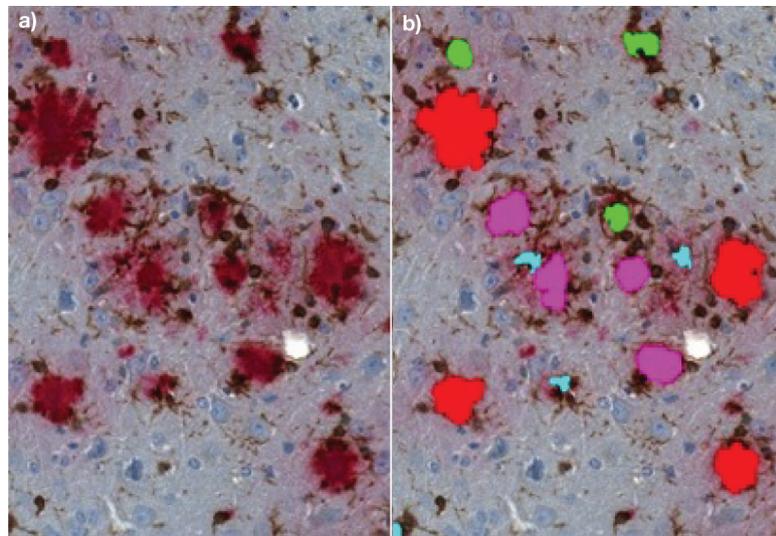
Extended Data Figure 4 | Brain penetration of aducanumab after a single intraperitoneal administration in 22-month-old Tg2576 transgenic mice. **a, b**, Aducanumab levels in plasma and brain (**a**), and plasma Aβ levels after a single dose (**b**; $n = 4-5$; mean \pm s.e.). **c, d**, *In vivo* binding of aducanumab to amyloid deposits detected using a human IgG-specific secondary antibody (**c**), and *ex vivo* immunostaining with a pan-Aβ antibody on consecutive section (**d**). Examples of a compact Aβ plaque (solid arrow), diffuse Aβ deposit (dashed arrow), and CAA lesion (dotted arrow). CAA, cerebral amyloid angiopathy.



Extended Data Figure 5 | Exposure following weekly dosing with ^{ch}aducanumab in 9.5- to 15.5-month-old Tg2576 transgenic mice.

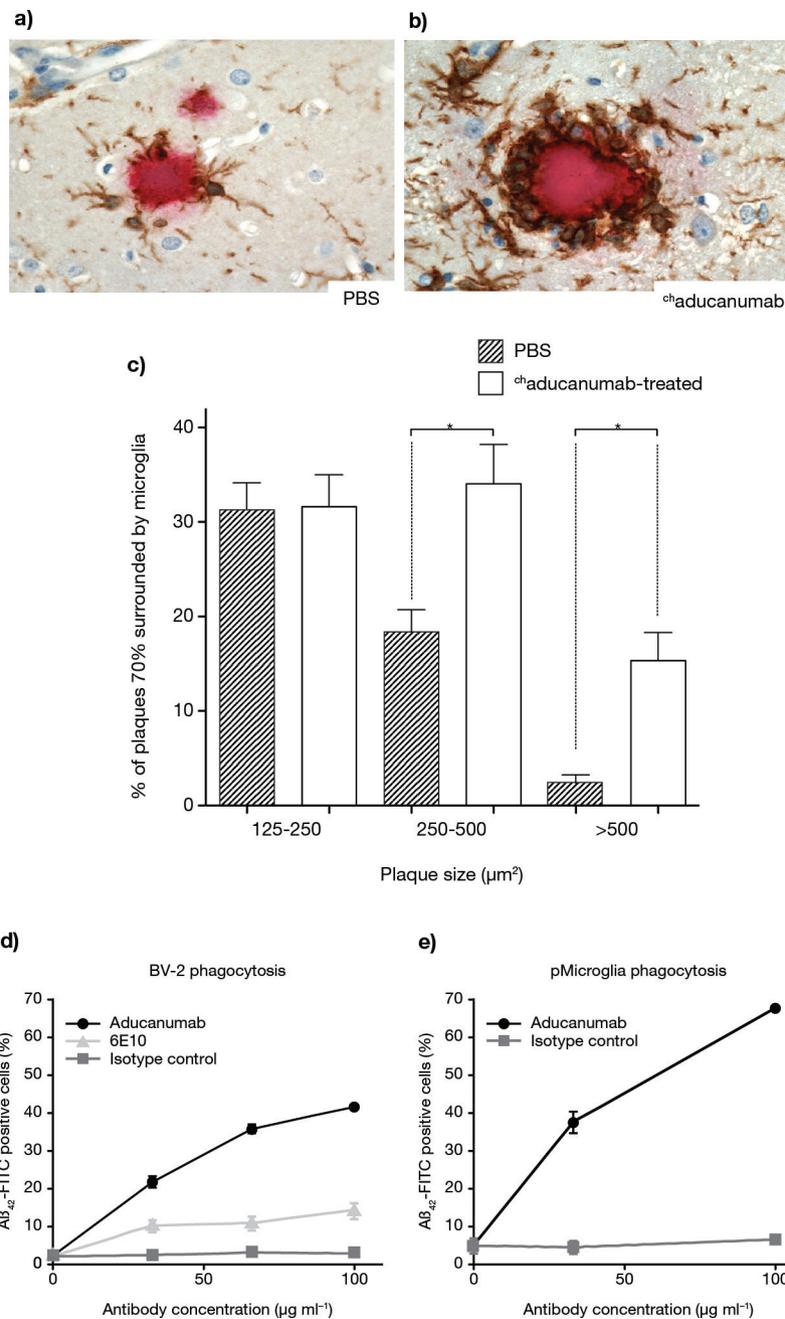
a, b, ^{ch}aducanumab concentrations in plasma (**a**), or DEA-soluble brain extract (**b**) were measured in samples collected 24 h after the last dose in the 'Chronic efficacy study'. Mean \pm s.e. Dotted lines represent the limits

of quantitation of each assay. **c,** Correlations of drug concentrations in plasma (open circles) or brain (open triangles) with administered dose. The average brain concentrations in the two groups receiving the lowest dose were below the limit of quantitation for that assay, which is indicated by a dotted line on the figure.



Extended Data Figure 6 | Treatment with chaducanumab affects plaques of all sizes. **a**, Following weekly dosing of chaducanumab in Tg2576 from 9.5–15.5 months of age, amyloid plaques were stained with 6E10 and quantified using Visiopharm software. **b**, Plaque size was defined by area, and coloured as follows: <125 μm^2 (cyan), 125–250 μm^2 (green), 250–500 μm^2 (pink), and >500 μm^2 (red). **c**, chaducanumab treatment was

associated with a significant decrease in plaque number in all size ranges relative to vehicle-treated controls, with reductions of 58%, 68%, 68%, and 53% in the number of plaques for the <125 μm^2 , 125–250 μm^2 , 250–500 μm^2 , and >500 μm^2 groups size, respectively. Mean \pm s.e.; statistically significant differences from vehicle for each size range are indicated with asterisks; * $P < 0.05$, Mann–Whitney test.



Extended Data Figure 7 | Enhanced recruitment of microglia to amyloid plaques following ch aducanumab treatment and engagement of $\text{Fc}\gamma$ receptors. **a, b**, Brain sections from either PBS- or ch aducanumab-treated mice ('Chronic efficacy study'; 3 mg kg^{-1} group) were immunostained for $\text{A}\beta$ (6E10; red) and a marker of microglia (Iba1; brown). **c**, The area of individual amyloid plaques was measured, and Iba1-stained microglia were grouped into two categories, either associated with plaques (within $25 \mu\text{m}$ of a plaque) or not associated with plaques ($>25 \mu\text{m}$ from a plaque). Plaques with circumferences $\geq 70\%$ surrounded by microglia were quantified and stratified based on plaque size. The fraction of plaques that were at least 70% surrounded

by microglia was significantly greater in the ch aducanumab-treated group (white bars) compared with the PBS control group (grey bars), for plaques $\geq 250 \mu\text{m}^2$. Mean \pm s.e.; statistically significant differences from vehicle for each size range are indicated with asterisks; $*P < 0.05$, Bonferroni's post hoc test following one-way analysis of variance. All quantifications were done using the Visiopharm software. **d, e**, FITC-labelled $\text{A}\beta_{42}$ fibrils were incubated with different concentrations of the antibodies before adding to BV-2 microglia cell line (**d**), or primary microglia (**e**) for phagocytosis experiment measuring uptake of $\text{A}\beta_{42}$ fibrils into the cells by FACS analysis. Mean \pm s.d.

Extended Data Table 1 | Change from baseline in amyloid PET SUVR values (a secondary endpoint at 6 months), and in exploratory clinical endpoints at the end of the placebo-controlled period (6-month data also shown for amyloid PET)

Adjusted mean \pm SE change from baseline for:	Aducanumab					p-value (dose-response)
	Placebo	1 mg kg ⁻¹	3 mg kg ⁻¹	6 mg kg ⁻¹	10 mg kg ⁻¹	
Amyloid PET SUVR values						
At 6 months	(n=34)	(n=26)	(n=27)	(n=23)	(n=27)	
	-0.005 \pm 0.018	-0.030 \pm 0.020	-0.087 \pm 0.020**	-0.143 \pm 0.022***	-0.205 \pm 0.020***	<0.0001
At 1 year [†]	(n=30)	(n=21)	(n=26)	(n=23)	(n=21)	
	0.003 \pm 0.021	-0.055 \pm 0.024	-0.135 \pm 0.022***	-0.210 \pm 0.024***	-0.268 \pm 0.025***	<0.0001
CDR-SB[‡]						
	(n=31)	(n=23)	(n=27)	(n=26)	(n=23)	
	1.87 \pm 0.41	1.72 \pm 0.46	1.37 \pm 0.43	1.11 \pm 0.44	0.63 \pm 0.47*	<0.05
MMSE[‡]						
	(n=32)	(n=25)	(n=26)	(n=26)	(n=25)	
	-2.81 \pm 0.67	-2.18 \pm 0.75	-0.70 \pm 0.75*	-1.96 \pm 0.75	-0.56 \pm 0.76*	<0.05
NTB overall Z score[†]						
	(n=29)	(n=23)	(n=26)	(n=24)	(n=24)	
	-0.11 \pm 0.08	-0.25 \pm 0.09	-0.13 \pm 0.08	-0.19 \pm 0.09	-0.10 \pm 0.09	NS
FCSRT: sum of free recall score[‡]						
	(n=31)	(n=23)	(n=25)	(n=25)	(n=25)	
	-2.33 \pm 1.07	-1.63 \pm 1.24	-1.25 \pm 1.20	-4.04 \pm 1.21	-0.69 \pm 1.20	NS

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus placebo; two-sided tests with no adjustments for multiple comparisons.

[†]At week 54.

[‡]At week 52.

Analyses using ANCOVA. ApoE $\epsilon 4$, apolipoprotein E $\epsilon 4$ allele; CDR-SB, Clinical Dementia Rating—Sum of Boxes; FCSRT, Free and Cued Selective Reminding Test; MMSE, Mini-Mental State Examination; NS, not significant; NTB, neuropsychological test battery; SE, standard error; SUVR, standard uptake value ratio.

Extended Data Table 2 | Incidence of ARIA based on MRI data and ARIA-E patient disposition

	Placebo	Aducanumab			
		1 mg kg ⁻¹	3 mg kg ⁻¹	6 mg kg ⁻¹	10 mg kg ⁻¹
Number of dosed subjects with at least one post-baseline MRI	38	31	32	30	32
ApoE ε4 carrier	24	19	21	21	20
ApoE ε4 non-carrier	14	12	11	9	12
ARIA-E, n (%)	0	1 (3)	2 (6)	11 (37)	13 (41)
By ApoE ε4					
ApoE ε4 carrier	0	1 (5)	1 (5)	9 (43)	11 (55)
ApoE ε4 non-carrier	0	0	1 (9)	2 (22)	2 (17)
ARIA-E and:					
Continued treatment	0	0	2 (6)	8 (27)	5 (16)
Same dose	0	0	0	2 (7)	0
Dose reduced	0	0	2 (6)	6 (20)	5 (16)
Discontinued treatment	0	1 (3)	0	3 (10)	8 (25)
ApoE ε4 carrier	0	1 (5)	0	2 (10)	7 (35)
ApoE ε4 non-carrier	0	0	0	1 (11)	1 (8)
Isolated ARIA-H, n (%)	2 (5)	2 (6)	3 (9)	0	2 (6)
ApoE ε4 carrier	2 (8)	1 (5)	2 (10)	0	2 (10)
ApoE ε4 non-carrier	0	1 (8)	1 (9)	0	0
ARIA-E and ARIA-H, n (%)	0	1 (3)	1 (3)	5 (17)	8 (25)
ApoE ε4 carrier	0	1 (5)	1 (5)	5 (24)	7 (35)
ApoE ε4 non-carrier	0	0	0	0	1 (8)

ApoE ε4, apolipoprotein E ε4 allele; ARIA, amyloid-related imaging abnormalities; ARIA-E (oedema); ARIA-H (micro-haemorrhages, macro-haemorrhages, or superficial siderosis); MRI, magnetic resonance imaging.

Extended Data Table 3 | Pharmacokinetic data

	Aducanumab			
	1 mg kg ⁻¹	3 mg kg ⁻¹	6 mg kg ⁻¹	10 mg kg ⁻¹
PK analysis population (intent-to-treat)*	<i>n</i> =31	<i>n</i> =32	<i>n</i> =30	<i>n</i> =32
Cumulative AUC (µg.h/mL, mean ± SD)	47,078 ± 17,555	143,395 ± 59,986	251,535 ± 122,883	346,163 ± 198,603
Subjects who received all 14 planned doses	<i>n</i> =18	<i>n</i> =18/19†	<i>n</i> =16	<i>n</i> =14
C _{max,ss} (µg/mL, mean ± SD)‡	21.2 ± 3.7	59.6 ± 19.6	123.8 ± 42.5	250.8 ± 33.5
Cumulative AUC (µg.h/mL, mean ± SD)	55,223 ± 11,529	169,457 ± 41,775	315,352 ± 76,300	524,511 ± 95,622

*Data include patients who missed doses.

†A total of 19 patients received all 14 doses but 1 patient missed the concentration measurement at Week 40 and so *n* = 18 for C_{max,ss} at 3 mg kg⁻¹ aducanumab.

‡The observed post-infusion concentrations at Week 40 were reported as steady-state C_{max}.

AUC, area under the concentration curve; C_{max,ss}, maximum concentration at steady state; PK, pharmacokinetic; SD, standard deviation.

Extended Data Table 4 | Change from baseline in amyloid PET SUVR values, CDR-SB, and MMSE at the end of the placebo-controlled period by absence/presence* of ARIA-E

Adjusted mean \pm SE for:	Treatment group (# without ARIA-E, # with ARIA-E)				
	Placebo	Aducanumab			
		1 mg kg ⁻¹	3 mg kg ⁻¹	6 mg kg ⁻¹	10 mg kg ⁻¹
Amyloid PET SUVR values [†]	(30, 0)	(21, 0)	(24, 2)	(17, 6)	(13, 8)
ARIA-E					
Absence	0.003 \pm 0.020	-0.056 \pm 0.024	-0.141 \pm 0.023	-0.243 \pm 0.027	-0.278 \pm 0.031
Presence	0.001 \pm 0.020	–	-0.069 \pm 0.075	-0.114 \pm 0.049	-0.263 \pm 0.040
CDR-SB [‡]	(31, 0)	(23, 0)	(25, 2)	(18, 8)	(14, 9)
ARIA-E					
Absence	1.84 \pm 0.42	1.72 \pm 0.48	1.33 \pm 0.47	1.11 \pm 0.54	0.78 \pm 0.61
Presence	1.95 \pm 0.35	–	2.04 \pm 1.38	1.18 \pm 0.73	0.67 \pm 0.67
MMSE [‡]	(32, 0)	(25, 0)	(24, 2)	(18, 8)	(16, 9)
ARIA-E					
Absence	-2.86 \pm 0.69	-2.20 \pm 0.77	-0.47 \pm 0.80	-1.82 \pm 0.91	-1.05 \pm 0.96
Presence	-2.60 \pm 0.69	–	-3.41 \pm 2.69	-1.95 \pm 1.42	0.83 \pm 1.35

*Since there were no ARIA-E events in the placebo group, the overall placebo group was used as the comparator in the subgroup analysis for presence of ARIA-E.

[†]At week 54.

[‡]At week 52.

Analyses based on observed data. Adjusted mean change and standard errors are based on an ANCOVA model for change from baseline with factors of treatment, laboratory ApoE ϵ 4 status (carrier and non-carrier), and baseline composite SUVR, CDR-SB, or MMSE, respectively. ARIA-E, amyloid-related imaging abnormalities (oedema); CDR-SB, Clinical Dementia Rating—Sum of Boxes; MMSE, Mini-Mental State Examination; PET, positron emission tomography; SE, standard error; SUVR, standard uptake value ratio.

ADDENDUM

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Addendum: The antibody aducanumab reduces A β plaques in Alzheimer's disease

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Figure 1 of our original Article illustrated that treatment with aducanumab reduced human brain amyloid- β plaques in a dose-dependent fashion as measured by florbetapir positron emission tomography (PET) imaging. The figure gave the visual appearance of standard uptake value ratio (SUVR) reduction in subcortical white matter as well as cortical regions, although statistically validated evidence of dose-dependent SUVR reduction was demonstrated only in cortical regions. We provide an updated figure (Fig. 1 of this Addendum), which includes colour bars and difference images to aid in the understanding and interpretation of the representative florbetapir PET images. An additional panel on the right illustrates the differences between baseline and week 54 images, computed by simple subtraction of the baseline from follow-up images, after co-registration to a common coordinate system. The difference images show that the SUVR reduction (which is unitless) occurs primarily in the cortical regions (highlighted in red) in patients treated with aducanumab.

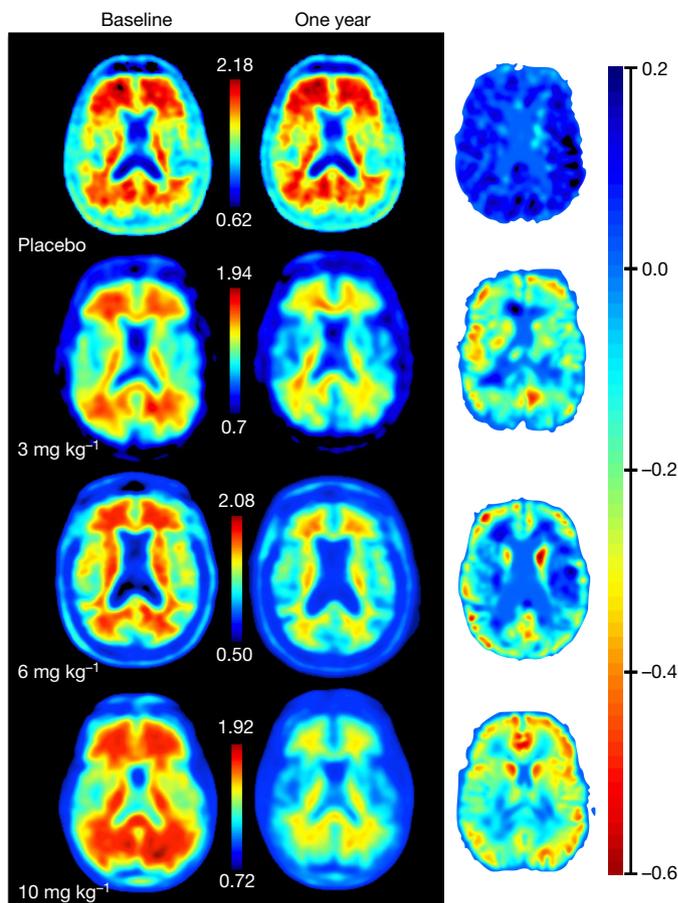


Figure 1 | This is the updated Fig. 1 of the original Article.

REVIEW

Open Access



The “rights” of precision drug development for Alzheimer’s disease

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Abstract

There is a high rate of failure in Alzheimer’s disease (AD) drug development with 99% of trials showing no drug-placebo difference. This low rate of success delays new treatments for patients and discourages investment in AD drug development. Studies across drug development programs in multiple disorders have identified important strategies for decreasing the risk and increasing the likelihood of success in drug development programs. These experiences provide guidance for the optimization of AD drug development. The “rights” of AD drug development include the right target, right drug, right biomarker, right participant, and right trial. The right target identifies the appropriate biologic process for an AD therapeutic intervention. The right drug must have well-understood pharmacokinetic and pharmacodynamic features, ability to penetrate the blood-brain barrier, efficacy demonstrated in animals, maximum tolerated dose established in phase I, and acceptable toxicity. The right biomarkers include participant selection biomarkers, target engagement biomarkers, biomarkers supportive of disease modification, and biomarkers for side effect monitoring. The right participant hinges on the identification of the phase of AD (preclinical, prodromal, dementia). Severity of disease and drug mechanism both have a role in defining the right participant. The right trial is a well-conducted trial with appropriate clinical and biomarker outcomes collected over an appropriate period of time, powered to detect a clinically meaningful drug-placebo difference, and anticipating variability introduced by globalization. We lack understanding of some critical aspects of disease biology and drug action that may affect the success of development programs even when the “rights” are adhered to. Attention to disciplined drug development will increase the likelihood of success, decrease the risks associated with AD drug development, enhance the ability to attract investment, and make it more likely that new therapies will become available to those with or vulnerable to the emergence of AD.

Keywords: Alzheimer’s disease, Drug development, Clinical trials, Biomarkers

Introduction

Alzheimer’s disease (AD) is rapidly increasing in frequency as the world’s population ages. In the USA, there are currently an estimated 5.3 million individuals with AD dementia, and this number is expected to increase to more than 13 million by 2050 [1, 2]. Approximately 15% of the US population over age 60 has prodromal AD and nearly 40% has preclinical AD [3]. Similar trends are seen globally with an anticipated worldwide population of AD dementia patients exceeding 100 million by 2050 unless means of delaying, preventing, or treating AD are found [4]. The

financial burden of AD in the USA will increase from its current \$259 billion US dollars (USD) annually to more than \$1 trillion USD by 2050 [5]. The cost of AD to the US economy currently exceeds that of cancer or cardiovascular disease [6].

Amplifying the demographic challenge of the rising numbers of AD victims is the low rate of success of the development of AD therapies. Across all types of AD therapies, the failure rate is more than 99%, and for disease-modifying therapies (DMTs), the failure rate is 100% [7, 8]. These numbers demand a re-examination of the drug development process. Success in other fields such as cancer therapeutics can be helpful in guiding better drug discovery and development practices of AD treatments. For example, 12 of 42 (28%) drugs approved by the US Food and Drug Administration (FDA) in 2017

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were oncology therapies (www.fda.gov); this contrasts with 0% of AD drugs in development. There are currently 112 new molecular entities in clinical trials in AD, whereas there are 3558 in cancer trials [9, 10]. Success in cancer drug development attracts funding and leads to more clinical trials, accelerating the emergence of new therapies. This model can assist in improving AD drug development.

Patient care increasingly demands precision medicine with the right drug, in the right dose, administered to the right patient, at the right time [11–13]. Precision medicine requires precision drug development. Effective medications, delivered in a correct dose, to a patient in the stage of the illness that can be impacted by therapy requires that these precision treatment characteristics be determined in a disciplined drug development program [14]. Drug development sponsors have developed systematic approaches to drug testing including the “rights” of drug development [15, 16], the “pillars” of drug development [17], model-based drug development [18, 19], and a translational medicine guide [20]. These approaches are appropriate across therapeutic areas, and none have been applied specifically to AD drug development. Building on these foundations, we describe a set of “rights” for AD drug development which are aligned with precision drug development. We consider lessons derived from drug development across several fields as well as learnings from recent negative AD treatment trials [14, 17, 21, 22]; we note the areas where success in the “right” principles is pursued. These “rights” for drug development are not all new innovations, but recent reviews of the AD drug pipeline show that they are often not implemented [16, 23, 24]. We consider how the “rights” will strengthen the AD drug discovery and development process, increase the likelihood of success, de-risk investment in AD therapeutic research, and spur interest in meeting the treatment challenges posed by the coming tsunami of patients.

Figure 1 provides an overview of the “rights of AD drug development.”

The right target

AD biology is complex, and only one target—the cholinergic system—has been fully validated through multiple successful therapies. Four cholinesterase inhibitors have been found to improve the dual outcomes of cognition plus function or cognition plus global status in patients with AD dementia [25, 26]. The successful development of memantine supports the validity of the *N*-methyl-D-aspartate (NMDA) receptor as a viable target, although only one agent has been shown to exert a therapeutic effect when modulating this receptor [27, 28]. A combination agent (Namzaric) addressing these two targets has been approved, establishing a precedent for combination

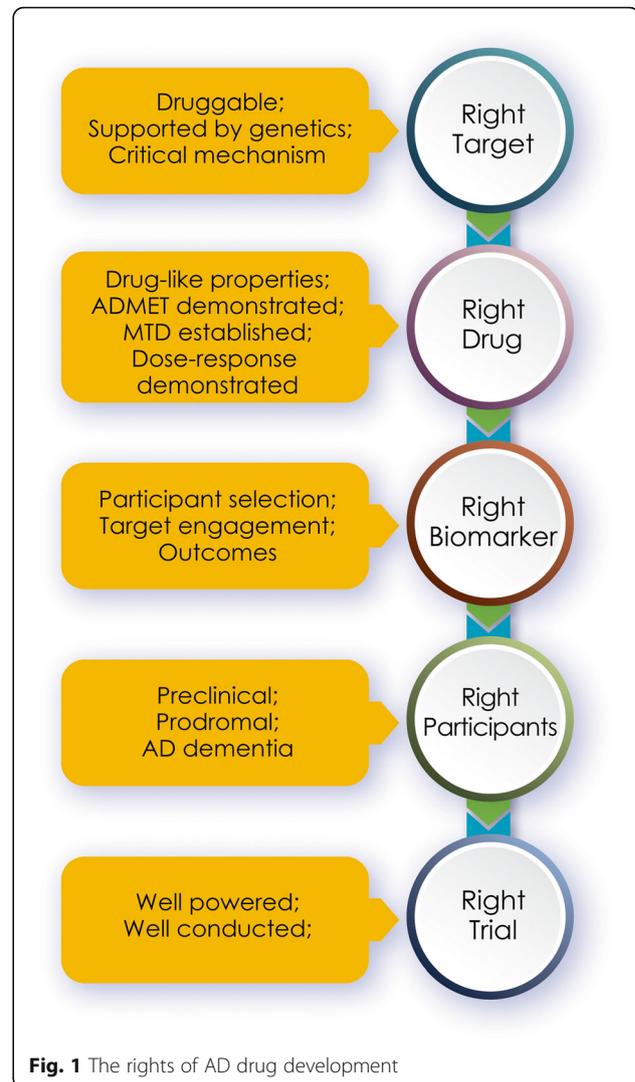


Fig. 1 The rights of AD drug development

therapy of two approved agents in AD [29]. Cholinesterase inhibitors have shown benefit in mild, moderate, and severe AD dementia [26]; memantine is effective in moderate and severe AD dementia [30]. No agent has shown benefit in prodromal AD (pAD), mild cognitive impairment (MCI), or preclinical AD [31].

No other target has been validated by successful therapy; all agents currently in development are unvalidated at the level of human benefit. Several targets are partially supported by biological and behavioral effects in animal models, and some agents have shown beneficial effects in preliminary clinical trials [32]. The lack of validation of a target by a specific trial does not disprove its worthiness for drug development; validation depends on concurrent conduct of other “rights” in the development program.

For an agent to be a DMT, the candidate drug treatment must meaningfully intervene in disease processes leading to nerve cell death [33] and be druggable (e.g.,

modifiable by a small molecule agent or immunotherapy [34, 35]). Viable targets must represent critical non-redundant pathways necessary for neuronal survival. Ideal targets have a proven function in disease pathophysiology, are genetically linked to the disease, have greater representation in disease than in normal function, can be assayed using high-throughput screening, are not uniformly distributed throughout the body, have an associated biomarker, and have a favorable side effect prediction profile [36]. Druggability relates to proteins, peptides, or nucleic acids with an activity that can be modified by a treatment [35].

A current National Institute of Health (NIH) ontology of candidate targets in AD includes amyloid-related mechanisms, tau pathways, apolipoprotein E e-4 (ApoE-4), lipid metabolism, neuroinflammation, autophagy/proteasome/unfolded protein response, hormones/growth factors, dysregulation of calcium homeostasis, heavy metals, mitochondrial cascade/mitochondrial uncoupling/antioxidants, disease risk genes and related pathways, epigenetics, and glucose metabolism [37, 38]. Other mechanisms may emerge; highly influential nodes in networks may be identified through systems pharmacology approaches; and opportunities or requirements for combination therapies may be discovered. Genetic editing techniques are increasingly used in experimental treatment paradigms, and RNA interference approaches show promise in non-AD neurodegenerative disorders [39]. With the recognition that late-life sporadic AD frequently has multiple contributing pathologies, identifying a single molecular therapeutic target whose manipulation is efficacious in all affected individuals may not be forthcoming [40–43].

Analysis of predictors of success in drug development programs shows that agents linked to genetically defined targets have a greater chance of being advanced from one phase to the next than drugs that address targets having no genetic links to the underlying disease [15, 21]. Transgenic (tg) animal models and knockout and knockin models of disease can add to the genetic evidence for a target. Genes can help prioritize drug candidates as well as support target validation [44]. Genes implicate potentially druggable pathways and networks involved in AD pathogenesis [45, 46]. Genetic linkages to amyloid precursor protein (APP), beta-site amyloid precursor protein cleavage enzyme (BACE), gamma-secretase, ApoE, tau metabolism, and immune function are elements within the pathophysiology of AD with identified genetic influences [47]. A coding mutation in the APP gene, for example, results in a 40% reduction in amyloid beta protein (A β) formation and a substantial reduction in the risk of AD [48]. This observation supports exploring the use of APP-modifying agents for the treatment and prevention of AD.

Defining the “right target” (or combination of targets) is currently the weakest aspect of AD drug discovery and development. The absence of a deep understanding of AD biology or focus on inappropriate targets will result in drug development failures regardless of how well the drug development program is conducted. This emphasizes the importance of investment by the National Institutes of Health (NIH), non-US basic biology initiatives, foundations, philanthropists, and others in the fundamental understanding of AD biology and identifying druggable targets and pathways [49].

The right drug

Clinical drug development is guided by defining a target product profile (TPP) describing the desirable and necessary features of the candidate therapy. The TPP establishes the goals of the development program, and each phase of a program is a step toward fulfilling the TPP [50, 51]. Drugs with TPP-driven development plans have a higher rate of regulatory success than those without [50].

Characterizing a candidate therapy begins with screening assays of the identified target in preclinical discovery campaigns, identifies a lead candidate or limited set of related candidates, continues through establishing the pharmacokinetic (PK) and pharmacodynamic (PD) features in non-clinical animal models, gains refined PK and safety information with first-in-human (FIH) exposure in phase 1 clinical trials, and accrues greater PD and dose-response information in phase 2 trials. Finally, fully powered trials for clinical efficacy are undertaken in phase 3 with efficacy confirmation [52]. Safety data are collected throughout the process.

Preliminary characterization of the molecule as a treatment candidate showing the desired effect in the screening assay starts by determining that it has drug-like properties including molecular weight of ≤ 500 Da, bond features that support membrane penetration including the blood-brain barrier (BBB), no “alerts” that predict toxicity [53, 54], and chemical properties that suggest scalable manufacture and formulation [55, 56]. If the molecule has these encouraging properties, its absorption, distribution, metabolism, excretion, and toxicity (ADMET) are determined in non-clinical models [57].

BBB penetration must be shown in humans in the course of the drug development program during phase 1 [53]. The human BBB has p-glycoprotein transporters and other mechanisms that may not be present in rodents, and central nervous system (CNS) penetration in animal models of AD is not a sufficient guide to human CNS entry [58]. Measurement of CNS levels in non-human primates more closely reflects the human physiology, but direct measures of cerebrospinal fluid (CSF) levels in phase 1 human

studies are required in a disciplined drug development program. CSF levels allow the determination of plasma/CSF ratios and help establish whether peripheral levels predict CNS exposures and whether CSF levels are compatible with those showing therapeutic effects in animal models of AD [59, 60]. CSF levels are an acceptable proxy for brain levels but leave some aspects of brain entry, neuronal penetration, and target exposure unassessed [61]. Understanding the PK/PD principles at the site of exposure of the agent to the target is one of the three pillars of drug development proposed by Morgan et al. [17]. Challenges in achieving target exposure is one reason for drug development failures in otherwise well-conducted programs. Tarenflurbil, for example, was shown to have poor BBB penetration after the development program was completed [62].

The “right drug” has shown efficacy in non-clinical models of AD. These models have not predicted success in human AD but advancing an agent to human testing without efficacy in animal models would add additional risk to the development program. A common strategy involves using genetic technologies to establish tg species bearing one or more human mutations leading to the overproduction of A β [63, 64]. These animals develop amyloid plaques similar to those of human AD but lack neurofibrillary tangles or cell death and are only partial simulacra of human AD [65]. They more closely resemble autosomal dominant AD with mutation-related overproduction of A β than typical late-onset AD where clearance of A β is the principal underlying problem [66, 67]. Activity in several AD models should be demonstrated to increase confidence in the robustness of the mechanism of the candidate agent [68]. There are recent efforts to more closely model human systems biology using human induced pluripotent stem cell (iPSC) disease models for drug screening [69–71].

Demonstration that the agent has neuroprotective effects is critical to the definition of DMT [33, 52], and interference in the processes leading to cell death should be established prior to human exposure. Many programs have shown effects on A β without documenting an impact on neuroprotection; more thorough exploration and demonstration of neuroprotection in non-clinical models may result in agents that exert greater disease modification in human trials.

Phase 1 establishes the PK features and ADMET characteristics of the candidate compound in humans. Several drug doses are assessed, first in single ascending dose (SAD) studies and then in multiple ascending dose (MAD) studies. A maximum tolerated dose (MTD) should be established in phase 1; without this, failure to show efficacy in later stages of development will invariably raise the question of whether the candidate agent

was administered at a too-low dose. In some cases, receptor occupancy studies with positron emission tomography (PET), saturation of active transport mechanisms, physical limits on the amount of drug that can be administered, or dose-response curves that remain flat above specific doses obviate the need or the ability to demonstrate an MTD. In all other circumstances, an MTD should be established during phase 1 [72]. MTDs have been difficult to establish for monoclonal antibodies (mAbs), and decisions are often based on feasibility rather than established PK/PD relationships [5]. The decision to increase the doses of mAbs by several folds in recent trials after phase 2 or 3 trials showed no drug-placebo difference (e.g., solanezumab, crenezumab, gantenerumab, aducanumab) demonstrates the difficulty of establishing dose and PK/PD relationships of mAbs; the absence of understanding of PK/PD for mAbs may have contributed to the failure of development programs for these agents. Formulation issues should be resolved prior to evaluating the MTD to ensure that formulation challenges do not prevent the assessment of a full range of doses.

Phase 2 studies establish dose and dose-response relationships. Showing a dose-response association increases confidence in the biological effects of an agent and de-risks further development. The response may be a clinical outcome or a target engagement biomarker linked to the mechanism of action (MOA) of the agent [73–75]. An acceptable dose-response approach includes a low dose with no or little effect, a middle dose with an acceptable biological or clinical outcome, and a high dose that is not well tolerated or raises safety concerns. After the exploration of the dose-response range in phase 2, one or two doses are advanced to phase 3 and will include the final dose(s) of the package insert of information for prescribers and patients. Using a Bayesian dose-finding approach to decide which of 5 BAN2401 doses to advance to phase 3 is an example of dose-finding in phase 2 of a development program [76].

The “right drug” has acceptable toxicity. Safety assessment begins with a review of structural alerts of the molecule predictive of toxicity such as hepatic injury assessed as part of lead candidate nomination and proceeds through evaluations of target organ toxicity in several animal species—typically a rodent species and a dog species [77, 78]. Given an acceptable non-clinical safety profile, the agent is advanced to phase 1 for a FIH assessment of safety in the clinical setting with the determination of the MTD. Safety and tolerability data continue to accrue in phase 2 and phase 3 trials. The number of human exposures remains relatively low until phase 3, and important toxicity observations may be delayed until the late phases of drug development. Semagestat, avagecestat, and verubecestat were all in phase 3

before cognitive toxicity was identified as an adverse event [79–81]. Some toxicities may not be identified until after approval and widespread human use. Vigilance for toxic effects of agents does not stop with drug approval and continues through the post-approval and marketing period [82]. AD is a fatal illness and—like life-extending cancer therapies—side effects of treatment may be an acceptable trade-off for slowing cognitive decline and maintaining quality of life [83].

The “right drug” at the end of phase 3 has demonstrated the specified features of the TPP, including efficacy and safety, and meets all the requirements for approval by the FDA, the European Medicines Agency (EMA), and other regulatory authorities as an AD therapy [50]. From an industry perspective, the “right” drug has substantial remaining patent life, is competitive with other agents with similar mechanisms, and will be acceptable to payers with reimbursement rates that make the development of the agent commercially attractive [15, 21]. The “right” features of the candidate agent can be scored with a translatability score that allows comparison and prioritization of agents for their readiness to proceed along the translational pathway to human testing and through the phases of clinical trials [84, 85]. Greater use of translational metrics may enhance the likelihood of drug development success [86].

The right biomarker

Biomarkers play many roles in drug development and are critical to the success of development programs (Table 1) [48]. Including biomarkers in development plans has been associated with greater success rates across therapeutic areas [15, 21, 87]. The use of several types of biomarkers (predictive, prognostic) in development programs is associated with higher success rates in trials compared to trials with no or few biomarkers [88].

The “right” biomarker varies by the type of information needed to inform a development program and the specific phase of drug development. Despite their importance, no biomarker has been qualified by the FDA for use across development programs [89].

The amyloid (A), tau (T), and neurodegeneration (N) framework provides an approach to diagnosis and monitoring of AD and helps guide the choice of biomarkers for drug development [90, 91]. “A” biomarkers (amyloid positron emission tomography [PET], CSF A β) support the diagnosis of AD; “A” and “T” (tau PET; CSF phospho-tau) biomarkers are pharmacodynamic biomarkers that can be used to demonstrate target engagement with A β or tau species; and “N” (magnetic resonance imaging [MRI], fluorodeoxyglucose PET, CSF total tau) biomarkers are pharmacodynamic markers of neurodegeneration that can provide evidence of neuroprotection and disease modification [33]. Additional markers for “N” are evolving, including neurofilament light (NFL) chain, which has shown promise in multiple sclerosis (MS) trials and preliminary AD trials [92]. Markers of synaptic degeneration such as neurogranin may also contribute to the understanding of therapeutic impact on “N” in AD. Emerging biomarkers are gaining credibility and will add to or amplify the ATN framework applicable to drug development [93].

In AD trials, biomarkers are needed to support the diagnosis. In prevention trials involving cognitively normal individuals, genetic trait biomarkers are used to establish the risk state of the individual or state biomarkers are employed to demonstrate the presence of AD pathology. In trials of treatments for autosomal dominant AD, demonstration of the presenilin 1, presenilin 2, or APP mutation is required in the trial participants [94, 95]. Similarly, in trials involving ApoE-4 homozygotes or

Table 1 Role of biomarkers in AD drug development

Role in trial	Examples of biomarker used
Identification of trial population	Presence of presenilin 1 (PS1), presenilin 2 (PS2), or amyloid precursor protein (APP) mutations; ApoE-4 plus TOMM40; trisomy 21
Confirmation of diagnosis; exclude non-AD diagnoses	Amyloid imaging; CSF AD signature
Prognosis and course projection	In MCI, ApoE-4 carriers progress more rapidly
Amyloid production and clearance (target engagement)	Stable isotope-labeled kinetics (SILK); BACE activity reduction with BACE inhibitor; CSF A β reduction by BACE inhibitor or gamma-secretase inhibitor
Impact of therapy on brain circuit and network function	fMRI; EEG
Impact of therapy on intermediate targets	Amyloid imaging; CSF amyloid; tau PET; CSF phospho-tau
Disease modification	MRI atrophy; CSF total tau; FDG PET; neurofilament light
Stratification for trial analysis	ApoE-4 genotype
Side effect monitoring	MRI surveillance for amyloid-related imaging abnormalities (ARIA); liver function tests; complete blood counts; electrocardiography

heterozygotes or AD in Down syndrome, appropriate testing of chromosome 19 polymorphisms or chromosome 21 triplication is required [96]. A combination of ApoE-4 and TOMM-40 has been used to attempt to show the risk and age of onset of AD [97]. State biomarkers useful in preclinical diagnosis include amyloid PET and the CSF A β /tau signature of AD [98, 99]. Tau PET may be useful in identifying individuals appropriate for tau-targeted interventions or for measuring success in reducing the propagation of tau pathology [100].

A substantial number of individuals with a clinical diagnosis of AD have been shown to lack amyloid plaque deposition when studied with amyloid imaging. Forty percent of patients diagnosed clinically with prodromal AD and 25% of those diagnosed with mild AD dementia lack evidence of amyloid pathology when studied with amyloid PET [52, 101]. Those with suspected non-amyloid pathology (SNAP) have undetermined underlying pathology and may not respond to proposed AD therapies. SNAPs may not decline in the expected manner in the placebo group, compromising the ability to demonstrate a drug-placebo difference [102]. SNAPs should be excluded from AD trials; the “right” biomarker for this includes amyloid imaging, the CSF AD signature, or tau imaging in patients with the AD dementia phenotype. In the idalopirdine development program, no enrichment strategies were used and power calculations showed that more than 1600 participants per arm would be needed to show a drug-placebo difference. With enrichment based on amyloid abnormalities, the decline was more rapid and the predicted sample size per arm to show a drug-placebo difference was 148 [103].

Target engagement biomarkers are the “missing link” in many development programs. Having shown that the candidate agent affects the target pathology in preclinical models and is safe in phase 1, sponsors have sometimes advanced through minimal phase 2 studies or directly to phase 3 [22] without showing that the drug treatment has meaningfully engaged the target in humans. Well-conducted phase 2 studies are a critical element of principled drug development and will provide two key pieces of information: target engagement and doses to be assessed in phase 3 [73, 74]. Phase 2 provides the platform for deciding if the candidate agent is viable for further development [75]. Target engagement may be shown directly, for example, with PET receptor occupancy studies or indirectly through proof-of-pharmacology [104, 105]. Examples of proof-of-pharmacology in AD drug development include the demonstration of reduced A β production using stable isotope-labeled kinetics (SILK) [106], reduced CSF A β with BACE inhibitors [107], glutaminyl cyclase enzyme activity with

phosphodiesterase inhibitors [108], and increased A β fragments in the plasma and CSF with gamma-secretase inhibitors and modulators [109]. Candidate target engagement/proof-of-pharmacology biomarkers include peripheral indicators of inflammation and oxidation for use in trials of anti-inflammatory and antioxidant compounds. Sponsors of drug development should advance markers of target engagement in concert with the candidate therapy; these may be used after regulatory approval as companion or complementary biomarkers [110, 111]. Demonstration of target engagement does not guarantee efficacy in later stages of development, but target engagement shown by the “right” biomarker provides important de-risking of a candidate treatment by showing biological activity that may translate into clinical efficacy. Semagecestat's effect on A β production in the CSF and aducanumab's plaque-lowering effect are examples where target engagement was demonstrated in phase 2 or phase 1B, and the agents still failed to show a beneficial drug-placebo difference in later-stage trials [32, 109]. Target engagement and proof-of-pharmacology are “pillars” of successful drug development [17].

Changes in the basic biology of AD—amyloid generation, tau aggregation, inflammation, oxidation, mitochondrial dysfunction, neurodegeneration, etc.—are linked to human cognition through neural circuits whose integrity is critical to normal memory and intellectual function [112]. Two techniques of assessing neural networks are electroencephalography (EEG) and functional magnetic resonance imaging (fMRI). In cognitively normal individuals with positive amyloid PET and low levels of tau as shown by tau PET, fMRI measures of the default mode network (DMN) reveal hyperactive circuit functions. In those with elevated amyloid and elevated tau levels, the circuits become hypoactive compared to age-matched controls [113, 114]. Decline in circuit function predicts progressive cognitive impairment [115]. Disrupted DMN function is present in prodromal AD and in AD dementia [116, 117]. Assessment of DMN integrity may be an important biomarker with predictive value for the impact of the intervention on clinical outcomes [112]. EEG is dependent on the intact network function and may have applications in AD drug development similar to, but more robustly, than those of fMRI [108, 118, 119]. Both EEG and fMRI require procedural and interpretative standardization to be implemented in multi-site trials. A recent alternative for the assessment of circuit integrity in AD is SV2A PET, targeting and visualizing the synaptic network and currently under study as a possible measure of target engagement for drugs aiming to influence synaptic function [120].

Amyloid imaging is a target engagement biomarker establishing reduction of plaque amyloid [111]. Several monoclonal antibodies have shown a dose and time-

dependent plaque reduction. In a phase 1B trial, aducanumab achieved both significant plaque reduction and benefit on some clinical measures with evidence of a dose-response relationship [32]. The beneficial effect was not recapitulated in a phase 3 trial. Bapineuzumab and gantenerumab decreased plaque A β but had no corresponding impact on cognition or function in the doses studied [121, 122]. Removal of plaque amyloid may be necessary but not sufficient for a therapeutic benefit of anti-amyloid agents or may be a coincidental marker of engagement of a broad range of amyloid species including those required for a therapeutic response. Tau PET assesses target engagement by anti-tau therapeutics; reduced tau burden or reduced tau spread would indicate a therapeutic response [123]. A β and tau signals do not measure neuroprotection and are not necessarily evidence of disease modification (DM).

Biomarkers play a critical role in demonstrating DM in DMT development programs. Evidence of neuroprotection is essential to support DM, and structural magnetic resonance imaging (MRI) is the current biomarker of choice for this purpose. Hippocampal atrophy has been linked to progressive disease and to nerve cell loss [124–126]. In clinical trials, MRI has often not fulfilled expectations, and atrophy has sometimes been greater in the treatment groups than in the placebo controls [127, 128]. Recent studies have shown drug-placebo differences on MRI in the anticipated direction suggesting that MRI may be an important DM marker depending on the underlying MOA of the agent. As noted, serum and CSF biomarkers of neurodegeneration such as NfL and synaptic markers have promise to assess successful DMTs but have been incorporated into relatively few AD trials [129]. CSF measures of total tau may be closely related to neurodegeneration and provide useful evidence of the impact on cell death [130, 131].

Biomarkers could eventually have a role as surrogate outcomes for AD trials if they are shown to be predictive of clinical outcomes. Currently, no AD biomarker has achieved surrogate status, and biomarkers are used in concert with clinical outcomes as measures of treatment effects.

Biomarkers have a role in monitoring side effects in the course of clinical trials. Liver, hematologic, and cardiac effects are monitored with liver function tests, complete blood counts, and electrocardiography, respectively. Atacabestat, for example, is a BACE inhibitor whose development was interrupted by the emergence of liver toxicity [132]. Amyloid-related imaging abnormalities (ARIA) of the effusion (ARIA-E) or hemorrhagic (ARIA-H) type may occur with MAbs and are monitored in trials with serial MRI [133]. ARIA has been observed with bapineuzumab, gantenerumab, aducanumab, and BAN2401 [32, 134, 135].

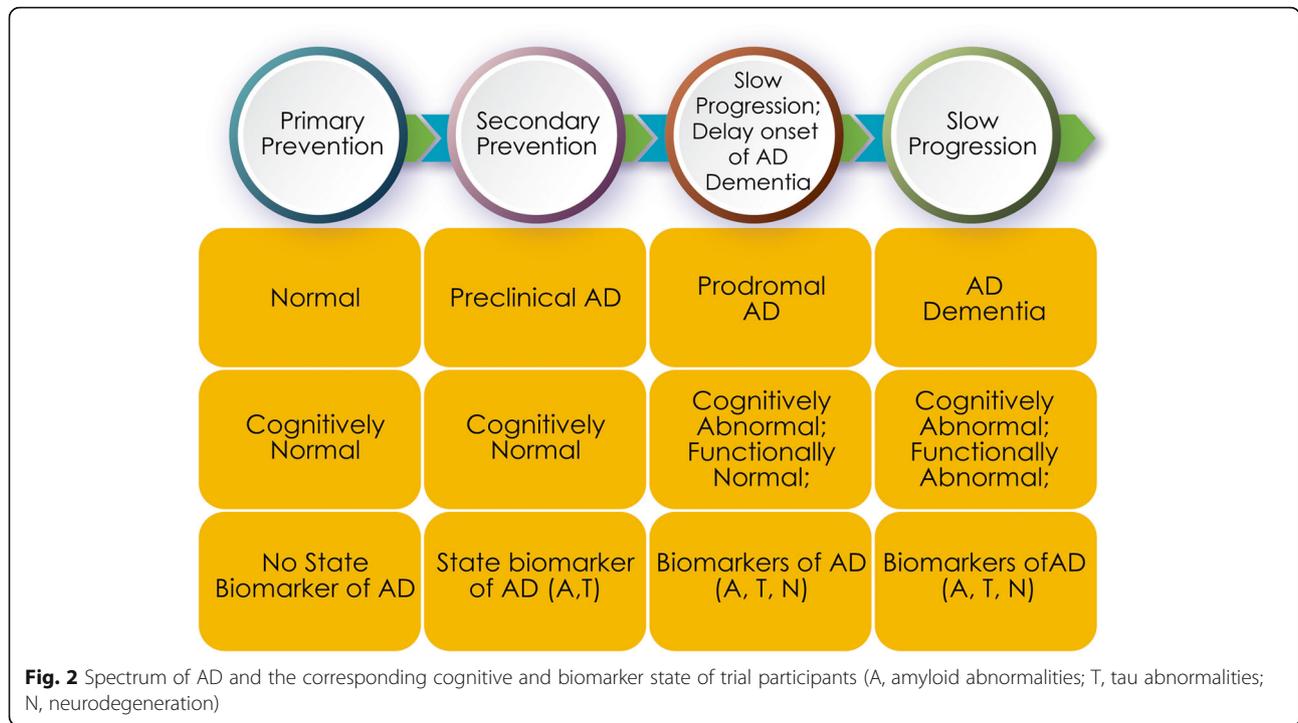
The right participant

AD progresses through a spectrum of severity from cognitively normal amyloid-bearing preclinical individuals, to those with prodromal AD or prodromal/mild AD dementia and, finally, to those with more severe AD dementia [136, 137] (Fig. 2). Based on this model, trials can target primary prevention in cognitively normal individual with risk factors for AD but no state biomarkers indicative of AD pathology, secondary prevention in preclinical AD participants who are cognitively normal but have positive state biomarkers (positive amyloid PET, low CSF A β), and treatment trials aimed at slowing disease progression in prodromal or prodromal/mild AD dementia or mild, moderate, and severe AD dementia (Fig. 2). Although AD represents a seamless progression from unaffected to severely compromised individuals, participants can be assigned to the progressive phases based on genetic markers, cognitive and functional assessments, amyloid imaging or CSF A β and tau measures, tau imaging, and MRI [52, 136, 137]. The ATN Framework is designed to guide the identification of the “right” participant for clinical trials [90, 91]. Early intervention has proven to be associated with better outcomes in other disorders such as heart failure [138] suggesting that early intervention in the “brain failure” of AD may have superior outcomes compared to later-phase interventions. However, available cognitive-enhancing agents have been approved for mild, moderate, and severe AD and have failed in trials with predementia participants; some DMT mechanisms may require use earlier in the disease process before pathologic changes are extensive [139–141].

The right participant also relates to the MOA of the agent being assessed. Cognitive enhancing agents will be examined in patients with cognitive abnormalities; agents reducing amyloid production may have the optimal chance of success in primary or secondary prevention; tau prevention trials may focus on the preclinical participants; tau removal agents might be appropriate for prodromal AD or AD dementia; combinations of agents may be assessed in trials with participants with corresponding biomarker changes. Experience with a greater array of agents in a variety of disease phases will help inform the match between the “right” participant and specific agent MOAs. Development of more biomarkers such as those indicating CNS inflammation, excessive oxidation, or the presence of concurrent pathologies such as TDP-43 or alpha-synuclein may assist in matching treatment MOA to the pathological form of AD.

The right trial

The “right trial” is a well-conducted clinical experiment that answers the central question regarding the superiority of the drug over placebo at the specified dose in the



time frame of observation in the defined population. Poorly conducted or underpowered trials do not resolve the central issue of drug efficacy and should not be conducted since they involve participant exposures and potential toxicity without the ability to provide valid informative scientific data. Trial sponsors incur the responsibility to report the results of trials to allow the field to progress by learning from the outcome of each experiment. Participants have accepted the risks of unknown drug effects and placebo exposure, and honoring this commitment requires that the learnings from the trial be made available publically [142].

A key element includes a sample size based on thoroughly vetted anticipated effect sizes. Trial simulations are available to model the results of varying effect sizes and the corresponding required population size [143].

Participation criteria critical to the trial success include defining an appropriate population of preclinical, prodromal, or AD dementia using biomarkers as noted above [136, 137]. Other key participation criteria include the absence of non-AD neurologic diagnoses, physical illness incompatible with trial requirements, or use of medications that may interact with the test agents. Fewer exclusions from trials lead to more generalizable results. Inclusion of diverse populations representative of the populations to which the agent will be marketed enhances the generalizability of trial results.

Clinical outcomes will be chosen based on the specific population included in the trial. The Preclinical Alzheimer Cognitive Composite (PACC) and the

Alzheimer Preclinical Cognitive Composite (APCC) used in the Alzheimer's Prevention Initiative, for example, are used as outcomes in studies of preclinical AD [137, 144, 145]. The Clinical Dementia Rating-Sum of Boxes (CDR-sb) is commonly used as an outcome in prodromal AD [146]. The AD Assessment Scale-Cognitive subscale (ADAS-cog) [147] or the neuropsychological test battery (NTB) [148] and the CDR-sb or Clinical Global Impression of Change with Caregiver Input (CIBIC+) are common dual outcomes in trials of mild-moderate AD dementia [40, 146]. The AD Composite Score (ADCOMS) is an analytic approach including items from the CDR-sb, ADAS-cog, and Mini-Mental State Examination (MMSE) that is sensitive to change and drug effects in prodromal AD and mild AD dementia [149]. The severe impairment battery (SIB) is the outcome assessment most commonly used in severe AD [150]. Having tools with sufficient sensitivity to detect drug-placebo differences in predementia phases of AD is challenging. Commonly used tools such as the ADAS-cog were developed for later stages of the disease. Newer instruments such as the PACC and APCC detect changes over time in natural history studies, but their performance in trials is unknown.

The Alzheimer's Disease Cooperative Study (ADCS) Activities of Daily Living (ADL) scale is commonly used to assess daily function in patients with MCI and mild to severe AD dementia [151]. The Amsterdam Instrumental Activities of Daily Living (IADL) Questionnaire is

increasingly employed for this purpose in MCI/prodromal AD and mild AD dementia [152, 153]. Table 2 summarizes the instruments currently used in trials of each major phase of AD.

The trial duration may vary from 12 months to 8 years for DMTs or 3–6 months for symptomatic agents based on the anticipated duration of exposure needed to demonstrate a drug-placebo difference. Preclinical trials may involve observing patients for up to 5 years to allow sufficient decline in the placebo group to be able to demonstrate a drug-placebo difference. These trial duration choices are arbitrary; a basic biological understanding linking the changes in the pathology to the duration of drug exposure is lacking. Using an adaptive design approach, it is possible to adjust trial durations based on emerging patterns of efficacy [76, 154]. Adaptive designs may be used to optimize sample size, trial duration, and dose selection and have been successful in trials of chemotherapy and in trials for treatments of diabetes [155]. Adaptive designs are currently in use in the European Prevention of AD (E-PAD), the Dominantly Inherited Alzheimer Network-Treatment Unit (DIAN-TU), and a study of oxytocin in frontotemporal dementia [156]; broad exploration of the approach is warranted [157, 158].

Globalization of clinical trials with the inclusion of trial sites in many countries is a common response to slow recruitment of trial participants. By increasing the number of trial sites, recruitment can be accelerated and drug efficacy demonstrated more promptly. Globalization, however, increases the number of languages and cultures of participants in the trials as well as increasing the heterogeneity of background experience among the trial sites and investigators. These factors may increase measurement variability and make it more difficult to demonstrate a drug-placebo difference [159–161]. The “right trial” will limit these factors by minimizing the number of regions, languages, and trial sites involved. Within diverse countries such as the USA, the inclusion of minority participants is key to insuring the generalizability of the findings from trials [162].

The right trial will include the right doses selected in phase 2 and the right biomarkers as noted above. The biomarker will be chosen to match the questions to be answered for each trial phase. Target engagement biomarkers are critical in phase 2, and DM biomarkers are critical in phase 3 of DMT trials.

The right trial is also efficiently conducted with rapid start-up, certified raters, a central institutional review board (IRB), and timely recruitment of appropriate subjects. Programs such as the Trial-Ready Cohort for Prodromal and Preclinical AD (TRC-PAD), Global Alzheimer Platform (GAP), and the EPAD initiative aim to enhance the efficiency with which trials are conducted [157, 163]. Development of online registries and trial-ready cohorts may accelerate trial recruitment and treatment evaluation [164–166]. Registries have been helpful in trial recruitment to non-AD disorders [167].

Inclusion of the right number of the right participants is of key importance in successfully advancing AD therapeutics. Compared to other fields, there is a reluctance by patients and physicians to participate in clinical trials for a disease that is considered by some to be a part of normal aging. Advocacy groups throughout the world strive to overcome this attitude; success in engaging participants in trials will become more pressing as more preclinical trials involving cognitively normal individuals are initiated. Sample size is related to the magnitude of the detectable effect which is in turn related to the effect size of the agent and the sensitivity of the measurement tool (clinical instruments or biomarkers); these factors require optimization to allow the conduct of trials with feasible sample sizes.

Hallmarks of poorly designed or conducted trials include failure of the placebo group to decline in the course of a trial (assuming an adequate observation period), failure to show separation of the placebo group from an active treatment arm such as donepezil, excessive measurement variability, or low levels of biological indicators of AD such as the percent of ApoE-4 carriers or the presence of fibrillar amyloid on amyloid imaging [22]. Trials with these features would not be expected to

Table 2 Instruments appropriate as the outcome assessments in different phases of AD

Domain	Prevention trials	Prodromal AD trials	AD dementia trials
Cognition	PACC; APCC	NTB	ADAS-cog in mild to moderate AD; SIB in moderate to severe AD
Global/composite	None	CDR-sb; ADCOMS; iADRS	CIBIC+ in shorter trials; CDR-sb in longer trials
Function	None	ADCS ADL MCI scale; Amsterdam IADL scale	ADCS ADL scale
Behavior	NPI	NPI	NPI

ADAS-cog Alzheimer's Disease Assessment Scale-cognitive subscale, ADCOMS Alzheimer's Disease Composite Scale, Alzheimer's Disease Cooperative Study Activities of Daily Living scale, APCC Alzheimer's Prevention Initiative (API) Composite Cognitive, CDR-sb Clinical Dementia Rating-Sum of Boxes, CIBIC+ Clinical Interview-Based Impression of Change with Caregiver Input, IADL Instrumental Activities of Daily Living, iADRS Integrated Alzheimer's Disease Rating Scale, NPI Neuropsychiatric Inventory, NTB neuropsychological test battery, PACC Preclinical Alzheimer Cognitive Composite, SIB severe impairment battery

detect drug-placebo differences or to inform the drug development agenda.

A well-designed phase 3 trial builds on observations made in phase 2. Drugs have often been advanced to phase 3 based on the interpretation of apparent effects observed in phase 2 unprespecified subgroup analyses that are derived from small non-randomized samples and are rarely if ever reproduced in phase 3 [22].

Summary and conclusions

AD drug development has had a high rate of failure [7]. In many cases, BBB penetration, dose, target engagement, or rigorous interrogation of early-stage data has not been adequately pursued. Agents have been advanced to phase 3 with little or no evidence of efficacy in phase 2. Better designed and conducted phase 2 studies will inform further development and enable stopping earlier and preserving resources that can be assigned to testing more drugs in earlier stages (preclinical and FIH), as well as promoting better drugs with a greater chance of success to phase 3 [168]. Deep insight into the biology of AD is currently lacking, and predicting drug success will continue to be challenging; optimizing drug development and clinical trial conduct will reduce this inevitable risk of AD treatment development. Table 3 provides a summary of the integration of the “rights” of AD drug development across the phases of the development cycle.

This “rights” approach to drug development will enable the precision medicine objective of the right drug, at the right dose, for the right patient, at the right time, tested in the right trial [11–13, 16]. Approaches such as these when used in other therapeutic areas

have improved the rate of success of drug development in other settings [15, 21]. Adhering to the “rights of AD drug development” will de-risk many of the challenges of drug development and increase the likelihood of successful trials of critically needed new treatments for AD.

Abbreviations

A: Amyloid; Aβ: Amyloid beta protein; AD: Alzheimer’s disease; ADAS-cog: AD Assessment Scale-Cognitive subscale; ADCOMS: AD Composite Score; ADCS: Alzheimer’s Disease Cooperative Study; ADL: Activities of daily living; ADMET: Absorption, distribution, metabolism, excretion, and toxicity; APOE: Apolipoprotein E; APP: Amyloid precursor protein; ARIA: Amyloid-related imaging abnormalities; ARIA-E: Amyloid-related imaging abnormalities effusion; ARIA-H: Amyloid-related imaging abnormalities hemorrhagic; BACE: Beta-site amyloid precursor protein cleavage enzyme; BBB: Blood-brain barrier; CDR-sb: Clinical Dementia Rating-Sum of Boxes; CIBIC+: Clinical Global Impression of Change with Caregiver Input; CNS: Central nervous system; CSF: Cerebrospinal fluid; DIAN-TU: Dominantly Inherited Alzheimer Network-Treatment Unit; DM: Disease modification; DMN: Default mode network; DMT: Disease-modifying therapy; EEG: Electroencephalography; EMA: European Medicines Agency; E-PAD: European Prevention of Alzheimer’s Disease; fMRI: Functional magnetic resonance imaging; FDA: US Food and Drug Administration; FIH: First-in-human; GAP: Global Alzheimer Platform; IADL: Instrumental Activities of Daily Living Questionnaire; iPSC: Induced pluripotent stem cell; IRB: Institutional review board; MABs: Monoclonal antibodies; MAD: Multiple ascending dose; MCI: Mild cognitive impairment; MMSE: Mini-Mental State Examination; MOA: Mechanism of action; MTD: Maximum tolerated dose; MRI: Magnetic resonance imaging; MS: Multiple sclerosis; N: Neurodegeneration; NfL: Neurofilament light; NIH: National Institutes of Health; NMDA: *N*-methyl-D-aspartate; NPI: Neuropsychiatric Inventory; NTB: Neuropsychological test battery; PACC: Preclinical Alzheimer Cognitive Composite; pAD: Prodromal Alzheimer’s disease; PD: Pharmacodynamic; PET: Positron emission tomography; PK: Pharmacokinetic; SAD: Single ascending dose; SIB: Severe impairment battery; SILK: Stable isotope-labeled kinetics; SNAP: Suspected non-amyloid pathology; T: Tau; tg: Transgenic; TOMM-40: Translocase of outer mitochondrial membrane 40; TPP: Target product profile; TRC-PAD: Trial-Ready Cohort for Prodromal and Preclinical AD; USD: US dollars

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Table 3 Five “rights” implemented across the spectrum of drug development

Right element	Target identification	Drug candidate optimization	Non-clinical assessment	Phase 1	Phase 2	Phase 3
Target	Druggable target identified in AD biology		PD effect supported	PD effect may be assessed with biomarkers	PD effect supported by biomarkers	PD effect supported by biomarkers and clinical outcomes
Drug		Chemical properties	ADME; toxicity; efficacy in animals	PK, ADME in healthy volunteers; MTD established; BBB penetration established	PK, PD in AD	PD in AD
Biomarker			Development of biomarkers useful in trials	Toxicity biomarkers	Patient selection; target engagement biomarkers	Patient selection; DM; toxicity; predictive biomarkers
Patient				Healthy volunteers; AD for immuno-therapy trials	Prodromal AD, AD dementia	High-risk normal subjects; prodromal AD; AD dementia
Trial				Single ascending dose; multiple ascending dose	Drug-placebo difference at endpoint; adaptive designs	Drug-placebo difference at endpoint; adaptive designs; delay to milestone

AD Alzheimer’s disease; ADME absorption, distribution, metabolism, excretion; DM disease modification; PK pharmacokinetics; PD pharmacodynamic

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BRIEF REPORT

Real-world evidence of stroke prevention in patients with nonvalvular atrial fibrillation in the United States: the REVISIT-US study

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ABSTRACT

Background: Little data exists regarding the effectiveness and safety of rivaroxaban or apixaban versus warfarin in nonvalvular atrial fibrillation (NVAf) patients treated outside of clinical trials.

Methods: This was a retrospective study using MarketScan claims from January 2012 to October 2014. We included adults, newly initiated on rivaroxaban, apixaban or warfarin, with a baseline CHA₂DS₂-VASc score ≥ 2 , ≥ 2 diagnosis codes for NVAf and ≥ 180 days of continuous medical and prescription benefits. Patients with a prior stroke, systemic embolism or intracranial hemorrhage (ICH) were excluded. Eligible rivaroxaban or apixaban users were 1:1 propensity-score matched individually to warfarin users. Cox regression was performed to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for rivaroxaban and apixaban versus warfarin for the combined endpoint of ischemic stroke or ICH and each endpoint individually.

Results: Upon matching 11,411 rivaroxaban to 11,411 warfarin users, rivaroxaban was associated with a significant reduction of the combined endpoint of ischemic stroke or ICH versus warfarin (HR = 0.61, 95% CI = 0.45–0.82). ICH was significantly (HR = 0.53, 95% CI = 0.35–0.79) and ischemic stroke nonsignificantly reduced (HR = 0.71, 95% CI = 0.47–1.07) by rivaroxaban versus warfarin. After matching 4083 apixaban and 4083 warfarin users, apixaban was found to nonsignificantly reduce the combined endpoint of ischemic stroke or ICH versus warfarin (HR = 0.63, 95% CI = 0.35–1.12) and to reduce ICH risk (HR = 0.38, 95% CI = 0.17–0.88). Ischemic stroke risk was nonsignificantly increased with apixaban (HR = 1.13, 95% CI = 0.49–2.63) versus warfarin.

Limitations: Sample size and number of combined events observed were relatively small. Residual confounding could not be ruled out.

Conclusions: Rivaroxaban and apixaban were associated with less ICH than warfarin and both are likely associated with reductions in the combined endpoint. Further investigation to validate the numerically higher rate of ischemic stroke with apixaban versus warfarin is required.

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Introduction

Nonvalvular atrial fibrillation (NVAf) is a common cardiac arrhythmia affecting up to 6.1 million persons in the US, and is associated with a ~5-fold increased risk of stroke¹. Current NVAf guidelines recommend initiation of oral anticoagulant (OAC) therapy based on validated stroke risk scores.

Randomized controlled trials (RCTs)^{2–4} have demonstrated favorable efficacy and safety profiles for the oral factor Xa inhibitors (rivaroxaban, apixaban, edoxaban) compared to warfarin. Most notably, these direct-acting OACs have been shown to significantly reduce patients' risk for intracranial hemorrhage (ICH) by 33–58%.

In routine clinical practice, OACs may be used differently than in their respective pivotal, phase III RCTs. When rigorously performed, real-world evidence studies

(including administrative claims database analyses) can offer valuable insight into the effectiveness and safety of OACs used outside of a well controlled clinical trial setting. The objective of the Real-world Evidence on Stroke prevention in patients with atrial fibrillation in the United States (REVISIT-US) study was to affirm the effectiveness and safety of previously OAC treatment naïve, newly initiated factor Xa inhibition with rivaroxaban or apixaban compared with warfarin in NVAf patients using data from a large, US administrative claims database.

Patients and methods

This manuscript was written in compliance with the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) statement⁵.

REVISIT-US was a retrospective administrative claims database study using US Truven MarketScan data spanning January 2012 through October 2014. MarketScan combines two separate databases, a commercial database and the Medicare supplemental database, to cover all age groups, and contains claims from ~100 employers, health plans and government and public organizations representing about 170 million covered lives in the US⁶. MarketScan captures health plan enrollment records, limited participant demographics, International Classification of Diseases, Ninth-Revision, Clinical Modification (ICD-9-CM) diagnosis and procedure codes, admission and discharge dates, inpatient mortality data and outpatient medical services and prescription drug dispensing records. All data included in the MarketScan database are de-identified and are in compliance with the Health Insurance Portability and Accountability Act of 1996 to preserve participant anonymity and confidentiality. For this reason, this study was exempt from institutional review board oversight.

To be included in REVISIT-US, patients had to be OAC treatment naïve in the 180 days prior to the day of the first qualifying OAC dispensing, newly initiated on rivaroxaban, apixaban, or warfarin, ≥ 18 years of age on the day of the first qualifying OAC dispensing (index date), with a baseline CHA₂DS₂-VASc score ≥ 1 , ≥ 2 ICD-9-CM diagnosis codes for NVAf (427.31) and ≥ 180 days of continuous medical and prescription coverage prior to initiation of OAC. Patients with valvular heart disease, a transient cause of NVAf, venous thromboembolism, hip or knee replacement surgery, malignant cancer or pregnancy, and patients receiving OAC before the index date, or prescribed >1 OAC agent on the index date or during follow-up were excluded. In addition, we excluded patients with a prior history of stroke, systemic embolism or ICH from the analysis to prevent misclassification of past events as new events.

Each eligible rivaroxaban user was 1:1 propensity-score matched (using greedy nearest neighbor matching and a caliper of 1%) to a warfarin user to minimize the presence of baseline differences between cohorts⁷. Similarly, each eligible apixaban user was 1:1 propensity-score matched to a warfarin user. As a result of the above described matching process, this study reports on two unique analyses (rivaroxaban versus warfarin and apixaban versus warfarin) with different sample sizes. We included rivaroxaban and apixaban patients starting at each agent's individual US Food and Drug Administration (FDA) approval date (November 2011 for rivaroxaban and December 2012 for apixaban)⁸ and only matched these patients to warfarin users initiating OAC during the same time frame. Residual differences in characteristics between matched cohorts were assessed by calculating the standardized differences, with differences $<10\%$ between cohorts considered balanced⁹. Patients were matched using age, gender, region, health plan type, CHADS₂, CHA₂DS₂-VASc, ATRIA, modified HAS-BLED (excluding the liable international normalized ratio criteria) and Deyo-Charlson Comorbidity Index scores^{1,10-12}, presence of comorbid heart failure, hypertension, diabetes mellitus, unstable angina or renal failure, use of anti-arrhythmic agents, beta-blockers, calcium channel blockers, angiotensin-converting enzyme

inhibitors, angiotensin receptor blockers, anti-platelet agents or nonsteroidal anti-inflammatory drugs and number of hospital days and office visits during the 180 day index period.

The primary endpoint evaluated in REVISIT-US was the combination of ischemic stroke or ICH (reflecting the most important efficacy and safety endpoints with comparable severity in OAC trials). Each component of this endpoint was also evaluated separately. Occurrence of these endpoints during the observation period was determined by the presence of an ICD-9-CM code as recommended by US FDA "Mini-Sentinel" post-marketing surveillance system¹³ coding schemas. Patients were followed until the occurrence of an ischemic stroke or ICH, discontinuation or switching to an alternative OAC, disenrollment from the insurance plan or end of study follow-up.

Baseline characteristics of patients were analyzed using descriptive statistics. Incidence rates of endpoints were reported as the number of events per 100 person-years (or %/year). Cox proportional hazard regression analysis was performed to estimate the hazard ratio (HR) with 95% confidence intervals (CIs) for developing each endpoint. Analyses were performed in Aetion Evidence Generation Platform – Effectiveness Evaluation Application version R2.0.20160113_2214-0-g6871884 (Aetion Inc., New York, NY, USA). Statistical testing was done in Aetion using R version 3.1.2 (The R Project for Statistical Computing, www.r-project.org)¹⁴. In all cases, a *P*-value $<.05$ was considered statistically significant.

Results

In total, 38,831 NVAf patients newly initiated on rivaroxaban or warfarin meeting inclusion criteria were identified (Figure 1). Of these, 10.5% of rivaroxaban patients and could not be adequately matched and were therefore excluded from the analyses. Following propensity scoring, 11,411 rivaroxaban (17.3% received the reduced 15 mg once daily) and 11,411 warfarin users were matched. Characteristics and person-years of follow-up of these rivaroxaban and warfarin cohorts are available in Table 1. The two cohorts were well matched, with no characteristic exhibiting a standardized difference $>10\%$. Seventy-three rivaroxaban and 103 warfarin users developed the combined endpoint, translating into a significant 39% (95% CI = 18–55%) lower hazard of developing ischemic stroke or ICH among rivaroxaban users (Figure 2). When analyzed separately, the hazard of both ICH and ischemic stroke were reduced with rivaroxaban use (47% and 29% lower) compared to warfarin, although reduction of the ischemic stroke endpoint did not reach statistical significance (42 versus 52 ischemic strokes and 38 versus 60 ICHs in rivaroxaban and warfarin users, respectively).

We identified 18,591 apixaban (15.5% received the reduced dose) or warfarin patients meeting inclusion criteria. Of these, 5.7% of apixaban patients could not be adequately matched and were therefore excluded from the analyses. Upon propensity scoring and matching, well matched (no standardized differences $>10\%$) cohorts consisting of 4083 apixaban and 4083 warfarin users were included (Table 2). Nineteen apixaban and 28 warfarin users experienced the

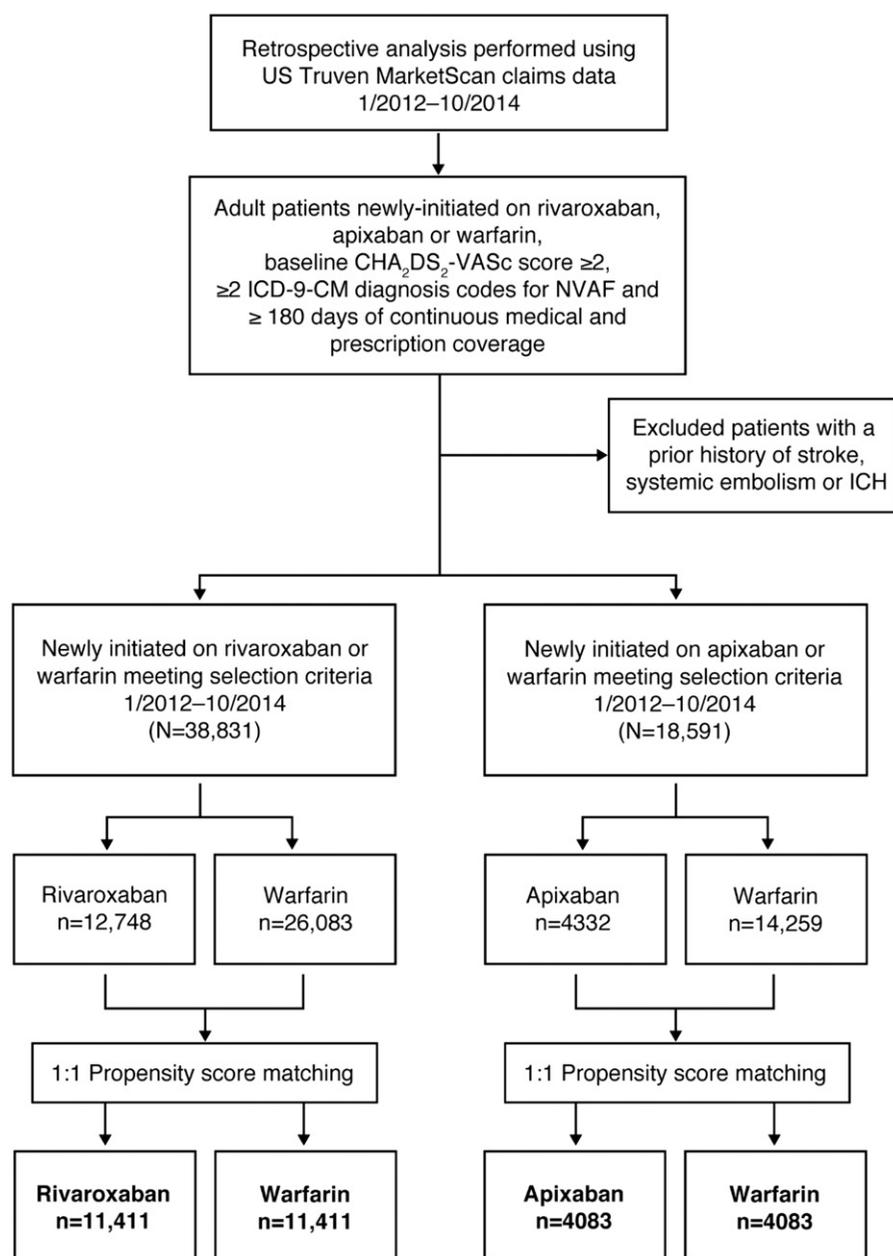


Figure 1. Patient flow diagram for the rivaroxaban versus warfarin and apixaban versus warfarin analyses. Abbreviations. ICD-9-CM, International Classification of Diseases, 9th Revision, Clinical Modification; ICH, intracranial hemorrhage; NVAF, nonvalvular atrial fibrillation.

combined endpoint of ischemic stroke or ICH (hazard reduction with apixaban = 37%, $P = \text{NS}$) (Figure 3). This finding was driven by a significant 62% reduction in ICH with apixaban (8 versus 19 events) and a 13% statistically nonsignificant increase in ischemic stroke hazard with apixaban (12 versus 10 events) compared to warfarin.

Discussion

This administrative claims database study affirmed that both rivaroxaban and apixaban use were associated with significant (47–62%) reductions in NVAF patients' hazard of developing ICH compared to warfarin in routine clinical practice. In the Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for

Prevention of Stroke and Embolism Trial in Atrial Fibrillation (ROCKET AF) trial², rivaroxaban was found to reduce the risk of ICH by 33% and in the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial³, apixaban reduced ICH by 58% versus warfarin ($P < .05$ for both). We believe our findings regarding ICH reduction should be reassuring to clinicians as they are generally consistent with those of the above-mentioned pivotal phase III trials. Notably, the reductions in ICH seen in REVISIT-US were the predominant drivers of the reductions in the combined endpoint observed with both rivaroxaban and apixaban versus warfarin (albeit only the rivaroxaban analysis reached statistical significance).

We found rivaroxaban to be associated with a nonsignificant reduced hazard of ischemic stroke versus warfarin in the present study. Apixaban was associated with a nonsignificant

Table 1. Baseline characteristics of propensity-score matched rivaroxaban and warfarin users.

Characteristic	Rivaroxaban (N = 11,411) n (%) (7715 PYs)	Warfarin (N = 11,411) n (%) (6271 PYs)	Standardized difference, %
Cohort entry (year)			0.9
2012	3132 (27.4)	3080 (27.0)	
2013	4764 (41.7)	4810 (42.2)	
2014	3515 (30.8)	3521 (30.9)	
Age, years (mean ± SD)	70.66 ± 10.99	70.72 ± 11.35	0.6
Male gender	6115 (53.6)	6145 (53.9)	
US region			0.5
Northeast	2342 (20.5)	2358 (20.7)	
North Central	3220 (28.2)	3212 (28.1)	
South	3936 (34.5)	3917 (34.3)	
West	1734 (15.2)	1745 (15.3)	
Unknown	179 (1.6)	179 (1.6)	
Health plan			1.3
Basic/major medical	0 (0)	0 (0)	
Comprehensive	3523 (30.9)	3516 (30.8)	
EPO	47 (.4)	51 (.4)	
HMO	1101 (9.6)	1117 (9.8)	
POS	544 (4.8)	533 (4.7)	
PPO	5517 (48.3)	5500 (48.2)	
POS with capitation	55 (.5)	53 (.5)	
CDHP	248 (2.2)	260 (2.3)	
HDHP	134 (1.2)	134 (1.2)	
Missing	242 (2.1)	247 (2.2)	
Stroke risk scores			
CHADS ₂ (mean ± SD)	1.92 ± 1.08	1.94 ± 1.08	1.7
CHA ₂ DS ₂ -VASc (mean ± SD)	3.46 ± 1.37	3.48 ± 1.35	1.8
Bleeding risk scores			
ATRIA score (mean ± SD)	1.76 ± 1.51	1.76 ± 1.55	0.4
HAS-BLED score (mean ± SD)	1.62 ± .69	1.62 ± .71	0.9
Comorbidities			
Deyo-Charlson Comorbidity Score (mean ± SD)	1.08 ± 1.10	1.09 ± 1.10	1.1
Heart failure	2259 (19.8)	2282 (20.0)	0.5
Hypertension	10,658 (93.4)	10,691 (93.7)	1.2
Diabetes mellitus	3913 (34.3)	3980 (34.9)	1.2
Renal failure	135 (1.2)	136 (1.2)	0.1
Medications			
Antiarrhythmics	1890 (16.6)	1912 (16.8)	0.5
Beta-blockers	5832 (51.1)	5866 (51.4)	0.6
Calcium channel blockers	3926 (34.4)	3953 (34.6)	0.5
Angiotensin-converting enzyme inhibitors	433 (3.8)	442 (3.9)	0.4
Angiotensin receptor blockers	2239 (19.6)	2297 (20.1)	1.3
Antiplatelet medications	1259 (11.0)	1239 (10.9)	0.6
Nonsteroidal anti-inflammatory drugs	1858 (16.3)	1823 (16.0)	0.8
Healthcare utilization (180 days prior to index date)			
Days in hospital (mean ± SD)	2.00 ± 4.37	2.02 ± 4.82	0.6
Number of office visits (mean ± SD)	7.37 ± 7.58	7.33 ± 7.68	0.4

Abbreviations. CDHP, consumer-driven health plan; EPO, exclusive provider organization; HDHP, high deductible health plan; HMO, health maintenance organization; PPO, preferred provider organization; POS, point-of-service; PY, person-year; SD, standard deviation.

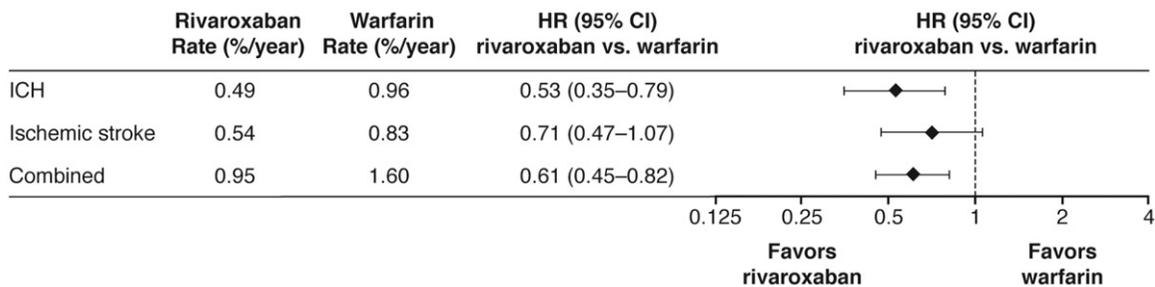


Figure 2. Impact of rivaroxaban versus warfarin on study endpoints. Abbreviations. CI, confidence interval; HR, hazard ratio; ICH, intracranial hemorrhage.

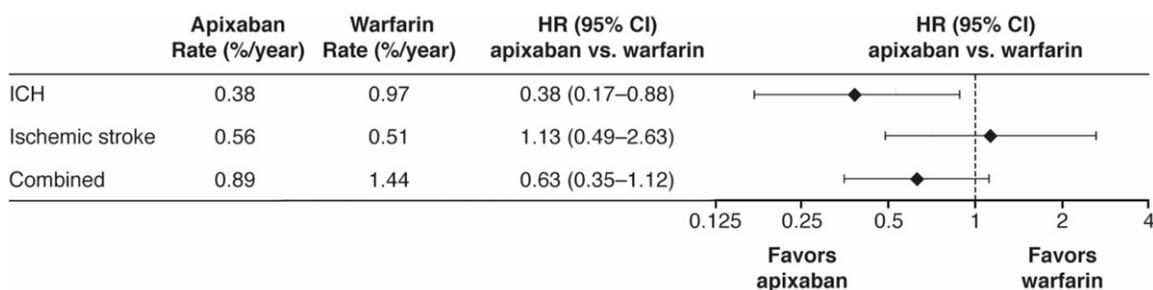
13% increased hazard of ischemic stroke. The reduction in ischemic stroke with rivaroxaban is generally consistent with ROCKET AF (HR = 0.94, 95% CI = 0.75–1.17)². The 13% increased hazard of ischemic stroke observed in apixaban

users compared to warfarin users is less consistent with ARISTOTLE (HR = 0.92, 95% CI = 0.74–1.13)³. This finding of a numerically higher ischemic stroke risk with apixaban in routine practice is supported by prior studies^{15,16}.

Table 2. Baseline characteristics of propensity-score matched apixaban and warfarin users.

Characteristic	Apixaban (N = 4083) n (%) (2125 PYs)	Warfarin (N = 4083) n (%) (1951 PYs)	Standardized difference
Cohort entry (year)			0.5
2012	0 (0)	0 (0)	
2013	1502 (36.8)	1493 (36.6)	
2014	2581 (63.2)	2590 (63.4)	
Age, years (mean ± SD)	71.00 ± 11.25	71.15 ± 11.32	1.3
Male gender	2172 (53.2)	2189 (53.6)	0.8
US region			2.4
Northeast	832 (20.4)	851 (20.8)	
North Central	1142 (28.0)	1158 (28.4)	
South	1496 (36.6)	1475 (36.1)	
West	538 (13.2)	532 (13.0)	
Unknown	75 (1.8)	67 (1.6)	
Health plan			4.2
Basic/major medical	0 (0)	0 (0)	
Comprehensive	1252 (30.7)	1260 (30.9)	
EPO	12 (.3)	6 (.1)	
HMP	361 (8.8)	347 (8.5)	
POS	190 (4.7)	205 (5.0)	
PPO	1996 (48.9)	2000 (49.0)	
POS with capitation	16 (.4)	14 (.3)	
CDHP	137 (3.4)	128 (3.1)	
HDHP	65 (1.6)	67 (1.6)	
Missing	54 (1.3)	56 (1.4)	
Stroke risk scores			
CHADS ₂ score (mean ± SD)	1.93 ± 1.07	1.92 ± 1.07	0.9
CHA ₂ DS ₂ -VAsC score (mean ± SD)	3.47 ± 1.38	3.47 ± 1.35	0.5
Bleeding risk scores			
ATRIA score (mean ± SD)	1.84 (1.59)	1.86 (1.66)	1.1%
HASBLED score (mean ± SD)	1.65 (.69)	1.66 (.72)	1.3%
Comorbidities			
Deyo–Charlson Comorbidity Score (mean ± SD)	1.05 ± 1.08	1.03 ± 1.08	1.7
Heart failure	778 (19.1)	776 (19.0)	0.1
Hypertension	3876 (94.9)	3863 (94.6)	1.4
Diabetes mellitus	1392 (34.1)	1381 (33.8)	0.6
Renal failure	72 (1.8)	73 (1.8)	0.2
Medications			
Antiarrhythmics	851 (20.8)	858 (21.0)	0.4
Beta-blockers	2285 (56.0)	2258 (55.3)	1.3
Calcium channel blockers	1514 (37.1)	1461 (35.8)	2.7
Angiotensin-converting enzyme inhibitors	157 (3.8)	152 (3.7)	0.6
Angiotensin receptor blockers	817 (20.0)	843 (20.6)	1.6
Antiplatelet medications	443 (10.8)	443 (10.8)	0
Nonsteroidal anti-inflammatory drugs	681 (16.7)	680 (16.7)	0.1
Healthcare utilization (180 days prior to index date)			
Hospital days (mean ± SD)	1.81 ± 4.22	1.81 ± 4.10	0
Office visits (mean ± SD)	7.54 ± 7.30	7.28 ± 7.60	3.5

Abbreviations. CDHP, consumer-driven health plan; EPO, exclusive provider organization; HDHP, high deductible health plan; HMO, health maintenance organization; PPO, preferred provider organization; POS, point-of-service; PY, person-year; SD, standard deviation.

**Figure 3.** Impact of apixaban versus warfarin on study endpoints. Abbreviations. CI, confidence interval; HR, hazard ratio; ICH, intracranial hemorrhage.

In an independent analysis, Noseworthy and colleagues¹⁵ found apixaban to be associated with a 27% (1.04 versus 0.73 events per 100 person-years, $P = .39$) increased hazard of ischemic stroke compared to rivaroxaban in an Optum Labs Data Warehouse claims study utilizing data from October 2010 to February 2015 (median age = 73 years;

CHA₂DS₂-VAsC score = 4 in both matched cohorts). Thus, one potential explanation for the numerical increase in ischemic stroke for apixaban versus warfarin seen in our analysis could be the more frequent use of the reduced 2.5 mg twice daily dose in routine clinical practice (15.5% received the reduced apixaban dose in REVISIT-US versus 4.6% in ARISTOTLE³).

For rivaroxaban the use of the reduced dose was more consistent with ROCKET AF (17.3% received the reduced 15 mg once daily rivaroxaban dose in REVISIT-US versus 20.7% in ROCKET AF)^{2,17}.

In addition, poor adherence to the twice daily dosing regimen of apixaban outside of controlled trials may also have contributed to our findings^{18–22}. Studies suggest that sub-optimal adherence (taking <80% of one's doses) among NVAf patients may be associated with a 50% increased risk of ischemic stroke (95% CI = 23–83%)¹⁸. Moreover, Shore and colleagues²² found that every 10% reduction in dabigatran adherence was associated with a 13% (95% CI = 8–19%) increased hazard of all-cause mortality or stroke. Available data from real-world evidence suggests that the use of apixaban in routine practice may be associated with more ischemic strokes versus warfarin, and this finding merits further investigation.

REVISIT-US was specifically designed to the extent possible within a claims database to optimize internal study rigor and, therefore, obtain the most unbiased HR estimates for rivaroxaban and apixaban compared to warfarin. In order to achieve our goal, we selected endpoints that were most likely to be accurately identified through ICD-9-CM coding in MarketScan and that were associated with similar degrees of morbidity and mortality to assist readers in drawing benefit–risk conclusions. Moreover, we used validated ICD-9-CM coding schemas¹⁴ and excluded patients with prior stroke, systemic embolism or ICH (which may have contributed to the low number of events)²³. Each of these methodological steps was taken to attenuate the risk of potential misclassification bias common to claims database analyses. Finally, because rivaroxaban and apixaban were approved at different times, and clinician experience and comfort with prescribing these agents likely grows over time potentially changing benefit and risk assessment, rivaroxaban and apixaban users were included starting at their respective US FDA approval dates and only matched to warfarin users initiating anticoagulation during the same time frame.

We feel it is also important for readers to be cognizant that two separate analyses (rivaroxaban versus warfarin and apixaban versus warfarin) were performed and presented in this paper. As these were statistically independent analyses, we discourage cross-comparison between the rivaroxaban and apixaban cohorts or between the two corresponding warfarin cohorts as this may not yield robust conclusions. The primary objective of our analyses was to show consistency between real-world claims database analysis and phase III RCTs, and not to draw comparisons between OACs that have not been rigorously compared in head-to-head RCTs. Direct comparison of rivaroxaban and apixaban in MarketScan is hampered by the database's insufficient reporting of laboratory (serum creatinine) and clinical data (body weight) which are required to determine whether rivaroxaban and apixaban prescribing was consistent with labeling.

This study has additional limitations worthy of discussion. First, while propensity-score matching generated cohorts that were comparable in key characteristics, only those variables measured in MarketScan could be matched upon and residual confounding cannot be excluded.

Second, MarketScan has a substantial lag in time to data availability. As a result, upon securing this data and performing analysis in early 2016, MarketScan data was only available through October 2014. This meant there were only about 4000 eligible apixaban users in this dataset. Given that RCTs of rivaroxaban² and apixaban³ have enrolled >7000 subject per study arm, it is likely that the apixaban analyses were somewhat underpowered and any apixaban versus rivaroxaban comparison would be more so. With this in mind, it is important to remember that failure to show a significant difference between agents, in studies such as the one presented, is not proof of equivalence or noninferiority. Finally, it was not possible to determine the duration of time warfarin users spent in the therapeutic international normalized ratio (INR) range of 2.0–3.0. Additional analyses evaluating the effectiveness and safety of the direct-acting OACs should be performed once sample sizes in claims databases grow larger.

Conclusion

In this real-world analysis of NVAf patients within the United States, both rivaroxaban and apixaban use was associated with less ICH than warfarin. This data confirms results of these agents' corresponding phase III clinical trials. Both rivaroxaban and apixaban appear likely associated with reductions in the combined endpoint of ICH or ischemic stroke versus warfarin as well. While only a preliminary finding based upon a relatively small number of events, further investigation into the numerically (but not statistically) higher rate of ischemic stroke with apixaban versus warfarin is required.

Transparency

Declaration of funding

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Declaration of financial/other relationships

C.I.C. has disclosed that he has received grant funding and consultancy fees from Janssen Pharmaceuticals, Bayer and Boehringer Ingelheim Pharmaceuticals. M.A. has disclosed that he has received consulting fees and speaker honoraria from Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo and Pfizer. E.P.S. has disclosed that he has no significant relationships with or financial interests in any commercial companies related to this study or article. T.E. and K.B. have disclosed that they are employees of Bayer Pharma AG. H.B. has disclosed that he has received honoraria for lectures from Advanced Circulatory Systems, Bayer, Biotronik, Boehringer Ingelheim, Boston Scientific, Bristol-Myers Squibb, Cardiome, Daiichi Sankyo, Impulse-Dynamics, Jolife, NayaMed, Medtronic, Lilly, MSD, Physiocontrol, Pfizer, Sanofi, Servier, Sorin and St. Jude Medical; honoraria for advisory board activities from Bayer, Boehringer Ingelheim, Biotronik, Biosense-Webster, Bristol-Myers Squibb, Boston

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Notice of correction

Please note that the abstract has been corrected since the article was first published online (20 September 2016)

Atrial fibrillation

XANTUS: a real-world, prospective, observational study of patients treated with rivaroxaban for stroke prevention in atrial fibrillation

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Aims

Although non-vitamin K antagonist oral anticoagulants are recommended for stroke prevention in patients with non-valvular atrial fibrillation (NVAF) based on clinical trial results, there is a need for safety and efficacy data from unselected patients in everyday clinical practice. XANTUS investigated the safety and efficacy of the Factor Xa inhibitor rivaroxaban in routine clinical use in the NVAF setting.

Methods and results

Consecutive consenting patients with NVAF newly started on rivaroxaban were eligible and were followed up at ~3-month intervals for 1 year, or for at least 30 days after permanent discontinuation. All adverse events (AEs) were recorded as AEs or serious AEs; major outcomes (including major bleeding, symptomatic thromboembolic events [stroke, systemic embolism, transient ischaemic attack, and myocardial infarction], and all-cause death) were centrally adjudicated. There were 6784 patients treated with rivaroxaban at 311 centres in Europe, Israel, and Canada. Mean patient age was 71.5 years (range 19–99), 41% were female, and 9.4% had documented severe or moderate renal impairment (creatinine clearance <50 mL/min). The mean CHADS₂ and CHA₂DS₂-VASc scores were 2.0 and 3.4, respectively; 859 (12.7%) patients had a CHA₂DS₂-VASc score of 0 or 1. The mean treatment duration was 329 days. Treatment-emergent major bleeding occurred in 128 patients (2.1 events per 100 patient-years), 118 (1.9 events per 100 patient-years) died, and 43 (0.7 events per 100 patient-years) suffered a stroke.

Conclusion

XANTUS is the first international, prospective, observational study to describe the use of rivaroxaban in a broad NVAF patient population. Rates of stroke and major bleeding were low in patients receiving rivaroxaban in routine clinical practice.

Trial registration number

Clinicaltrials.gov: NCT01606995.

Keywords

Anticoagulants • Atrial fibrillation • Real world • Rivaroxaban • Stroke • Thromboembolism

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Introduction

Atrial fibrillation (AF) affects ~2% of the European population, and its prevalence is rising due to concomitant conditions and ageing populations.¹ Stroke is one serious consequence of AF,² but oral anticoagulation can prevent most cases of AF-related stroke.^{3,4} Although the evidence supporting the use of anticoagulation for stroke prevention in AF has been generated with dose-adjusted vitamin K antagonists (VKAs), four non-VKA oral anticoagulants (NOACs) have been found to be at least as effective and safer than VKAs for stroke prevention in patients with non-valvular AF (NVAF).^{5–8} These NOACs have been approved for use in this indication and are recommended as alternatives to VKAs in international guidelines.^{3,4} Further information on the effectiveness of NOACs is still accumulating in the form of retrospective registries and additional randomized clinical trials. A high volume of prospectively collected information in large patient groups is still lacking. This was recognized by the European Medicines Agency (EMA), leading to a requirement to conduct observational studies as part of the post-approval plan. Here, outcomes are reported from the XANTUS study, which assessed the safety and efficacy of rivaroxaban in routine, 'real-world' clinical practice.

Methods

The design of the international, non-interventional, observational XANTUS study was approved by the EMA and details have been published previously.⁹

Study population and screening

Eligible patients had a diagnosis of NVAF, were aged ≥ 18 years, started rivaroxaban therapy to reduce the risk of stroke or systemic embolism (SE), and provided written informed consent. All patients were screened sequentially and documented in an anonymous log file independent of therapy.

Medication and follow-up

Decisions about rivaroxaban prescription were at the discretion of the treating physician, including dose and duration of therapy. Label-recommended rivaroxaban doses for stroke prevention in NVAF are 20 mg once daily (od) for patients with normal renal function or mild impairment (creatinine clearance [CrCl] ≥ 50 mL/min) and 15 mg od for patients with moderate or severe renal impairment (CrCl 15–49 mL/min; e.g. in Europe). After the screening visit, follow-up data collection was at the time of hospital discharge, if applicable, and approximately every 3 months thereafter. The overall follow-up period was 1 year. For patients who discontinued therapy before the end of the 1-year follow-up, the observation period ended ~30 days after the last dose of rivaroxaban.

Study outcomes

The primary outcomes were related to the safety of rivaroxaban, recorded as adverse events (AEs) or serious AEs (SAEs), and included major bleeding events (defined using International Society on Thrombosis and Haemostasis [ISTH] criteria), all-cause death, and any other AEs and SAEs.⁹ Secondary outcomes included symptomatic thromboembolic events (stroke, non-central nervous system SE, transient ischaemic attack [TIA], and myocardial infarction [MI]) and non-major bleeding events (defined as any bleeding event not meeting the criteria for a

major haemorrhage) across patients with different baseline risk profiles for stroke or bleeding. Intracranial bleeding that met the definition of stroke was included in both stroke and major bleeding endpoints. Haemorrhagic transformations of ischaemic stroke were counted as a major bleeding event regardless of whether symptomatic or not. Other outcomes included treatment persistence, patient satisfaction (reported by patients using standardized questionnaires), healthcare resource use, and details of treatment interruption and interventions such as management of bleeding events and stroke.

Study conduct

XANTUS applied several quality assurance measures. Physicians were specifically requested to document at every visit if bleeding, stroke, SE, TIA, MI, or other AEs had occurred, and this was captured as a 'yes/no' response for each event of interest. To detect unreported events, the database was searched for concomitant medications, interventions, other key words, and laboratory findings potentially associated with a bleeding or thromboembolic event. Questionable findings from this search triggered medical queries to the investigator and potentially central adjudication.

An independent Central Adjudication Committee (CAC) of five expert physicians (not directly involved in the care of XANTUS study patients) adjudicated major bleeding, stroke, SE, TIA, MI, and all-cause death. The CAC had access to all patient records. Bleeding events were documented by the investigators as AEs. A verified algorithm was used to search the database for all recorded bleeding AEs that were associated with transfusions, were fatal, occurred at a critical site, were associated with an intervention, or were assessed as 'major' by the investigator. The algorithm also identified any recorded haemoglobin decreases of ≥ 2 g/dL regardless of the documentation of an AE. All cases identified via this algorithm were adjudicated by the CAC. Thromboembolic events were also recorded as AEs. In cases of potential stroke, SE, TIA, or MI from either investigator assessment or a database search, central adjudication was performed. The CAC also adjudicated the type of stroke and occurrence of a haemorrhagic transformation of ischaemic stroke. Clinical cause of death was centrally adjudicated. To ensure reporting standards, quality assessment and source data verification visits were performed at 61 (19.6%) sites between Q4–13 and Q3–14, and documentation related to 581 patients (8.6%) was reviewed.

Study governance

The study complied with the Declaration of Helsinki, was approved by the appropriate Health Authorities, independent Ethics Committees, and Independent Review Boards as required, and was conducted in accordance with Good Pharmacoepidemiological Practice (GPP). An independent academic Steering Committee oversaw the design, execution, and conduct of the study, was responsible for manuscript development, had full access to all data, and approved all versions of the manuscript.

Patients' informed consent included the permission for data collection and analysis. To minimize the risk of loss to follow-up, in countries where this is permitted, patients could voluntarily provide an alternative contact to the investigator/independent patient management team. In compliance with Good Clinical Practice (GCP) standards, data management and statistical analyses were overseen by the sponsor. The lead statistician oversaw programming and validation of the statistical analyses.

Statistical analysis

Events were considered 'treatment-emergent' if they occurred from the day of the first dose of rivaroxaban, and up to 2 days after the last dose in the event of permanent discontinuation. Statistical analyses of the

events were descriptive, exploratory, and generally limited to frequency tables or summary statistics (e.g. mean \pm standard deviation [SD] or median \pm quartiles). The primary analysis population was the full safety population, defined as all patients who had taken at least one dose of rivaroxaban. Both raw incidence proportions (patients with events/number of treated patients) and incidence rates (events per 100 patient-years) are presented, with corresponding 95% confidence intervals.

Results

Patient demographics and clinical characteristics

A total of 10 934 patients were screened between June 2012 and December 2013, of whom 6785 were enrolled from 311 centres in Europe, Israel, and Canada. Most patients (5287 [77.9%]) were from Western Europe, Canada, and Israel, with 1497 (22.1%) patients from Eastern Europe. One patient did not take rivaroxaban; therefore, the analysis reported here is based on 6784 patients; of whom, 5336 (78.7%) received rivaroxaban 20 mg od, 1410 (20.8%) received 15 mg od, and 35 (0.5%) received another dose (information on dosing was missing in three patients; *Figure 1*).

The mean observation period was 329 (SD 115, median 366) days. In total, 45.5% of patients had previous VKA use, 54.5% were categorized as VKA naive, 18% had previously used acetylsalicylic acid (excluding combination therapies) for stroke prevention, and 1.0% had received dual antiplatelet therapy alone. The baseline demographics and clinical characteristics of patients are summarized in *Table 1*. Mean patient age was 71.5 years; 37% of all patients were aged >75 years, and 59% were male. Co-morbidities were common: 74.7% of patients had hypertension; 19.6% had diabetes; 19.0% had experienced a prior stroke, SE, or TIA; and 18.6% had congestive heart failure. The mean CHADS₂ score was 2.0 (median 2.0) and the mean CHA₂DS₂-VASc score was 3.4 (median 3.0). There were 12.7% of patients who had a CHA₂DS₂-VASc score of either 0 or 1; furthermore, 18.5% of patients were first diagnosed with NVAF, 40.6% with paroxysmal AF, and 40.7% with persistent or permanent AF.

Outcomes

In the cohort of 6784 patients, the overall numbers of major bleeding and thromboembolic events and all-cause deaths were low and increased progressively over time (*Figure 2A*). Most patients

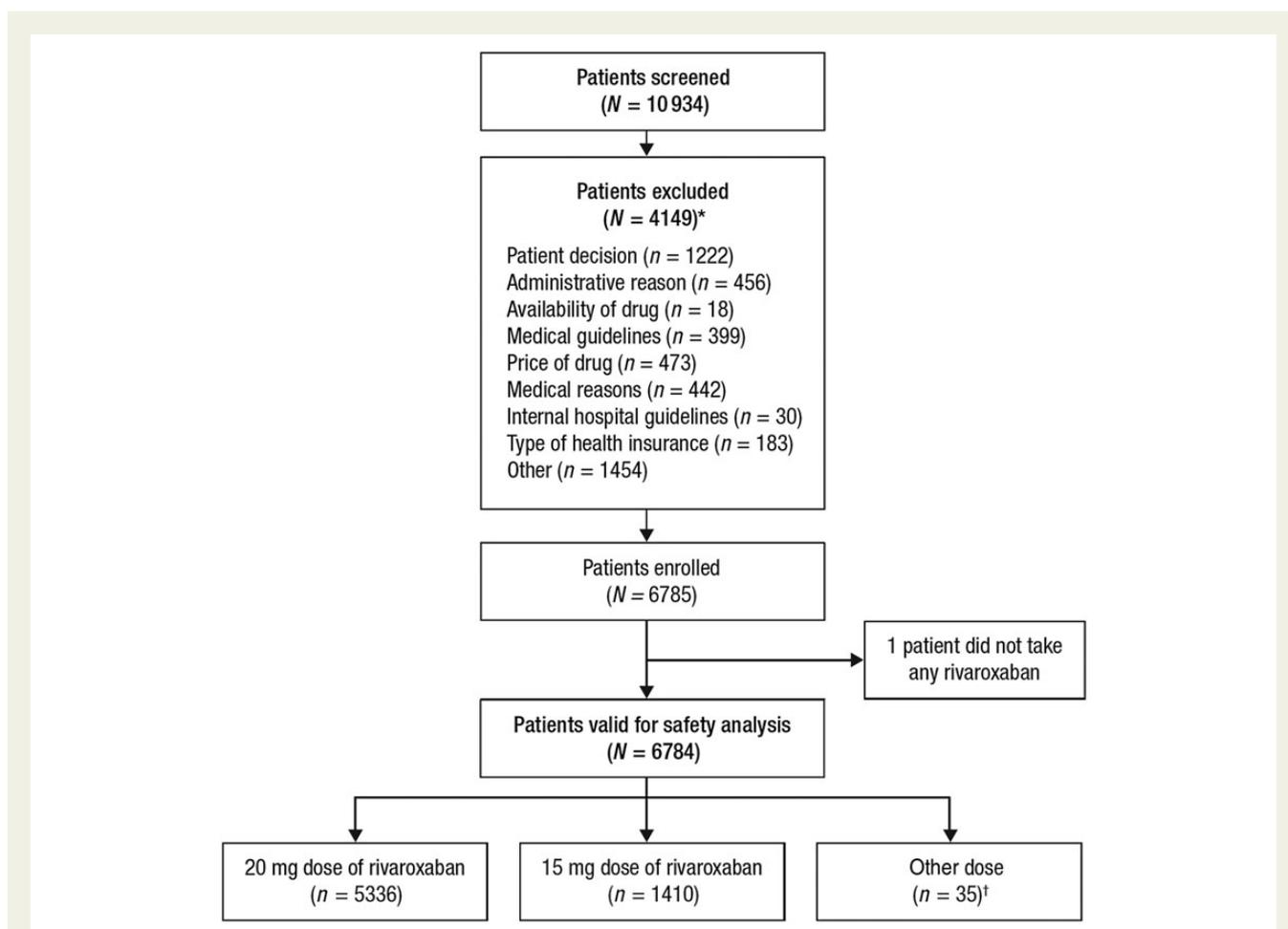


Figure 1 Patient disposition during the study. *Reasons for not continuing in the study included, but were not limited to, patient decision and administrative and medical reasons. Some patients could have more than one reason for exclusion. †Other dose includes any initial rivaroxaban dose besides 15/20 mg once daily (excluding missing information, $n = 3$).

Table 1 Baseline demographics and clinical characteristics of patients in the XANTUS study

	Rivaroxaban (N = 6784)
Age (years), mean ± SD	71.5 ± 10.0
Age <65, n (%)	1478 (21.8)
Age ≥65 to ≤75, n (%)	2782 (41.0)
Age >75, n (%)	2524 (37.2)
Gender (male), n (%)	4016 (59.2)
Weight (kg), mean ± SD	83.0 ± 17.3
BMI (kg/m ²), mean ± SD	28.3 ± 5.0
BMI >30, n (%)	1701 (25.1)
Creatinine clearance (mL/min), n (%)	
<15	20 (0.3)
≥15 to <30	75 (1.1)
≥30 to <50	545 (8.0)
≥50 to ≤80	2354 (34.7)
>80	1458 (21.5)
Missing	2332 (34.4)
Existing co-morbidities, n (%)	
Hypertension	5065 (74.7)
Diabetes mellitus	1333 (19.6)
Prior stroke/non-CNS SE/TIA	1291 (19.0)
Congestive HF	1265 (18.6)
MI	688 (10.1)
Hospitalization at baseline, n (%)	1226 (18.1)
AF, n (%)	
First diagnosed	1253 (18.5)
Paroxysmal	2757 (40.6)
Persistent	923 (13.6)
Permanent	1835 (27.0)
Missing	16 (0.2)
CHADS ₂ score	
Mean score ± SD	2.0 ± 1.3
Score, n (%)	
0	703 (10.4)
1	2061 (30.4)
2	2035 (30.0)
3	1111 (16.4)
4	618 (9.1)
5	222 (3.3)
6	34 (0.5)
Missing	0 (0.0)
CHA ₂ DS ₂ -VASc score	
Mean score ± SD	3.4 ± 1.7
Score, n (%)	
0	174 (2.6)
1	685 (10.1)
2	1313 (19.4)
3	1578 (23.3)
4	1405 (20.7)
5	837 (12.3)
6–9	789 (11.6)
Missing	3 (<0.05)

Continued

Table 1 Continued

	Rivaroxaban (N = 6784)
Prior use of antithrombotics, n (%)	
VKA	2767 (40.8)
Direct thrombin inhibitor	208 (3.1)
Acetylsalicylic acid (excluding dual antiplatelet therapy)	1224 (18.0)
Dual antiplatelet therapy	68 (1.0)
Factor Xa inhibitor (excluding rivaroxaban)	10 (0.1)
Heparin group	217 (3.2)
Other	55 (0.8)
Multiple	410 (6.0)
VKA	
Experienced	3089 (45.5)
Naive	3695 (54.5)

CrCl calculated using the Cockcroft–Gault formula.

AF, atrial fibrillation; BMI, body mass index; CNS, central nervous system;

CrCl, creatinine clearance; HF, heart failure; MI, myocardial infarction; SD, standard deviation; SE, systemic embolism; TIA, transient ischaemic attack; VKA, vitamin K antagonist.

(6522 [96.1%]) did not experience any of the outcomes of treatment-emergent major bleeding, all-cause death, or stroke/SE (freedom from events; Figure 2B).

A total of 2709 patients (39.9%) had a treatment-emergent AE and 1200 (17.7%) had a treatment-emergent SAE. There were 142 treatment-emergent major bleeding events in 128 patients (2.1 events per 100 patient-years). The incidence rate of fatal bleeding was 0.2 events per 100 patient-years; critical organ bleeding occurred at a rate of 0.7 events per 100 patient-years, including intracranial haemorrhage (0.4 events per 100 patient-years). The incidence of major gastrointestinal bleeding was 0.9 events per 100 patient-years (Table 2). Stroke occurred in 43 (0.7 events per 100 patient-years) patients, with SE occurring in a further 8 patients (0.1 events per 100 patient-years). Eleven patients (0.2%) had a haemorrhagic stroke and 32 (0.5%) an ischaemic stroke. Left atrial thrombus was recorded in six patients (0.1 events per 100 patient-years). All-cause death occurred in 118 patients (1.9 events per 100 patient-years) within the study treatment period, with the adjudicated cause of death due primarily to cardiovascular causes, mainly heart failure, followed by cancer (Table 3).

The incidence of major bleeding events increased with age and occurred at a rate of 0.9 events per 100 patient-years in patients aged <65 years, 1.7 events per 100 patient-years in patients aged ≥65 to ≤75 years, and 3.2 events per 100 patient-years in those aged >75 years. The corresponding rates for symptomatic thromboembolic events (stroke/SE, TIA, and MI) were 0.8, 1.8, and 2.3 events per 100 patient-years, respectively. Outcome analysis according to CHADS₂ and CHA₂DS₂-VASc risk scores showed that stroke/SE, major bleeding, and all-cause death generally increased with higher risk scores (Figure 3).

Creatinine clearance values were reported in 4452 (65.6%) patients. Of these, 14.4% had CrCl <50 mL/min and 85.6% had

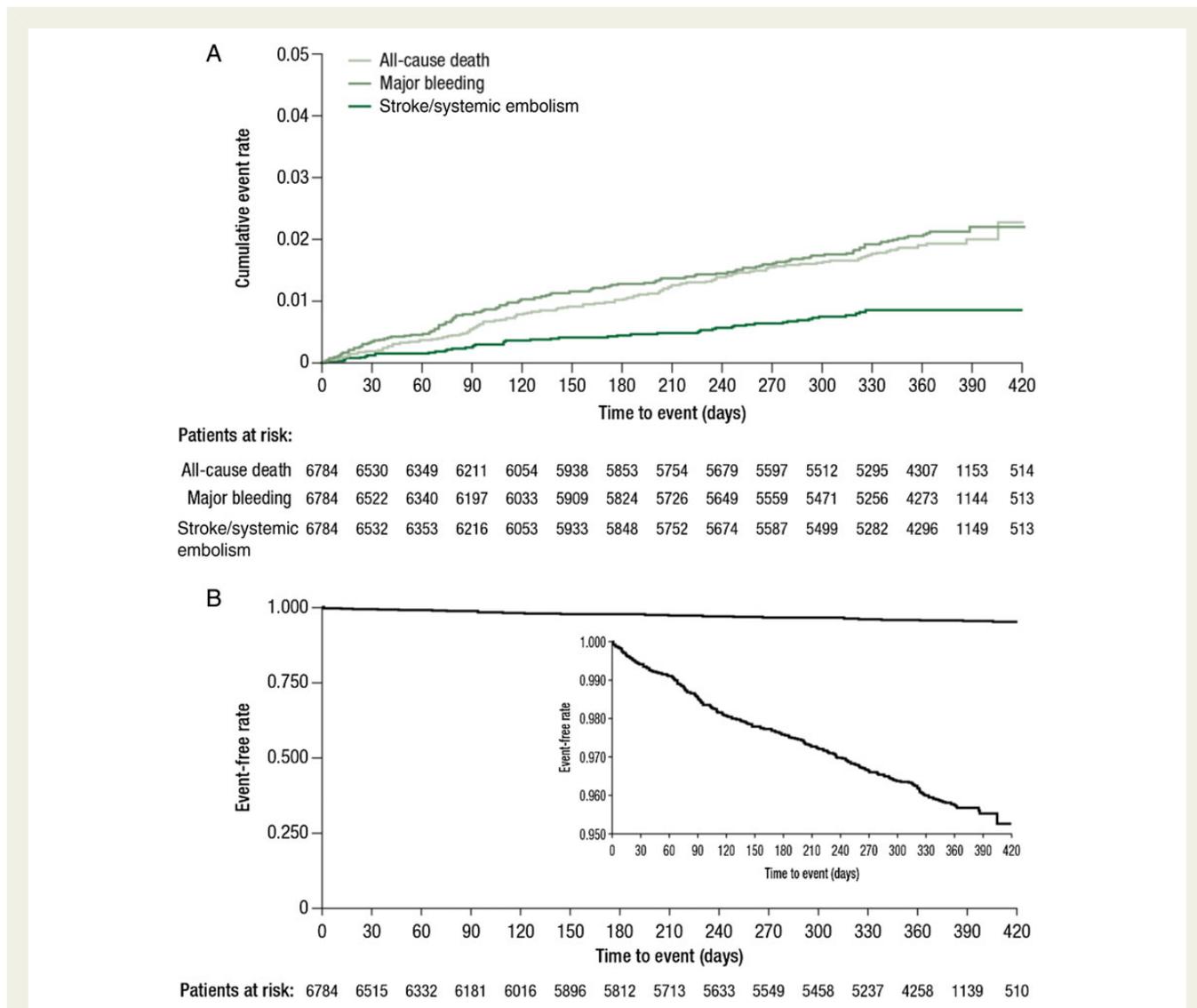


Figure 2 (A) Cumulative rates (Kaplan–Meier) for treatment-emergent all-cause death, major bleeding events, and stroke/systemic embolism. (B) Event-free rate (Kaplan–Meier) for treatment-emergent all-cause death, major bleeding events, and stroke/systemic embolism. In total, 6522 (96.1%) patients did not experience any of the outcomes of treatment-emergent all-cause death, major bleeding, or stroke/systemic embolism. Safety analysis set.

CrCl ≥ 50 mL/min. As expected, rates of major bleeding were highest in patients with documented reduced renal function (3.4% in patients with CrCl < 50 mL/min). The lowest incidence proportion for major bleeding (0.6%) was observed in patients for whom no CrCl test results were recorded. Of 3812 patients with a documented CrCl of ≥ 50 mL/min, 15% received the lower rivaroxaban dose of 15 mg od; conversely, the 20 mg od dose was received by 36% of the 640 patients who had moderate or severe renal impairment documented at any time during the study. Outcomes of major bleeding, all-cause death, or thromboembolic events (stroke, SE, TIA, and MI combined) showed numerically higher incidence rates for the 15 mg od dose compared with the 20 mg od dose: 3.1 vs. 1.8 events per 100 patient-years for major bleeding, 3.7 vs. 1.4 events per 100 patient-years for all-cause death, and 2.3 vs. 1.6 events per 100 patient-years for thromboembolic events, respectively.

Additional outcomes

In total, 598 patients (8.8%) had at least one interruption of rivaroxaban therapy, which was most commonly because of a need for surgery, or because of bleeding or another AE. The median treatment interruption period was 4 days (Q1–Q3; 2–12 days). Among all patients with treatment interruption, major bleeding was recorded in 5.2% during the interruption period or within 2 days of the end of this period; thromboembolic events occurred in 2.0% of patients. Interventions for stroke were rare: among 32 patients with ischaemic stroke, no thrombectomies were performed and two patients underwent thrombolysis. Among 27 patients with MI, no thrombolysis was performed, but percutaneous intervention and coronary artery bypass grafting were performed in 11 patients and two patients, respectively. Major bleeding was mostly treated using conservative methods and non-specific reversal agents were rarely

Table 2 Adjudicated treatment-emergent thromboembolic and bleeding events and all-cause death

	Rivaroxaban (N = 6784)	
	Incidence proportion, n (%)	Incidence rate, events per 100 patient-years (95% CI)
All-cause death	118 (1.7)	1.9 (1.6–2.3)
Thromboembolic events (stroke, SE, TIA, and MI)	108 (1.6)	1.8 (1.5–2.1)
Stroke/SE	51 (0.8)	0.8 (0.6–1.1)
Stroke	43 (0.6)	0.7 (0.5–0.9)
Primary haemorrhagic	11 (0.2)	
Primary ischaemic	32 (0.5)	
Haemorrhagic transformation	3 (<0.05)	
No haemorrhagic transformation	29 (0.4)	
Uncertain	0	
SE	8 (0.1)	0.1 (0.1–0.3)
TIA	32 (0.5)	0.5 (0.4–0.7)
MI	27 (0.4)	0.4 (0.3–0.6)
Major bleeding	128 (1.9)	2.1 (1.8–2.5)
Fatal	12 (0.2)	0.2 (0.1–0.3)
Critical organ bleeding	43 (0.6)	0.7 (0.5–0.9)
Intracranial haemorrhage	26 (0.4)	0.4 (0.3–0.6)
Intraparenchymal	6 (0.1)	
Subarachnoid	5 (0.1)	
Intraventricular	6 (0.1)	
Subdural haematoma	6 (0.1)	
Epidural haematoma	1 (<0.05)	
Haemorrhagic transformation of ischaemic stroke	3 (<0.05)	
Missing	2 (<0.05)	
Mucosal bleeding ^a	60 (0.9)	1.0 (0.7–1.3)
Gastrointestinal	52 (0.8)	0.9 (0.6–1.1)
Haemoglobin decrease ≥ 2 g/dL ^b	52 (0.8)	0.9 (0.6–1.1)
Transfusion of ≥ 2 units of packed red blood cells or whole blood ^b	53 (0.8)	0.9 (0.6–1.1)
Non-major bleeding events	878 (12.9)	15.4 (14.4–16.5)

CI, confidence interval; MI, myocardial infarction; SE, systemic embolism; TIA, transient ischaemic attack.

^aThe numbers shown here are for major mucosal and gastrointestinal bleeding events. Mucosal bleeding events include gingival, epistaxis, gastrointestinal, rectal, macroscopic haematuria, and increased or prolonged menstrual or abnormal vaginal bleeding.

^bRepresents major bleeding.

used; the use of prothrombin complex concentrate was documented in two patients, tranexamic acid in three patients, and etamsylate in one patient. Treatment persistence remained high over the 1-year study period, with a discontinuation rate at the end of the

Table 3 Causes of treatment-emergent adjudicated death

Adjudicated causes of death	Number of patients (N = 118 ^a), n (%)
Cardiovascular	49 (41.5)
Cardiac decompensation, heart failure	24 (20.3)
Sudden or unwitnessed death	14 (11.9)
MI	6 (5.1)
Non-haemorrhagic stroke	4 (3.4)
Dysrhythmia	1 (0.8)
Venous thromboembolism	0
Other vascular event	0
Cancer	23 (19.5)
Other	16 (13.6)
Bleeding	12 (10.2)
Extracranial haemorrhage	5 (4.2)
Intracranial bleeding	7 (5.9)
Infectious disease	10 (8.5)
Unexplained	9 (7.6)

MI, myocardial infarction.

^aMultiple reasons were recorded for the cause of treatment-emergent adjudicated death of some patients.

observation period of 20.1%. This finding coincided with 5096 (75.1%) patients reporting to their physicians that they were 'very satisfied' or 'satisfied' with their treatment. The main reason for premature discontinuation (7.9% of all patients) was the occurrence of an AE.

Discussion

Studies such as XANTUS complement the outcomes of pivotal trials through the use of unselected real-world populations and conditions. XANTUS is the first international, prospective, non-interventional study describing the use of a NOAC for stroke prevention in a broad NVAf patient population. Whereas patients in the phase III ROCKET AF trial had a mean CHADS₂ score of 3.5, with 55% having experienced prior stroke/SE or TIA,⁷ patients studied in XANTUS had a lower risk of stroke, with a mean CHADS₂ score of 2.0 and 19.0% experiencing prior stroke/TIA or SE. The baseline stroke risk of patients in XANTUS is, therefore, similar to that of other NOAC trials, such as RE-LY and ARISTOTLE, in which the mean CHADS₂ score was 2.1, and the percentages of patients with a prior stroke were 20.0 and 19.4%, respectively.^{5,6} XANTUS included patients at a slightly lower baseline stroke risk, with a lower percentage of patients with prior stroke than ENGAGE AF-TIMI 48, for which values were 2.8 and 28.3%, respectively.⁸

With the distribution of stroke risk scores in XANTUS, real-world stroke incidence was low in patients receiving anticoagulation, with an annual stroke rate of 0.7% (vs. 1.7 events per 100 patient-years in the ROCKET AF on-treatment population). The incidence rates of other thromboembolic events and for all-cause

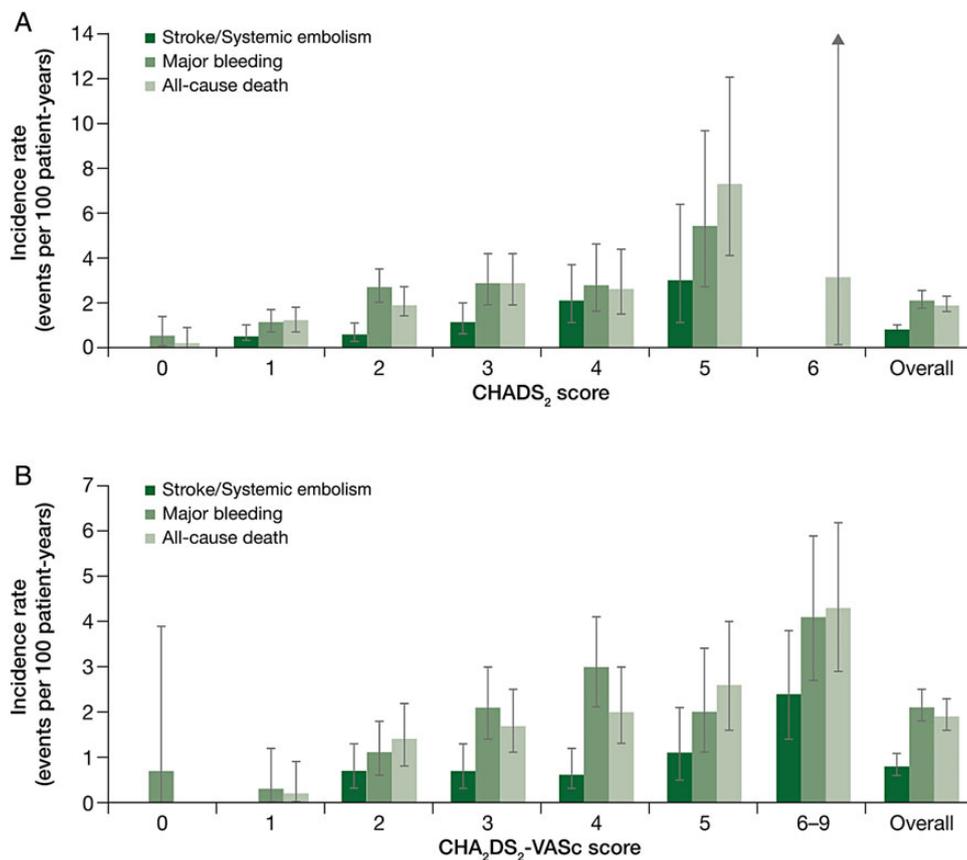


Figure 3 Outcomes as a function of (A) CHADS₂ and (B) CHA₂DS₂-VASc scores.

death were also low in this unselected patient population. The incidence rate of major bleeding was 2.1 events per 100 patient-years, which is lower than in ROCKET AF (3.6 events per 100 patient-years)⁷ and similar to that seen in a large US study of electronic medical records of 27 467 patients (2.9 events per 100 patient-years), although this study used a different bleeding definition.¹⁰ The major bleeding rate was also similar to published data from the smaller, real-world, observational Dresden NOAC Registry involving 1200 AF patients treated with rivaroxaban (3.1 events per 100 patient-years).¹¹ The incidence rates of fatal bleeding, critical organ bleeding, and intracranial haemorrhage were similar to those observed in ROCKET AF (XANTUS vs. ROCKET AF 0.2 vs. 0.2 events per 100 patient-years, 0.7 vs. 0.8 events per 100 patient-years, and 0.4 vs. 0.5 events per 100 patient-years, respectively), whereas major gastrointestinal bleeding occurred less frequently (0.9 events per 100 patient-years) than that seen in ROCKET AF (2.0 events per 100 patient-years).¹²

Throughout the study, the use of non-specific reversal agents (such as prothrombin complex concentrate) was low. This finding is in line with outcomes from ROCKET AF and the Dresden NOAC Registry,^{11,13} and suggests that these agents are rarely used in clinical practice. The lowest incidence proportion of major bleeding (0.6%) was observed in patients for whom no CrCl test results were recorded throughout the study, suggesting that laboratory testing may have been reserved for patients at higher risk,

and clinical assessment may have been judged appropriate in patients with overall acceptable health. Because this was an observational study, it is also possible that CrCl tests may have been performed but not documented. The Executive Steering Committee specifically asked all investigators in a letter to document renal function; however, it cannot be excluded that measured CrCl has not been documented and this would contribute to the missing data. In addition, major bleeding, all-cause death, and thromboembolic events (stroke, SE, TIA, and MI combined) occurred at higher incidence rates for the 15 mg od vs. the 20 mg od dose, which indicated that dosing decisions might have been based on other clinical considerations besides impaired renal function.

Drug persistence is a major concern in stroke prevention because anticoagulant discontinuation potentially leaves patients unprotected from the risk of stroke. Recent data obtained with VKAs showed discontinuation rates of 32 and 38% at 6 and 12 months, respectively.^{14,15} Available data on NOACs suggest higher persistence rates. Persistence with rivaroxaban in XANTUS was 80% at 1 year, which is higher than recent US studies^{14,15} but in line with that observed in other real-world studies such as the Dresden NOAC Registry, in which discontinuations of ~15% were recorded in the first year.¹⁶

There are some limitations to this real-world study. XANTUS was a single-arm study and, as with any open-label study, the study design can introduce bias related to knowledge about treatment.

In addition, patients agreeing to participate in the study may, to some extent, have self-selected for risk of stroke or bleeding, and conscientious participation, and a selection bias based around intact cognitive function could have arisen with the investigator. Owing to the observational design, interference with patient management, such as reinforcement of laboratory and other investigations, was not allowed. This led, for example, to a large number of patients with unknown CrCl values. Although it was possible to assess persistence, there was no possibility to assess drug adherence in a standardized fashion in an observational study. Finally, outcomes per rivaroxaban dose were not adjusted for baseline risk factors for this analysis.

Strengths of this study include its meaningful sample size and a prospective design allowing for greater completeness of data and potentially better data quality compared with retrospective designs. The independent endpoint adjudication is expected to have reduced reporting bias.

Conclusion

XANTUS is the first large, international, prospective study describing the use of rivaroxaban for stroke prevention in a broad NVAF patient population. The rates of major bleeding and stroke with rivaroxaban were found to be low in routine clinical practice.

Authors' contributions

S.K.: performed statistical analysis; S.Ha., M.v.E., A.J.C., P.K., S.He., A.G.G.T., and P.A.: conceived and designed the research; A.J.C., P.K., and S.He.: drafted the manuscript; S.Ha., A.G.G.T., P.A., and M.v.E.: made critical revision of the manuscript for key intellectual content.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: A.J.C. has served as a consultant for AstraZeneca, Bayer HealthCare, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, Pfizer, Sanofi, Aryx, and Johnson & Johnson. P.A. has served as a consultant for Bayer HealthCare Pharmaceuticals, Bristol-Myers Squibb, Pfizer, Boehringer Ingelheim, Daiichi Sankyo, AstraZeneca, Sanofi, Boston Scientific, Edwards, Lundbeck, Merck, and Kowa Pharmaceutical.

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ORIGINAL ARTICLE

Five-Year Outcomes after PCI or CABG for Left Main Coronary Disease

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ABSTRACT

BACKGROUND

Long-term outcomes after percutaneous coronary intervention (PCI) with contemporary drug-eluting stents, as compared with coronary-artery bypass grafting (CABG), in patients with left main coronary artery disease are not clearly established.

METHODS

We randomly assigned 1905 patients with left main coronary artery disease of low or intermediate anatomical complexity (according to assessment at the participating centers) to undergo either PCI with fluoropolymer-based cobalt–chromium everolimus-eluting stents (PCI group, 948 patients) or CABG (CABG group, 957 patients). The primary outcome was a composite of death, stroke, or myocardial infarction.

RESULTS

At 5 years, a primary outcome event had occurred in 22.0% of the patients in the PCI group and in 19.2% of the patients in the CABG group (difference, 2.8 percentage points; 95% confidence interval [CI], –0.9 to 6.5; $P=0.13$). Death from any cause occurred more frequently in the PCI group than in the CABG group (in 13.0% vs. 9.9%; difference, 3.1 percentage points; 95% CI, 0.2 to 6.1). In the PCI and CABG groups, the incidences of definite cardiovascular death (5.0% and 4.5%, respectively; difference, 0.5 percentage points; 95% CI, –1.4 to 2.5) and myocardial infarction (10.6% and 9.1%; difference, 1.4 percentage points; 95% CI, –1.3 to 4.2) were not significantly different. All cerebrovascular events were less frequent after PCI than after CABG (3.3% vs. 5.2%; difference, –1.9 percentage points; 95% CI, –3.8 to 0), although the incidence of stroke was not significantly different between the two groups (2.9% and 3.7%; difference, –0.8 percentage points; 95% CI, –2.4 to 0.9). Ischemia-driven revascularization was more frequent after PCI than after CABG (16.9% vs. 10.0%; difference, 6.9 percentage points; 95% CI, 3.7 to 10.0).

CONCLUSIONS

In patients with left main coronary artery disease of low or intermediate anatomical complexity, there was no significant difference between PCI and CABG with respect to the rate of the composite outcome of death, stroke, or myocardial infarction at 5 years. (Funded by Abbott Vascular; EXCEL ClinicalTrials.gov number, NCT01205776.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Stone at the Cardiovascular Research Foundation, 1700 Broadway, 8th Fl., New York, NY 10019, or at gstone@crf.org.

*A complete list of investigators, institutions, and research organizations participating in the EXCEL trial is provided in the Supplementary Appendix, available at NEJM.org.

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PERCUTANEOUS CORONARY INTERVENTION (PCI) with drug-eluting stents has emerged as an acceptable treatment for selected patients with left main coronary artery disease.¹⁻⁸ However, long-term data from randomized trials comparing PCI involving contemporary drug-eluting stents with coronary-artery bypass grafting (CABG) in these patients are lacking. In the Evaluation of XIENCE versus Coronary Artery Bypass Surgery for Effectiveness of Left Main Revascularization (EXCEL) trial,² which involved patients with left main coronary artery disease of low or intermediate anatomical complexity, PCI with everolimus-eluting stents was noninferior to CABG with respect to the primary composite outcome measure of death, stroke, or myocardial infarction at a median 3-year follow-up. However, although the incidence of periprocedural adverse events (within 30 days) was lower in the PCI group, patients in the CABG group had fewer adverse events between 30 days and 3 years after the procedure. To further characterize the long-term outcomes of PCI as compared with CABG in patients with left main coronary artery disease, we report the final 5-year outcomes from this trial.

METHODS

TRIAL DESIGN

The trial design has been described previously.^{2,9} In brief, we performed an international, open-label, multicenter, randomized trial that compared PCI involving thin-strut cobalt–chromium fluoropolymer-based everolimus-eluting stents (XIENCE, Abbott Vascular) with CABG in patients with left main coronary artery disease. The organization of the trial is described and participating centers are listed in the Supplementary Appendix, available with the full text of this article at NEJM.org. The protocol, also available at NEJM.org, was designed by the principal investigators and trial committees, in which interventional cardiologists and cardiac surgeons were represented equally. The trial was approved by the investigational review board or ethics committee at each participating center, and written informed consent was obtained from all the patients. The trial was sponsored by Abbott Vascular, which participated in the design of the protocol and in the selection and management of the sites but

was not involved in the management or analysis of the data or preparation of the manuscript, although it had the right to a nonbinding review of the manuscript. The principal investigators had unrestricted access to the data, prepared the manuscript, and vouch for the completeness and accuracy of the data and analyses and for the fidelity of the trial to the protocol.

ENROLLMENT, RANDOMIZATION, AND FOLLOW-UP

Patients were eligible to participate in the trial if they had stenosis of the left main coronary artery of 70% or more (as estimated visually) or stenosis of 50% to less than 70% (if determined by means of noninvasive or invasive testing to be hemodynamically significant) and if a consensus among the members of the heart team had been reached regarding eligibility for revascularization with either PCI or CABG (Table S1 in the Supplementary Appendix).^{2,9} In addition, eligible patients had low or intermediate anatomical complexity of coronary artery disease, as assessed at the participating center and defined by a Synergy between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery (SYNTAX) score of 32 or less.¹⁰ The SYNTAX score reflects a comprehensive angiographic assessment of the coronary vasculature, with 0 as the lowest score, and higher scores (no upper limit) indicating more complex coronary anatomy.

Randomization was performed with the use of an interactive voice-based or Web-based system in block sizes of 16, 24, or 32, with stratification according to the presence or absence of diabetes, SYNTAX score, and trial center. Clinical follow-up was performed at 1 month, 6 months, and 1 year and then annually through 5 years. Guideline-directed medical therapy and management of risk factors were recommended for all the patients, as previously described.⁹ Dual antiplatelet therapy was administered before PCI and for a minimum of 1 year thereafter. Aspirin was administered before and after CABG, and the use of clopidogrel during follow-up was allowed but not mandatory. Routine angiographic follow-up was not permitted.

OUTCOMES

The primary outcome was the composite of death from any cause, stroke, or myocardial infarction at 3 years. Major secondary outcomes included

the primary outcome measure at 30 days and the composite of death, stroke, myocardial infarction, or ischemia-driven revascularization at 3 years. The cause of death was adjudicated as definite cardiovascular, definite noncardiovascular, or undetermined, and undetermined cases were conservatively classified as cardiovascular. Long-term additional secondary outcomes included these measures and their components at 5 years, as well as therapy failure (definite stent thrombosis or symptomatic graft stenosis or occlusion), all revascularizations, and all cerebrovascular events (stroke or transient ischemic attack). Outcomes are defined in Table S2. Trial monitors collected source documents of all primary and secondary outcome events for adjudication by an independent events committee. The extent of disease and SYNTAX score were assessed at an angiographic core laboratory.

STATISTICAL ANALYSIS

The trial was powered to show the noninferiority of PCI to CABG with respect to the primary outcome at 3 years.^{2,9} The 5-year secondary outcomes were prespecified but were not explicitly powered or adjusted for multiple comparisons. The principal analyses were performed in the intention-to-treat population, which included all patients according to the group to which they were randomly assigned, regardless of the treatment received. Sensitivity analyses were performed in the per-protocol and as-treated populations,⁹ with multiple imputation to account for missing follow-up data.¹¹

Event rates were based on Kaplan–Meier estimates in time-to-first-event analyses. However, the underlying assumption of proportional hazards in the Cox model for the primary and major secondary outcomes from randomization through 5 years was not met (treatment–time interaction, $P < 0.001$). Principal comparisons between treatments were therefore performed by logistic regression with follow-up time included as a log-transformed offset variable (no other covariates were included), with the use of an estimated standard error for the difference. In a post hoc analysis, we also evaluated piecewise hazards models separately within 0 to 30 days (the periprocedural period), 30 days to 1 year (the major risk period for stent restenosis), and 1 year to 5 years (long-term follow-up) — intervals during

which proportional hazards were preserved. Given the presence of nonproportional hazards, net treatment effects were also examined with the use of post hoc milestone and restricted mean survival time analyses (Table S3). For milestone analysis, the percentage of patients with an event in each group was estimated with the Kaplan–Meier method, and Greenwood’s formula was used to estimate standard errors. The difference between groups in milestone event rates that occurred each day during the 5-year follow-up period is reported. Restricted mean event-free survival time is the mean time free from an outcome event adjusted for loss to follow-up, reflecting the area under the survival curve.¹² The difference between groups in the restricted mean survival time over the 5-year follow-up period is reported.

Categorical variables were compared with the use of the chi-square test or Fisher’s exact test. Continuous variables were compared with the use of Student’s *t*-test or the Wilcoxon rank-sum test for non-normally distributed data. For superiority, a two-sided *P* value of less than 0.05 was considered to indicate statistical significance. The 95% confidence intervals for secondary outcomes were not adjusted for multiple comparisons, and therefore inferences drawn from these intervals may not be reproducible. All statistical analyses were performed with the use of SAS software, version 9.4 (SAS Institute).

RESULTS

PATIENTS AND PROCEDURES

From September 29, 2010, to March 6, 2014, a total of 1905 patients with left main coronary artery disease were randomly assigned at 126 sites in 17 countries to PCI (948 patients) or CABG (957 patients) (Fig. S1). Baseline clinical and angiographic characteristics were well balanced between the groups (Tables S4 and S5). The mean (\pm SD) age of the patients was 66.0 ± 9.6 years, 76.9% of patients were male, and 29.1% had diabetes. The mean SYNTAX score was 20.6 ± 6.2 according to assessment at local sites and 26.5 ± 9.3 according to the angiographic core laboratory analysis, and 80.5% of the patients had distal left main bifurcation disease. Procedural data are shown in Table S6. Adherence to guideline-directed medical therapy was high,

Table 1. Primary and Secondary Clinical Outcomes at 5 Years.*

Outcome	PCI (N=948)		CABG (N=957)		Difference in Event Rates (95% CI) percentage points	Odds Ratio (95% CI)
	Events	Event Rate	Events	Event Rate		
	no.	%	no.	%		
Primary outcome						
Death, stroke, or myocardial infarction	203	22.0	176	19.2	2.8 (−0.9 to 6.5)	1.19 (0.95 to 1.50)
Secondary outcomes						
Death, stroke, myocardial infarction, or ischemia-driven revascularization	290	31.3	228	24.9	6.5 (2.4 to 10.6)	1.39 (1.13 to 1.71)
Death from any cause	119	13.0	89	9.9	3.1 (0.2 to 6.1)	1.38 (1.03 to 1.85)
Cardiovascular	61	6.8	49	5.5	1.3 (−0.9 to 3.6)	1.26 (0.85 to 1.85)
Definite cardiovascular	45	5.0	40	4.5	0.5 (−1.4 to 2.5)	1.13 (0.73 to 1.74)
Undetermined cause	16	1.9	9	1.1	0.9 (−0.3 to 2.0)	1.78 (0.78 to 4.06)
Noncardiovascular	58	6.6	40	4.6	2.0 (−0.2 to 4.2)	1.47 (0.97 to 2.23)
Stroke	26	2.9	33	3.7	−0.8 (−2.4 to 0.9)	0.78 (0.46 to 1.31)
Myocardial infarction	95	10.6	84	9.1	1.4 (−1.3 to 4.2)	1.14 (0.84 to 1.55)
Periprocedural	37	3.9	57	6.1	−2.1 (−4.1 to −0.1)	0.63 (0.41 to 0.96)
Nonperiprocedural	59	6.8	31	3.5	3.2 (1.2 to 5.3)	1.96 (1.25 to 3.06)
Ischemia-driven revascularization	150	16.9	88	10.0	6.9 (3.7 to 10.0)	1.84 (1.39 to 2.44)
PCI	125	14.1	80	9.1	4.9 (1.9 to 7.9)	1.65 (1.22 to 2.22)
CABG	38	4.3	8	0.9	3.4 (1.9 to 4.9)	4.90 (2.27 to 10.56)
Additional outcomes						
Any revascularization	153	17.2	92	10.5	6.7 (3.5 to 9.9)	1.79 (1.36 to 2.36)
Stent thrombosis	16	1.8	0	0	—	—
Definite	10	1.1	0	0	—	—
Probable	6	0.7	0	0	—	—
Symptomatic graft stenosis or occlusion	0	0	58	6.5	—	—
Therapy failure†	10	1.1	58	6.5	−5.4 (−7.2 to −3.6)	0.16 (0.08 to 0.32)
Cerebrovascular events‡	29	3.3	46	5.2	−1.9 (−3.8 to 0)	0.61 (0.38 to 0.99)
Transient ischemic attack	3	0.3	14	1.6	−1.3 (−2.2 to −0.4)	0.21 (0.06 to 0.74)

* Event rates were based on Kaplan–Meier estimates in time-to-first-event analyses; thus, the rate is not the same as the ratio of the numerator and denominator. Odds ratios and 95% confidence intervals were estimated from logistic regression with follow-up time included as a log-transformed offset variable. The 95% confidence intervals for secondary outcomes have not been adjusted for multiple comparisons, and therefore inferences drawn from these intervals may not be reproducible. CABG denotes coronary-artery bypass grafting, and PCI percutaneous coronary intervention.

† Therapy failure was defined as definite stent thrombosis or symptomatic graft stenosis or occlusion.

‡ Cerebrovascular events were stroke or transient ischemic attack.

although medication use differed between the groups during follow-up (Table S7).

PRIMARY OUTCOME

Five-year follow-up was achieved in 93.2% and 90.1% of the PCI and CABG groups, respectively.

The primary composite of death, stroke, or myocardial infarction at 5 years occurred in 22.0% of the patients in the PCI group and 19.2% of the patients in the CABG group (difference, 2.8 percentage points; 95% confidence interval [CI], −0.9 to 6.5; $P=0.13$) (Table 1 and Fig. 1A). The

hazard ratios (PCI vs. CABG) for the primary outcome varied in the three periods between 0 to 30 days (hazard ratio, 0.61; 95% CI, 0.42 to 0.88), 30 days to 1 year (hazard ratio, 1.07; 95% CI, 0.68 to 1.70), and 1 year to 5 years (hazard ratio, 1.61; 95% CI, 1.23 to 2.12) (Table 2 and Fig. S2). Analyses of milestones and restricted mean survival time showed that the early benefit of PCI was gradually diminished over time by increased postprocedural risk (Fig. S3). Mean event-free survival through 5 years was 5.2 days (95% CI, -46.1 to 56.5) longer after PCI than after CABG. The treatment effect for the primary outcome in prespecified subgroups is shown in Figure 2. Results were similar in the per-protocol and as-treated populations and after multiple imputation accounting for missing follow-up data (Tables S8 and S9).

SECONDARY OUTCOMES

The secondary composite outcome of death, stroke, myocardial infarction, or ischemia-driven revascularization at 5 years occurred in 31.3% of the patients in the PCI group and 24.9% of the patients in the CABG group (difference, 6.5 percentage points; 95% CI, 2.4 to 10.6) (Table 1 and Fig. 1B). The incidences of the individual components of the primary and secondary composite outcomes are shown in Table 1 and Figure 3. Death from any cause occurred in 13.0% of the patients in the PCI group and 9.9% of the patients in the CABG group (difference, 3.1 percentage points; 95% CI, 0.2 to 6.1). Eighteen of the 30 excess deaths in the PCI group were adjudicated as noncardiovascular deaths, 5 as definite cardiovascular deaths, and 7 as being of undetermined cause (Table S10). The results were similar after accounting for patients who were lost to follow-up (Table S9). The incidences of stroke and myocardial infarction at 5 years did not differ significantly between the PCI group and the CABG group. Ischemia-driven revascularization within 5 years was performed more frequently after PCI than after CABG, whereas the incidences of all cerebrovascular events and definite stent thrombosis or symptomatic graft stenosis or occlusion at 5 years were less frequent with PCI than with CABG. The hazard ratios for the secondary outcomes between 0 to 30 days, 30 days to 1 year, and 1 year to 5 years are provided in Table 2.

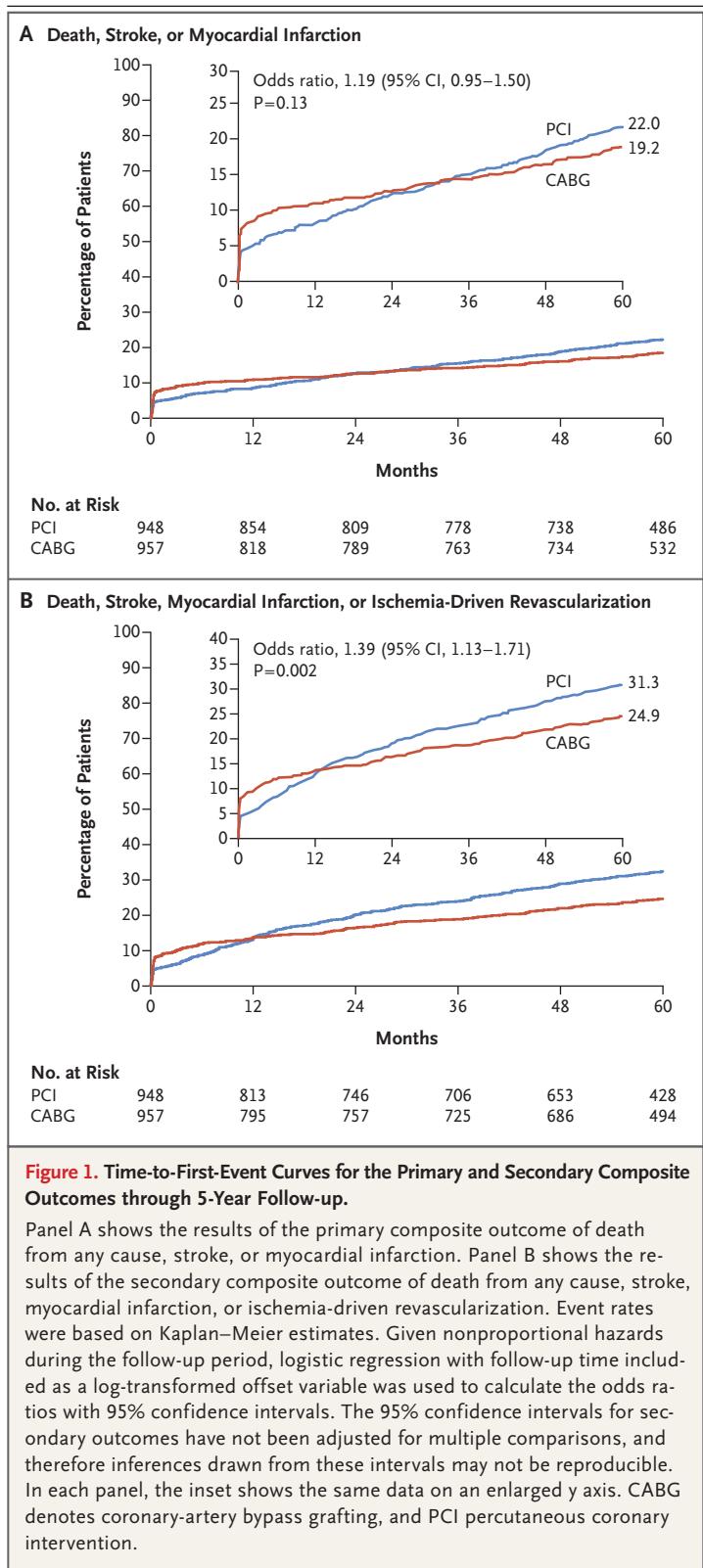


Figure 1. Time-to-First-Event Curves for the Primary and Secondary Composite Outcomes through 5-Year Follow-up.

Panel A shows the results of the primary composite outcome of death from any cause, stroke, or myocardial infarction. Panel B shows the results of the secondary composite outcome of death from any cause, stroke, myocardial infarction, or ischemia-driven revascularization. Event rates were based on Kaplan–Meier estimates. Given nonproportional hazards during the follow-up period, logistic regression with follow-up time included as a log-transformed offset variable was used to calculate the odds ratios with 95% confidence intervals. The 95% confidence intervals for secondary outcomes have not been adjusted for multiple comparisons, and therefore inferences drawn from these intervals may not be reproducible. In each panel, the inset shows the same data on an enlarged y axis. CABG denotes coronary-artery bypass grafting, and PCI percutaneous coronary intervention.

Table 2. Primary and Secondary Outcomes over Three Periods.*

Variable	PCI		CABG		Hazard Ratio (95% CI)
	Events	Event Rate	Events	Event Rate	
	no./no. of patients	%	no./no. of patients	%	
Outcomes at 30 days					
Death, stroke, or myocardial infarction	46/948	4.9	75/957	8.0	0.61 (0.42–0.88)
Death	9/948	1.0	10/957	1.1	0.90 (0.37–2.21)
Stroke	6/948	0.6	12/957	1.3	0.50 (0.19–1.32)
Myocardial infarction	37/948	3.9	59/957	6.3	0.63 (0.42–0.94)
Death, stroke, myocardial infarction, or ischemia-driven revascularization	46/948	4.9	80/957	8.5	0.57 (0.40–0.82)
Ischemia-driven revascularization	6/948	0.6	13/957	1.4	0.46 (0.17–1.21)
Definite stent thrombosis or symptomatic graft stenosis or occlusion	3/948	0.3	11/957	1.2	0.27 (0.08–0.97)
Outcomes from 30 days to 1 yr					
Death, stroke, or myocardial infarction	38/948	4.1	35/957	3.8	1.07 (0.68–1.70)
Death	22/948	2.4	23/957	2.5	0.94 (0.53–1.69)
Stroke	5/948	0.5	7/957	0.8	0.71 (0.22–2.23)
Myocardial infarction	16/948	1.7	10/957	1.1	1.58 (0.72–3.48)
Death, stroke, myocardial infarction, or ischemia-driven revascularization	83/948	8.9	56/957	6.1	1.48 (1.05–2.07)
Ischemia-driven revascularization	59/948	6.4	28/957	3.1	2.10 (1.34–3.30)
Definite stent thrombosis or symptomatic graft stenosis or occlusion	0/948	0	22/957	2.4	—
Outcomes from 1 yr to 5 yr					
Death, stroke, or myocardial infarction	133/933	15.1	83/929	9.7	1.61 (1.23–2.12)
Death	88/933	10.0	56/929	6.6	1.57 (1.12–2.19)
Stroke	16/933	1.9	15/929	1.8	1.06 (0.52–2.15)
Myocardial infarction	43/933	5.1	20/929	2.4	2.16 (1.27–3.67)
Death, stroke, myocardial infarction, or ischemia-driven revascularization	198/933	22.4	118/929	13.8	1.74 (1.38–2.18)
Ischemia-driven revascularization	100/933	11.6	49/929	5.8	2.10 (1.49–2.95)
Definite stent thrombosis or symptomatic graft stenosis or occlusion	7/933	0.8	25/929	3.0	0.28 (0.12–0.64)

* Event rates were based on Kaplan–Meier estimates in time-to-first-event analyses; thus, the rate is not the same as the ratio of the numerator and denominator. The landmark period from 30 days to 5 years includes all randomly assigned patients at day 30 except those who died before day 30. Thus, some patients with a stroke, myocardial infarction, or ischemia-driven revascularization within 30 days may have had a second event between 30 days and 5 years. The 95% confidence intervals for secondary outcomes have not been adjusted for multiple comparisons, and therefore inferences drawn from these intervals may not be reproducible.

DISCUSSION

Patients with left main coronary artery disease have a poor prognosis because of the large amount of myocardium at risk.¹³ Survival among patients with left main coronary artery disease is longer after revascularization with either PCI

or CABG than with medical therapy alone.¹⁴ In six randomized trials involving patients with left main coronary artery disease, PCI with drug-eluting stents was associated with more favorable outcomes at 1 year than CABG, with fewer periprocedural adverse events and more rapid recovery.^{3,4,15} Conversely, conflicting long-term

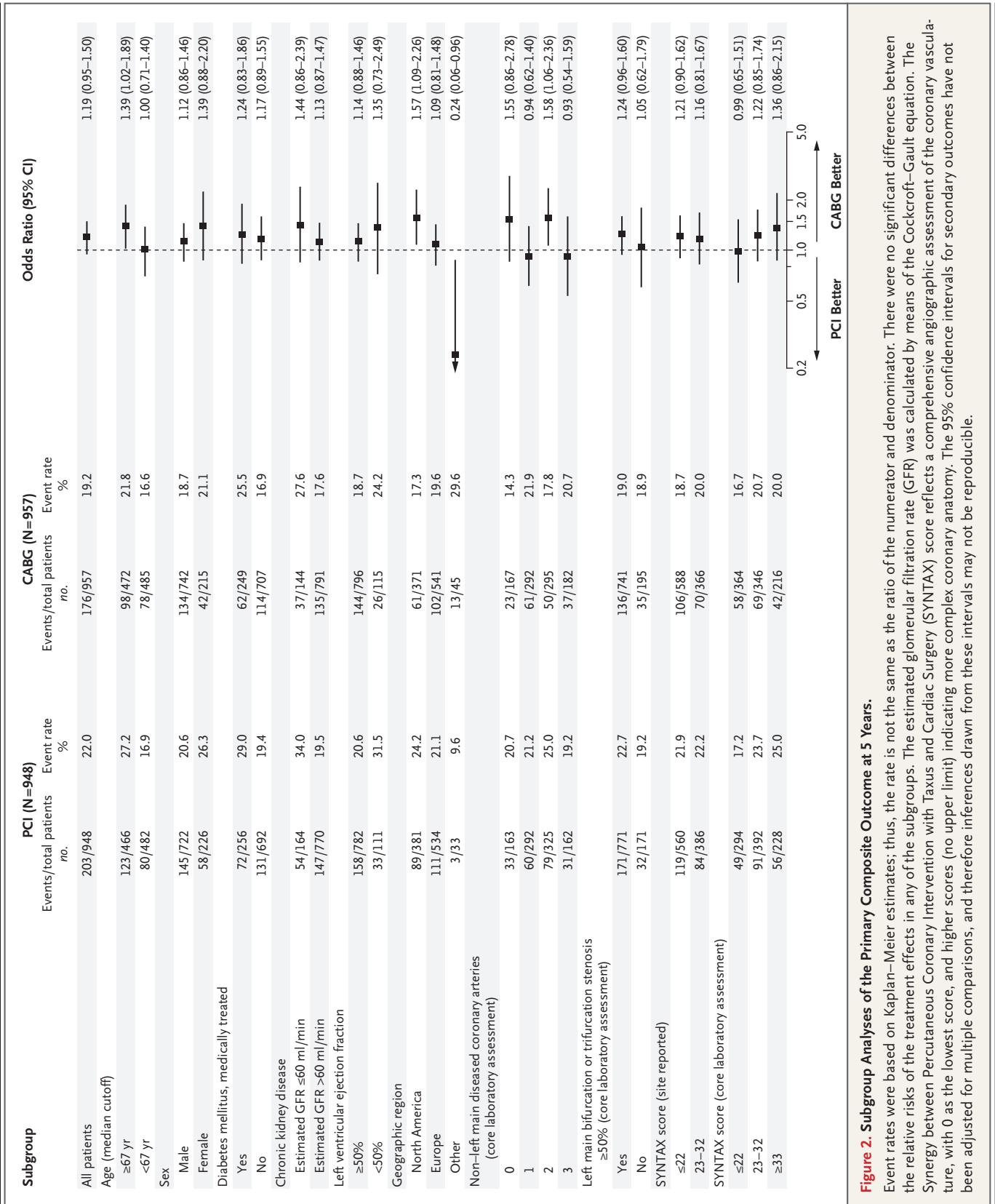
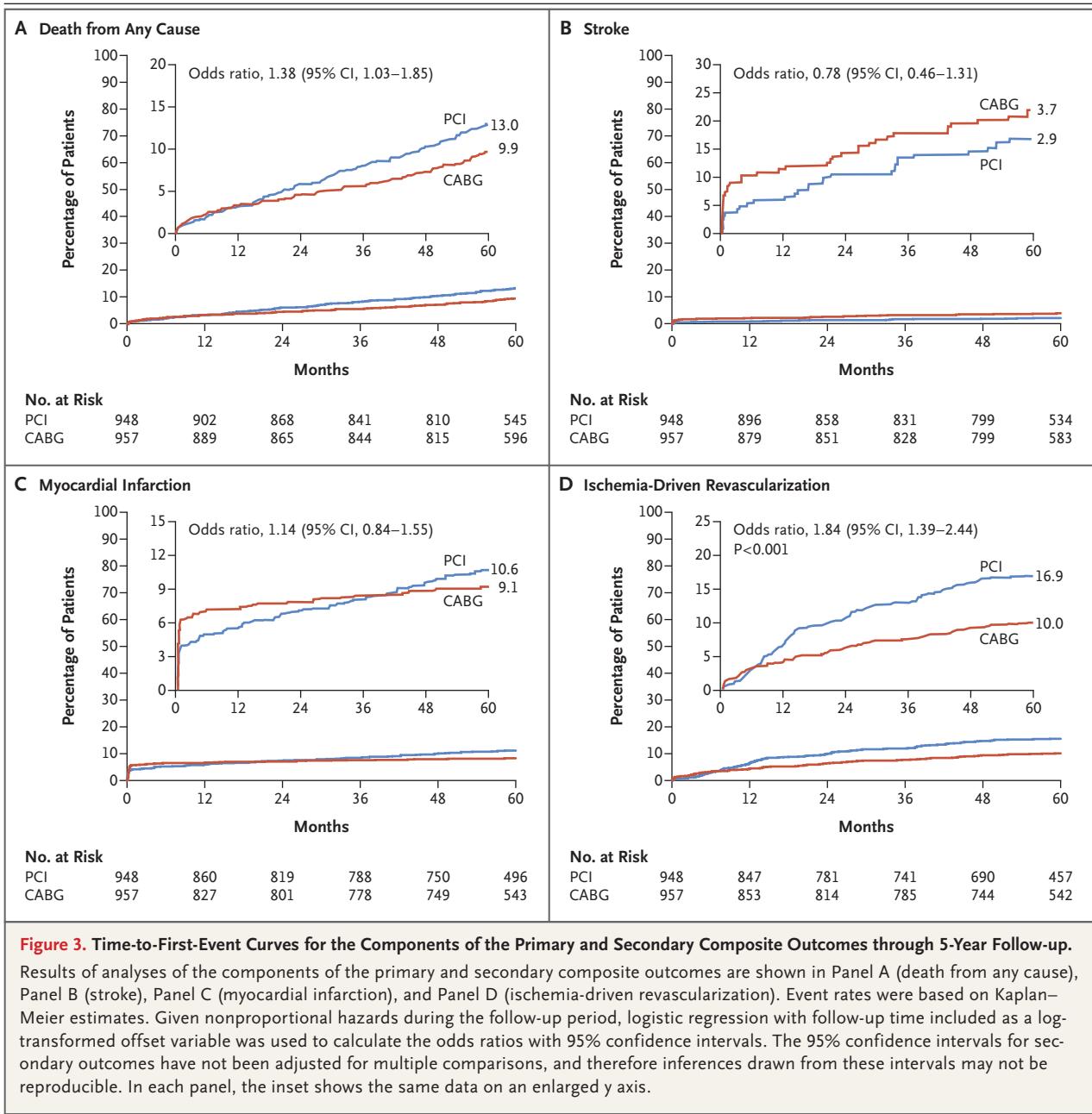


Figure 2. Subgroup Analyses of the Primary Composite Outcome at 5 Years.

Event rates were based on Kaplan–Meier estimates; thus, the rate is not the same as the ratio of the numerator and denominator. There were no significant differences between the relative risks of the treatment effects in any of the subgroups. The estimated glomerular filtration rate (GFR) was calculated by means of the Cockcroft–Gault equation. The Synergy between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery (SYNTAX) score reflects a comprehensive angiographic assessment of the coronary vasculature, with 0 as the lowest score, and higher scores (no upper limit) indicating more complex coronary anatomy. The 95% confidence intervals for secondary outcomes have not been adjusted for multiple comparisons, and therefore inferences drawn from these intervals may not be reproducible.



findings from these trials have been reported.^{1,3–5} However, to achieve adequate power, these trials relied on differences in the incidence of repeat revascularization, an outcome of lesser importance to physicians and patients than death, stroke, and myocardial infarction.¹⁶ The degree of deterioration in health status that triggers repeat revascularization is also greater after CABG than after PCI; this calls into question the parity of this outcome.^{17,18} In addition, previous

trials did not use contemporary drug-eluting stents, which have a better safety profile than that of earlier devices.

The present trial was powered to examine the composite rate of death, stroke, or myocardial infarction. Current-generation fluoropolymer-based thin-strut cobalt–chromium everolimus-eluting stents, which are associated with a low incidence of stent thrombosis, were used,^{19,20} and contemporary CABG techniques were incorpo-

rated.²⁹ We did not detect a significant difference in the composite rate of death, stroke, or myocardial infarction at 5 years between patients with left main coronary artery disease and low or intermediate anatomical complexity (as defined by a site-reported SYNTAX score of ≤ 32) who underwent PCI and those who underwent CABG. This finding was consistent across important subgroups, including patients with diabetes and those without diabetes and patients with lower and higher SYNTAX scores. However, interpreting these results as showing no difference between treatments is simplistic. As shown by the piecewise hazards analysis, three distinct periods of relative risk were present: from 0 to 30 days, when PCI resulted in fewer primary outcome events than CABG; from 30 days to 1 year, when the incidence of the events was similar among patients in each treatment group; and from 1 to 5 years, when primary outcome events were less common after CABG than after PCI. Consideration of the differential timing of risk is clinically relevant, since earlier exposure to adverse events has a more profound influence on the long-term burden of disease than exposure to events occurring later. As shown by the analysis of milestones and restricted mean survival time, by 5 years, the early benefit of PCI due to reduced periprocedural risk was attenuated by the greater number of events that occurred during follow-up than with CABG, such that the cumulative mean time free from adverse events was similar in the two treatment groups.

There were numerical differences between PCI and CABG in several nonpowered secondary outcomes. The event rates of death from any cause (a 3.1-percentage-point difference between the groups) and repeat revascularization (a 6.9-percentage-point difference) favored CABG, whereas event rates of cerebrovascular events (a 1.9-percentage-point difference) and therapy failure (a 5.4-percentage-point difference) favored PCI. Rates of myocardial infarction at 5 years were similar in the two groups, but they favored PCI in the periprocedural period and CABG during long-term follow-up. Although some of these findings may indicate true treatment effects, they must be interpreted cautiously, since more than 20 secondary outcomes were assessed and analyses were not adjusted for multiple comparisons. Nonetheless, several findings warrant comment.

Although the cause of death can sometimes be ambiguous, rates of adjudicated definite cardiovascular death were similar among patients who underwent PCI and those who underwent CABG, consistent with the similar rates of myocardial infarction at 5 years. The difference in all-cause mortality between the groups was driven by noncardiovascular deaths, especially those from cancer and infection, which occurred more commonly after PCI during late follow-up. The finding of a possible excess of deaths from any cause after PCI is at odds with the similar rates of death at 5 years among patients who underwent PCI and among those who underwent CABG in the contemporary Nordic–Baltic–British Left Main Revascularization (NOBLE) trial,³ an individual patient-data pooled analysis of six randomized trials involving 4478 patients with left main coronary artery disease, and in other meta-analyses^{4,21} and with the similar mortality at 10 years after PCI and CABG among patients with left main coronary artery disease in the SYNTAX trial.²²

Whereas previous studies have shown higher rates of stroke after CABG than after PCI,²³ the excess of cerebrovascular events after CABG in the present trial was driven more by transient ischemic attacks than by strokes. The greater observed incidence of repeat revascularization after the use of drug-eluting stents than after CABG is consistent with previous analyses, but most revascularization events were repeat PCI procedures; only 1 of 25 patients initially treated with everolimus-eluting stents underwent CABG within 5 years. Nonetheless, repeat revascularization procedures may be associated with myocardial infarction and death.²⁴ These considerations notwithstanding, the absolute 5-year differences between the groups with respect to all the secondary outcomes were relatively modest, and some may have been due to chance. This perspective should be considered in discussions between the heart team and the patient when weighing the pros and cons of the different therapies.²⁵

Additional limitations of this trial should be considered. First, bias in event ascertainment cannot be ruled out given the open-label trial design. Second, although the trial excluded patients with high SYNTAX scores, approximately 25% of the patients met this criterion according to the core laboratory analysis. Although the primary outcome results were consistent in this

subgroup, further studies are needed to determine the most appropriate treatment for patients with left main coronary artery disease and high anatomical complexity. Third, a specific biomarker-based definition of large periprocedural myocardial infarction was used in the present trial^{2,6}; this definition differs from the criteria used in the Third Universal Definition of Myocardial Infarction²⁶ (which was developed while the current trial was ongoing) and the Fourth Universal Definition of Myocardial Infarction²⁷ (which was developed subsequently). The definition used in the EXCEL trial is based on previously established criteria that have been shown to be prognostically relevant after PCI and after CABG and that minimize ascertainment bias.²⁸ As previously reported, the occurrence of periprocedural myocardial infarction according to this protocol definition was independently predictive of late death from cardiovascular causes and death from any cause after PCI and after CABG, whereas lesser degrees of elevated levels of biomarkers were not.²⁹ Fourth, patients who underwent PCI, as compared with those who underwent CABG, more commonly received dual antiplatelet therapy and inhibitors of the renin-angiotensin axis during follow-up, whereas pa-

tients who underwent CABG more commonly received oral anticoagulants, beta-blockers, diuretics, and antiarrhythmic agents; these differences reflect inherent differences between the procedures and their resulting complications. The extent to which variability in medication use contributed to the present results is uncertain. Finally, follow-up was limited to 5 years, and at this time point the hazard curves were continuing to diverge. Ten-year (or longer) follow-up is needed to characterize the very late safety profiles of PCI and CABG, since both stents and bypass grafts progressively fail over time.

In conclusion, in the present trial we did not find a significant difference between PCI and CABG with respect to rates of the composite outcome of death, stroke, or myocardial infarction at 5 years among patients with left main coronary artery disease and low or intermediate anatomical complexity (as defined by the SYNTAX score) according to assessment at the participating centers.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

APPENDIX

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Transcatheter Aortic-Valve Replacement with a Balloon-Expandable Valve in Low-Risk Patients

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ABSTRACT

BACKGROUND

Among patients with aortic stenosis who are at intermediate or high risk for death with surgery, major outcomes are similar with transcatheter aortic-valve replacement (TAVR) and surgical aortic-valve replacement. There is insufficient evidence regarding the comparison of the two procedures in patients who are at low risk.

METHODS

We randomly assigned patients with severe aortic stenosis and low surgical risk to undergo either TAVR with transfemoral placement of a balloon-expandable valve or surgery. The primary end point was a composite of death, stroke, or rehospitalization at 1 year. Both noninferiority testing (with a prespecified margin of 6 percentage points) and superiority testing were performed in the as-treated population.

RESULTS

At 71 centers, 1000 patients underwent randomization. The mean age of the patients was 73 years, and the mean Society of Thoracic Surgeons risk score was 1.9% (with scores ranging from 0 to 100% and higher scores indicating a greater risk of death within 30 days after the procedure). The Kaplan–Meier estimate of the rate of the primary composite end point at 1 year was significantly lower in the TAVR group than in the surgery group (8.5% vs. 15.1%; absolute difference, –6.6 percentage points; 95% confidence interval [CI], –10.8 to –2.5; $P < 0.001$ for noninferiority; hazard ratio, 0.54; 95% CI, 0.37 to 0.79; $P = 0.001$ for superiority). At 30 days, TAVR resulted in a lower rate of stroke than surgery ($P = 0.02$) and in lower rates of death or stroke ($P = 0.01$) and new-onset atrial fibrillation ($P < 0.001$). TAVR also resulted in a shorter index hospitalization than surgery ($P < 0.001$) and in a lower risk of a poor treatment outcome (death or a low Kansas City Cardiomyopathy Questionnaire score) at 30 days ($P < 0.001$). There were no significant between-group differences in major vascular complications, new permanent pacemaker insertions, or moderate or severe paravalvular regurgitation.

CONCLUSIONS

Among patients with severe aortic stenosis who were at low surgical risk, the rate of the composite of death, stroke, or rehospitalization at 1 year was significantly lower with TAVR than with surgery. (Funded by Edwards Lifesciences; PARTNER 3 ClinicalTrials.gov number, NCT02675114.)

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THE ROLE OF TRANSCATHETER AORTIC-valve replacement (TAVR) in the treatment of patients with severe, symptomatic aortic stenosis has evolved on the basis of evidence from clinical trials.¹⁻¹¹ Previous randomized trials of TAVR with both balloon-expandable valves¹⁻⁷ and self-expanding valves⁸⁻¹¹ showed that, in patients who were at intermediate or high risk for death with surgery, TAVR was either superior or noninferior to standard therapies, including surgical aortic-valve replacement; these results led to an expansion of guideline recommendations for TAVR.^{12,13} Moreover, technological enhancements and procedural simplification have contributed to increased use of TAVR, such that more patients now undergo TAVR than isolated surgery for aortic-valve replacement in the United States.¹⁴ However, most patients with severe aortic stenosis are at low surgical risk,¹⁵ and there is insufficient evidence regarding the comparison of TAVR with surgery in such patients.^{16,17} We report the findings of the Placement of Aortic Transcatheter Valves (PARTNER) 3 trial, in which TAVR was compared with surgery in low-risk patients.

METHODS

TRIAL DESIGN AND OVERSIGHT

The PARTNER 3 trial was a multicenter, randomized trial in which TAVR with transfemoral placement of a third-generation balloon-expandable valve was compared with standard surgical aortic-valve replacement in patients with severe aortic stenosis and a low risk of death with surgery. A list of participating sites and investigators is provided in the Supplementary Appendix, available with the full text of this article at NEJM.org. The trial protocol, available at NEJM.org, was designed by the trial sponsor (Edwards Lifesciences) and the steering committee, with guidance from the Food and Drug Administration. The protocol was approved by the institutional review board at each site. The sponsor funded all trial-related activities and participated in site selection, data collection and monitoring, and statistical analysis. The principal investigators (the first two authors) and steering committee monitored all aspects of trial conduct. The principal investigators had unrestricted access to the data, prepared all drafts of the manuscript, and vouch for the completeness and accuracy of the data and analyses and the fidelity of the trial to the protocol. De-

tails regarding the trial design and administrative data are provided in Sections A and B and Figure S1 in the Supplementary Appendix.

PATIENTS

Patients were eligible for inclusion in the trial if they had severe calcific aortic stenosis and were considered to be at low surgical risk according to the results of clinical and anatomical assessment, including a Society of Thoracic Surgeons Predicted Risk of Mortality (STS-PROM) score of less than 4% (with scores ranging from 0 to 100% and higher scores indicating a greater risk of death within 30 days after the procedure) and agreement by the site heart team and the trial case review committee. Patients had to be eligible for TAVR with transfemoral placement of the balloon-expandable SAPIEN 3 system (Edwards Lifesciences). Patients with clinical frailty (as determined by the heart team), bicuspid aortic valves, or other anatomical features that increased the risk of complications associated with either TAVR or surgery were excluded. Details regarding inclusion and exclusion criteria are provided in Section C in the Supplementary Appendix. All the patients provided written informed consent.

RANDOMIZATION AND PROCEDURES

Eligible patients were randomly assigned, in a 1:1 ratio, to undergo either TAVR with the SAPIEN 3 system or surgical aortic-valve replacement with a commercially available bioprosthetic valve. Randomization was conducted with the use of an electronic system, with block sizes of four, and was stratified according to site.

The SAPIEN 3 system and the procedures for TAVR and surgery have been described previously¹⁸; details are provided in Section D in the Supplementary Appendix. All TAVR procedures used the transfemoral access route. Balloon aortic valvuloplasty before and after TAVR was performed at the operator's discretion. Patients received aspirin (81 mg) and clopidogrel (≥ 300 mg) before TAVR and were advised to continue taking these medications for at least 1 month after the procedure.

END POINTS

The primary end point was a composite of death from any cause, stroke, or rehospitalization at 1 year after the procedure. All the patients underwent neurologic examinations at baseline and

at 30 days. Patients who had suspected stroke after the procedure underwent serial neurologic examinations, including assessment with the National Institutes of Health Stroke Scale and the modified Rankin scale at 90 days after the event. Rehospitalization was defined as any hospitalization related to the procedure, the valve, or heart failure.

Key secondary end points were prespecified for hierarchical testing to control type 1 error. These included stroke, a composite of death or stroke, and new-onset atrial fibrillation at 30 days, as well as the length of the index hospitalization and a poor treatment outcome, which was a composite of death or a low Kansas City Cardiomyopathy Questionnaire (KCCQ) overall summary score (with scores ranging from 0 to 100 and higher scores indicating fewer physical limitations and a greater feeling of well-being) at 30 days. Analyses of change in New York Heart Association (NYHA) functional class, 6-minute walk-test distance, and KCCQ summary score were also performed. A list of all the secondary safety and effectiveness end points and their definitions are provided in Sections E and F in the Supplementary Appendix. All components of the primary end point and key secondary end points were adjudicated by a clinical events committee whose members were aware of the treatment assignments.

STATISTICAL ANALYSIS

We estimated that a sample of 864 patients would provide the trial with 90% power to show the noninferiority of TAVR to surgery with regard to the primary end point at 1 year, assuming a Kaplan–Meier estimate of the rate of 14.6% in the TAVR group and 16.6% in the surgery group. A sample size of 1000 patients was chosen to allow for withdrawals, crossovers, and loss to follow-up. To test for noninferiority, we determined whether the upper boundary of the 95% confidence interval for the difference in the rate of the primary end point between the TAVR group and the surgery group was less than the prespecified noninferiority margin of 6 percentage points.

If the requirement for noninferiority was met, testing for the superiority of TAVR to surgery with regard to the primary end point was to be performed at a two-sided alpha level of 0.05. The primary analysis was performed in the as-treated population, which included patients who underwent randomization and in whom the index procedure was initiated. Sensitivity analyses of the

primary end point were performed in the intention-to-treat population, as well as with the use of multiple imputation to account for missing data (Section G in the Supplementary Appendix). An analysis of the hierarchical composite of death, stroke, or rehospitalization was performed with the use of the win ratio method.¹⁹ Prespecified subgroup analyses, with tests for interaction, were also performed.

There were two categories of secondary end points. For key secondary end points, testing for superiority was performed in a prespecified hierarchical order with the use of a gatekeeping method to control for multiple comparisons; P values are presented with claims of significance. For other secondary end points, analyses were performed without correction for multiple comparisons; hazard ratios and 95% confidence intervals are presented without P values or claims of significance, and inferences drawn from these 95% confidence intervals may not be reproducible.

Continuous variables, which are presented as means with standard deviations or medians with interquartile ranges, were compared with the use of Student's t-test or the Wilcoxon rank-sum test. Categorical and ordinal variables, which are presented as proportions, were compared with the use of Fisher's exact test or the Wilcoxon rank-sum test. Continuous variables obtained after baseline were compared with the use of analysis of covariance with adjustment for the baseline measurement. Time-to-event analyses were performed with the use of Kaplan–Meier estimates and were compared with the use of the log-rank test. Echocardiographic analyses were performed in the valve-implant population, which included patients in whom the intended valve was implanted. All statistical analyses were performed with the use of SAS software, version 9.4 (SAS Institute).

RESULTS

PATIENTS

From March 2016 through October 2017, a total of 1000 patients were enrolled at 71 sites; 979 of the patients were from the United States, 8 from Canada, 7 from Australia or New Zealand, and 6 from Japan. The patients were randomly assigned to undergo either TAVR (503 patients) or surgery (497 patients). The assigned procedure was performed in 950 patients (496 in the TAVR group and 454 in the surgery group), who com-

posed the as-treated population, and the intended valve was implanted in 948. Among the patients who did not undergo the assigned procedure (7 in the TAVR group and 43 in the surgery group), the most common reason was withdrawal from the trial (in 41 patients), mainly owing to the decision not to undergo surgery or the preference to undergo surgery at a nontrial site. Details regarding enrollment, randomization, and follow-up are provided in Figure S2 in the Supplementary Appendix.

Characteristics of the patients at baseline were balanced in the two groups (Table 1, and Fig. S3 in the Supplementary Appendix), except for a higher percentage of patients with an NYHA class of III or IV in the TAVR group than in the surgery group (31.2% vs. 23.8%). The patients enrolled in this trial were younger (mean age, 73 years), included more men (69.3%), and had lower STS-PROM scores (mean score, 1.9%) and fewer coexisting conditions than patients enrolled in previous randomized trials of TAVR.¹⁻³ Baseline characteristics were similar in the as-treated population and in patients who underwent randomization and were not included in the as-treated population (Table S1 in the Supplementary Appendix).

PROCEDURAL OUTCOMES

The median time from randomization to the index procedure was 11 days. One TAVR procedure was converted to surgery, and one surgical procedure was aborted. Concomitant procedures were performed in 7.9% of the patients in the TAVR group and in 26.4% of the patients in the surgery group. Concomitant coronary revascularization was performed in 6.5% and 12.8%, respectively. In the TAVR group, conscious sedation was used in 65.1% of the patients. In the surgery group, minimally invasive surgery was performed in 24.3% of the patients, and the surgical valve was 23 mm in diameter or larger in 79.9%. Details regarding the procedures are provided in Tables S2 and S3 and Figure S4 in the Supplementary Appendix.

There were six deaths during the index hospitalization, which occurred in two patients in the TAVR group and in four patients in the surgery group. Other serious intraprocedural complications that occurred in the TAVR group included implantation of a second valve, annulus rupture, coronary-artery obstruction, and ventricular perforation (in one patient each) (Tables S4 and S5 in the Supplementary Appendix).

PRIMARY END POINT

At 1 year, data regarding the primary end point were available for 98.4% of the patients. The composite of death from any cause, stroke, or rehospitalization had occurred in 42 patients (8.5%) in the TAVR group as compared with 68 patients (15.1%) in the surgery group. The requirements for both noninferiority and superiority were met, with an absolute difference between the TAVR group and the surgery group of -6.6 percentage points (95% confidence interval [CI], -10.8 to -2.5; $P < 0.001$ for noninferiority) and a hazard ratio of 0.54 (95% CI, 0.37 to 0.79; $P = 0.001$ for superiority) (Fig. 1A).

Results of an analysis performed with the use of the hierarchical win ratio method (win ratio, 1.88; 95% CI, 1.29 to 2.76) were consistent with those of the primary analysis. Results of sensitivity analyses of the primary end point performed in the intention-to-treat population and with the use of multiple imputation for missing data were also consistent with those of the primary analysis, as were results of analyses involving patients who underwent revascularization or other concomitant procedures and those who did not. Subgroup analyses of the primary end point at 1 year showed no heterogeneity of treatment effect in any of the subgroups that were examined (Fig. 2). Details regarding these analyses are provided in Tables S6, S7, and S8 and Figure S5 in the Supplementary Appendix.

Data regarding the individual components of the primary end point are shown in Figure 1B, 1C, and 1D, and in Table S9 in the Supplementary Appendix. At 1 year, the Kaplan-Meier estimate of the rate was 1.0% in the TAVR group as compared with 2.5% in the surgery group (hazard ratio, 0.41; 95% CI, 0.14 to 1.17) for death from any cause, 1.2% as compared with 3.1% (hazard ratio, 0.38; 95% CI, 0.15 to 1.00) for stroke, and 7.3% as compared with 11.0% (hazard ratio, 0.65; 95% CI, 0.42 to 1.00) for rehospitalization.

SECONDARY END POINTS

For key secondary end points, results of prespecified hierarchical testing are shown in Table 2. At 30 days, TAVR resulted in a lower rate of stroke than surgery (0.6% vs. 2.4%; hazard ratio, 0.25; 95% CI, 0.07 to 0.88; $P = 0.02$) and in lower rates of death or stroke (1.0% vs. 3.3%; hazard ratio, 0.30; 95% CI, 0.11 to 0.83; $P = 0.01$) and new-onset atrial fibrillation (5.0% vs. 39.5%; hazard

Table 1. Characteristics of the Patients at Baseline.*

Characteristic	TAVR (N=496)	Surgery (N=454)
Age — yr	73.3±5.8	73.6±6.1
Male sex — no. (%)	335 (67.5)	323 (71.1)
Nonwhite race or ethnic group — no. (%)†	38 (7.7)	45 (9.9)
Body-mass index‡	30.7±5.5	30.3±5.1
STS score§	1.9±0.7	1.9±0.6
EuroSCORE II score¶	1.5±1.2	1.5±0.9
NYHA class III or IV — no. (%)	155 (31.2)	108 (23.8)
Coronary artery disease — no./total no. (%)	137/494 (27.7)	127/454 (28.0)
Previous myocardial infarction — no./total no. (%)	28/495 (5.7)	26/452 (5.8)
Previous stroke — no./total no. (%)	17/496 (3.4)	23/453 (5.1)
Carotid disease — no./total no. (%)	61/481 (12.7)	50/442 (11.3)
Peripheral vascular disease — no./total no. (%)	34/494 (6.9)	33/453 (7.3)
COPD — no./total no. (%)	25/495 (5.1)	28/454 (6.2)
Creatinine >2 mg/dl — no. (%)	1 (0.2)	1 (0.2)
Diabetes — no./total no. (%)	155/496 (31.2)	137/453 (30.2)
Atrial fibrillation — no./total no. (%)	78/496 (15.7)	85/453 (18.8)
Permanent pacemaker — no. (%)	12 (2.4)	13 (2.9)
Left bundle-branch block — no./total no. (%)	15/495 (3.0)	15/453 (3.3)
Right bundle-branch block — no./total no. (%)	51/495 (10.3)	62/453 (13.7)
Overall frailty — no./total no. (%)**	0/495	0/453
Pulmonary hypertension — no./total no. (%)	23/495 (4.6)	24/454 (5.3)
Aortic-valve area — cm ²	0.8±0.2	0.8±0.2
Aortic-valve gradient — mm Hg	49.4±12.8	48.3±11.8
Left ventricular ejection fraction — %	65.7±9.0	66.2±8.6
Moderate or severe regurgitation — no./total no. (%)		
Aortic	19/484 (3.9)	11/446 (2.5)
Mitral	6/477 (1.3)	14/437 (3.2)
Tricuspid	8/473 (1.7)	10/430 (2.3)
Systolic annular perimeter on CT — mm	78.1±6.9	78.6±7.2
Systolic annular area on CT — mm ²	473.5±83.3	479.6±87.6

* Plus–minus values are means ±SD. There were no significant between-group differences in baseline characteristics, except for New York Heart Association (NYHA) class III or IV ($P<0.05$). Data on aortic-valve area were available for 459 patients in the TAVR group and 424 patients in the surgery group; aortic-valve gradient, 484 and 442, respectively; left ventricular ejection fraction, 472 and 436; and systolic annular perimeter and area on computed tomography (CT), 486 and 441. COPD denotes chronic obstructive pulmonary disease, and TAVR transcatheter aortic-valve replacement.

† Race or ethnic group was reported by the patient.

‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.

§ Society of Thoracic Surgeons Predicted Risk of Mortality (STS-PROM) scores range from 0 to 100%, with higher scores indicating a greater risk of death within 30 days after the procedure. STS-PROM uses an algorithm that is based on the presence of coexisting illnesses in order to predict 30-day operative mortality. The STS-PROM score equals the predicted mortality expressed as a percentage. Less than 5% of patients in the population on which the STS-PROM algorithm is based had a predicted operative mortality (score) of more than 10%.

¶ Scores on the European System for Cardiac Operative Risk Evaluation (EuroSCORE) II range from 0 to 100, with higher scores indicating a greater risk of death within 30 days after the procedure.

|| To convert the values for creatinine to micromoles per liter, multiply by 88.4.

** Overall frailty was defined as the presence of three or more of the following criteria: grip strength of less than 18 kg, 5-meter walk-test time of more than 6 seconds, serum albumin level of less than 3.5 g per deciliter, and Katz Activities of Daily Living total score of 4 or less (with scores ranging from 0 to 6 and higher scores indicating greater independence in performing activities of daily living).

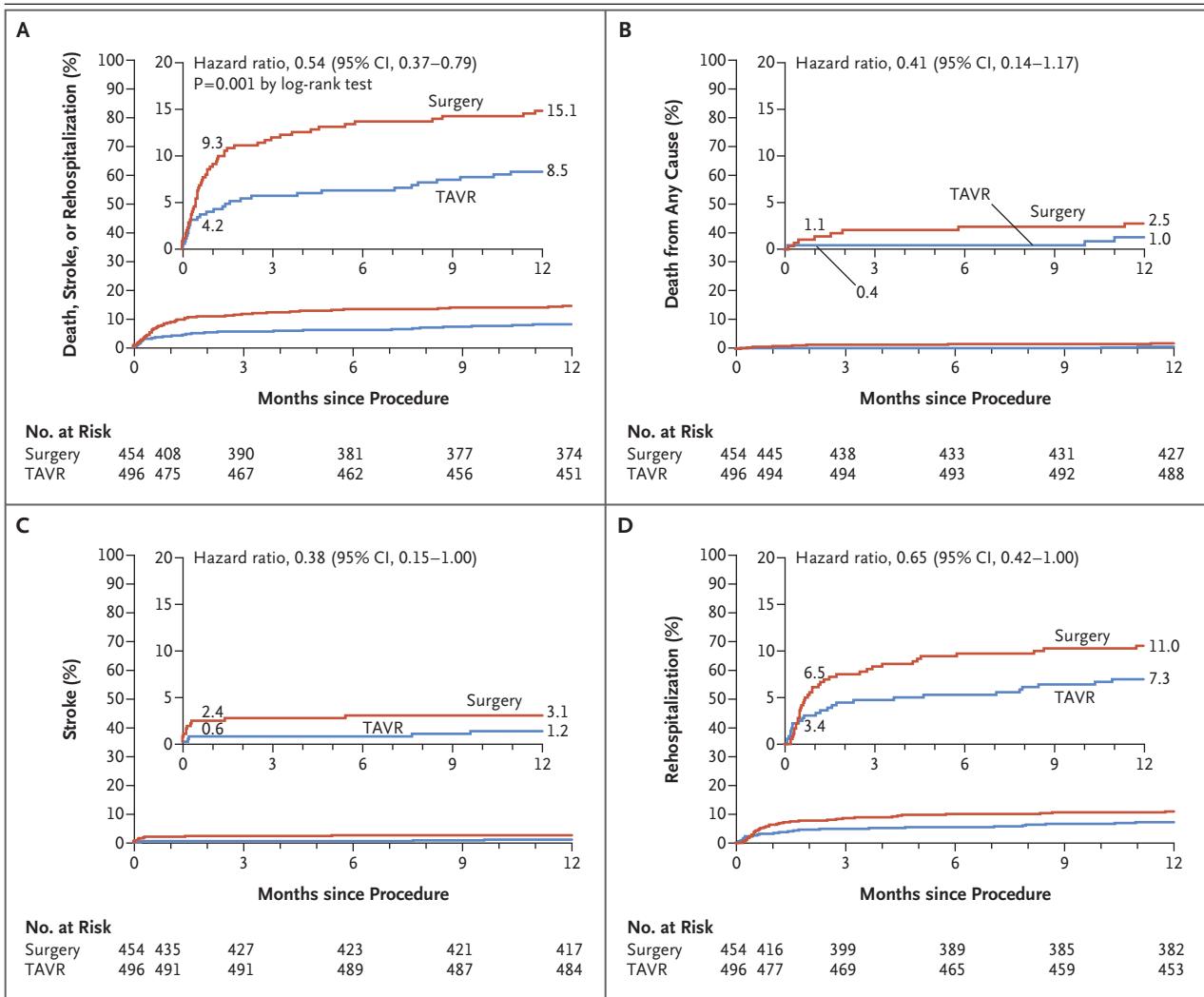


Figure 1. Time-to-Event Curves for the Primary Composite End Point and the Individual Components of the Primary End Point.

Shown are Kaplan–Meier estimates of the rate of the primary composite end point (Panel A) and the individual components of the primary end point, which are death from any cause (Panel B), stroke (Panel C), and rehospitalization (Panel D), in patients who underwent transcatheter aortic-valve replacement (TAVR) and those who underwent surgical aortic-valve replacement. The insets show the same data on an enlarged y axis.

ratio, 0.10; 95% CI, 0.06 to 0.16; $P < 0.001$). TAVR also resulted in a shorter index hospitalization than surgery (3 days vs. 7 days, $P < 0.001$) and in a lower risk of a poor treatment outcome (death or a low KCCQ score) at 30 days (3.9% vs. 30.6%, $P < 0.001$), a result that was confirmed with the use of multiple imputation for missing data (Table S10 in the Supplementary Appendix). At 1 year, the rate of death or disabling stroke was 1.0% in the TAVR group as compared with 2.9% in the surgery group (hazard ratio, 0.34; 95% CI, 0.12 to 0.97).

Complete data regarding secondary end points at 30 days and 1 year are provided in Tables S9 and S11 through S16 and Figures S6 through S9 in the Supplementary Appendix. The percentage of patients who were discharged to home or self-care was 95.8% in the TAVR group as compared with 73.1% in the surgery group. There were no significant differences between the two groups with regard to most safety end points at 30 days, including major vascular complications and new permanent pacemaker insertions. The percentage of patients with new left

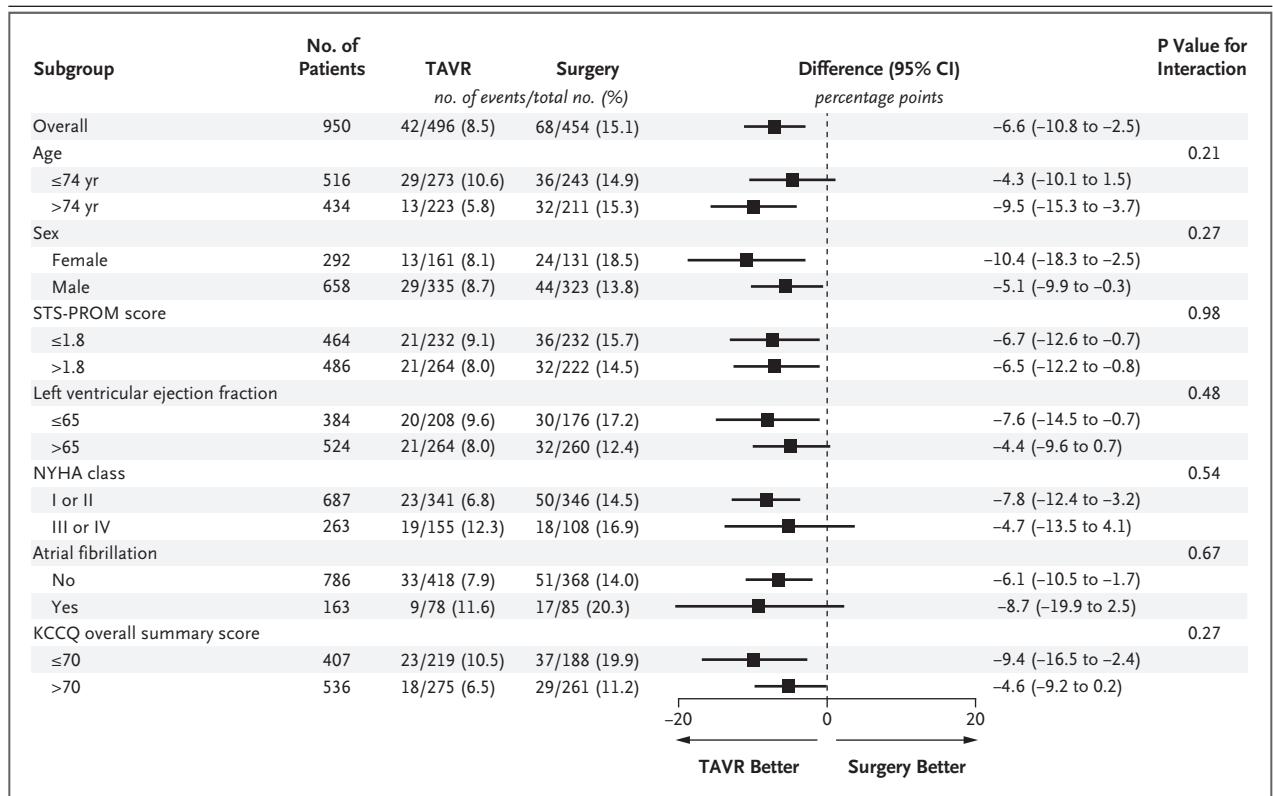


Figure 2. Subgroup Analyses of the Primary Composite End Point of Death from Any Cause, Stroke, or Rehospitalization.

All percentages are Kaplan–Meier estimates. Society of Thoracic Surgeons Predicted Risk of Mortality (STS-PROM) scores range from 0 to 100%, with higher scores indicating a greater risk of death within 30 days after the procedure. Kansas City Cardiomyopathy Questionnaire (KCCQ) overall summary scores range from 0 to 100, with higher scores indicating fewer physical limitations and a greater feeling of well-being. NYHA denotes New York Heart Association.

bundle-branch block at 1 year was 23.7% in the TAVR group as compared with 8.0% in the surgery group (hazard ratio, 3.43; 95% CI, 2.32 to 5.08). The percentage of patients with life-threatening or major bleeding was 3.6% in the TAVR group as compared with 24.5% in the surgery group (hazard ratio, 0.12; 95% CI, 0.07 to 0.21). Changes from baseline in the NYHA class, 6-minute walk-test distance, and KCCQ score at 30 days and 1 year are shown in Figure 3.

ECHOCARDIOGRAPHIC FINDINGS

At 30 days, the mean aortic-valve gradient was 12.8 mm Hg in the TAVR group and 11.2 mm Hg in the surgery group. The mean aortic-valve area was 1.7 cm² and 1.8 cm², respectively. The percentage of patients with moderate or severe paravalvular regurgitation did not differ significantly between the TAVR group and the surgery group (0.8% and none, respectively, at 30 days;

0.6% and 0.5% at 1 year). The percentage of patients with mild paravalvular regurgitation at 1 year was higher with TAVR than with surgery (29.4% vs. 2.1%). There were no episodes of valve thrombosis associated with clinical events. Six asymptomatic patients (five in the TAVR group and one in the surgery group) had findings suggestive of valve thrombosis, including increased valve gradients and evidence on imaging of restricted leaflet motion. Details regarding echocardiographic findings are provided in Tables S17 and S18 and Figures S10 through S13 in the Supplementary Appendix.

DISCUSSION

There are three main findings of the PARTNER 3 trial. First, TAVR, performed by means of transfemoral placement of the balloon-expandable SAPIEN 3 system, was superior to surgery

Table 2. Key Secondary End Points.*

End Point	TAVR (N=496)	Surgery (N=454)	TAVR vs. Surgery (95% CI)†‡	P Value‡
New-onset atrial fibrillation at 30 days — no./total no. (%)§¶	21/417 (5.0)	145/369 (39.5)	0.10 (0.06 to 0.16)	<0.001
Length of index hospitalization — median no. of days (interquartile range)	3.0 (2.0 to 3.0)	7.0 (6.0 to 8.0)	−4.0 (−4.0 to −3.0)	<0.001
Death from any cause, stroke, or rehospitalization at 1 year — no. (%)§	42 (8.5)	68 (15.1)	0.54 (0.37 to 0.79)	0.001
Death, KCCQ score of <45, or decrease from baseline in KCCQ score of ≥10 points at 30 days — no./total no. (%)	19/492 (3.9)	133/435 (30.6)	−26.7 (−31.4 to −22.1)	<0.001
Death or stroke at 30 days — no. (%)§	5 (1.0)	15 (3.3)	0.30 (0.11 to 0.83)	0.01
Stroke at 30 days — no. (%)§	3 (0.6)	11 (2.4)	0.25 (0.07 to 0.88)	0.02

* Key secondary end points were tested in a prespecified hierarchical order with the use of a gatekeeping method to control for multiple comparisons.

† For the first, third, fifth, and sixth end points, the value is a hazard ratio. For the second end point, the value is a difference in medians estimated with the use of bootstrap techniques. For the fourth end point, the value is a difference in proportions and is presented in percentage points.

‡ For the first, third, fifth, and sixth end points, the P value was based on the log-rank test. For the second end point, the P value was based on the Wilcoxon rank-sum test. For the fourth end point, the P value was based on Fisher's exact test.

§ The percentages are Kaplan–Meier estimates.

¶ Patients who had atrial fibrillation before the procedure were excluded from the analysis.

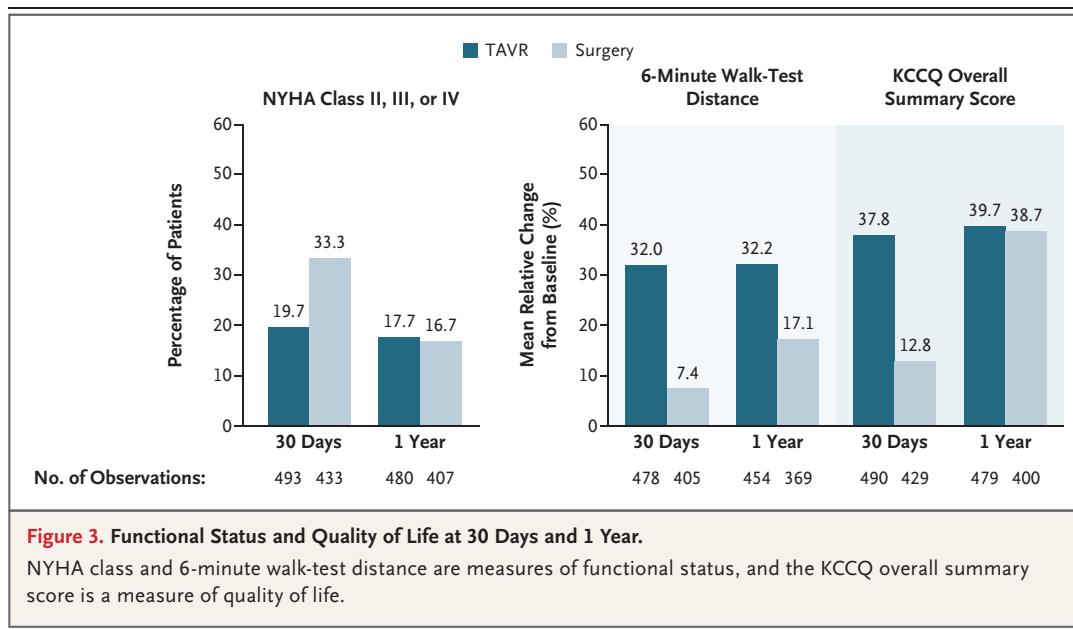
|| Kansas City Cardiomyopathy Questionnaire (KCCQ) overall summary scores range from 0 to 100, with higher scores indicating fewer physical limitations and a greater feeling of well-being.

with regard to the primary composite end point of death, stroke, or rehospitalization at 1 year. Multiple sensitivity analyses confirmed the robustness of the results of the primary analysis. Results for the three components of the primary end point favored TAVR at both 30 days and 1 year. Second, analyses of key secondary end points, which were adjusted for multiple comparisons, showed that TAVR was associated with a significantly lower rate of new-onset atrial fibrillation at 30 days, a shorter index hospitalization, and a lower risk of a poor treatment outcome (death or a low KCCQ score) at 30 days than surgery. Third, patients who underwent TAVR had more rapid improvements in the NYHA class, 6-minute walk-test distance, and KCCQ score than those who underwent surgery.

During the past decade, recommendations for TAVR in patients with severe, symptomatic aortic stenosis have been expanded to include strata with incrementally lower surgical risk.^{12,13,20,21} Current clinical practice has restricted the use of TAVR in patients who are at low risk and in younger patients, for whom surgery is standard therapy. Previous research that supports the use of TAVR in low-risk patients is limited, mostly consisting of retrospective, observational studies.²²⁻²⁷ One randomized trial of TAVR with an

early-generation self-expanding valve in 280 patients at all risk levels (>80% with an STS-PROM score of <4%) showed that TAVR was noninferior to surgery with more than 5 years of follow-up.¹⁶ A recent prospective series of TAVR with balloon-expandable and self-expanding valves in 200 low-risk patients without frailty from 11 U.S. centers showed no deaths or disabling strokes at 30 days.¹⁷

In the PARTNER 3 trial, surgical outcomes were excellent: in the surgery group, the rate of death at 30 days was 1.1%, and the rate of a composite of death or disabling stroke at 1 year was 2.9%. Nevertheless, in the TAVR group, the rate of death at 30 days was even lower (0.4%), and the rate of death or disabling stroke at 1 year was only 1.0%. Complications that were more frequent with TAVR than with surgery in previous trials^{1-3,6,28-32} occurred with similar frequency in the two groups in this trial, including major vascular complications, new permanent pacemaker insertions, moderate or severe paravalvular regurgitation, and coronary-artery obstruction. Life-threatening or major bleeding occurred less frequently with TAVR than with surgery. Results for other secondary end points, including new left bundle-branch block and mild paravalvular regurgitation, favored surgery. Between-group differences in transvalvular aortic-valve gradients



also favored surgery, although this was not the case in previous randomized trials of TAVR^{2,3,5}; this result was probably due to the greater use of larger surgical valves in this trial.

The most important limitation of this trial is that our current results reflect only 1-year outcomes and do not address the problem of long-term structural valve deterioration.^{33,34} Definitive conclusions regarding the advantages and disadvantages of TAVR as compared with surgery (with either bioprosthetic or mechanical valves) depend on long-term follow-up. In this trial involving younger, low-risk patients, the protocol requires clinical and echocardiographic follow-up to continue for at least 10 years.

This trial has several other limitations. First, in this trial, as in previous TAVR trials, adjudication of end points was not blinded, which could have resulted in bias in outcome assessment. Second, the results apply only to the defined trial population, which excluded patients with poor transfemoral access, bicuspid aortic valves, or other anatomical or clinical factors that increased the risk of complications associated with either TAVR or surgery. Third, the findings cannot be extrapolated to TAVR performed with other systems or by less experienced operators.^{35,36} Fourth, more patients in the surgery group than in the TAVR group withdrew from the trial (both early and late). Fifth, missing data regarding NYHA class, 6-minute walk-test distance, KCCQ

score, and follow-up echocardiograms were not fully accounted for with multiple imputation. Sixth, this analysis did not examine the rate and relevance of asymptomatic valve thrombosis.^{37,38}

This issue is being examined in a randomized subtrial, in which 435 patients are undergoing serial computed tomographic angiography for the detection of abnormalities in valve-leaflet function, with investigators unaware of imaging findings.

The proof-of-concept first case of TAVR performed by Cribier and colleagues in 2002³⁹ was intended to open a treatment pathway for the highest-risk patients with limited therapeutic options. Our findings in low-risk patients suggest that the value of TAVR as compared with surgery may be independent of risk profiles.

In conclusion, among patients with severe aortic stenosis who were at low risk for death with surgery, the rate of the composite of death, stroke, or rehospitalization at 1 year was significantly lower with TAVR than with surgical aortic-valve replacement.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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APPENDIX

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A Phase IIIb Study to Evaluate the Safety of Ranibizumab in Subjects with Neovascular Age-related Macular Degeneration

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Objective: To evaluate the safety and efficacy of intravitreal ranibizumab in a large population of subjects with neovascular age-related macular degeneration (AMD).

Design: Twelve-month randomized (cohort 1) or open-label (cohort 2) multicenter clinical trial.

Participants: A total of 4300 subjects with angiographically determined subfoveal choroidal neovascularization (CNV) secondary to AMD.

Methods: Cohort 1 subjects were randomized 1:1 to receive 0.3 mg (n = 1169) or 0.5 mg (n = 1209) intravitreal ranibizumab for 3 monthly loading doses. Dose groups were stratified by AMD treatment history (treatment-naïve vs. previously treated). Cohort 1 subjects were retreated on the basis of optical coherence tomography (OCT) or visual acuity (VA) criteria. Cohort 2 subjects (n = 1922) received an initial intravitreal dose of 0.5 mg ranibizumab and were retreated at physician discretion. Safety was evaluated at all visits.

Main Outcome Measures: Safety outcomes included the incidence of ocular and nonocular adverse events (AEs) and serious adverse events (SAEs). Efficacy outcomes included changes in best-corrected VA over time.

Results: Some 81.7% of cohort 1 subjects and 49.9% of cohort 2 subjects completed the 12-month study. The average total number of ranibizumab injections was 4.9 for cohort 1 and 3.6 for cohort 2. The incidence of vascular and nonvascular deaths during the 12-month study was 0.9% and 0.7% in the cohort 1 0.3 mg group, 0.8% and 1.5% in the cohort 1 0.5 mg group, and 0.7% and 0.9% in cohort 2, respectively. The incidence of death due to unknown cause was 0.1% in both cohort 1 dose groups and cohort 2. The number of vascular deaths and deaths due to unknown cause did not differ across cohorts or dose groups. Stroke rates were 0.7%, 1.2%, and 0.6% in the 0.3 mg and 0.5 mg groups and cohort 2, respectively. At month 12, cohort 1 treatment-naïve subjects had gained an average of 0.5 (0.3 mg) and 2.3 (0.5 mg) VA letters and previously treated subjects had gained 1.7 (0.3 mg) and 2.3 (0.5 mg) VA letters.

Conclusions: Intravitreal ranibizumab was safe and well tolerated in a large population of subjects with neovascular AMD. Ranibizumab had a beneficial effect on VA. Future investigations will seek to establish optimal dosing regimens for persons with neovascular AMD.

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Neovascular age-related macular degeneration (AMD) is characterized by new vessel growth and leakage in the choroidal vascular network beneath the macula, with extension and leakage into the subretinal space. Although the pathologic events that precede choroidal neovascularization (CNV) are not clearly understood, disrupting the activity of vascular endothelial growth factor A (VEGF-A), a diffusible cytokine that promotes angiogenesis and vascular permeability, effectively treats CNV secondary to AMD.

Ranibizumab (LUCENTIS, Genentech, Inc., South San Francisco, CA) is a recombinant, humanized monoclonal antibody antigen-binding fragment (Fab) that neutralizes all active forms of VEGF-A. In 2 pivotal phase III trials—

Minimally Classic/Occlude Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular Age-Related Macular Degeneration (MARINA)¹ and Anti-Vascular Endothelial Growth Factor (VEGF) Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization (CNV) in Age-related Macular Degeneration (ANCHOR)²—monthly intravitreal injections of 0.3 mg or 0.5 mg ranibizumab not only prevented vision loss but also improved visual acuity (VA) in patients with minimally classic or occult without classic and predominantly classic CNV, respectively. In those studies, ranibizumab treatment was associated with a low rate of serious adverse events (SAEs), including those attributable to systemic VEGF inhibition.

The Safety Assessment of Intravitreal Lucentis for AMD (SAILOR) study was a phase IIIb follow-up study to the MARINA and ANCHOR studies to evaluate the long-term safety and efficacy of ranibizumab in a large population of subjects with all subtypes (minimally classic, occult without classic, and predominantly classic) of neovascular AMD. SAILOR included more than 5 times as many ranibizumab-treated subjects as the MARINA and ANCHOR studies combined. Thus, it is the largest multicenter randomized study to date to evaluate safety and efficacy outcomes of anti-VEGF treatment in wet AMD, and it is the only phase III study to examine individualized, criteria-based retreatment.

Materials and Methods

SAILOR was a 12-month, multicenter, phase IIIb study intended to further characterize the safety and efficacy profiles of intravitreal ranibizumab. Protocols were approved by the institutional review board at each study site, and the study was conducted according to the International Conference on Harmonisation E6 Guideline for Good Clinical Practice and any national requirements. All subjects provided informed consent before participation in the study. The SAILOR study is registered at www.clinicaltrials.gov (NCT00251459; accessed February 5, 2009).

Two study cohorts were enrolled. Cohort 1 subjects were randomized 1:1 to receive 0.3 mg or 0.5 mg intravitreal ranibizumab. Cohort 2 subjects received open-label 0.5 mg intravitreal ranibizumab. Eligible subjects were ≥ 50 years of age with 20/40 to 20/400 (Snellen equivalent) best-corrected VA in the study eye. Cohort 1 VA was assessed with the Early Treatment Diabetic Retinopathy Study (ETDRS) chart. In the interest of conserving time and resources, VA for cohort 2 (under a less rigorous treatment and assessment schedule) was assessed using Snellen charts. All subjects had angiographically determined subfoveal CNV (minimally classic, occult without classic, predominantly classic) secondary to AMD (as determined by the investigating physician), with evidence of recent disease progression defined by any of the following: loss of ≥ 5 ETDRS letters (or ≥ 1 Snellen line) within 6 months before study initiation (i.e., day 0); 10% increase in the CNV lesion area determined by comparing a fluorescein angiogram performed within 1 month before day 0 with an angiogram performed within 6 months before day 0; subretinal hemorrhage associated with CNV within 1 month before day 0; or classic CNV comprising $>50\%$ of the CNV lesion area.

Key exclusion criteria included verteporfin photodynamic therapy, pegaptanib sodium, or other AMD therapy within 30 days before day 0; previous submacular surgery or other surgical intervention for AMD in the study eye; participation in an investigational drug (except vitamins and minerals) study within 30 days before day 0; previous participation in a ranibizumab clinical trial; intravitreal administration of bevacizumab within 30 days before day 0; or current use of systemic anti-VEGF agents. Also excluded were subjects with fibrosis or atrophy involving the foveal center of the treated eye in the absence of a new lesion; CNV in either eye due to other causes, such as ocular histoplasmosis, trauma, or pathologic myopia; a tear in the retinal pigment epithelium of the study eye involving the macula; or any current intraocular condition in the study eye (e.g., cataract or diabetic retinopathy) that, in the investigating physician's opinion, would require medical or surgical intervention during the 12-month study period or, if allowed to progress untreated, would likely contribute to the loss of at least 2 Snellen equivalent lines of VA over the 12-month study

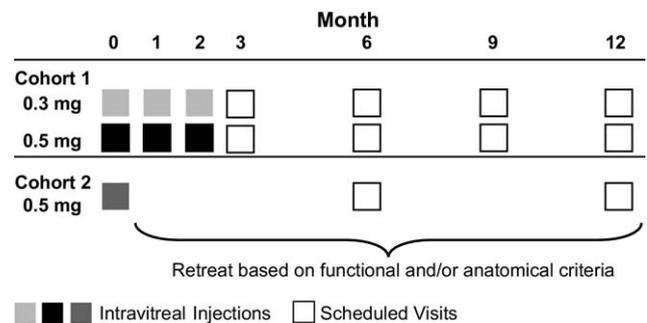


Figure 1. Study treatment and assessments. Cohort 1 subjects received 3 loading doses of ranibizumab and were retreated on the basis of VA (>5 letter decrease in VA from highest score at prior visits) or VA and/or OCT ($>100 \mu\text{m}$ increase in CFT from the lowest measurement at prior visits) criteria. Cohort 2 subjects received 1 dose of ranibizumab on day 1 and were retreated at physician discretion. CFT = central foveal thickness; OCT = optical coherence tomography; VA = visual acuity.

period. Subjects with a history of cardiovascular disease were not excluded if their disease was controlled.

Cohort 1 subjects were randomized 1:1 to receive 0.3 mg or 0.5 mg intravitreal ranibizumab. To prevent bias in reporting AEs, subjects were masked to treatment dose. (Because SAILOR was not designed with efficacy as an objective, physicians and study monitors were not masked.) Randomization was stratified according to treatment history. "Previously treated" subjects had previously received treatment AMD. "Treatment-naïve" subjects were newly diagnosed with neovascular AMD. Cohort 1 subjects received 3 monthly loading doses of intravitreal ranibizumab (day 0, month 1, and month 2) with scheduled follow-up visits at months 3, 6, 9, and 12 (Fig 1). If, at any time, the investigating physician believed that the between-visit interval was too long for a patient to go without being assessed, an unscheduled visit could occur. After the 3 loading doses, retreatment was based on (1) VA (a >5 ETDRS letter decrease in VA compared with the highest VA score at any prior scheduled visit) or (2) VA (same as above) and/or optical coherence tomography (OCT) (a $>100\text{-}\mu\text{m}$ increase in central foveal thickness [CFT] compared with the lowest measurement at any previous scheduled study visit, with intraretinal or subretinal fluid present). Thus, OCT assessment was required only for retreatment option 2, in which case OCT data were consistently obtained at all study visits. Retreatments were to occur no more frequently than every 30 days. Before randomization, the investigating physician selected the retreatment criterion for each subject that was to be used throughout the study.

Cohort 1 subjects were evaluated with a full ocular examination and best-corrected VA (ETDRS chart at a distance of 4 m) and safety assessments on day 0 and at all scheduled (months 1, 2, 3, 6, 9, and 12) visits. Visual acuity assessments were required at unscheduled visits if a subject was being evaluated for retreatment. Safety assessments were required at all unscheduled visits.

Cohort 2 included both previously treated and treatment-naïve subjects. Subjects received 0.5 mg of ranibizumab, with an initial injection on day 0 and retreatment at the investigating physician's discretion, no more frequently than every 30 days. Cohort 2 subjects were evaluated for Snellen VA at day 0 and months 6 and 12. At unscheduled visits, VA was assessed at the investigating physician's discretion. Serious adverse events and adverse events (AEs) were assessed at scheduled and unscheduled visits, with formal safety assessments scheduled for months 6 and 12.

Adverse events included any unfavorable or unintended sign, symptom, or disease temporally associated with use of study drug or other protocol-imposed intervention. An AE was classified as an SAE if it caused or led to death, required or prolonged subject hospitalization, resulted in persistent or significant disability or incapacitation, or was considered to be a significant medical event by the investigating physician.

One eye per subject (i.e., the study eye) was treated. After thoroughly cleansing the lid, lashes, periorbital area, and conjunctiva with povidone iodine, local anesthesia and antimicrobials (ofloxacin ophthalmic solution, trimethoprim-polymyxin B ophthalmic solution, moxifloxacin ophthalmic solution, or gatifloxacin ophthalmic solution) were administered to the study eye. A 30-gauge, 0.5-inch needle attached to a low-volume syringe containing 50 μ L of ranibizumab solution was inserted through the conjunctiva and sclera, 3.5 to 4.0 mm posterior to the limbus, avoiding the horizontal meridian and aiming toward the center of the globe. The injection volume was delivered slowly. The needle was slowly removed, ensuring that all drug solution was in the eye. Immediately after the injection, antimicrobial drops were administered, and the subject was instructed to self-administer antimicrobial drops 4 times daily for 3 days. The study eye was assessed with a finger count test and intraocular pressure within 15 and 70 minutes, respectively, of the ranibizumab injection.

The primary safety end point for cohort 1 was incidence of ocular and nonocular SAEs evaluated through month 12. A secondary safety end point was incidence of ocular and nonocular AEs evaluated through month 12. Efficacy end points for cohort 1 included change from baseline VA, proportion of subjects who gained ≥ 15 VA letters from baseline, and change from baseline CFT across the study period.

The primary safety end points for cohort 2 were the incidence of ocular and nonocular SAEs and AEs evaluated through month 12. Efficacy outcomes for cohort 2 included median change in Snellen VA from baseline and the proportion of subjects with Snellen 20/200 or worse at baseline compared with months 6 and 12.

Statistical Analysis

Safety and efficacy analyses included all subjects who received at least 1 injection of ranibizumab. Incidence of ocular and nonocular SAEs and AEs and 95% 2-sided confidence intervals for key SAEs were determined for both cohorts and each dose group. No formal hypothesis testing was conducted to compare cohorts, dose groups, or treatment-naïve and previously treated subjects. A sample of 2378 cohort 1 subjects and 1922 cohort 2 subjects was considered sufficient to estimate rates of uncommon SAEs and AEs.

Efficacy results for cohort 1 were stratified by dose group and treatment history. Estimated proportions were obtained for dichotomous end points. Continuous end points were evaluated using descriptive statistics, including mean, median, standard deviation, standard error, and range.

To further evaluate stroke rates across cohorts and dose groups, each subject's medical history was reviewed, and subjects were classified by preexisting conditions that may have been associated with the incidence of stroke during the 12-month study. These included prior stroke, myocardial infarction (MI), hypertension, transient ischemic attack, coronary artery disease, arrhythmias, valve malfunction, congestive heart failure, angioplasty, deep vein thrombosis, diabetes, endocardectomy, cardiac inflammation, prior stent, and use of aspirin, lipid-lowering drugs, anticoagulants, or platelet aggregation inhibitors. A univariate Cox proportional hazard regression model was used to identify which of those were significant (i.e., $P \leq 0.05$) risk factors for stroke in SAILOR. In

addition, models that included the interaction of dose with each of the significant risk factors were fit separately.

Missing Data

Missing data were not imputed for safety end points. For cohort 1, missing values for efficacy end points were imputed using the last-observation-carried-forward method. For cohort 2, missing Snellen values were not imputed.

Results

From November 2005 to June 30, 2006 (when ranibizumab was approved for the treatment of neovascular AMD by the Food and Drug Administration), 2378 cohort 1 subjects were randomly assigned to receive 0.3 mg ($n = 1169$) or 0.5 mg ($n = 1209$) intravitreal ranibizumab at 105 US centers. Cohort 1 subjects had an average age of 79 years, and 59% were female (Table 1). Approximately 60% of cohort 1 subjects in each dose group had been previously treated for AMD. The types of previous treatment were similar across dose groups and included photodynamic therapy (33%), intravitreal pegaptanib sodium (30%), intravitreal triamcinolone acetonide (17%), and laser photocoagulation (10%). Investigating physicians elected to use the VA plus OCT retreatment criterion for approximately 81% of the subjects in each dose group.

Previously treated and treatment-naïve subjects had similar baseline ocular characteristics, with the exception that previously treated subjects had a longer time since first diagnosis and lower baseline VA (Table 2). Approximately 18% of cohort 1 subjects in each dose group discontinued the study before the month 12 visit (Table 3). Baseline ocular characteristics of subjects who com-

Table 1. Subject Baseline Characteristics

Characteristic	Cohort 1		Cohort 2
	0.3 mg ($n = 1169$)	0.5 mg ($n = 1209$)	0.5 mg ($n = 1922$)
Age (yrs)			
Mean \pm SD	78.7 \pm 7.6	78.7 \pm 8.6	78.7 \pm 8.1
Range	51–97	52–101	45–99
Sex			
Female	59.9	58.1	61.6
Race			
Caucasian	96.6	97.1	96.2
AMD treatment history			
Treatment naïve	39.5	40.5	—
Previously treated	60.5	59.5	—
Retreatment criteria			
VA	19.3	18.4	—
VA plus OCT	80.7	81.6	—
Systolic BP			
Mean \pm SD	137.4 \pm 17.3	137.8 \pm 18.0	—
Range	90–213	80–220	—
Diastolic BP			
Mean \pm SD	76.2 \pm 9.7	77.0 \pm 9.7	—
Range	48–118	48–110	—

AMD = age-related macular degeneration; BP = blood pressure; OCT = optical coherence tomography; SD = standard deviation; VA = visual acuity.

Values are percentages except where otherwise noted.

Table 2. Baseline Ocular Characteristics

	Cohort 1				Cohort 2 (n = 1922)
	Treatment Naive		Previously Treated		
	0.3 mg (n = 462)	0.5 mg (n = 490)	0.3 mg (n = 707)	0.5 mg (n = 719)	
Age at diagnosis (yrs)	79.9±7.9	75.8±8.0	79.9±7.5	79.9±7.5	—
Time since diagnosis (yrs)	0.3±1.4	0.3±0.7	1.4±2.0	1.3±1.7	—
CNV type (%)					
Predominantly classic	32.0	29.4	30.6	31.7	—
Minimally classic	19.7	20.2	26.2	23.5	—
Occult without classic	45.5	48.6	38.6	40.6	—
VA					
ETDRS letters	55.0±12.5	48.9±13.8	53.8±13.8	50.0±14.3	—
Snellen					
Median	20/80	20/80	20/100	20/100	20/100
20/200 or worse (%)	12.2	15.0	22.9	23.0	39
Central foveal thickness (µm)	312±104	322±116	315±113	310±113	—
Intraocular pressure (mmHg)	15.3±3.2	15.3±3.2	15.7±3.3	15.4±3.4	—

CNV = choroidal neovascularization; ETDRS = Early Treatment Diabetic Retinopathy Study; VA = visual acuity. Values are mean ± standard deviation except where otherwise noted.

pleted the study and those who discontinued were similar. All cohort 1 subjects received their assigned dose of ranibizumab on day 0, and approximately 96% of cohort 1 subjects received their assigned dose at months 1 and 2 (Fig 2). Cohort 1 subjects received an average of 4.6 injections during the 12-month study (the protocol required 3 initial injections). The average number of visits was 8.8 (the protocol required 7 scheduled visits). During months that visits were not scheduled (i.e., months 4, 5, 7, 8, 10, and 11), approximately 40% of the subjects made unscheduled visits, and approximately 16% of those subjects received an injection of ranibizumab at the unscheduled visit (relative to the number of subjects remaining in the study that month) (Fig 2).

From March 2006 to June 30, 2006, 1922 cohort 2 subjects were enrolled at 104 US centers and received 0.5 mg intravitreal ranibizumab (Table 1). Approximately 50% of cohort 2 subjects discontinued the study before the month 12 visit (Table 3). All cohort 2 subjects received the protocol-required injection on day 0 and received an average of 3.6 injections during the 12-month study (the protocol required 3 scheduled visits). During months that visits were not required

(i.e., all but months 6 and 12), the percentage of subjects who remained in the study that made unscheduled visits ranged from 65% at month 2 to 17.4% at month 11. The percentage of subjects receiving injections ranged from 64% at month 2 to 16.5% at month 11.

Table 3. Reasons for Discontinuation

	Cohort 1		Cohort 2
	0.3 mg (n = 1169)	0.5 mg (n = 1209)	0.5 mg (n = 1922)
Discontinued early (%)	18.6	18.0	50.1
Reason for early discontinuation (%)			
Death	1.7	2.3	1.5
Adverse event	2.6	2.2	1.8
Loss to follow-up	0.7	0.9	2.0
Subject decision	6.7	5.8	29.0
Physician decision	3.4	2.8	9.4
Sponsor decision	0.2	0.1	0.3
Subject noncompliance	0.6	0.9	0.9
Subject's condition mandated other therapeutic intervention	2.7	3.1	5.3
Reason not provided	0.1	0	0

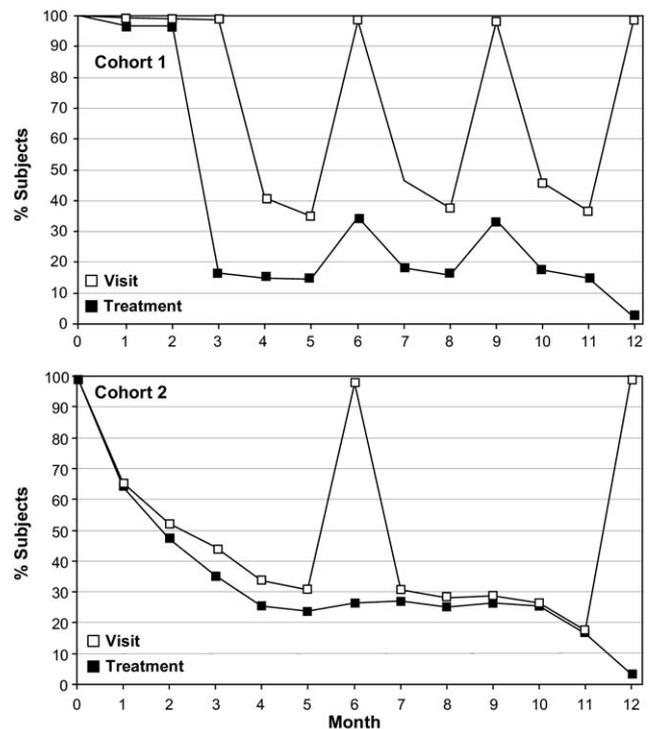


Figure 2. Visits and treatment. The percentage of cohort 1 (upper) and cohort 2 (lower) patients making visits and receiving ranibizumab treatment during each month of the 12-month study are shown. Cohort 1 visits were scheduled for day 0 and months 1, 2, 3, 6, 9, and 12. Cohort 2 visits were scheduled for day 0 and months 6 and 12. Data from cohort 1 0.3 and 0.5 mg dose groups are combined. Values are based on the percentage of subjects remaining in the study at each time point. Treatment received at month 12 was in violation of the protocol.

Table 4. Key Ocular Serious Adverse Events

Event, %	Cohort 1		Cohort 2
	0.3 mg (n = 1169)	0.5 mg (n = 1209)	0.5 mg (n = 1922)
Presumed endophthalmitis*	0.2	0.4	0.1
Uveitis	0.1	0.2	0
Retinal detachment	0.1	0	0.1
Retinal tear	0	0.1	0
Retinal hemorrhage	0.9	0.9	0.3
Detachment of retinal pigment epithelium	0	0.2	0.1
Vitreous hemorrhage	0.3	0.1	0.2
Cataract	0.1	0.1	0.1

*Includes 2 cases of uveitis and 1 case of iridocyclitis that were treated with antibiotics.

Safety

Ocular safety. The rates of individual key ocular SAEs in cohort 1 were <1% and similar across dose groups (Table 4). Two subjects (0.2%) in the 0.3 mg group and 5 subjects (0.4%) in the 0.5 mg group developed endophthalmitis or presumed endophthalmitis (i.e., ocular infection treated with antibiotics). One subject in each cohort 1 dose group had a serious cataract event. The rates of individual key ocular SAEs in cohort 2 were <1%. One cohort 2 subject developed endophthalmitis, and 1 subject had a serious cataract event (Table 4).

The incidence of ocular inflammation AEs, including iritis, uveitis, vitritis, and iridocyclitis, was 1.0% in the 0.3 mg group, 1.5% in the 0.5 mg group, and 0.5% in cohort 2. The overall incidence of cataract AEs was 5.4% in the 0.3 mg group, 6.0% in the 0.5 mg group, and 2.8% in cohort 2, and was similar when broken down by nuclear, subcapsular, and cortical subtypes.

Nonocular safety. The rates of key nonocular SAEs were similar across cohort 1 dose groups (Fig 3; Table 5). Nonvascular death, stroke, and hemorrhage rates were numerically higher in the 0.5 mg group. Eight subjects (0.7%) in the 0.3 mg group and 15 subjects (1.2%) in the 0.5 mg group had a stroke during the

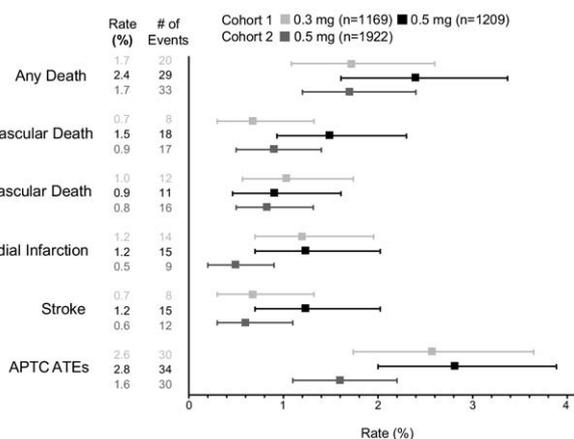


Figure 3. Key nonocular SAEs. The rates of individual events are depicted as point estimates with 2-sided Blyth-Still-Casella 95% confidence intervals. Antiplatelet Trialists' Collaboration ATEs include vascular deaths and deaths due to unknown cause, nonfatal MI, and nonfatal stroke. APTC = Antiplatelet Trialists' Collaboration; ATE = arterial thromboembolic event; SAE = serious adverse events.

Table 5. Nonocular Adverse Events Potentially Related to Anti-Vascular Endothelial Growth Factor Therapy

Classification, %	Cohort 1		Cohort 2
	0.3 mg (n = 1169)	0.5 mg (n = 1209)	0.5 mg (n = 1922)
Arterial thromboembolic events			
All	3.8	4.1	2.4
Serious	2.5	3.1	1.6
Hypertension			
All	9.0	10.3	3.0
Serious	0.1	0.1	0
Nonocular hemorrhage			
All	2.9	3.1	1.4
Serious	0.9	1.5	0.6
Proteinuria			
All	0.1	0	0
Serious	0	0	0
Other			
All	0.7	0.4	0.1
Serious	0.3	0.2	0.1

VEGF = vascular endothelial growth factor.

12-month study period. The incidence of MI and Antiplatelet Trialists' Collaboration (APTCC)³ arterial thromboembolic events (ATEs), which include vascular death and death of unknown cause, nonfatal MI, and nonfatal cardiovascular accidents, were similar across cohort 1 dose groups.

Rates of key nonocular SAEs in cohort 2 were generally lower than those in cohort 1, which may be a result of under-reporting because of the large number of cohort 2 subjects who discontinued. The incidence of nonocular AEs potentially related to anti-VEGF therapy was low and comparable across cohorts and dose groups.

Prior stroke, history of arrhythmias, and history of congestive heart failure were significant risk factors for stroke (Fig 4). Although the numbers were small, there was a nonstatistically significant trend toward higher incidence of stroke in the cohort 1 0.5 mg group subjects with a history of stroke. Seven of the 73 subjects (9.6%) with a history of stroke in the 0.5 mg group experienced a stroke during the study compared with 2 of the 73 subjects (2.7%) with a history of stroke in the 0.3 mg group. None of the cohort 2 subjects with a history of stroke experienced a stroke during the study (Fig 4).

Twenty subjects (1.7%) in the cohort 1 0.3 mg group, 29 subjects (2.4%) in the cohort 1 0.5 mg group, and 33 subjects (1.7%) in cohort 2 died during the 12-month study (Table 6). The number of vascular deaths and deaths due to unknown cause did not differ across cohorts or dose groups.

Efficacy

Cohort 1 efficacy results were stratified by dose and previous treatment for AMD. For all groups, study eye VA increased with 3 loading doses of ranibizumab (day 0, month 1, month 3) (Fig 5). At month 3, treatment-naïve subjects in the 0.3 mg group had gained an average of 5.8 VA letters and those in the 0.5 mg group had gained an average of 7.0 VA letters. From months 3 to 12, with protocol-defined retreatment, VA tended to decrease. At month 12, treatment-naïve subjects in the 0.3 mg group had gained an average of 0.5 VA letters and those in the 0.5 mg group had gained an average of 2.3 letters. A similar pattern was observed for previ-

ously treated subjects. At month 3, previously treated subjects in the 0.3 mg group had gained an average of 4.6 VA letters and those in the 0.5 mg group had gained an average of 5.8 VA letters. At month 12, previously treated subjects in the 0.3 mg group had gained an average of 1.7 VA letters and those in the 0.5 mg group had gained an average of 2.3 letters.

In all cohort 1 groups, the proportion of subjects who gained ≥ 15 letters from baseline VA increased with 3 loading doses of ranibizumab (Fig 6). At month 3, 19.4% of treatment-naïve subjects in the 0.3 mg group and 20.1% in 0.5 mg group had gained ≥ 15 letters. The proportion of those who gained ≥ 15 letters tended to be maintained for the duration of the 12-month study, with 14.6% of 0.3 mg group subjects and 19.3% of 0.5 mg subjects gaining ≥ 15 VA letters at month 12. A similar pattern was observed for previously treated subjects. At month 3, 16.0% of previously treated subjects in the 0.3 mg group and 18.6% in the 0.5 mg group had gained ≥ 15 letters; and at month 12, 15.8% of 0.3 mg group subjects and 16.5% of 0.5 mg group subjects had gained ≥ 15 VA letters.

Study eye CFT of cohort 1 subjects for whom OCT data were available decreased with 3 loading doses of ranibizumab, increased from months 3 to 6, and remained stable from months 6 to 12 (Fig 7). For treatment-naïve subjects, CFT had decreased an average of 107.0 μm in the 0.3 mg group and 122.0 μm in the 0.5 mg group at month 3. At month 12, the average decrease from baseline CFT was 72.0 μm in the 0.3 mg group and 92.0 μm in the 0.5 mg group. For previously treated subjects, CFT had decreased an average of 98.0 μm in the 0.3 mg group and 108.0 μm in the 0.5 mg group at month 3. At month 12, the average decrease from baseline CFT was 71.0 μm in the 0.3 mg group and 76.0 μm in the 0.5 mg group.

Because of the large number of cohort 2 subjects who discontinued, the last-observation-carried-forward method was not used to impute missing efficacy values, and observed results are reported. This should be kept in mind when interpreting the results.

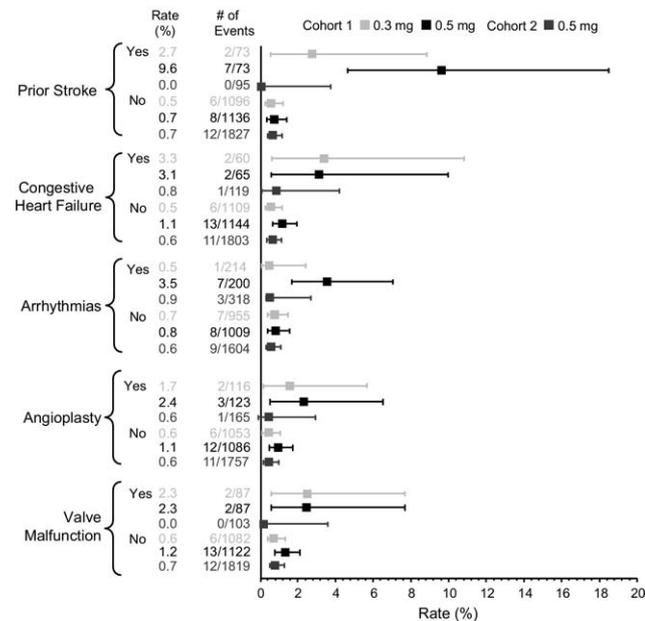


Figure 4. Stroke rate by risk factor. Point estimates and 2-sided Blyth-Still-Casella 95% confidence intervals for stroke rate when the risk factor was present or absent are shown. We evaluated the impact of 21 factors on the incidence of stroke. The 5 risk factors that had the greatest effect on stroke rates are presented.

Table 6. Cause of Death

	Cohort 1		Cohort 2
	0.3 mg (n = 1169)	0.5 mg (n = 1209)	0.5 mg (n = 1922)
All deaths, %	1.7	2.4	1.7
Deaths due to unknown cause, %	0.1	0.1	0.1
Vascular deaths, %	0.9	0.8	0.7
Cardiovascular ^a	0.8	0.5	0.7
Stroke ^b	0.2	0.3	0.1
Nonvascular deaths, %	0.7	1.5	0.9
Respiratory: pneumonia, respiratory failure, pulmonary failure, pulmonary edema	0.3	0.6	0.5
Accident, injury, intracranial bleed secondary to fall	0	0.2	0.1
Renal failure	0	0.1	0
Cancer	0	0.4	0.3
Infection (septic shock, sepsis, urosepsis), liver failure due to hepatitis	0.3	0.2	0
Postoperative bowel obstruction	0.1	0	0
Vasculitis	0	0	0.1

^aIncludes ischemic cardiomyopathy, coronary heart disease, cardiac arrest, MI, saddle pulmonary embolism, and heart failure.
^bIncludes stroke, acute ischemic stroke, intracerebral hemorrhage, cerebrovascular disease, and brain hemorrhage secondary to fall. Three 0.5 mg subjects with preexisting cancer had previously received cancer treatment.

Snellen VA in cohort 2 subjects improved from a median of 20/100 at baseline to 20/80 at months 6 and 12. The proportion of subjects with a Snellen equivalent of 20/200 or worse decreased from approximately 39% at baseline to 31% at month 6 and 32% at month 12.

Discussion

SAILOR is the largest study to date to evaluate safety (primary objective) and efficacy (secondary objective) of

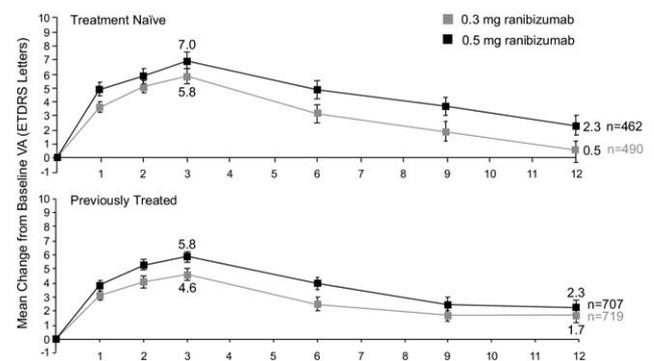


Figure 5. Change from baseline VA (cohort 1). For all groups, VA increased with 3 loading doses of ranibizumab (day 0, month 1, month 3). From months 3 to 12, with protocol-defined retreatment, VA tended to decrease. Error bars are ± 1 standard error. ETDRS = Early Treatment Diabetic Retinopathy Study; VA = visual acuity.

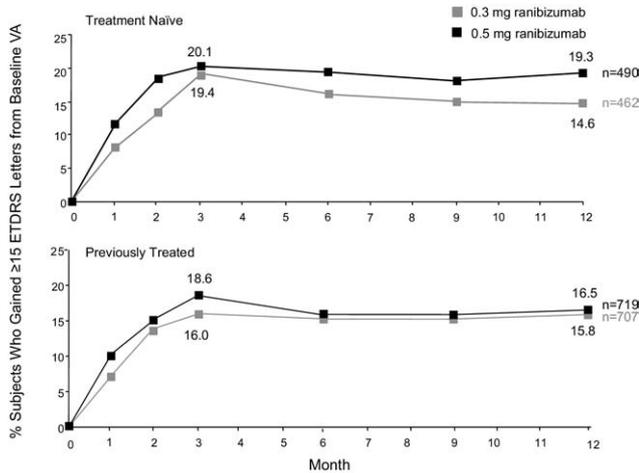


Figure 6. Subjects gaining ≥ 15 letters from baseline VA (cohort 1). The proportion of cohort 1 subjects who gained ≥ 15 letters increased with 3 loading doses of ranibizumab and was then maintained for the duration of the 12-month study. ETDRS = Early Treatment Diabetic Retinopathy Study; VA = visual acuity.

intravitreal ranibizumab in a population of subjects with CNV secondary to AMD. Ranibizumab was well tolerated, and the incidence of ocular SAEs and AEs was low and unrelated to dose. The rates of key nonocular SAEs and AEs, including APTC ATEs, MI, and vascular death, were similar across cohorts and dose groups.

The incidence of stroke in SAILOR was similar to that observed in previous ranibizumab studies.^{1,2,4,5} An interim analysis of SAILOR cohort 1 safety data (October 2006) suggested a higher incidence of stroke in subjects who received 0.5 mg ranibizumab compared with those who received 0.3 mg ranibizumab and triggered a “Dear Doctor” letter in January 2007. The interim safety analysis was based on an incomplete data set, and the difference between doses was less pronounced in the final study data.

The final study data showed a difference in stroke rate between doses, with a higher rate in the 0.5 mg dose group compared with the 0.3 mg dose group. The total number of events was small, and the difference was not confirmed statistically. However, there is potentially a higher stroke rate associated with the 0.5 mg dose, which is being monitored via postmarketing surveillance and ongoing trials of ranibizumab in neovascular AMD.

A more comprehensive data set exists with regard to safety when SAILOR data are combined with data from the studies designated A Phase IIIb, Multicenter, Randomized, Double Masked, Sham Injection Controlled Study of the Efficacy and Safety of Ranibizumab in Subjects with Subfoveal Choroidal Neovascularization (CNV) with or without Classic CNV Secondary to Age-Related Macular Degeneration (PIER)⁵ and RhuFab V2 Ocular Treatment Combining the Use of Visudyne to Evaluate Safety,⁶ MARINA, and ANCHOR trials. Further evaluation from a meta-analysis of these studies evaluating the incidence of strokes and overall APTC ATEs will be conducted and will include additional clinical trial data as they become available.

In SAILOR there was not a difference between doses in APTC ATEs overall, which is consistent with our current understanding of ranibizumab pharmacology. As a Fab, ranibizumab has low systemic bioavailability ($\sim 1/90,000$ of intravitreal concentration) and a half-life of only several hours (Kubler P, Xu L, Jumbe N, et al. Population pharmacokinetics of ranibizumab in patients with age-related macular degeneration. Presented at: American Society of Retina Specialists Annual Meeting, December 1–5, 2007; Indian Wells, California).

Certain subgroups of subjects (e.g., those with prior cardiovascular accidents) may experience higher rates of systemic SAEs. We observed that the incidence of stroke was greater for cohort 1 subjects who had a history of stroke, congestive heart failure, or arrhythmias. However, the low incidence of stroke in SAILOR made it difficult to draw meaningful conclusions about the relationship between risk factors and stroke. Although the results of clinical trials cannot be directly compared with epidemiology studies in AMD, epidemiology stroke rates can provide a reference that aids in understanding stroke rates in SAILOR. The annual stroke rate for new-onset neovascular AMD in a large sample of Medicare subjects was 3.8%, and the annual ischemic stroke rate was 56.4% for those subjects who had experienced an ischemic stroke in the year before study entry.⁷ Both of these rates are higher than those observed in SAILOR.

Ranibizumab treatment was associated with a net gain in VA in the cohort 1 0.3 mg and 0.5 mg dose groups. However, consistent with the results of MARINA and ANCHOR, 0.5 mg doses of ranibizumab tended to have a slightly greater VA benefit than 0.3 mg doses in subjects with neovascular AMD. Ranibizumab also tended to be

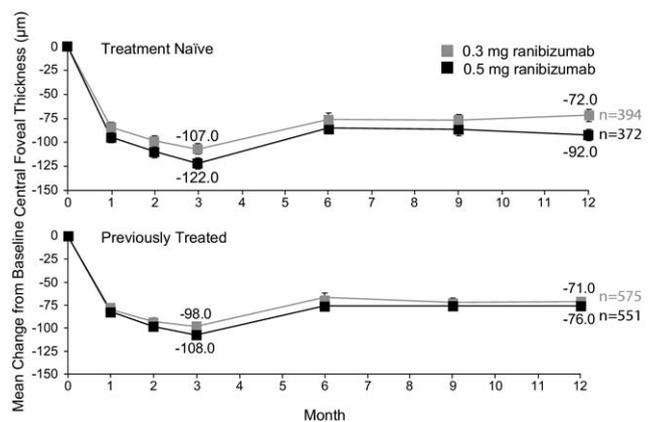


Figure 7. Change from baseline CFT (cohort 1). In cohort 1 subjects with OCT data, CFT decreased with 3 loading doses of ranibizumab. Central foveal thickness then increased from months 3 to 6 and remained stable from months 6 to 12. Error bars are ± 1 standard error. CFT = central foveal thickness; ETDRS = Early Treatment Diabetic Retinopathy Study; OCT = optical coherence tomography.

more efficacious in treatment-naïve subjects than in previously treated subjects.

The VA changes observed after month 3 on the SAILOR dosing regimen were not as great as those observed with continual monthly dosing in the MARINA and ANCHOR studies, in which VA increased throughout the first study year. In the SAILOR study, VA increased with 3 loading doses of ranibizumab and then decreased from month 3 to 12. A similar trend was observed in the PIER study, in which subjects received 3 loading doses of ranibizumab followed by quarterly injections. Thus, SAILOR and PIER subjects made fewer visits and were treated less frequently than subjects in MARINA and ANCHOR, which may account for the reduced VA benefits observed with less-than-monthly dosing.

The protocol-defined retreatment criteria in SAILOR may have permitted too much disease progression before retreatment was permitted. For example, for cohort 1 subjects who were retreated according to VA and OCT criteria (81%), retreatment was not permitted until a 100 μm increase in CFT or a loss of >1 line of best-corrected VA, relative to the lowest previously recorded value, occurred. Given that the largest average decrease in CFT ranged from 98 to 122 μm , it is possible that subjects lost nearly all of their prior anatomic improvement before qualifying for retreatment.

The Prospective Optical Coherence Tomography Imaging of Patients with Neovascular AMD Treated with Intra-Ocular Ranibizumab (Lucentis) (PrONTO) study, a nonrandomized, single-institute study with more flexible retreatment criteria,⁴ demonstrated that VA benefits similar to those of ANCHOR and MARINA could be obtained with less-than-monthly dosing when retreatment was based on qualitative and quantitative OCT, VA, hemorrhage, and fluid criteria. A future goal is to develop less-than-monthly treatment regimens that will prove optimal for physicians and subjects while realizing the full VA benefits of ranibizumab.

Study Limitations

Because the study did not include a control arm, safety could not be evaluated in terms of events related to ranibizumab treatment and events inherent to the elderly SAILOR subject population. Although differences in subject populations and dosing regimens prevent direct comparison across ranibizumab studies, the rates of safety events in the SAILOR study were low and similar to those of previous ranibizumab studies. Likewise, although the true benefit of ranibizumab could not be evaluated in the absence of a control group, SAILOR efficacy results were consistent with those in other controlled ranibizumab studies.

Eligibility for the SAILOR study was contingent on angiographically determined CNV. However, angiography was evaluated by individual investigators rather than a central reading center. Thus, investigator bias may have been introduced in subject selection across study sites.

Approximately 18% of cohort 1 subjects in each dose group discontinued the study before the month 12 visit, and

approximately 50% of cohort 2 subjects discontinued before the end of the 12-month study. The primary reason for discontinuation from each cohort was "subject decision," and although case report forms did not provide specific reasons that subjects opted to discontinue, one can speculate reasons for doing so. For instance, subjects may have discontinued so that their fellow eye could be treated with ranibizumab. Also, subjects who did not fulfill the retreatment criteria may have discontinued the study so that they could follow a less-conservative retreatment regimen. Ranibizumab became commercially available, and bevacizumab (Avastin, Genentech, Inc.) became widely used for AMD treatment during the study period; therefore, subjects were not required to remain in the study to receive ranibizumab/anti-VEGF-A therapy. Furthermore, ranibizumab was provided to cohort 2 subjects for only 30 days after it became commercially available on June 30, 2006. Thus, many cohort 2 subjects may have discontinued the study to pursue other treatment options.

In conclusion, intravitreal ranibizumab was safe and well tolerated in a large population of subjects with neovascular AMD. Ranibizumab had a beneficial effect on VA and retina anatomy. Future investigations will seek to establish optimal dosing regimens for persons with neovascular AMD.

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Footnotes and Financial Disclosures

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JAMA Ophthalmology | Original Investigation | CLINICAL TRIAL

Outcomes of the Veterans Affairs Low Vision Intervention Trial II (LOVIT II)

A Randomized Clinical Trial

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IMPORTANCE Randomized clinical trials are needed to compare effectiveness and cost-effectiveness of different low-vision (LV) programs.

OBJECTIVE To determine the value of adding LV rehabilitation with a therapist compared with LV services without intervention.

DESIGN, SETTING, AND PARTICIPANTS A randomized clinical trial was conducted from September 27, 2010, to July 31, 2014, of 323 veterans with macular diseases and best-corrected distance visual acuity (BCDVA better-eye) of 20/50 to 20/200. Masked interviewers administered questionnaires by telephone before and after LV treatment. Using an intention-to-treat design, participants were randomized to receive LV devices with no therapy or LV devices with a rehabilitation therapist providing instruction and homework on the use of LV devices, eccentric viewing, and environmental modification. Visual ability was measured in dimensionless log odds units (logits) (0.14-logit change in visual ability corresponds to ability change expected from a 1-line change in visual acuity).

INTERVENTIONS Low-vision devices without therapy and LV devices with therapy.

MAIN OUTCOMES AND MEASURES Comparison of changes (baseline to 4 months) in overall visual ability and in 4 functional domains (reading, visual information, visual motor, and mobility) estimated from responses to the Veterans Affairs Low Vision Visual Functioning Questionnaire (higher scores indicates more ability or less difficulty in performing activities), and comparison of MNREAD changes (baseline to end of treatment) in maximum reading speed, critical print size, and reading acuity (higher number indicates lower visual acuity).

RESULTS Of the 323 participants, 314 were male (97.2%); mean (SD) age, 80 (10.5) years. Basic LV was effective in improving visual ability. However, the LV rehabilitation group improved more in all visual function domains except mobility. Differences were 0.34-logit reading (95% CI, 0.0005 to 0.69; $P = .05$), 0.27-logit visual information (95% CI, 0.01 to 0.53; $P = .04$), 0.37-logit visual motor (95% CI, 0.08 to 0.66; $P = .01$), and 0.27-logit overall (95% CI, 0.06 to 0.49; $P = .01$). For MNREAD measures, there was more improvement in reading acuity (difference, -0.11 logMAR, 95% CI, -0.15 to -0.07 ; $P < .001$) and maximum reading speed (mean increase of 21.0 words/min; 95% CI, 6.4 to 35.5; $P = .005$), but not critical print size for the LV rehabilitation group (-0.06 logMAR; 95% CI, -0.12 to 0.002; $P = .06$). In stratified analyses, the LV rehabilitation group with BCDVA better-eye worse than 20/63 to 20/200 improved more in visual ability (reading, visual motor, and overall). Differences were 0.56-logit reading ability (95% CI, 0.08-1.04; $P = .02$), 0.40-logit visual motor (95% CI, 0.03-0.78; $P = .04$), 0.34-logit overall (95% CI, 0.06-0.62; $P = .02$). There was no significant difference between treatment groups for those with BCDVA better-eye of 20/50 to 20/63.

CONCLUSIONS AND RELEVANCE Both basic LV alone and combined with LV rehabilitation were effective, but the added LV rehabilitation increased the effect only for patients with BCDVA better-eye worse than 20/63 to 20/200. Basic LV services may be sufficient for most LV patients with mild visual impairment.

TRIAL REGISTRATION clinicaltrials.gov Identifier: [NCT00958360](https://clinicaltrials.gov/ct2/show/study/NCT00958360)

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Group Information: The LOVIT II Study Group members are listed at the end of this article.

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Low vision (LV) is defined as any chronic, uncorrectable visual impairment that affects daily life.¹ Low vision interferes with performance of activities such as reading, mobility, recognizing faces, and interacting with family and friends.²⁻⁷ Persons with LV can also experience loss of self-esteem⁸ and personal independence,⁹ as well as decreased quality of life¹⁰ accompanied by a decline in general health¹¹ and an increased risk of depression,¹²⁻¹⁴ injury,¹⁵⁻¹⁷ and mortality.¹⁸

Low vision programs include a variety of devices and therapies to improve patients' performance of tasks limited by visual impairment.² Despite the wide variation in the range and intensity of services provided, different LV programs may demonstrate successful outcomes based on the specific services they provide.² In a systematic literature review, Binns et al³ concluded that there is good evidence that LV devices improve reading ability and are valued by patients, that rehabilitation programs provided by the Veterans Affairs (VA) have a large positive effect, and that other rehabilitation programs have a medium to large effect in improving functional ability.

Veterans Affairs services for blind and visually impaired veterans include comprehensive inpatient rehabilitation programs, as well as multidisciplinary and basic LV programs.¹⁹ Randomized clinical trials are needed to compare the effectiveness and cost-effectiveness of LV programs, guide policy, and identify individuals who benefit most from different services.²⁰ The Veterans Affairs Low Vision Intervention Trial (LOVIT) evaluated an intense outpatient LV rehabilitation program for legally blind veterans with macular diseases (habitual distance visual acuity better-eye worse than 20/100 to 20/400) compared with a waiting-list control group.²¹ LOVIT II complemented LOVIT by comparing the outcomes of 2 types of LV programs for veterans less severely visually impaired from macular diseases (best-corrected distance visual acuity better-eye [BCDVA better-eye] 20/50-20/200).²⁰

Methods

Study Population

The inclusion criteria were eligible for VA benefits, diagnosis of any macular disease, and BCDVA better-eye of 20/50 to 20/200. Exclusion criteria were no access to telephone, less than fifth grade level achieved on the Dolch English Literacy Test,²² and no vision loss since previous LV rehabilitation, Telephone Interview for Cognitive Status²³ screening score less than 30, unable or unwilling to attend clinic visits, hearing impairment that interferes with telephone questionnaires, visual field better-eye less than 20° in diameter, vitreous hemorrhage affecting line of sight, cataract extraction planned within 4 months, receiving macular disease treatment expected to improve vision,²⁰ and participating in another study that does not allow dual enrollment.

LOVIT II was conducted at 9 VA medical facilities. The study rationale and methods have been published.^{20,24} The protocol (available in the [Supplement](#)) and written informed consent were approved by the VA Central Institutional Review Board. Participants gave written informed consent

Key Points

Question Are low-vision devices with low-vision rehabilitation (including therapy and homework to teach device use, eccentric viewing, and environmental modification) more effective than basic low-vision services (low-vision devices dispensed without therapy) for veterans with macular diseases and visual acuity of 20/50 to 20/200?

Findings In a multicenter randomized clinical trial, both treatments were found to be effective, but low-vision rehabilitation was more effective than basic low-vision services only for patients with visual acuity worse than 20/63 to 20/200.

Meaning Basic low-vision services are sufficient for most patients with low vision who have mild visual impairment.

after the purpose and procedures of the trial were explained, and financial compensation was provided. Study oversight was provided by an independent data and safety monitoring committee and the VA Cooperative Studies Program Coordinating Center.

Protocol Design

Patients with macular diseases were screened by medical records review for major inclusion and exclusion criteria. Eligible patients received study information from clinical health care professionals or a letter sent by mail. Site coordinators approved patients for enrollment after screening to determine eligibility using the Early Treatment of Diabetic Retinopathy Study visual acuity chart,²⁵ Dolch English Literacy Test words,²² and the Telephone Interview for Cognitive Status.²³

Low vision devices were prescribed based on standard of care after a LV examination.²⁶ Contrast sensitivity was measured with the Pelli-Robson Contrast Sensitivity Test.²⁷ Central and juxtafixational scotomas (defined as 4 contiguous points not seen) were measured with the Johns Hopkins University and Erickson Visual Field Test.²⁸ The MNREAD test²⁹ was administered at baseline. On the test, a higher number for reading acuity indicates lower visual acuity; maximum reading speed is patient's reading speed when reading is not limited by print size (units are words/min), and critical print size is the smallest print size the patient can read with their maximum reading speed at 20 cm with +5.00 diopters adjusted for nonstandard viewing distances; a higher number indicates larger critical print size; positive changes from baseline indicate worsening and negative changes indicate improvement. The VA Low Vision Visual Functioning Questionnaire (VA LV VFQ-48),³⁰⁻³⁴ Short Form-36,³⁵ and EuroQol-5D³⁶ were administered by telephone before randomization. A higher score on the VA LV VFQ-48 indicates better ability or less difficulty in performing activities, Higher scores on both the Short Form-36 and EuroQol-5D indicate better quality of life.

Eligible and consenting patients were assigned randomly to the treatment groups. In basic LV services, the optometrist dispensed LV devices without therapy or assigned homework. In LV rehabilitation, patients received basic LV services plus 1 to 3 therapy sessions including instruction in eccentric

viewing, use of LV devices (at near, intermediate, and far distances), environmental modification, integration of LV devices into lifestyle, and assigned homework to practice using LV devices for everyday tasks.

Changes in visual ability and quality of life were assessed by telephone 4 months from baseline with the VA LV VFQ-48, Short Form-36, and EuroQol-5D. Changes in MNREAD measures were assessed after treatment.

Randomization

The coordinating center created a computer-generated permuted block randomization with random block sizes. Study site coordinators received assignments from the online randomization system and informed patients and clinical staff of the treatment assignments. Preplanned stratification was by participating site and BCDVAbetter-eye (20/50 to 20/63 and worse than 20/63 to 20/200).

Masking

Interviewers who were certified and masked read a script to inform participants that questionnaire responses and treatment assignments were anonymous and confidential. Outcomes data were not shared with investigators or clinical staff until the study concluded.

Assessment of Outcomes

The primary outcome measure was comparison of the changes in reading ability (measured with responses to 10 items in the 48-item VA LV VFQ) at baseline compared with 4 months later in the treatment groups. The questionnaire was validated previously in LV populations.^{30,32,33,37} Patients rated their difficulty performing 48 daily activities using ordered response categories or they responded that they do not perform the activity for nonvisual reasons. Comparison of changes between groups for overall visual ability (from responses to all 48 items) and the other VA LV VFQ-48 visual ability domains (mobility, visual information processing, and visual motor skills from responses to different subsets of items) from baseline to 4 months, and changes in MNREAD measures of maximum reading speed, critical print size (smallest print that can be read at the maximum speed), and reading acuity from baseline to completion of treatment were secondary outcomes.

Statistical Analysis

A 0.35-treatment effect, 5% type I error, and 85% power were selected. This effect size corresponds to a clinically significant change of 2.5 lines due to the strong linear trend between visual ability person measures (measured in dimensionless log odds units [logits]) and visual acuity (in logMAR units).²⁰ A 0.14-logit visual ability change corresponds to the ability change expected from a 1-line change in visual acuity.³⁸ With a 2-sided *t* test for 2 independent groups, a sample size of 300 (150 per group) was calculated.²⁰ A 10% withdrawal rate was estimated based on previous studies, yielding a sample size of 330 patients (165 per group). Statistical guidelines for early stopping were not used because both groups received treatment, the study

duration was short, and the interventions were low risk. The data and safety monitoring committee reviewed the interim progress report biannually.

Rasch analyses³⁹ of responses to the 48 items and different subsets of items were used to estimate linear item and person measures in logits (with the origin arbitrarily set to the mean of the 48-item measures) for each of the 4 functional domains and overall visual ability for each participant. Comparisons of visual function person measures and subgroup analyses based on stratification were analyzed according to the intention-to-treat principle. The 2-sample *t* test was used to compare the differences in the primary and secondary outcomes between the treatment groups. Cohen *d* was used to calculate the magnitude of treatment effects as small (0.2), medium (0.5), or large (0.8).⁴⁰ Analysis of covariance was used to compare mean changes in the outcomes between the 2 arms adjusting for age, baseline measures (visual ability, BCDVAbetter-eye, contrast sensitivity, maximum reading speed, critical print size, and reading acuity), presence of visual fluctuations, history of receiving anti-vascular endothelial growth factor (VEGF) injections in the year before the study, and presence of central or juxtafixational scotomas.

The paired *t* test was used to test within-group changes. The differences between the treatment groups in quality of life from baseline to 4 months and changes in MNREAD measures of maximum reading speed, critical print size, and reading acuity, measured from baseline to completion of treatment, were also analyzed using 2-sample *t* tests.

Stepwise linear regression models were used to determine whether the mean changes in overall visual ability person measures and person measures for each functional domain from baseline to 4 months can be determined by baseline measures of visual impairment, baseline BCDVAbetter-eye, maximum reading speed, critical print size, reading acuity, presence of scotomas, visual fluctuations, treatment with anti-VEGF injections, age, and treatment group.

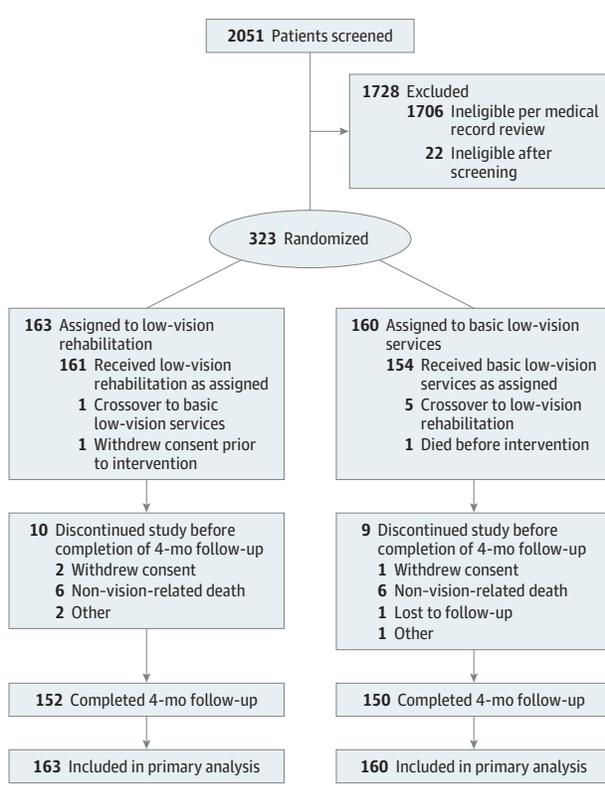
All analyses were 2-sided; $P \leq .05$ was considered statistically significant. SAS software, version 9.4, was used to perform all analyses.⁴¹

Results

Patient Characteristics

Enrollment began September 27, 2010; accrual was completed July 31, 2014; and follow-up ended February 17, 2015. The **Figure** describes the flow of participants through the trial. A total of 2051 patients were screened, of whom 1728 were excluded (1706 ineligible per medical records review and 22 ineligible after screening). Randomization was completed for 323 patients, with 163 assigned to LV rehabilitation and 160 assigned to basic LV services. The most frequent reason for exclusion was that the BCDVAbetter-eye did not fall into the required range. Nineteen patients discontinued the study before completion. The actual withdrawal rate was 6%, lower than the 10% withdrawal estimate used for sample size calculation. Based on actual attrition rate, recruitment exceeded the

Figure. CONSORT Diagram



required sample size of 320 patients. Final analyses included all 323 randomized patients.

Baseline characteristics, patients' health status, changes in visual function (baseline to 4 months), and changes in reading performance measures (baseline to end of treatment) are presented in Tables 1, 2, and 3. Overall, 97.2% of the participants were male and 90.4% were white; mean (SD) age was 80 (10.5) years. The most frequent eye diagnoses (better-seeing eye) were nonexudative age-related macular degeneration (AMD) (LV rehabilitation group, 65 [39.9%]; basic LV group, 70 [43.8%]) and exudative AMD (LV rehabilitation group, 37 [22.7%]; basic LV group, 41 [25.6%]). Anti-VEGF injections were received in the year before the study by 53 patients (32.5%) in the LV rehabilitation group and 60 (37.5%) in the basic LV group. Vision fluctuations were experienced by 50 patients (30.7%) in the LV rehabilitation group and 59 (36.9%) in the basic LV group. Mean BCDVA better-eye was 0.6 (0.2) logMAR (20/80 Snellen equivalent) for both groups; mean contrast sensitivity (better-seeing eye) was 1.1 (0.8) for both groups. There were no differences in baseline health status between the groups. Mean MREAD reading performance measures at baseline were reading acuity, 0.9 (0.4) logMAR (20/160 Snellen equivalent) for both groups; maximum reading speed was 109.1 (68.6) words per minute for the LV rehabilitation group and 120.2 (72.2) words per minute for the basic LV group. Critical print size was 1.2 (0.3) logMAR (20/300 Snellen equivalent) for both groups.

Table 1. Baseline Characteristics and Health Status of Patients

Characteristic	No. (%)	
	LV Rehabilitation (n = 163)	Basic LV Services (n = 160)
Age, mean (SD), y	80.1 (10.8)	79.2 (10.1)
Male	158 (96.9)	156 (97.5)
Race		
White	148 (90.8)	144 (90.0)
African American	15 (9.2)	10 (6.3)
Other	0	6 (3.8)
Ethnicity (non-Hispanic origin)	157 (96.3)	152 (95.0)
Educational level (≤high school)	72 (44.2)	79 (49.4)
Living situation	47 (28.8)	43 (26.9)
Alone		
With family	102 (62.6)	103 (64.4)
With nonfamily	7 (4.3)	7 (4.4)
Nursing home/assisted living	7 (4.3)	7 (4.4)
Employment status		
Employed	11 (6.7)	7 (4.4)
Unemployed or retired	152 (93.3)	152 (95.0)
Income, \$		
<39 999	88 (54.0)	99 (61.9)
40 000-59 999	32 (19.6)	22 (13.8)
>60 000	10 (6.1)	12 (7.5)
No answer	33 (20.2)	27 (16.9)
Diabetes	61 (37.4)	70 (43.8)
Pulmonary disease	35 (21.5)	32 (20.0)
Arthritis	93 (57.1)	84 (52.5)
Depression	30 (18.4)	26 (16.3)
Hypertension	101 (62.0)	92 (57.5)
Heart problems	77 (47.2)	74 (46.3)
Need walking assistance	72 (44.2)	81 (50.6)
Hand grip		
Strong	87 (53.4)	88 (55.0)
Intermediate	61 (37.4)	53 (33.1)
Weak	15 (9.2)	18 (11.3)
Unable to grip	0	1 (0.6)
Other hand problems	65 (39.9)	67 (41.9)
Motion limitation	23 (14.1)	22 (13.8)
Endurance limits	91 (55.8)	103 (64.4)
Memory		
No problems	58 (35.6)	51 (31.9)
Occasionally forgetful	99 (60.7)	92 (57.5)
Frequently forgetful	6 (3.7)	16 (10.0)
Confused	0	1 (0.6)
Age at developing vision problem, y		
≤40	14 (8.6)	12 (7.5)
41-60	31 (19.0)	31 (19.4)
>60	118 (72.4)	117 (73.1)
Vision fluctuation	50 (30.7)	59 (36.9)
Anti-VEGF injections last year	53 (32.5)	60 (37.5)
Use hearing aid	67 (41.1)	57 (35.6)
Best corrected distance visual acuity in better seeing eye, mean (SD), logMAR ^a	0.6 (0.2)	0.6 (0.2)

(continued)

Table 1. Baseline Characteristics and Health Status of Patients (continued)

Characteristic	No. (%)	
	LV Rehabilitation (n = 163)	Basic LV Services (n = 160)
Contrast sensitivity in better seeing eye, mean (SD)	1.1 (0.7)	1.1 (0.8)
MNREAD, mean (SD)		
Reading acuity, logMAR ^b	0.9 (0.4)	0.9 (0.4)
Maximum reading speed, s	109.1 (68.6)	120.2 (72.2)
Critical print size, logMAR ^c	1.2 (0.3)	1.2 (0.3)

Abbreviations: LV, low vision; VEGF, vascular endothelial growth factor.

^a Snellen equivalent: 20/80.

^b Snellen equivalent: 20/160.

^c Snellen equivalent: 20/300.

Treatment

The LV rehabilitation group received a mean (SD) of 1.9 (0.3) therapy sessions and completed 9.8 (4.8) homework assignments. Mean therapy time was 234.2 (145.9) minutes. Therapy was longest for eccentric viewing skills at 64.1 (48.8) minutes; the time varied for LV devices, but was longest for portable electronic magnifiers (62.3 [28.2] minutes) and desktop video magnifiers (68.6 [39.2] minutes). Dispensing time for the basic LV group varied based on LV devices prescribed (54.2 [11.1] minutes). There was no difference in the percentages of LV devices prescribed for both groups. The LV rehabilitation group (n = 163) received 56 (34.4%) monocular telescopes, 82 (50.3%) telescopes, 102 (62.6%) pocket magnifiers, 76 (46.6%) stand magnifiers, 48 (29.4%) intermediate distance devices, 51 (31.3%) reading glasses, 52 (31.9%) desktop electronic magnifiers, 40 (24.5%) portable electronic magnifiers, 95 (58.3%) filters or prescription sunglasses, and 18 (11.0%) filters to control glare indoors. The basic LV services group (n = 160) received 73 (45.6%) monocular telescopes, 90 (56.3%) telescopes, 122 (76.3%) pocket magnifiers, 91 (56.9%) stand magnifiers, 44 (27.5%) intermediate distance devices, 54 (33.8%) reading glasses, 55 (34.4%) desktop electronic magnifiers, 56 (35.0%) portable electronic magnifiers, 101 (63.1%) filters or prescription sunglasses, and 25 (15.6%) filters to control glare indoors.

Outcomes

Primary and Secondary Outcomes—VA LV VFQ-48

Change of Visual Ability | Table 2 presents the comparison of the mean changes in primary and secondary outcomes in logits from baseline to 4 months between the treatment groups. A 0.14-logit change in visual ability corresponds to the ability change expected from a 1-line change in visual acuity. Compared with those in the LV group who received basic services, patients in the LV rehabilitation group who received basic LV services plus LV rehabilitation reported greater improvement in visual ability (reading, visual information processing, visual motor skills, and overall). Within groups, improvement was found in all functional domains and overall visual ability in the LV rehabilitation group and for all functional domains and overall visual ability except mobility in the basic LV group.

The outcomes comparisons between the treatment groups were not altered after adjusting for all covariates (n = 272).

Table 2 reports subgroup analyses based on the pre-planned stratification by BCDVAbetter-eye. Patients with BCDVAbetter-eye worse than 20/63 to 20/200 assigned to the LV rehabilitation group who received basic LV plus LV rehabilitation experienced more improvement in visual ability (reading, visual motor, and overall) than those assigned to basic LV services. There was no difference in outcomes between treatment groups for patients with BCDVAbetter-eye 20/50 to 20/63. Compared with patients with worse visual acuity, those with better BCDVAbetter-eye received fewer anti-VEGF injections in the year before the study (35 [26.5%] vs 78 [40.8%]; $P = .005$); they had fewer central or juxtafoveal scotomas (48 [44.9%] vs 125 [74.0%]; $P < .001$), higher contrast sensitivity (1.2 [0.8%] vs 1.0 [0.7%]; $P = .04$), and better MNREAD reading performance measures (reading acuity, 0.65 logMAR vs 1.03 logMAR; $P < .001$; maximum reading speed, 142.6 vs 94.8 words/min; $P < .001$; critical print size, 1.09 logMAR vs 1.31 logMAR; $P = .03$). In addition, patients with better BCDVAbetter-eye were prescribed fewer desktop electronic magnifiers (20 [15.2%] vs 87 [45.5%]; $P < .001$) or portable video magnifiers (28 [21.2%] vs 68 [35.6%]; $P = .004$).

Indicators of Change of Visual Ability | Improvements in visual ability domains and overall visual ability were indicated by lower baseline scores (B coefficients from stepwise linear regression models: reading, -0.52 ; mobility, -0.41 ; visual information, -0.42 ; visual motor, -0.32 ; overall, -0.30 ; $P < .001$). Improvement in all domains except visual information processing was indicated by LV rehabilitation group assignment (B coefficients from stepwise linear regression models: reading, 0.36; mobility, 0.25; visual motor, 0.44; overall, 0.29; $P < .05$).

Changes in Reading Performance Measures | Table 3 presents the mean changes in MNREAD reading performance measures for all patients. The LV rehabilitation group demonstrated more improvement in reading acuity ($P < .001$) and maximum reading speed (differences: -0.11 logMAR reading acuity [equivalent to 1 line]; 95% CI, -0.15 to -0.07 ; $P < .001$; mean increase of 21.0 words/min in maximum reading speed, 95% CI, 6.4 to 35.5; $P = .005$). There were no changes in critical print size within or between groups.

Changes in Quality-of-Life Scores | Comparison of changes in quality-of-life scores from baseline to 4 months between treatment groups found no differences in Short Form-36 subscale scores for physical functioning, physical role limitations, bodily pain, vitality, social functioning, emotional role limitations, mental health, general health, and physical or mental components. There was also no change in the EuroQol-5D scores from baseline to 4 months between the treatment groups.

Adverse Events

A total of 11 adverse events were reported from inception of the study to the 4-month follow-up. None of these adverse events was related to the study intervention.

Table 2. Mean Changes in Primary and Secondary Outcome Measures^a

VA LV VFQ-48 ^b	Mean (SD)		LV Rehabilitation vs Basic LV Services		
	LV Rehabilitation (n = 163)	Basic LV Services (n = 160)	Difference (95% CI)	P Value	Effect Size ^c
All Patients					
Reading ability					
Baseline	0.51 (1.43)	0.51 (1.44)			
Change from baseline to 4 mo	1.29 (1.66) ^d	0.95 (1.46) ^d	0.34 (0.0005 to 0.69)	.05	0.22
Mobility					
Baseline	0.71 (1.31)	0.60 (1.16)			
Change from baseline to 4 mo	0.31 (1.15) ^d	0.12 (1.16)	0.19 (-0.06 to 0.45)	.13	0.17
Visual information processing					
Baseline	0.62 (1.35)	0.72 (1.26)			
Change from baseline to 4 mo	0.67 (1.21) ^d	0.40 (1.13) ^d	0.27 (0.01 to 0.53)	.04	0.23
Visual motor skill					
Baseline	0.73 (1.38)	0.67 (1.32)			
Change from baseline to 4 mo	0.77 (1.47) ^d	0.40 (1.19) ^d	0.37 (0.08 to 0.66)	.01	0.28
Overall visual ability					
Baseline	0.61 (1.10)	0.61 (1.02)			
Change from baseline to 4 mo	0.70 (1.06) ^d	0.43 (0.89) ^d	0.27 (0.06 to 0.49)	.01	0.28
Stratum With BCVA better-eye 20/50 to 20/63					
Reading ability					
Baseline	1.17 (0.99)	1.05 (1.23)			
Change from baseline to 4 mo	0.83 (1.41) ^d	0.81 (1.21) ^d	0.02 (-0.43 to 0.47)	.93	0.02
Mobility					
Baseline	1.03 (1.33)	0.80 (1.12)			
Change from baseline to 4 mo	0.27 (1.21)	0.15 (1.15)	0.12 (-0.29 to 0.52)	.57	0.02
Visual information processing					
Baseline	1.16 (1.29)	1.22 (1.32)			
Change from baseline to 4 mo	0.55 (1.24) ^d	0.31 (1.10) ^e	0.24 (-0.17 to 0.64)	.25	0.20
Visual motor skill					
Baseline	1.19 (1.32)	1.04 (1.44)			
Change from baseline to 4 mo	0.76 (1.63) ^d	0.43 (1.06) ^e	0.33 (-0.15 to 0.80)	.18	0.24
Overall visual ability					
Baseline	1.07 (1.00)	0.99 (1.00)			
Change from baseline to 4 mo	0.57 (1.09) ^d	0.40 (0.85) ^d	0.17 (-0.17 to 0.51)	.32	0.17
Stratum With BCVA better-eye Worse Than 20/63 to 20/200					
Reading ability					
Baseline	0.06 (1.51)	0.13 (1.46)			
Change from baseline to 4 mo	1.60 (1.75) ^d	1.04 (1.61) ^d	0.56 (0.08 to 1.04)	.02	0.33
Mobility					
Baseline	0.49 (1.25)	0.47 (1.17)			
Change from baseline to 4 mo	0.34 (1.11) ^e	0.09 (1.17)	0.25 (-0.08 to 0.57)	.14	0.22
Visual information processing					
Baseline	0.25 (1.26)	0.37 (1.09)			
Change from baseline to 4 mo	0.76 (1.18) ^d	0.47 (1.16) ^d	0.29 (-0.04 to 0.63)	.09	0.25
Visual motor skill					
Baseline	0.41 (1.34)	0.40 (1.18)			
Change from baseline to 4 mo	0.78 (1.36) ^d	0.38 (1.28) ^e	0.40 (0.03 to 0.78)	.04	0.30
Overall visual ability					
Baseline	0.30 (1.06)	0.34 (0.94)			
Change from baseline to 4 mo	0.79 (1.04) ^c	0.45 (0.92) ^c	0.34 (0.06 to 0.62)	.02	0.35

Abbreviations: BCVA better-eye, best-corrected visual acuity; LV, low-vision; VA LV VFQ-48, Veterans Affairs Low Vision Visual Functioning Questionnaire.

^a Changes in visual ability (reading, mobility, visual information processing, visual motor skills, and overall ability) from baseline to 4 months measured with the VA LV VFQ-48 scale for the LV rehabilitation and basic LV service groups. Logits are the dimensionless units of measurement on the visual ability scale. A 0.14-logit change in visual ability corresponds to the ability

change expected from a 1-line change in visual acuity.

^b Higher score indicates better ability or less difficulty in performing activities.

^c Effect size characterizes the magnitude of the treatment effect as small (0.2), medium (0.5), or large (0.8).

^d $P < .001$ for within-group change.

^e $P < .05$ for within-group change.

Table 3. Mean Changes in MNREAD Measures for All Patients

MNREAD Measures	Mean (SD)		LV Rehabilitation vs Basic LV	
	LV Rehabilitation (n = 134)	Basic LV (n = 149)	Difference (95% CI)	P Value
Reading Acuity^a				
Baseline	0.89 (0.35)	0.86 (0.35)		
Change from baseline to completion of treatment	-0.10 (0.18) ^b	0.01 (0.14)	-0.11 (-0.15 to -0.07)	<.001
Maximum Reading Speed^c				
Baseline	109.1 (68.6)	120.2 (72.2)		
Change from baseline to completion of treatment	19.5 (79.6) ^d	-1.5 (40.1)	21.0 (6.4 to 35.5)	.005
Critical Print Size^e				
Baseline	1.23 (0.27)	1.21 (0.28)		
Change from baseline to completion of treatment	-0.04 (0.27)	0.02 (0.25)	-0.06 (-0.12 to 0.002)	.06

Abbreviations: D, diopter; LV, low-vision.

^a Reading acuity is smallest print size read at 20 cm with +5.00 D adjusted for nonstandard viewing distances; units are logMAR (Snellen equivalent: 20/160). Higher number indicates lower visual acuity.

^b $P < .001$ for within-group change.

^c Maximum reading speed is patient's reading speed when reading is not limited by print size; units are words per minute.

^d $P < .01$ for within-group change.

^e Critical print size is smallest print size the patient can read with their maximum reading speed at 20 cm with +5.00 D adjusted for nonstandard viewing distances; units are logMAR (Snellen equivalent: 20/300). Higher number indicates larger critical print size; positive changes from baseline indicate worsening and negative changes indicate improvement.

Discussion

This RCT demonstrated that both basic LV services and basic LV services plus LV rehabilitation provided for veterans with macular diseases, most of whom were white, male, and covered by Medicare,⁴² had improved visual ability (reading, visual information processing, visual motor skills, and overall) at 4-month follow-up. Basic LV services plus LV rehabilitation also improved mobility. In preplanned stratified analyses, visual ability (reading, visual motor skills, and overall) improved more in the LV rehabilitation group than in the basic LV services group for patients with BCDVA better-eye worse than 20/63 to 20/200; there were no differences between treatment groups for those with BCDVA better-eye 20/50 to 20/63. There were differences in the number of anti-VEGF injections received in the year before the study, contrast sensitivity, presence of central or juxtafoveal scotomas, and LV devices prescribed between the stratified groups. These results led us to conclude that patients with mild LV (20/50 to 20/63) benefit from basic LV services except for mobility but gain no additional benefit from LV rehabilitation, whereas patients with moderate LV (worse than 20/63 to 20/200), also with the exception of mobility, benefit from basic LV services but gain even greater benefit with the addition of LV rehabilitation.

As expected from the relationship of visual ability with visual acuity, patients with better BCDVA better-eye had more overall visual ability at baseline than did patients with lower BCDVA better-eye. The patients with more visual ability were closer to the measurement ceiling of the VA LV VFQ-48; therefore, they had less room for improvement compared with those with lower BCDVA better-eye and less visual ability. Confirming this observation, the stepwise linear regression showed that improvement in all visual ability domains and overall visual ability is indicated by lower baseline scores and with the addition of LV rehabilitation.

LOVIT was a multicenter RCT²¹ that evaluated the effectiveness of an LV program rehabilitation for legally blind veterans with macular diseases similar to but more intense than the LV rehabilitation provided in LOVIT II. LOVIT demonstrated that LV rehabilitation significantly improved the visual ability of veterans compared with patients similarly impaired in the waiting-list control group who lost visual ability during the same 4-month interval.²¹ Patients in the LOVIT treatment group demonstrated more improvement in reading ability at 4-month follow-up than did patients in the LV rehabilitation group in the present study. Consistent with their more severe visual impairment, baseline reading ability for patients in LOVIT was less than that of patients in LOVIT II, so participants in LOVIT had more room for improvement. Another difference is that patients in LOVIT II received less therapy and homework and fewer desktop electronic magnifiers were prescribed compared with patients in LOVIT.

Changes in reading acuity and maximum reading speed from baseline to completion of treatment in the LV rehabilitation group compared with the basic LV services group (Table 3) mirrored changes in self-reported functional reading ability. Instruction in eccentric viewing, word and letter recognition skills, scanning techniques, and homework to practice skills may have contributed to these changes. There were no significant changes in critical print size between groups or within groups.

Three previous studies reported in the literature did not find differences in outcomes between basic and multidisciplinary or enhanced LV service delivery models in the United Kingdom, the Netherlands, or New Zealand.⁴³⁻⁴⁷ It is a challenge to compare these LV effectiveness studies because (1) different outcome measures were used; (2) protocols differed with regard to inclusion criteria for severity of impairment, diagnosis, and follow-up time; and (3) access to LV devices and therapies was based on health services policies that vary among countries.³

Strengths and Limitations

Both LOVIT studies had many strengths: a RCT design, a well-defined treatment protocol guided by therapy and home-work manuals that was consistently followed at all sites, the same validated questionnaires used to assess outcomes and health status, and scientific oversight and monitoring provided by a coordinating center and data and safety monitoring committee.^{20,21} The LOVIT studies were conducted in the VA system where veterans are eligible for LV services and provided LV devices without charge. The primary weakness is that study results cannot be generalized to the US private sector where Medicare covers LV therapy prescribed by physicians

and provided by occupational therapy, but does not cover the cost of LV devices.⁴²

Conclusions

Basic LV services alone or with the addition of LV rehabilitation was effective in this trial, but basic LV services plus LV rehabilitation was more effective than basic LV services alone only for patients with BCDVA better-eye worse than 20/63 to 20/200. These findings suggest that basic LV services are sufficient for most patients with LV who have mild visual impairment.

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Electrophysiological and transcriptomic correlates of neuropathic pain in human dorsal root ganglion neurons

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Neuropathic pain encompasses a diverse array of clinical entities affecting 7–10% of the population, which is challenging to adequately treat. Several promising therapeutics derived from molecular discoveries in animal models of neuropathic pain have failed to translate following unsuccessful clinical trials suggesting the possibility of important cellular-level and molecular differences between animals and humans. Establishing the extent of potential differences between laboratory animals and humans, through direct study of human tissues and/or cells, is likely important in facilitating translation of preclinical discoveries to meaningful treatments. Patch-clamp electrophysiology and RNA-sequencing was performed on dorsal root ganglia taken from patients with variable presence of radicular/neuropathic pain. Findings establish that spontaneous action potential generation in dorsal root ganglion neurons is associated with radicular/neuropathic pain and radiographic nerve root compression. Transcriptome analysis suggests presence of sex-specific differences and reveals gene modules and signalling pathways in immune response and neuronal plasticity related to radicular/neuropathic pain that may suggest therapeutic avenues and that has the potential to predict neuropathic pain in future cohorts.

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Abbreviation: DRG = dorsal root ganglion

Introduction

Significant effort has been placed on development of molecularly targeted therapies for neuropathic pain given the tremendous unmet need and consequent expanding chronic pain epidemic (van Hecke *et al.*, 2014). Yet, numerous promising therapeutics derived from discoveries in animal models have failed in clinical trials (Hill, 2000; Gavva *et al.*, 2008). A variety of factors have been proposed as possible causes for these failures with basic cellular-level and molecular differences between animals and humans commonly implicated (Borsook *et al.*, 2014; Gereau *et al.*, 2014). More efficient translation may be facilitated through direct study of human tissues and/or cells. Prior laboratory studies with human dorsal root ganglion (DRG) neurons from foetal tissue, post-mortem organ donation, and patients undergoing surgical treatments for chronic pain have attempted to make such confirmations by probing a wide array of basic histochemical and electrophysiological parameters (Baumann *et al.*, 1996; Borsook *et al.*, 2014; Davidson *et al.*, 2014; Li *et al.*, 2015, 2017). However, a key gap in knowledge is direct comparison of DRG neuron electrophysiology and paired gene expression profiling from patients with and without chronic neuropathic pain. Using a unique cohort of patients, here we provide detailed electrophysiological characterization and RNA sequencing (RNA-seq) of DRG neurons and tissue, respectively, from people with neuropathic pain. Our results provide clear evidence of spontaneous activity in sensory neurons as a driver of neuropathic pain; and our RNA-seq data suggest key pathways for targeted therapeutics and reveal potential biomarkers for neuropathic pain.

Materials and methods

Study approval

Written informed consent for participation, including use of tissue samples, was obtained from each patient prior to inclusion. The protocol was reviewed and approved by the M.D. Anderson and The University of Texas at Dallas Institutional Review Boards and all experiments conform to relevant guidelines and regulations.

Clinical data collection

Clinical data were obtained from patients undergoing treatment at MD Anderson Cancer Center for malignant tumours involving the spine through a combination of retrospective review of medical records and prospective data collection at the time of study enrolment. These data included basic patient demographics, medical history, and clinical symptoms. Preoperative MRI was evaluated for radiographic evidence of spinal cord or nerve root compression. Spinal cord compression was evaluated according to the epidural spinal cord compression scale (Bilsky *et al.*, 2010). Presence of nerve root compression was determined based on a documented report

from a neuroradiologist or review by a neurosurgeon. Axial spine pain was defined as present if there was a documented history of pain complaint in the midline in the neck or back, or if physical exam findings indicative of the axial spine as a pain generator was present. Axial spine pain was determined as absent if there was no documentation of a history of midline pain in the neck/back and a documented denial of axial pain, nor any physical exam findings indicative of axial spine as a pain generator. Determination of presence or absence of radicular/neuropathic pain was performed for each dermatome associated with a harvested dorsal root ganglion and consistent with the guidelines for probable or definite neuropathic pain from the Assessment Committee of the Neuropathic Pain Special Interest Group of the International Association for the Study of Pain (IASP) (Haanpaa *et al.*, 2011). Specifically, pain was deemed present if the patient had documented symptoms of spontaneous pain, sensory loss, paraesthesia, dysaesthesia, hyperalgesia, or allodynia in a distribution at or within two classically defined dermatomes of the harvested ganglion. Neuropathic pain was considered absent if the patient had no history of any symptoms defined in part 1 or if the ganglion was harvested from the side contralateral to reported pain in a patient with only unilateral symptoms. Any remaining scenario was categorized as indeterminate and neurons from these ganglia excluded from analysis of associations with clinical data. Of note, although some patients had a history of chemotherapy treatment, the DRG collected here were outside the dermatomes affected by length-dependent neuropathy. Detailed clinical characteristics for the entire cohort are found in Supplementary Table 1.

Human dorsal root ganglion neuron preparation

Human DRG neurons were prepared as described previously (Li *et al.*, 2015, 2017) and based largely on additional prior work (Davidson *et al.*, 2014). Briefly, each donor was undergoing surgical treatment that necessitated ligation of spinal nerve roots to facilitate tumour resection or spinal reconstruction. Spinal roots were ligated proximal to the DRG, spinal root sharply cut both proximal and distal to the DRG, and excised DRG transferred immediately into cold (~4°C) and sterile balanced salt solution containing nutrients. DRG were transported to the laboratory on ice in a sterile, sealed 50-ml centrifuge tube. Upon arrival to the laboratory, each ganglion was carefully dissected from the surrounding connective tissues and sectioned into three to four parts. One section was immediately frozen in RNAlater (Ambion) and saved for subsequent RNA sequencing. One or two sections of DRG were further cut into several ~1–2-mm pieces and cells dissociated for electrophysiology recording. Further details on the DRG cell dissociation, recording procedures can be found in the Supplementary material.

RNA sequencing

Total RNA from 21 quartered DRG samples from 15 patients were purified using TRIzolTM (ThermoFisher) and subjected to ribosomal RNA depletion and total RNA Tru-seq library preparation according to the manufacturer's instructions (Illumina). Tru-seq total RNA library kit with ribosomal RNA depletion

(Illumina) was used to generate sequencing libraries. Fifty cycle, single-end sequencing of these RNA-seq libraries was performed on the Illumina Hi-Seq sequencing platform. Obtained sequencing reads were mapped to the reference genome in a strand-aware fashion, retaining only uniquely mapped reads, based on the reference transcriptome annotations and the reference human genome hg19 in the NCBI Entrez/RefSeq database (Maglott *et al.*, 2005). The bowtie2 tool (with maximum allowed alignment mismatch ≤ 2) (Langmead and Salzberg, 2012) was used for mapping reads and the Subread package was used for counting mapped reads (Liao *et al.*, 2013). Read counts were normalized to transcripts per million for downstream analysis.

Random Forest-based prediction of cohort membership

We performed a proof-of-principle analysis for predicting the pain categorization of each sample based solely on the RNA abundance profile using the predictive classification model Random Forest, which uses an ensemble of decision trees to classify samples, and which has been used successfully in whole genome assay studies (Chen and Ishwaran, 2012). We built separate classifiers to discriminate between male-pain and male-no pain samples; and between male-pain and female-pain samples, solely based on the RNA profile of the autosomal gene expression profile of the corresponding sample. We performed leave-one-out cross validation analysis, by training our Random Forest model on all but one of the samples. We then blinded ourselves to the cohort membership of the held out sample (referred to as the test sample), and then predicted the label of the test sample using its RNA profile. This analysis was performed on every sample in turn to generate a cohort membership prediction for every sample based on their individual RNA profiles. Our leave-one-out cross validation approach provides an alternative to an independent validation cohort for confirming whether conclusions drawn from our present cohort about discriminative gene sets can be successfully applied to new datasets.

Statistics and computation: clinical, electrophysiological and RNA-seq data analysis

Clinical and electrophysiological data were analysed with GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA). Unless otherwise specified, data are expressed as mean \pm standard error of mean (SEM). Continuous variables were analysed with Mann-Whitney U-test. Fisher's exact test was used for analysis of contingency tables. Details of the computational analysis of the RNA-seq data (including the random forest classification algorithm) can be found in the Supplementary material.

Data availability

The neurophysiological data can be shared on request. The full transcriptomic dataset and code for analysis is available at: <https://www.utdallas.edu/bbs/painneurosciencelab/sensoryomics/hdrclinical/>. Raw sequencing datasets are available from the dbGaP repository as single-end read libraries (phs001158.v2.p1).

Results

Sixty-six DRG were collected from 26 (eight female and 18 male) patients whose clinical data, including opioid consumption, are summarized in Supplementary Table 1. The donor cohort in this study is unique in that all donors had a complete medical history available for review allowing us to make clear distinctions between pain and no pain samples for electrophysiological and RNA-seq analyses. The majority of patients ($n = 17$) were afflicted with metastatic carcinoma to the spine versus primary malignancies of bone ($n = 7$) or local extension of a primary carcinoma ($n = 2$); and most ($n = 25$) had a history consistent with axial spine pain. Presence versus absence of associated dermatomal radicular/neuropathic pain was determined for all donated ganglia. These criteria defined three patient groups. The first was composed of six patients with isolated axial spine pain, but without any radicular/neuropathic pain (Fig. 1A–C). Group 2 included 15 patients with unilateral radicular/neuropathic pain (Fig. 1D and E) and Group 3 included five patients with bilateral radicular/neuropathic pain (Fig. 1G–I). Radicular/neuropathic pain was strongly associated with radiographic evidence of nerve root compression (Fig. 1K, 35/39 compressed ganglia with pain, $P < 0.001$). At maximal intensity, median visual analogue pain scale (VAS) was 7.72 for the entire cohort and no statistically significant difference between VAS score for patients with versus without radicular/neuropathic symptoms (7.72 versus 7.53, $P = 0.85$). The majority of patients' radicular/neuropathic symptoms were present for more than 6 months (12/20) and there were no patients without symptoms dating back at least 1 month.

Whole-cell patch clamp recordings were performed on samples from 17 patients from a total of 28 DRG after dissociation and > 24 h in culture. The median patched cells per patient was nine (range 2–26). Spontaneous activity was recorded in 13% of neurons (20/149), from 39% of donated DRG (11/28), and in 59% of patients (10/17). Representative analogue traces show the baseline membrane potential in a non-spontaneous activity neuron was stable (Fig. 1J); whereas the exploded view of the baseline membrane potential (Fig. 1K) and compressed time base (Fig. 1L) for a neuron with spontaneous activity show the occurrence of spontaneous depolarizations of membrane potential was only observed in cells with spontaneous activity (Fig. 1K) and these cells typically showed an irregular pattern of action potentials (Fig. 1L). Statistical analysis relating the clinical parameters to electrophysiology revealed significant associations of spontaneous activity and neuronal hyperexcitability [hyperpolarization of action potential threshold (Fig. 1M) and decrease in step rheobase (Fig. 1N)] with both radicular/neuropathic pain and radiographic nerve root compression (Fig. 1L and M, spontaneous activity: $P < 0.05$, spike threshold $P < 0.05$, rheobase: $P < 0.05$). Spontaneous activity was noted in 19% (20/106) of neurons from DRG with corresponding

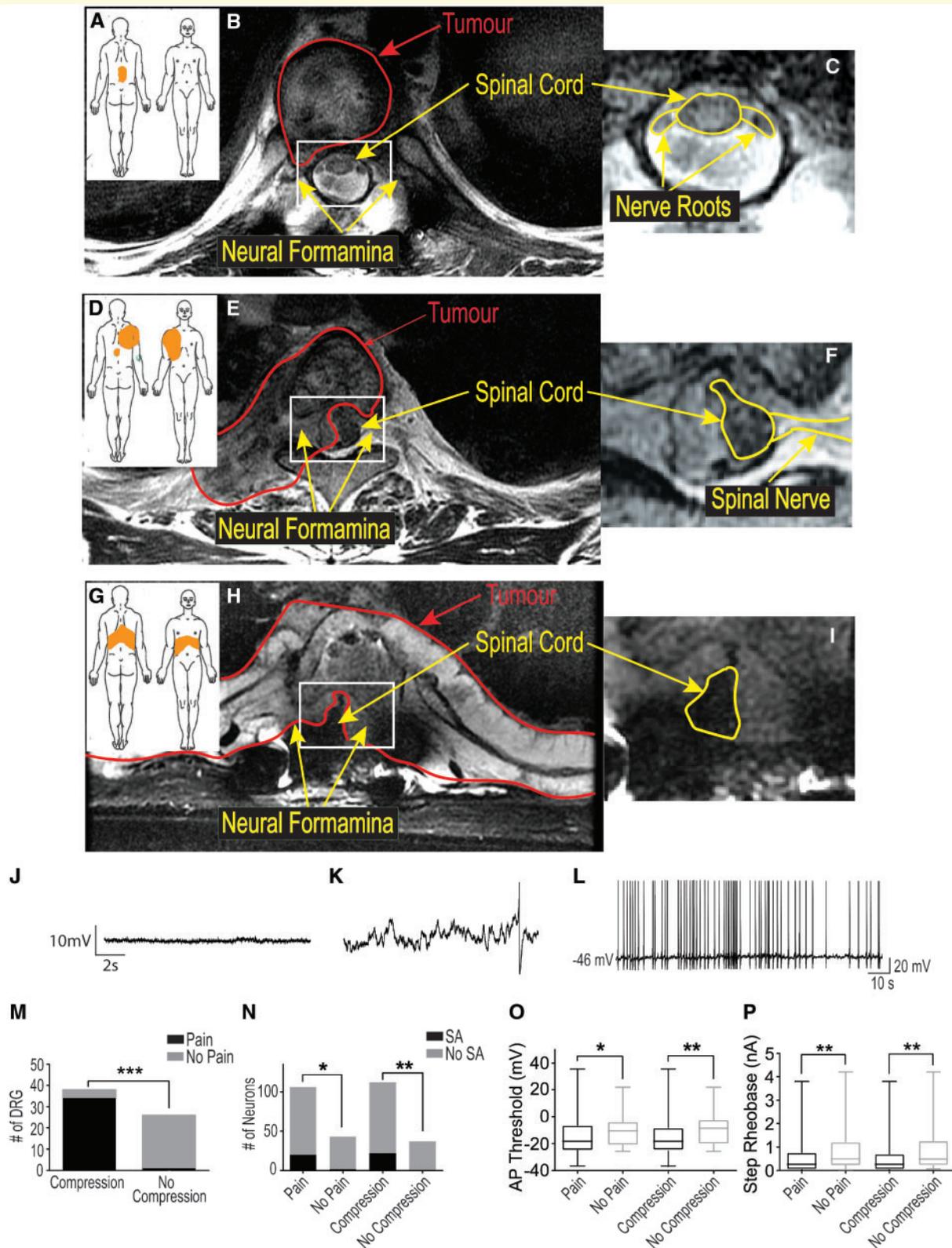


Figure I DRG neurons from dermatomes with radicular/neuropathic pain show ectopic spontaneous activity and hyperexcitability. Pain diagrams and MRI spinal images for three categories of patients are shown in A–I. The orange shaded area in A, D and G indicate where patients marked the location of their pain. This was either localized to the spine without signs of radicular/neuropathic pain (axial pain only, A); showed radiation only to one side (unilateral radicular/neuropathic pain, D); or pain that radiated to both sides of the body (bilateral radicular/neuropathic pain). The large MRI scan in B shows that patients with axial pain often only had tumours (outlined in red) that did not compress the nerve roots or spinal cord. Patients with unilateral neuropathic pain (E) typically had tumours that compressed one or more nerve roots on one

(continued)

Table 1 Detailed neurophysiological parameters for DRG neurons organized by groups

Group	Diameter, μm	RMP, mV	C, pF	Rheobase, nA	Spike threshold, mV	AP peak, mV	AP overshoot, mV	AP rise time, ms	AP fall time, ms	AHP amplitude, mV	Tau, ms
All neurons	43.7 \pm 0.8	-57.9 \pm 0.9	190 \pm 10	0.7 \pm 0.08	-14.1 \pm 1.3	77.0 \pm 1.4	62.4 \pm 1.7	1.9 \pm 0.1	6.0 \pm 0.3	15.7 \pm 0.5	41.1 \pm 3.0
With pain	42.1 \pm 0.8	-57.8 \pm 1.0	200 \pm 20	0.6 \pm 0.09*	-14.8 \pm 1.7*	78.0 \pm 1.5	63.4 \pm 1.8	1.8 \pm 0.1	6.1 \pm 0.4	15.9 \pm 0.6	40.2 \pm 3.5
No pain	45.1 \pm 1.3	-58.2 \pm 1.8	180 \pm 20	0.9 \pm 0.17	-11.3 \pm 2.1	75.3 \pm 3.5	60.9 \pm 4.2	2.0 \pm 0.3	5.5 \pm 0.7	15.2 \pm 1.2	45.3 \pm 6.9

AHP = after-hyperpolarization; AP = action potential; C = capacitance; RMP = resting membrane potential.

*P < 0.05.

dermatomal pain and in 20% (22/112) of neurons with associated radiographic nerve root compression. Spontaneous activity was noted in only 4.6% (2/43) of neurons from DRG without associated dermatomal pain and in none (0/37) of the neurons from DRG without radiographic nerve root compression. Differences in resting membrane potential, neuron size, capacitance, action potential profile and kinetics were not significantly correlated with either radicular/neuropathic pain or nerve root compression (Table 1). No significant relationships for these same parameters were found for age, sex, axial spine pain, radiographic spinal cord compression, prior chemotherapy, prior radiation treatment, or a history of length-dependent peripheral neuropathy (this latter symptom affected dermatomes that were not sampled).

Pairwise distances between 21 sample transcriptomes were calculated from RNA-seq data (Supplementary Table 2). Samples were separated into two groups: those with associated dermatomal radicular/neuropathic pain and those without. Distribution of distances between pain and non-pain samples was higher on average (Fig. 2A). Twelve of 21 samples were from six donors with two sequenced DRGs each. The pairwise distance between donor-controlled pairs was smaller for their respective groups (Fig. 2A). Hierarchical clustering of pain and non-pain groups revealed that only a small number of genes are consistently differentially expressed between the groups. However, female pain samples were well correlated with each other (Fig. 2B) suggesting that sex of the sample is also influential in shaping the DRG transcriptome. Based on these insights, our 21 samples were partitioned into four

cohorts by sex and pain state (male, female, pain, and no-pain).

The three male donor DRG pairs with pain in one dermatome, but not the other, were each analysed for differentially-expressed genes (Table 2 and Supplementary Tables 3 and 4). Several signalling pathways were enriched in the gene set upregulated in pain samples, including the TNF-alpha, TGF-beta, MAPK and TLR pathways (Letterio and Roberts, 1998; Morikawa *et al.*, 2004; Wei *et al.*, 2013; Cevikbas *et al.*, 2014). Transcription factors linked to neuropathic pain in preclinical models, including FOS, FOSB and ATF3, and a number of well-known cytokine ligands including TNF, IL6 and CCL3 were also upregulated in at least two of the three pairs.

The analysis was broadened further to contrast the male-pain and male-no pain cohorts. The comparison yielded 70 genes that were upregulated and 52 genes that were down-regulated in the male-pain cohort (Fig. 2C and Supplementary Table 5). Gene set enrichment analysis (Supplementary Table 6) showed an upregulated signature of genes related to spinal cord injury, and enrichment of several important signalling pathways (MAPK, TGFB, OSM and corticotrophin hormone pathways) that were similar to observations in the paired samples. Genes upregulated in pain samples include well known neuro-immune genes (*CD93*, *CCL4*, *SOCS3*), Schwann cell genes involved in rodent models of nerve injury (*NR4A1*, *EGR1*, *EGR3*), and genes known to be expressed in the human DRG and mouse sensory neurons (*ARC*, *OMP*, *CHST1*), suggesting crosstalk between immune cells and neurons/glia (Usoskin *et al.*, 2015; Ray *et al.*, 2018).

Figure 1 Continued

side and part of the spinal cord. Patients with bilateral neuropathic pain typically had compression of one or more roots on both sides and the spinal cord (H). The area in B, E and H outlined in white are magnified in C, F, and I to show the spinal cord and nerve roots better (outlined in yellow). A representative recording of the resting membrane potential with an expanded time base for a cell without spontaneous activity is shown in J while a similar recording for a cell with spontaneous activity is shown in K to illustrate the spontaneous depolarizing fluctuations (DSFs) in membrane potential that occurred in these cells. A single action potential is shown at the right of this trace occurring atop one of the larger of these DSFs. The representative trace shown in L illustrates the irregular pattern of action potentials typically seen in cells with spontaneous activity. The bar graphs in M show that radiological evidence of nerve compression was strongly associated with signs of radicular/neuropathic pain; while in N the bar graphs show the relationship of radicular/neuropathic pain and nerve compression with spontaneous activity (SA). The box and whisker plots in O and P show that DRG neurons from a dermatome with pain and/or nerve compression had a more depolarized spike threshold potential and lower rheobase, respectively.

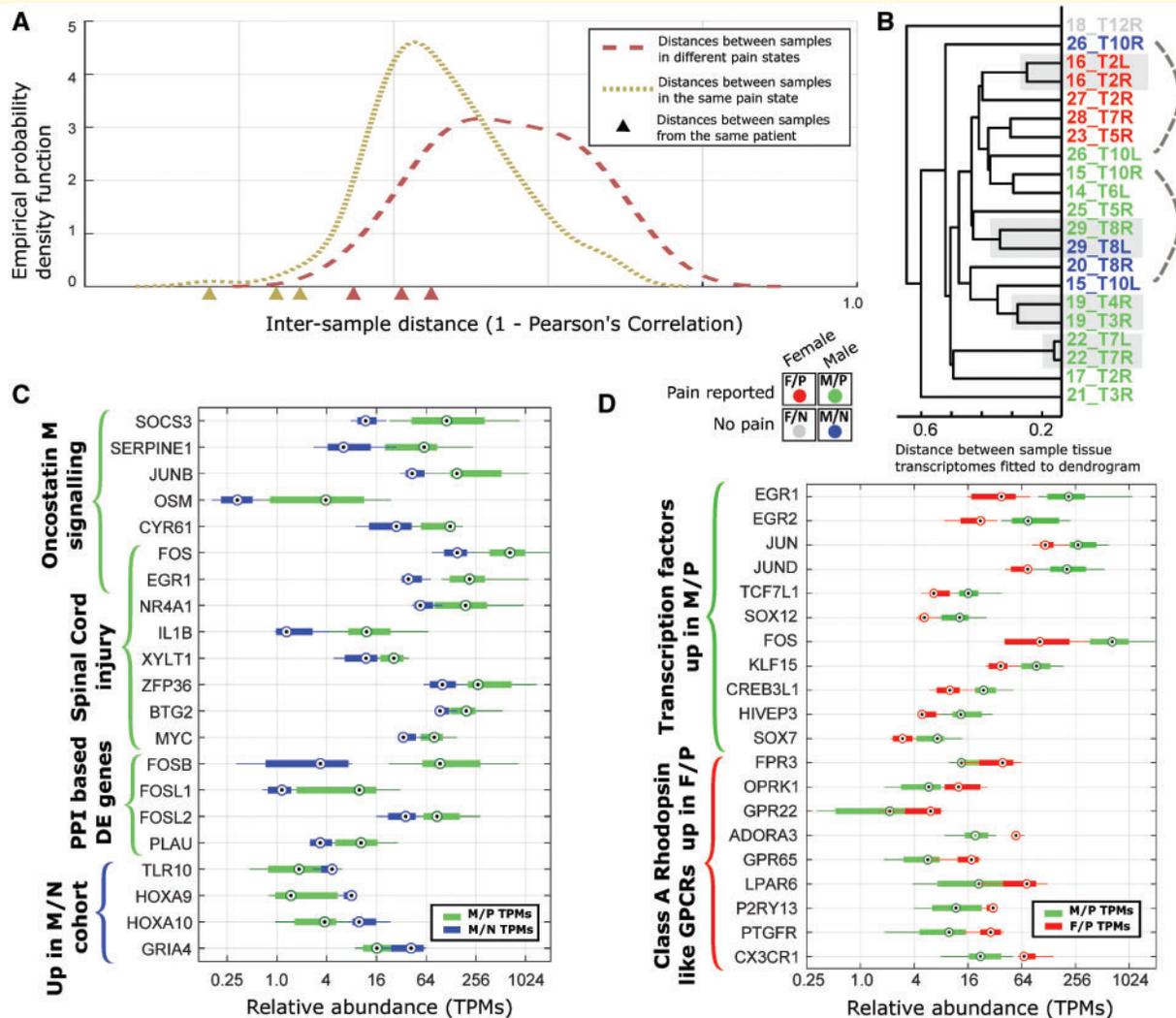


Figure 2 Differential expression analysis for human DRG transcriptomes. **(A)** Empirically estimated density function for pairwise transcriptome distances between samples with the same pain state and between samples with different pain states show overlap but a clear increase overall. Inter-sample distances for samples from the same patient (shown by triangles on the x-axis) are comparatively low with respect to the set of all distances. **(B)** Hierarchical clustering of RNA profiles for all samples, showing close distances between female pain samples. Standard hierarchical clustering was performed for all RNA-seq samples using expression patterns of high variability (entropy < 3.5, see Fig. 3), expressed [transcripts per million (TPM) > 1.5 in at least one sample] genes with distance metric = $1 - \text{Pearson's correlation coefficient}$, and average linkage. Four cohorts [male-pain (M/P), male-no pain (M/N), female-pain (F/P), and female-no pain (F/N)] are colour-coded. **(C and D)** Several representative differentially-expressed gene sets for the male-pain versus male-no pain **(C)** and for the male-pain versus female-pain **(D)** comparison.

Interestingly, comparison of the male-pain and female-pain cohorts (Fig. 2D and Supplementary Table 7) yielded a more extensive set of differentially-expressed genes (426 autosomal genes upregulated in male-pain and 149 upregulated in female-pain cohorts). This could occur because some of the detected genes have sex-differential expression in baseline DRG while others could potentially underlie a sex-specific neuropathic pain pathology. It is interesting to note that based on gene set enrichment analysis (Supplementary Table 6), a different set of spinal cord injury-associated genes were upregulated in the female-pain cohort (*TLR4*, *AIF1*, *OMG*, *CIQB*) as compared to

the male-enriched genes (*EGR1*, *NR4A1*, *ZFP36*, *BTG2*, *MYC* and others). Overlap with known lineage-specific gene modules in human macrophage lineages (Xue *et al.*, 2014) suggests that some of the sex-differential gene expression in pain samples may be driven by macrophages (Supplementary Table 7). Human macrophage lineage-enriched genes up in the male-pain cohort (136 out of 426 autosomal genes) include *CXCL2*, *TNF*, and several transcription factors of the FOS-JUN family (*FOS*, *FOSB*, *JUNB*, *JUND*), while genes up in the female-pain cohort (75 of 149 autosomal genes) include several class A rhodopsin like G-protein coupled receptors (*CX3CR1*,

Table 2 Fold change in transcripts per million in paired single patient samples (pain:no pain)

	Patient 15	Patient 29	Patient 26	MAPK signalling	TNF- α signalling	TGF- β signalling	TLR signalling	OSM signalling	AHR signalling	SCI
Transcription factors and co-factors										
<i>JUN</i>	2.62	1.24	1.83	✓	✓	-	✓	-	✓	-
<i>FOS</i>	2.60	2.00	10.40	✓	-	-	✓	✓	-	✓
<i>NFKB2</i>	2.00	1.06	0.92	-	✓	-	✓	-	-	-
<i>RUNX2</i>	2.75	1.22	1.93	-	-	✓	-	-	-	-
<i>FOSB</i>	13.55	13.53	283.90	-	-	✓	-	-	-	-
<i>ATF3</i>	7.58	1.99	1.76	-	-	✓	-	-	-	-
<i>JUNB</i>	3.39	1.38	4.90	-	-	✓	-	✓	✓	-
<i>EGR1</i>	6.28	2.47	10.19	-	-	-	-	✓	-	✓
<i>KLF10</i>	2.35	1.28	1.47	-	-	✓	-	-	-	-
<i>NR4A1</i>	3.21	1.01	1.76	✓	-	-	-	-	-	✓
<i>HES1</i>	2.41	1.17	1.36	-	-	-	-	-	✓	-
<i>AHR</i>	2.02	1.26	1.04	-	-	-	-	-	✓	-
<i>BTG2</i>	1.23	1.11	2.79	-	-	-	-	-	-	✓
RNA binding proteins										
<i>ZFP36</i>	2.29	1.31	4.12	-	-	-	-	-	-	✓
Cytokine ligands										
<i>TNF</i>	3.71	LE	19.66	✓	✓	-	✓	-	✓	✓
<i>IL1B</i>	8.72	LE	9.09	✓	-	-	✓	-	✓	✓
<i>IL6</i>	9.13	LE	4.68	-	✓	-	✓	-	-	✓
<i>IL12A</i>	3.01	1.40	1.31	-	-	-	✓	-	✓	-
<i>CCL2</i>	3.04	1.00	0.93	-	-	-	-	✓	-	✓
<i>CCL3</i>	12.52	3.45	75.40	-	-	-	✓	-	-	-
<i>CCL4</i>	23.60	1.60	3035.20	-	-	-	✓	-	-	-
<i>CXCL2</i>	1.92	LE	2.99	-	-	-	-	-	-	✓
<i>OSM</i>	10.39	LE	37.07	-	-	-	-	✓	-	-
<i>TGFB1</i>	2.13	1.09	1.81	✓	-	✓	-	-	✓	✓
<i>TGFB3</i>	4.77	1.35	1.81	✓	-	✓	-	-	-	-
<i>GDNF</i>	8.47	LE	LE	-	-	-	-	-	-	✓
Cytokine regulators										
<i>SOCS3</i>	14.41	2.84	4.64	-	-	-	✓	✓	-	-
Neurotrophins										
<i>NGF</i>	2.30	1.04	1.39	✓	-	-	-	-	-	-

Genes involved in important signalling pathways or spinal cord injury, and their fold-change in the three pairs of samples from the same patients with differing pain states show several key transcription factors and cytokines to be upregulated in the pain state. LE = low expression; SCI = spinal cord injury.

✓ = gene set membership.

ADORA3, *P2RY13* and *GPR65*). While DRG-specific ion channels have been shown to be differentially expressed in mouse and rat models of neuropathic pain (Lacroix-Fralish *et al.*, 2011; Zhang and Dougherty, 2014) and in human neuropathic pain (Li *et al.*, 2018), we do not find statistically significant differences in abundances for ion channels expressed in human DRG (Supplementary Table 8), possibly due to regulation in translation or post-translation phases that are not reflected in RNA-seq data. Based on our cohort analysis, we found a set of ion channels (*ANO8*, *GRIK5*, *GRIN1*, *HCN2*, *KCNAB2*, *KCNC1*, *KCNG1*, *KCNH2*, *KCNK3*, *PANX2*) that have higher expression in the male-pain cohort compared to the female-pain cohort, again suggesting sexually dimorphic mechanisms (Supplementary Table 8).

Multiple control analyses were performed on the data. The distribution of gene relative abundances (in transcripts

per million) were plotted to ensure a similar distribution and comparable inflexion points in the distribution across samples (Fig. 3A). Genes with higher variability across samples in our dataset were identified in a cohort-agnostic manner using the notion of Shannon's entropy (Fig. 3B). The sex of each sample was validated based on reads mapping to the *XIST* locus (Fig. 3C). For pain and non-pain samples derived from the same patient, the distribution of fold change in gene expression was quantified to identify the genes with the biggest change in abundance (Fig. 3D).

We predicted cohort membership for each sample (with the exception of the sole female-no pain sample) based on trained Random Forest classifiers. The cross validation training and testing batches we used are shown in Fig. 4A. We classified 11 male-pain and five female-pain samples using the male-pain versus female-pain classifier, and classified 11 male-pain and four male-no pain samples

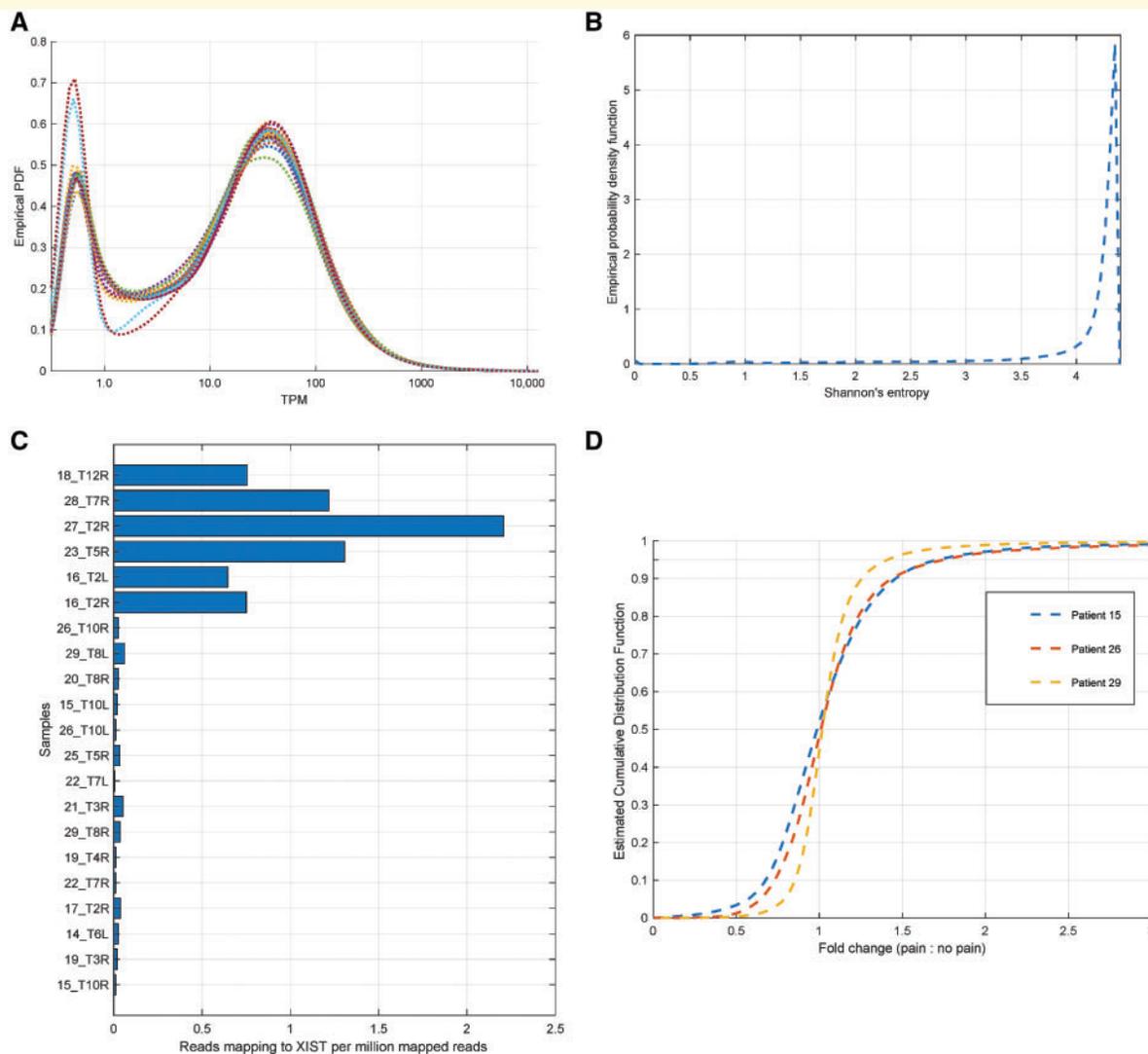


Figure 3 *In silico* controls for RNA-seq analysis. **(A)** The estimated probability density function for transcripts per million (TPMs) (smoothed by adding 0.5 to each value) show that all samples have approximately similar distributions over coding gene TPMs, along with a consistent number of genes expressed at 1.5 TPM or higher in each sample (between 13 850 and 14 715). **(B)** Genes with high variability in TPM across our datasets were identified in a fashion agnostic to clinical information by calculating Shannon's entropy for each gene's TPMs across RNA-seq samples, identifying genes with high variability (based on low entropy values in the left tail of the estimated distribution, value < 3.5). Higher values correspond to more generic expression patterns. **(C)** Some well-known marker genes were checked in RNA-seq samples. The reported sex for each sample was independently verified using reads mapping to the *XIST* non-coding gene. **(D)** Estimated density function for the gene expression (for genes with transcripts per million > 3.0 in either sample) fold change between pain and non-pain samples derived from the same patient, showing that a 2-fold change corresponds to the top 5th percentile.

using the male-pain versus male-no pain classifier. This process was repeated 20 times (using random seeds to initialize the classifier training) to evaluate our classification algorithm. Of the 620 (31 classifications over 20 trials) predictions, we obtained a high (94.7%) accuracy in cohort membership prediction, suggesting that the gene expression changes we see are consistent and correlated and our classifier is able to harness this signal to perform classification (Fig. 4B). Random Forests are trained by identifying a set of discriminative features (in this case, genes) used to construct decision trees. We identified the genes that were most

frequently chosen by the algorithm to construct Random Forests, since these were putatively the most reliable genes for discriminating across cohorts. For genes used in > 15% of the trained Random Forests, we find that a majority of these genes overlap with the genes we identified in our cohort analysis in the previous section. They include genes coding for transcriptional regulators (like members of the FOS/JUN and EGR family), post transcriptional and translational regulators (*ZFP36*, *EEF2K*), transferases (*WNK2*, *SOCS3*, *MAPK7*), and signalling molecules (*ISLR2*, *OSM*, *CD93*, *IL1B*) (Fig 4C). Regulatory and

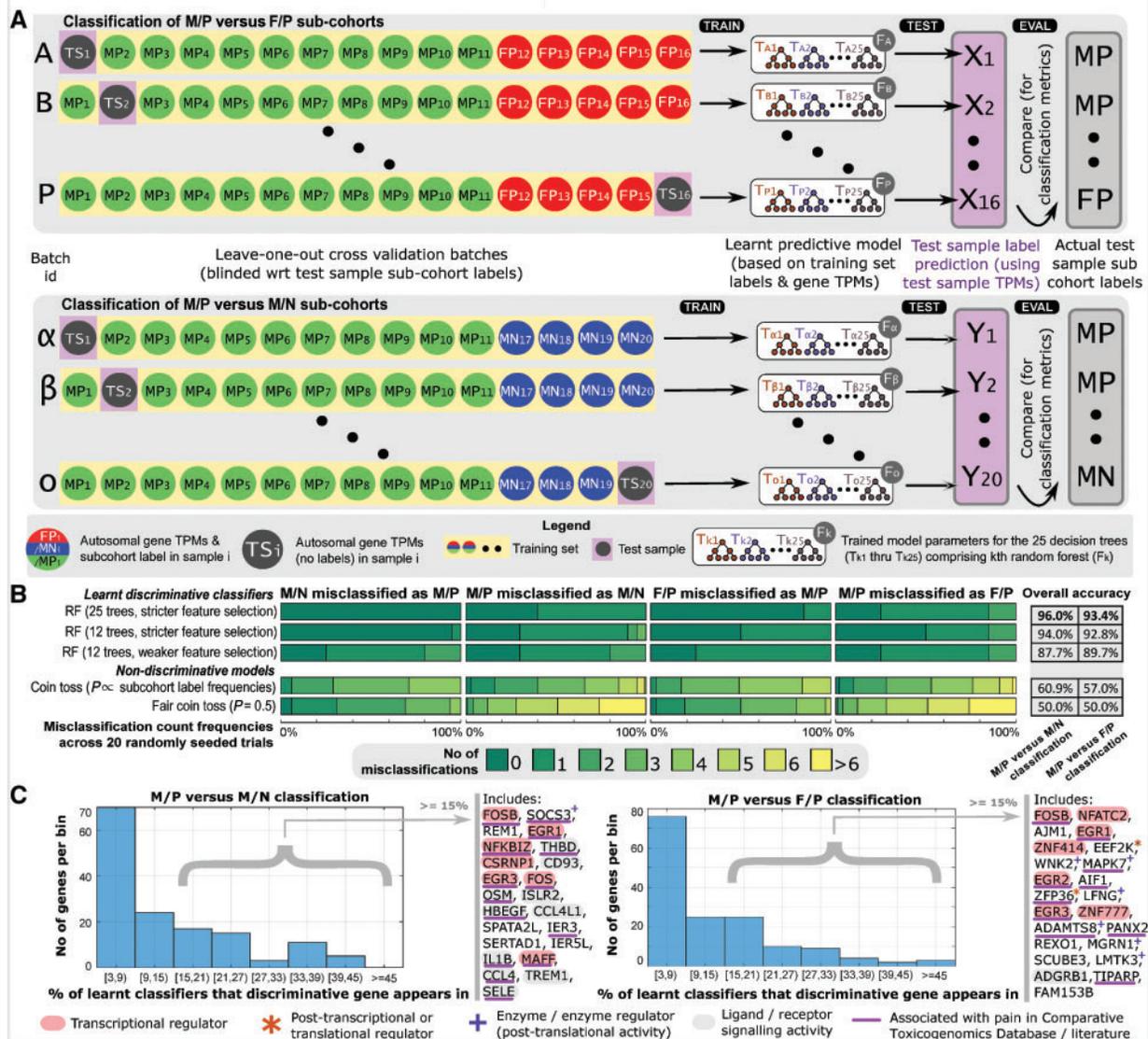


Figure 4 Sample cohort prediction using Random Forests. (A) Leave one out cross validation schema is shown, with one sample (test sample) held out from training in each batch. The RNA profile of the test sample is then used by the trained classifier to predict its cohort membership, and the predicted cohort label is compared to actual cohort membership to evaluate the quality of classification. (B) Classification metrics for our optimal Random Forest model, using 25 decision trees, with no more than five decisions per tree, and using an input set of discriminative candidate genes is shown on top. Metrics from random forests built using 12 trees; as well as from random forests using 12 trees and a larger input set of candidate genes are also shown. Our classifier achieves discriminative results across a range of training parameters. We also show expected classification metrics for classifiers with no discriminative ability: based on models of biased and unbiased coin tosses. (C) A small set of genes are chosen for many of the random forests that we trained, suggesting a high predictive ability of these genes. Histograms show the number of genes that are chosen most frequently (in 3% to 45% of trained random forests) for both male-pain (M/P) versus female-pain (F/P) and male-pain versus male-no pain (M/N) classification. Genes chosen in > 15% of the random forests include transcriptional/post transcriptional regulators, enzymes, and signalling molecules, many of which are associated with pain.

signalling molecules in the discriminative gene set clearly suggests consistent usage of specific regulatory programs and signalling pathways, which could yield molecular signatures underlying human pain states in the future.

Finally, we compiled a list of studies that identified gene-neuropathic pain associations in humans or model species

for the list of differentially expressed genes that we identified (Supplementary Table 9). Of ~750 differently expressed genes across our analysis, 220 were identified in existing databases of pain-associated genes in humans. Therefore, while our dataset has substantial overlap with an existing knowledgebase in the field, we have identified a large cohort of new potential targets to investigate for

neuropathic pain mechanisms based entirely on molecular investigation on patient samples.

Discussion

A key aspect of this study is the pairing of electrophysiology with RNA-seq for discovery of transcriptomic signatures of neuropathic pain. Though limited by a relatively small cohort and the multifactorial nature of each patient's dermatomal pain (with potential contributions from local effects such as direct neural compression, peritumoral inflammation, tumour-derived soluble factors, and systemic conditions such as diabetes mellitus and/or prior treatments of patient's malignancies), our findings allow several important conclusions.

First, there is a strong correlation between both radicular/neuropathic pain and radiographic nerve root compression to the presence of spontaneous activity and electrophysiological measures of hyperexcitability. Our results are similar to incidence of spontaneous activity reported in the literature for animal experiments with 10.3–20.5% for injured nerves versus 1.6–2.8% in controls (Liu *et al.*, 2002; Ma and LaMotte, 2007; Li *et al.*, 2017). Three physiological maladaptations were noted in recent work on the mechanisms underlying spontaneous activity in a model of spinal cord injury neuropathic pain. These included the development of a more positive resting membrane potential; a more hyperpolarized action potential threshold; and the occurrence of depolarizing spontaneous fluctuations in membrane potential (Odem *et al.*, 2018). We found two of these occurring in human neurons with spontaneous activity, a more hyperpolarized action potential threshold (Table 1) and depolarizing spontaneous fluctuations (Fig. 1K). Therefore, we establish that the emergence of DRG neuron spontaneous activity and hyperexcitability are fundamental shared features between animal models of radicular/neuropathic pain and humans with clinically defined radicular/neuropathic pain.

It is perhaps surprising that significant changes in specific ion channels were only observed for the paired samples but not in the overall population analysis. There are a number of potential reasons for this. RNA-seq data measures the steady-state abundance of RNA species. This means that only changes at the transcriptional and post-transcriptional levels will be reflected in the data. There is clear evidence that specific ion channels contribute to ectopic spontaneous activity in human DRG neurons as shown by increased protein abundance changes and suppression of spontaneous activity using specific ion channel inhibitors (Li *et al.*, 2017, 2018). But this can occur because of changes in translational regulation. Additionally, post translational regulation can also affect ion channel function. These changes would not be apparent in our datasets. Moreover, we performed bulk RNA-seq, with input coming from neuronal and non-neuronal cells, thus the signal for changes in a single sensory neuronal subpopulation (as would be the case for an

ion channel such as $\text{Na}_v1.7$) would be diluted in the bulk RNA-seq data. Future single cell assays (like imaging studies for *in situ* hybridization, or single cell RNA-seq) may be sufficiently sensitive to adequately capture such changes. Alternatively, changes in ion channel abundance may be temporally transient during the development of neuropathic pain. Electrophysiological recordings and RNA-sequencing are performed on the same donor DRG, but patients are at different times in the disease pathology. This cannot be controlled for in a clinical cohort like ours, but is always controlled for in animal studies, where much of the evidence for such changes originates. Finally, a combination of these points is likely.

The second broad conclusion that can be drawn here is that in male DRGs from painful dermatomes a transcriptional signature associated with spinal cord injury and enriched in signalling factors that converge on gp-130 receptors can be clearly identified. Given the known role of gp-130 expression in the DRG in preclinical pain models (Andratsch *et al.*, 2009), our findings validate this pathway but unexpectedly implicate OSM and its receptor, OSMR, which forms a signalling complex with gp-130, in human neuropathic pain. This would not be predicted based on the preclinical literature which has predominately focused on IL6 as the primary mechanism for activating gp-130 in chronic pain. This finding has obvious implications for biological (e.g. antibody) development targeting this signalling system. We also uncover preliminary evidence of differences in transcriptomic signatures in the DRGs of males and females with neuropathic pain. While our cohort sizes are relatively modest and require further validation, this is consistent with emerging lines of evidence for sex differential neuroimmune response in preclinical models (Sorge *et al.*, 2015; Lopes *et al.*, 2017) and suggests the potential of sex-specific mechanisms for the development of neuropathic pain and spontaneous activity in DRG neurons.

Importantly, our study identifies sets of genes that are differentially expressed in the male-pain, male-no pain and female-pain cohorts. Our machine learning approach, which used a Random Forest model, finds that these genes have good predictive ability for identifying these cohorts, suggesting consistent changes in gene expression. We propose that this experimental framework will be useful in new datasets that are generated from independent projects to test if pain phenotypes can be reliably predicted from RNA-seq data. A limitation of this approach is that DRGs are not readily available from most clinical cohorts. However, some previous experiments in animal models have shown that certain immune cells can be predictive of transcriptomic changes in other nervous system areas in neuropathic pain (Massart *et al.*, 2016). If this is also true in humans, it may eventually be possible to use a specific immune cell population as a proxy for transcriptomic changes in the DRG. This idea can be tested in ongoing studies with the clinical cohort described here.

Finally, while many of the genes we identified are known from previous human or (mostly) rodent studies, the

majority of these have been understudied or not been studied in the context of neuropathic pain (e.g. *OSM*, discussed in the ‘Results’ section). Another excellent example is *ISLR2*. This mRNA encodes a protein called Linx that is known to play a role in the development of nociceptors (Mandai *et al.*, 2009). Linx interacts with two well-known tyrosine receptor kinases, TrkA and TrkC, and previous work has shown a clear effect of this gene product in regulating how NGF signals through the TrkA receptor (Mandai *et al.*, 2009). No previous studies have investigated the role of this gene in neuropathic pain but our machine learning approach identifies this gene as predictive of neuropathic pain phenotypes. Given the well-known role of NGF and TrkA signaling in pain, and the expanding clinical literature based on anti-NGF therapeutics with mixed results in neuropathic pain trials (Bannwarth and Kostine, 2014), we propose that this is an excellent example of a high-quality target for further exploration as a therapeutic intervention point.

In conclusion, our work provides the first evidence that neuropathic pain in humans is associated with spontaneous activity in the soma of DRG neurons. Combining this electrophysiological approach with bulk RNA-seq gives extensive new insight into mechanisms of neuropathic pain based entirely on clinical samples. Two important features of neuropathic pain emerging from this approach are marked sexual dimorphisms and nuances in known mechanisms that have important implications for therapeutic development. A caveat in consideration of these results is that the possibility exists that some of the results seen here could also be due to the influence of tumour-derived factors in addition to nerve injury.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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A Quantitative Sensory Analysis of Peripheral Neuropathy in Colorectal Cancer and Its Exacerbation by Oxaliplatin Chemotherapy

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Abstract

Peripheral neuropathy caused by cytotoxic chemotherapy, especially platins and taxanes, is a widespread problem among cancer survivors that is likely to continue to expand in the future. However, little work to date has focused on understanding this challenge. The goal in this study was to determine the impact of colorectal cancer and cumulative chemotherapeutic dose on sensory function to gain mechanistic insight into the subtypes of primary afferent fibers damaged by chemotherapy. Patients with colorectal cancer underwent quantitative sensory testing before and then prior to each cycle of oxaliplatin. These data were compared with those from 47 age- and sex-matched healthy volunteers. Patients showed significant subclinical deficits in sensory function before any therapy compared with healthy volunteers, and they became more pronounced in patients who received chemotherapy. Sensory modalities that involved large A β myelinated fibers and unmyelinated C fibers were most affected by chemotherapy, whereas sensory modalities conveyed by thinly myelinated A δ fibers were less sensitive to chemotherapy. Patients with baseline sensory deficits went on to develop more symptom complaints during chemotherapy than those who had no baseline deficit. Patients who were tested again 6 to 12 months after chemotherapy presented with the most numbness and pain and also the most pronounced sensory deficits. Our results illuminate a mechanistic connection between the pattern of effects on sensory function and the nerve fiber types that appear to be most vulnerable to chemotherapy-induced toxicity, with implications for how to focus future work to ameliorate risks of peripheral neuropathy. *Cancer Res*; 74(21); 5955–62. ©2014 AACR.

Introduction

Neuropathy induced by chemotherapy can seriously impede successful treatment for many cancers as it often leads to reduction or cessation of frontline treatment; and can drastically impact patients' quality of life both during and following therapy (1). The mechanism for chemotherapy-induced peripheral neuropathy (CIPN) is poorly understood. Primary afferent neurons seem to be the most vulnerable as most commonly sensory symptoms alone start in the tips of the

toes and fingers and then advance over time proximally in a "stocking-glove" distribution (2–5). More specifically, pain is typically reported in the tips of the toes and fingers; numbness and tingling, but not necessarily pain is present in the soles of the feet and palms; while hairy skin is typically outside the areas of patient complaint (3, 6). Yet specific data concerning the vulnerability of primary afferent fiber subtypes to toxic insult by cancer and its treatment are not known. Here, quantitative sensory tests (QST) were conducted in patients before and after various cumulative doses of oxaliplatin to fill this gap in knowledge. QST is a psychophysical method used to study human somatic sensory physiology including pain perception (7). Small-fiber sensory function is assessed by measuring the threshold to detect warmth, hot, and cold pain; thinly myelinated fibers are assessed by measurement of threshold to detect skin cooling and sharpness; and large-fiber sensory function is measured by detection thresholds for cutaneous mechanical stimuli (8). The pattern of effects of chemotherapy on sensory function has clear mechanistic implications for the fiber types that are vulnerable to the toxicity of chemotherapy.

Patients and Methods

Patients

Patients starting initial chemotherapy with oxaliplatin for stage II, III, or IV colorectal cancer at MD Anderson (Houston, TX) were recruited for the study that was approved by the

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Institutional Review Board. Seventy-eight patients were enrolled and gave informed consent. Patients with any history of neuropathy or other factors known to contribute to neuropathy including diabetes mellitus, history of alcohol intake more than 100 g per week, vitamin deficiency, nerve compression, or any central nervous system metastasis were excluded. The typical chemotherapy protocol consisted of oxaliplatin administered at a dose of 85 mg/m² on 2-week cycles. Patients underwent QST before each treatment with oxaliplatin. In addition, 47 age- and sex-matched volunteers were recruited to provide comparative data.

QST

QST data were collected with the patients comfortably seated in a quiet, dedicated psychophysics laboratory in the daytime hours with the subjects not on analgesics that might interfere with the tasks. Three sites were tested in each subject, the tip of the index finger, the thenar eminence, and the volar surface of the forearm to encompass the areas of skin that are typically affected in chronic CIPN patients (2–5). QST were conducted by two clinical data coordinators with many years of experience and with previously verified excellent inter-rater reliability (2–5). The specific tests that were performed included the following and were performed in the order described.

Basal skin temperature. Basal skin temperature was measured using an infrared thermistor positioned against the skin at each site.

The Slotted Pegboard test. The Slotted Pegboard test was used to evaluate sensorimotor function (9). Participants filled a 5×5 slotted pegboard with spindles in nonrandom fashion by one row or column at a time with the dominant hand and then with the nondominant hand (10). The time for each participant to complete the task was recorded with a 5-minute (300 seconds) cutoff.

Bumps detection. Bumps detection was used to assess low threshold mechanosensation (11, 12). Participants used their index finger to probe a smooth plate that was divided into nine blocks, with each block marked by five colored circles. Over one of the circles in each block, a bump of varying height (500 μm in diameter, 2.5–22.5 μm tall) was concealed such that it was not visible to the patient (3 plates total in the set). The threshold was defined as the lowest height bump correctly detected with the next two higher bumps also correctly detected.

Touch detection threshold. Touch detection threshold was determined using von Frey monofilaments (Semmes-Weinstein) in an up/down manner as previously described (2). The filaments were applied for 1 second at each testing site starting with a force of 0.5 g and the patients were unable to see the stimulus application. If a participant did not feel a given filament, the next higher force filament was applied. If a participant felt a stimulus, the next lower force filament was applied. Threshold was defined as the first filament force detected by the participant three times.

Sharpness detection threshold. Sharpness detection threshold was determined using blunted 30-gauge needles with force determined by weights graded from 8 to 128 g (10, 13). Weighted needles were applied in order from lightest

to heaviest at each site for 1 second, and participants were asked to report each stimulus as touch, pressure, sharp, or pain. The lowest force at which the report of "sharp" or "painful" was given determined the endpoint for each trial. The final threshold was the mean of three trials separated by 30 to 90 seconds. The starting weight was modified between trials to manage errors in anticipation.

Thermal detection threshold. Thermal detection threshold was determined using a 3.6 × 3.6 cm Peltier probe set at a baseline temperature of 32°C (2). The probe temperature was ramped upward at a rate of 0.30°C/second for detection of warmth and heat pain thresholds, whereas cool detection and cold pain threshold were determined using a decreasing ramp of 0.50°C/second. Participants were not given any cue to the onset of a given trial, nor whether the probe would heat or cool. Participants were instructed to indicate when they could first detect a change in temperature and then when the temperature became painful; at that point, the probe was immediately returned to the baseline temperature. The final threshold was the average of three heating and cooling trials separated by 30 to 90 seconds.

Descriptors of symptoms. Descriptors of symptoms were assessed using questionnaires and a standardized body map presented to the participants at each meeting (2). The participants marked areas where they felt pain with a red pen and areas where they felt tingling or numbness with a green pen. Participants also selected descriptors for their symptoms from a standardized list (2) that was previously validated (14).

Data analysis

Analysis of the data was based on total cumulative oxaliplatin dose that patients received before each test. In this manner, patient data were stratified into baseline (cumulative dose 0), low (115.7–345.1 mg), medium (347.1–737.8 mg), and high dose (739.5–2328.2 mg) categories established by empirical analysis. Patients only contributed one set of data per dose category with that included at the highest dose if sampled more than once within a given category. Finally, patients were also tested at approximately 6 months after chemotherapy. The nonparametric Kruskal-Wallis test was applied to all data. Patient data were compared with that from the health volunteers only for the baseline time point. The patient data collected at the time points during and following chemotherapy were compared with the patient baseline dataset. Significance was defined as any *P* value < 0.05.

Results

Patient demographics and clinical data are shown in Table 1. The breakdown by treatment category resulted in 51 patients in the baseline group because some had not agreed to take part in the study before starting chemotherapy. Sixty-two patients were included in the low-dose category; 54 in the medium-dose category; and 49 in the high cumulative dose category. Finally, 27 patients underwent QST at a postchemotherapy follow-up examination at a mean of 165 (±12) days after the last chemotherapy. The

Table 1. Demographic and clinical characteristics

	Patients (N = 78)	Volunteers (N = 47)
Age (mean ± SD)	55.7 ± 1.45 y	53.87 ± 2.02 y
Male (%)	48 (62)	29 (62)
Female (%)	30 (39)	18 (38)
White (%)	47 (60)	30 (64)
Black (%)	14 (18)	13 (28)
Hispanic or Latino (%)	12 (15)	4 (8)
Asian (%)	4 (5)	0 (0)
Unknown (%)	1 (1)	0 (0)
Smoke > 10 pack years (%)	19 (24)	0 (0)
Married	54 (70)	35 (74)
TNM stage ^a		
I	0 (0)	NA
II	11 (14)	NA
III or IV	67 (86)	NA
Mean cycles (mean ± SD)	6.7 ± 3.18	NA

^aAmerican Joint Committee on Cancer Staging Manual (7th edition) criteria.

numbers vary due to missed visits and/or loss of subjects to the study over time.

Pegboard test

Patients at baseline took significantly more time to complete the pegboard test with the dominant hand than volunteers did (Fig. 1A, $P < 0.001$). The same was observed for the nondominant hand (Fig. 1B, $P < 0.001$). Subsequent pegboard tests collected during chemotherapy did not show any differences from the patient baseline value. Indeed, there was a trend in both hands for a slight decline in pegboard time, most likely reflecting a training effect (none of the volunteers were allowed training before their data collection).

Bumps detection

The Bumps test is an assessment of large diameter A β fiber mechanoreceptor function best correlated to transduction by Meissner's corpuscles (12, 15). The patients showed a significantly elevated Bumps threshold at baseline compared with healthy volunteers (Fig. 1C, $P < 0.01$). There was a clear trend for the impairment seen at the patient baseline QST to worsen during therapy, with this difference becoming statistically significant from the patient baseline in the high-dose chemotherapy group (Fig. 1C, $P < 0.05$). The mean bump detection score in patients who had undergone high cumulative doses of oxaliplatin was 2.1 times that of volunteers (6.33 ± 0.68 in patients vs. 3.32 ± 0.32 in healthy volunteers; $P < 0.05$; Fig. 2A). However, patients in the follow-up group displayed the most significant impairment in bump detection compared

with all other groups ($P < 0.01$ – <0.0001) consistent with the coating phenomenon often attributed to platin-based chemotherapeutics.

Touch detection

The detection of touch using von Frey filaments engages large diameter A β slowly adapting Merkel complex mechanoreceptors (16, 17). There was no difference between touch detection threshold between healthy volunteers and patients before therapy. Touch threshold did show increasing deficit with dose during chemotherapy that became statistically significant in the fingertips at middle and high chemotherapy doses (Fig. 2A, $P < 0.05$). Similarly, touch threshold in the thenar eminence gradually increased with chemotherapy dose and was statistically significant between the patient

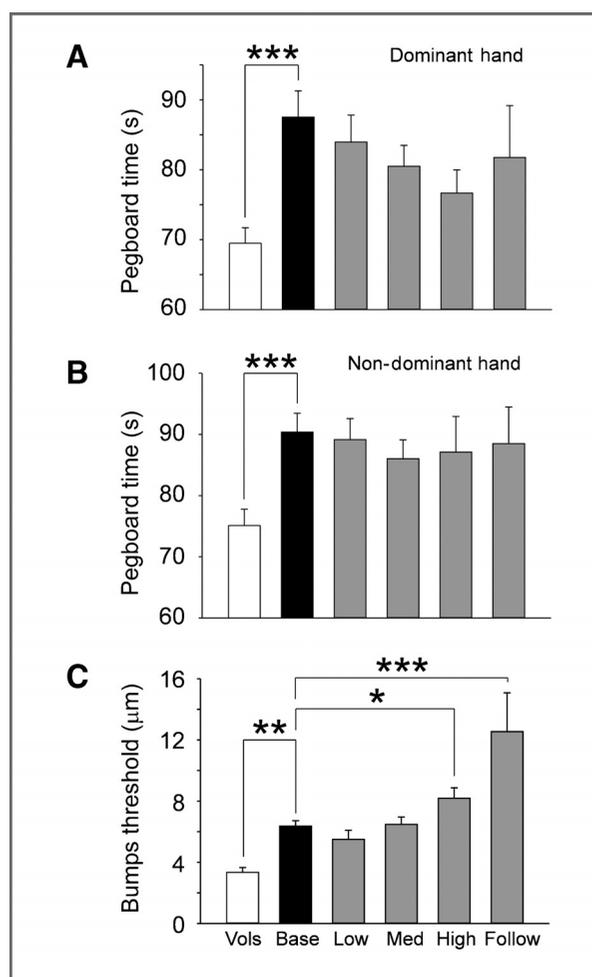


Figure 1. The bar graphs show the mean and SE for the slotted pegboard times in the dominant (A) and nondominant (B) hand and the Bumps detection threshold (C) for the healthy volunteers (Vols, open bars) and the patients at the pretreatment baseline (Base, black bars) and at each cumulative dose treatment category [gray bars, Low, up to 370.6 mg; medium (Med), up to 795.6 mg; High, up to 2328.2 mg; Follow, 6 months after treatment]. The horizontal lines indicate the comparisons made. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

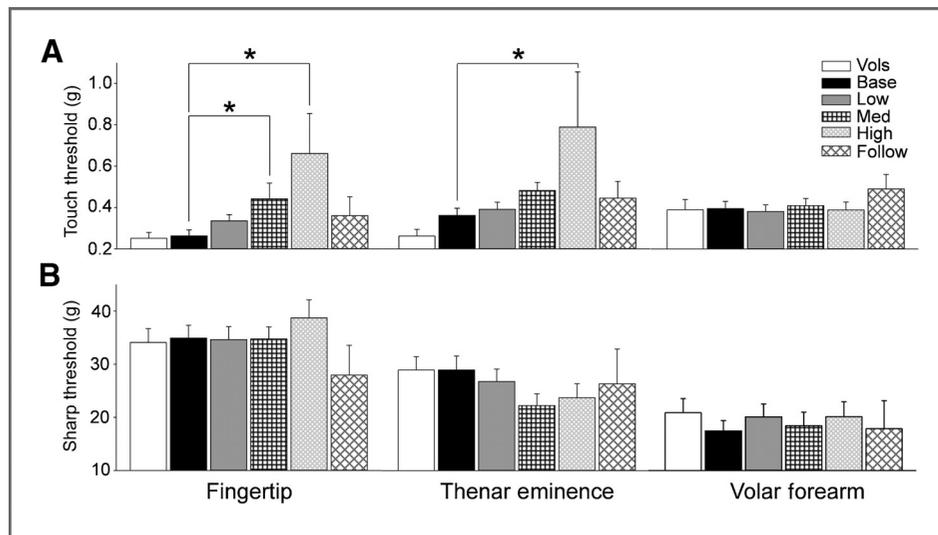
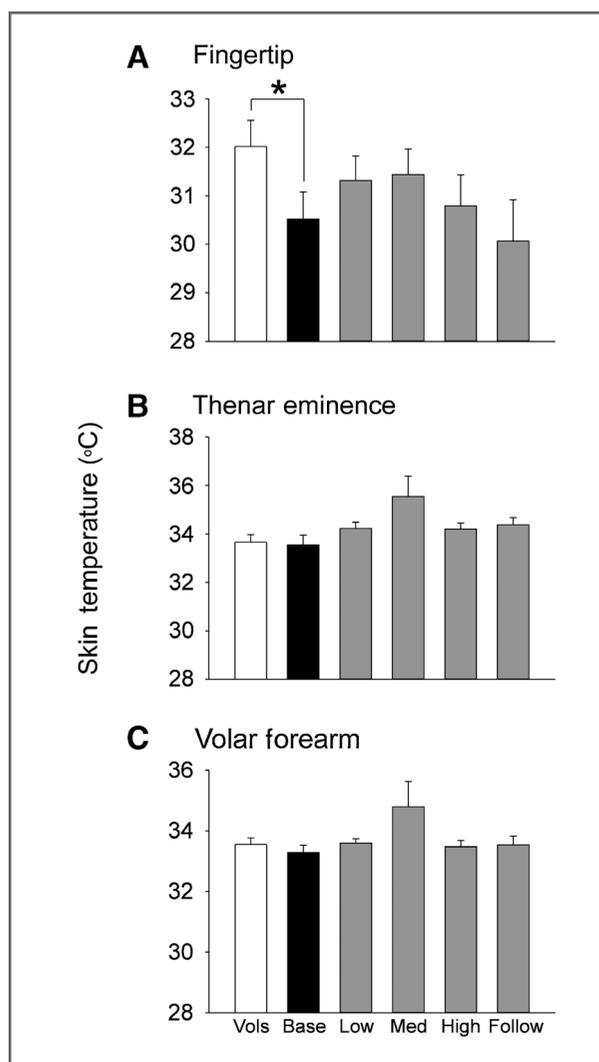


Figure 2. The bar graphs show the mean and SE for the VonFrey touch detection threshold (A) and the sharpness detection threshold (B) for the healthy volunteers (open bars) and the patients at the pretreatment baseline (black bars) and at each cumulative dose treatment category [Low, up to 370.6 mg, gray bars; medium (Med), up to 795.6 mg, black cross hatch; High, up to 2328.2 mg, gray diagonal narrow cross hatch; Follow, 6 months after treatment, black diagonal wide cross hatch]. The horizontal lines indicate the comparisons made. *, $P < 0.05$.



baseline for those patients receiving high-dose chemotherapy (Fig. 2A, $P < 0.05$). Interestingly, these deficits resolved at the 6-month follow-up test. Touch threshold in hairy skin showed no change at any time point.

Sharpness detection

Sharpness detection threshold is an assessment of thinly myelinated A δ fibers. There were no differences observed in sharpness detection between healthy volunteers and patients before chemotherapy (Fig. 2B). There was also no change shown in sharpness detection in patients at the various cumulative doses of chemotherapy compared with the patient baseline.

Basal skin temperature

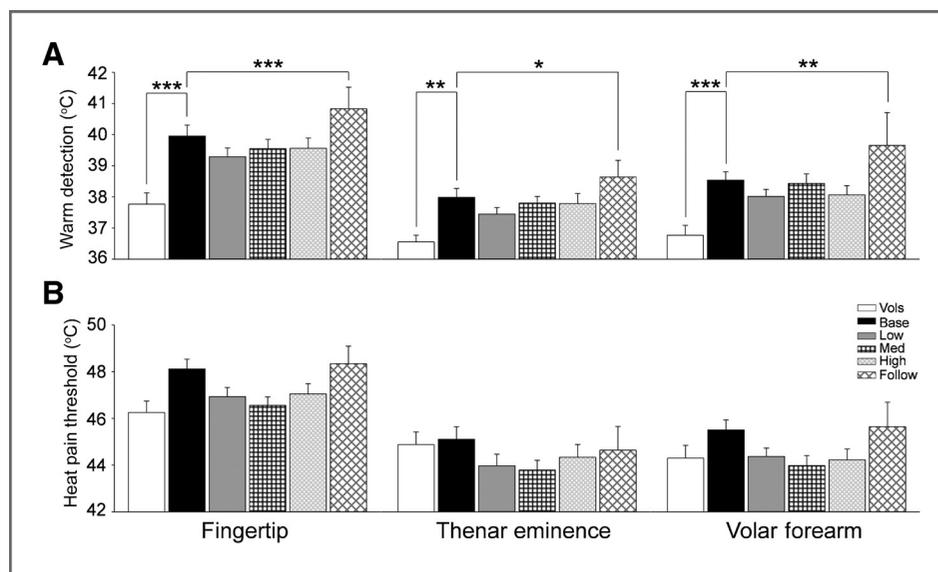
The patient group showed significantly cooler skin temperature in the fingertips before chemotherapy than that found in the healthy volunteer group (Fig. 3). Interestingly, skin temperature returned toward that of healthy volunteers in the treatment groups, but this change was not significant compared with the patient baseline group.

Temperature detection

The detection of warming is dependent on inputs from subgroups of unmyelinated C fibers given the relatively slow heat ramps that were used in this study. The patient baseline warm detection threshold was significantly elevated from that of healthy volunteers at all three test sites (Fig. 4A, $P <$

Figure 3. The bar graphs show the mean and SE for skin temperature in the index finger (A), the thenar eminence (B), and the volar forearm (C) for the healthy volunteers (Vols, open bars) and the patients at the pretreatment baseline (Base, black bars) and at each cumulative dose treatment category [gray bars, Low, up to 370.6 mg; medium (Med), up to 795.6 mg; High, up to 2328.2 mg; Follow, 6 months after treatment]. The horizontal lines indicate the comparisons made. *, $P < 0.05$.

Figure 4. The bar graphs show the mean and SE for warm detection threshold (A) and heat pain threshold (B) for the healthy volunteers (open bars) and the patients at the pretreatment baseline (black bars) and at each cumulative dose treatment category [Low, up to 370.6 mg, gray bars; medium (Med), up to 795.6 mg, black cross hatch; High, up to 2328.2 mg, gray diagonal narrow cross hatch; Follow, 6 months after treatment, black diagonal wide cross hatch]. The horizontal lines indicate the comparisons made. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

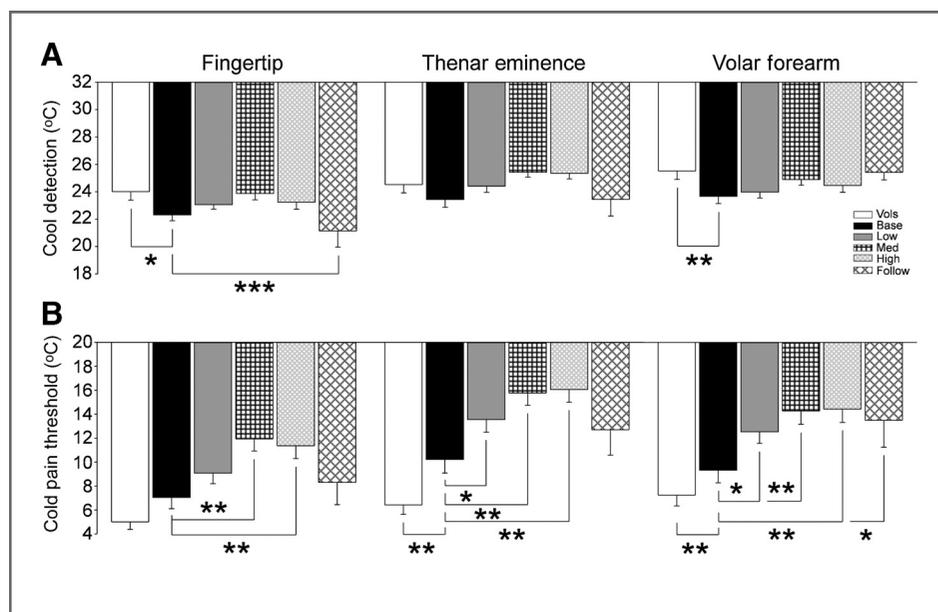


0.01–0.001). Warm detection in the treatment groups showed no change from the patient baseline. However, warm detection threshold was significantly increased from the patient baseline in the 6-month follow-up group (Fig. 4A, $P < 0.05$ –0.001). Heat pain threshold also mediated by unmyelinated C fibers tended to be higher in the patient group at baseline compared with that of the healthy volunteers, but this difference did not achieve statistical significance. No change in heat pain threshold from the patient baseline was observed in any of the treatment groups or at 6-month follow-up (Fig. 4B).

Cool and cold pain detection is mediated by activity in thinly myelinated A δ and unmyelinated C fibers, respectively. The patients had a significantly lower threshold to detect skin

cooling at the baseline measure before chemotherapy than the healthy volunteers in both the fingertips and the hairy skin of the volar forearm (Fig. 5A, $P < 0.05$ –0.01). This threshold showed a further deficit at 6-months follow-up compared with the patient baseline. The patient baseline cold pain threshold was significantly elevated compared with the healthy volunteers in both the thenar eminence and volar forearm (Fig. 5B, $P < 0.01$). Chemotherapy treatment increased this deficit such that the moderate and high doses resulted in pain at significantly warmer temperatures than at the patient baseline (Fig. 5B, $P < 0.05$ –0.01). This deficit showed some resolution at 6-months follow-up back toward the original patient baseline, though cold pain in the volar forearm remained significantly different.

Figure 5. The bar graphs show the mean and SE for cool detection threshold (A) and cold pain threshold (B) for the healthy volunteers (open bars) and the patients at the pretreatment baseline (black bars) and at each cumulative dose treatment category [Low, up to 370.6 mg, gray bars; medium (Med), up to 795.6 mg, black cross hatch; High, up to 2,328.2 mg, gray diagonal narrow cross hatch; Follow, 6 months after treatment, black diagonal wide cross hatch]. The horizontal lines indicate the comparisons made. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.



Neuropathy score and symptom complaints

Figure 6A shows the overall neuropathy scores for the healthy volunteers and for each of the patient groups tabulated by determining the number of measures in the QST battery for each subject that were 2 SDs or more outside the healthy volunteer mean values. The mean neuropathy score for the healthy volunteers was predictably very low, and as detailed in the previous sections, the value for the patients at baseline was significantly higher (Fig. 6A, $P < 0.01$). The mean neuropathy scores showed significant further increases from the patient baseline value with chemotherapy dose and had a peak at the 6-month follow-up (Fig. 6A, $P < 0.05$ – 0.01). Figure 6B shows the percentage of subjects in each group that had QST measures that were 2 SDs or more outside the healthy volunteer mean values (filled circles). Approximately 25% of the healthy volunteers had at least one out-of-range measure compared with roughly three quarters of the patients ($P < 0.01$). Notably, the QST deficits in the patients at baseline were subclinical as none reported any numbness or pain at the baseline measure (Fig. 6B, open circles and filled triangles). The percentage of patients with abnormal QST measures showed a continuous increase across the treatment groups with the final peak at the 6-month follow-up (Fig. 6B, filled circles). The decay in sensory function was paralleled by increasing reports of numbness and pain in the patient groups such that by 6-months follow-up, roughly three quarters of the patients reported numbness and roughly one fifth reported pain. Finally, Fig. 6C shows the rates of symptom complaint that developed during chemotherapy or present at follow-up based on whether the patients had a baseline QST deficit. The frequency of both numbness and pain was significantly increased in the patients who presented with subclinical neuropathy versus those who did not.

Discussion

This is the first study to use repeated QST in the study of the development of CIPN bringing a highly sensitive method to detect sensory impairments to this field (8). A key finding from this approach was the detection of preexisting subclinical sensory deficits in a large cohort of patients with colorectal cancer before treatment that seems to be disease driven and that when present seems to increase the risk for the later development of clinical CIPN. This observation, therefore, provides a generalization of a correlation between apparent subclinical pretreatment neuropathy and risk for CIPN as previously suggested in patients with multiple myeloma (10, 11, 15). A caveat, however, is that QST was not performed on the feet for convenience of the patients, yet CIPN often first presents in the feet. Hence, this study may present an overly conservative survey of QST deficits, particularly those that remained subclinical, that occurred in this patient cohort. It should be noted, however, that all of our subjects who became symptomatic complained of symptoms in the hands as well as the feet.

Perhaps the most important findings of this study are the mechanistic implications for impact of chemotherapy on

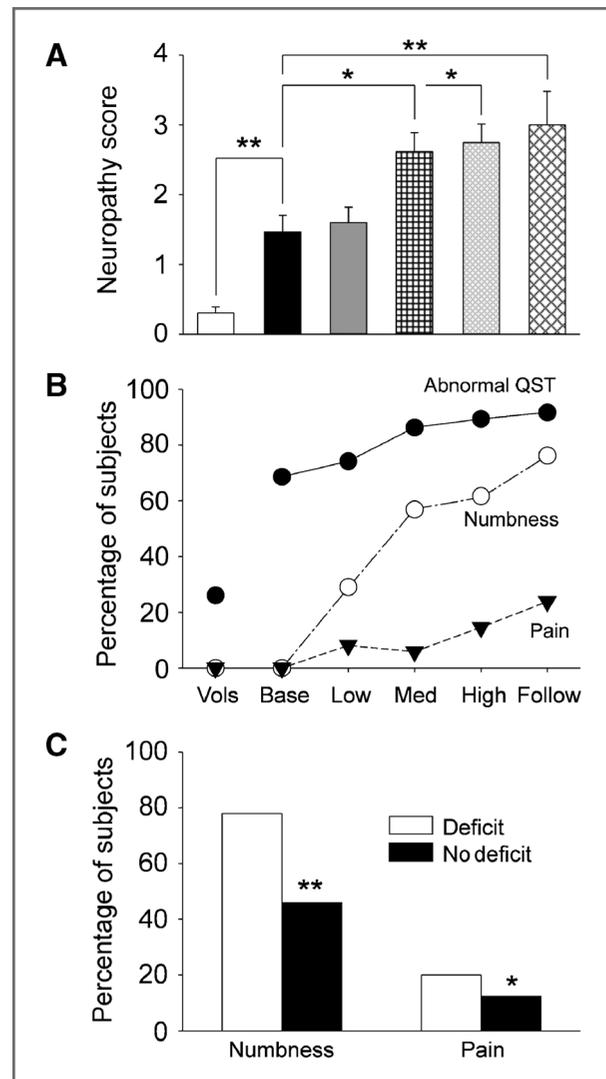


Figure 6. The bar graphs in A show the mean and SE for the neuropathy score (total number of QST measures two SDs or more from the healthy volunteer mean) for the healthy volunteers (Vols, open bars) and the patients at the pretreatment baseline (Base, black bars) and at each cumulative dose treatment category [Low, up to 370.6 mg, gray bars; medium (Med), up to 795.6 mg, black cross hatch; High, up to 2328.2 mg, gray diagonal narrow cross hatch; Follow, 6 months after treatment, black diagonal wide cross hatch]. The horizontal lines indicate the statistical comparisons made. The scatter and line plot in B shows the percentage of healthy volunteers and patients at each treatment category (abbreviations as above) that had abnormal QST measures (out of range measures as defined above, black circles) or that reported numbness (open circles) or pain (filled triangles). Finally, the bar graphs in C show the percentage of patients that reported numbness or pain at the end of treatment categorized on whether they had any QST deficit (open bars) or not (black bars) at the pretreatment baseline assessment. *, $P < 0.05$; **, $P < 0.01$.

specific groups of primary afferent fibers. Touch detection using the Bumps and von Frey assays is transduced by large diameter myelinated axons that terminate in or near Meissner's corpuscles (15) and Merkel disk complexes (16–18), respectively. The slotted pegboard task, although also

dependent to a degree on cutaneous mechanoreceptors, is more dependent on sensorimotor coordination involving neural inputs from muscle and joint mechanoreceptors that engage spinocerebellar and cortical cognitive processing. The pegboard test was significantly worse for patients at baseline compared with healthy controls, but then did not show any decay from that level and even a trend toward improvement. On the other hand, patient mechanoreceptor function tested using the Bumps test not only showed a difference at baseline from healthy volunteers, but also showed significant further deterioration with increasing chemotherapy doses and evidence of coasting following the termination of chemotherapy. Mechanoreceptor function assessed using von Frey monofilaments showed much of the same results as in the Bumps test. The difference between the pegboard to the Bumps and von Frey tests could be explained by assuming that the patients learned to cognitively overcome mechanoreceptor deficits in the former, whereas the lack of learning cues in the latter tests prevented this compensation. Alternatively, this paradox in results could also indicate that the myelinated fibers innervating the different tissues involved in these tasks show differential toxic deficit to oxaliplatin.

The A δ fiber-dependent tasks seem to provide clear psychophysical evidence in support of a differential susceptibility of primary afferent fibers to toxic insult by chemotherapy. Although the percept of sharpness/sharp pain evoked by the weighted needles showed little change at baseline or with chemotherapy, the detection of skin cooling showed a clear impairment. Similarly, various groups of C fibers are recruited in the detection of warm, heat, and cold pain, yet the patients showed preserved heat pain in the context of altered warmth and cold pain detection. Thus, for each group of fibers, psychophysical evidence indicates that the mechanism of toxicity engaged by chemotherapeutics differentially impacts function in different subtypes of primary afferent fibers, resulting in the clinical phenotype that is observed.

One possible explanation that fits this criterion well is the recent demonstration of an interaction of the chemotherapeutic paclitaxel with Toll-like receptor 4 (TLR4; ref. 19). Not all, but only subsets of small (C-fiber) and medium sized (A δ fiber) DRG neurons express TLR4 following chemotherapy treatment and show signs of an activated innate immune response including an increase in the expression of proinflam-

matory cytokines such as MCP-1 (20). The effects of MCP-1 and other cytokines on peripheral nerves could account for the clinical presentation of CIPN and the known risk and protective factors. Schwann cells express cytokine receptors that when activated lead to dedifferentiation and downregulation of myelin synthesis (21–24). This would consequently have pronounced functional impact on A β fibers that require extensive myelination, but less so on C-fibers, thus generating a clinical picture like that observed in the patient studies as described here. Finally, this mechanism would explain the observed effects of anticytokine treatments, such as minocycline, in preventing CIPN (25, 26) and suggests a clear short-term target for clinical evaluation in preventing a major complication of cancer treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Writing, review, and/or revision of the manuscript: M. de Carvalho Barbosa, A.K. Kosturakis, C. Eng, C.S. Cleeland, P.M. Dougherty

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. de Carvalho Barbosa, A.K. Kosturakis, P.M. Dougherty

Study supervision: X.S. Wang, P.M. Dougherty

Other (provision of the metrology used, especially "the Bumps," and review and suggestions for changes/corrections of the manuscript): W.R. Kennedy, D.A. Simone

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Session One B: Identifying Research

After learning about clinicaltrials.org, look for research on your health care condition of interest. Identify a trial with which you are not familiar and answer the following questions:

1. What is the research question?
2. What can you determine about the design of the trial?
3. Who is eligible? What are the exclusion criteria and why might they be there?

4. How long will the study take? Why do you think it will take this amount of time?

5. From a patient or caretaker perspective, what questions or concerns do you have about this trial?



Alliance for Aging Research Research Advocacy Action Plan November 2019

Name _____

We hope that you will take the next step and seek out opportunities in your community to engage in research. Working with your colleagues at your table, please identify one or two goals, and what it will take to accomplish those goals.

FIRST GOAL

1. What do you want to accomplish?

2. Who has information or other resources that can help you accomplish this goal? This could be people you are already know, or do not know.

3. What is the first step to making this happen?

4. What barriers might you face? If this happens, how will you respond?

5. How can we at the Alliance for Aging Research support your efforts?

3. What is the first step to making this happen?

4. What barriers might you face? If this happens, how will you respond?

5. How can we at the Alliance for Aging Research support your efforts?



Evaluation Forms

We would be very grateful if you could complete these evaluations. We will use this feedback to improve our program.

3. What suggestion do you have for improving this evening's session?

4. Is there anything else you would like to tell us about your experience?

Session One, Parts A and B: Clinical Trials **(8:40 am-2:30 pm)**

Wednesday, November 20, 2019

Includes researcher presentations, extracting key information from journal articles, exploration of current clinical trials

1. What is one thing you learned from this session?

2. What did you appreciate about this session?

3. What suggestion do you have for improving this session?

4. Is there anything else you would like to tell us about your experience?

Session Two: Patient-Centered Outcomes Research (2:40 pm-4:00 pm)

Includes small group brainstorming and exploration of study design

1. What is one thing you learned from this session?

2. What did you appreciate about this session?

3. What suggestion do you have for improving this session?

4. Is there anything else you would like to tell us about your experience?



Session Three: Advocate to Advocate and Action Plan (8:30 am-Noon)

Thursday, November 21, 2019

Includes panel discussion, small group meetings, creation of action plans

1. What is one thing you learned from this session?

2. What did you appreciate about this session?

3. What suggestion do you have for improving this session?

4. Is there anything else you would like to tell us about this experience?



Talk NERDY to Me Network Advocacy Training
Nurturing Engagement in Research and Development with You
November 19-21, 2019
Biographies

Patient-Centered Outcomes Research Institute (PCORI) Representative

Lia Hotchkiss, M.P.H.

Director of the Eugene Washington PCORI Engagement Awards Program, PCORI

Lia is Director of the Eugene Washington PCORI Engagement Awards Program at the Patient-Centered Outcomes Research Institute (PCORI). She sets the strategic direction and oversees the implementation of the PCORI Engagement Awards Program, which aims to support active integration of patient, research, and other stakeholder communities in the patient-centered outcomes research process. Before joining PCORI, Lia was at the UPMC Health Plan, where she initiated, planned, and executed projects and programs for the Consumer Innovation Department. She also served as Director of the Agency for HealthCare Research and Quality's Comparative Effectiveness Research Portfolio.

Talk NERDY to Me Advisory Council

Penney Cowan

Founder and Chief Executive Officer, American Chronic Pain Association

Penney is the founder and CEO of the American Chronic Pain Association (ACPA). As a chronic pain sufferer herself, she established the ACPA in 1980 to help others living with the condition and has since been an advocate and consumer representative for pain issues. She also served as a Consumer Representative for the Food and Drug Administration (FDA)/Center for Drug Evaluation and Research (CDER) Anesthetic and Analgesic Drug Products Advisory Committee (AADPAC)).

George Perry, Ph.D.

Professor of Biology and Chemistry, The University of Texas at San Antonio

George is Professor of Biology and Chemistry and former dean of the College of Sciences at the University of Texas at San Antonio. He is recognized in the field of Alzheimer's disease research, particularly for his work on oxidative stress. George is distinguished as one of the top Alzheimer's disease researchers, with over 1,000 publications, and is recognized as one of the 100 most-cited scientists in Neuroscience & Behavior.

Mellanie True Hills, CSP

Founder and CEO, StopAfib.org

Mellanie is an atrial fibrillation patient who is now 14 years a-fib free. She is the author of the multiple award-winning book, *A Woman's Guide to Saving Her Own Life: The HEART Program*

for Health & Longevity, and founder of the non-profit American Foundation for Women's Health and StopAfib.org, a patient advocacy organization for those living with atrial fibrillation. In carrying out her mission to rid the world of atrial fibrillation-related strokes, she created Atrial Fibrillation Awareness Month and worked with other organizations to gain U.S. Senate designation of September as National Atrial Fibrillation Awareness Month. She hosts the annual Get In Rhythm. Stay In Rhythm.®Atrial Fibrillation Patient Conference, speaks at medical conferences worldwide, brings the voice of the atrial fibrillation patient community to think tanks and health policy discussions, co-chaired the global Sign Against Stroke in Atrial Fibrillation Task Force, and co-created MyAFibExperience.org with the American Heart Association.

Talk NERDY to Me Corporate Advisory Council

DeAnna DuBose

Director of Patient Engagement, Edwards Lifesciences, Irvine, CA

DeAnna joined Edwards in September 2019 as the Director of Patient Engagement. Based in Irvine, California, she serves as a key strategic resource in developing the patient community to help Edwards better understand and serve patient needs to improve patient outcomes. Her focus is stewarding the inclusion of patients into the organization. Prior to joining Edwards, DeAnna spent more than 15 years in healthcare public affairs. For the last decade, she served in progressively senior leadership positions focused on pharmaceutical and medical device public affairs, with a particular focus on patient engagement.

Mary Slomkowski, Pharm.D.

Senior Director, Clinical Management CNS, Otsuka

Mary Slomkowski, Pharm.D. is a senior director in the clinical management group at Otsuka. She is responsible for executing central nervous system (CNS) clinical trials at Otsuka, mainly focusing in Alzheimer's disease. She is working on employing strategies to combine the use of technology and therapeutics to deliver high-quality clinical trial data. Mary is also responsible for building a more robust patient engagement practice within the organization by establishing a framework to enable data-driven decisions by incorporating an element of patient engagement into all Phase 1-3 clinical trials.

Talk NERDY to Me Disease-Specific Experts and Presenters

Carolyn Carman, O.D.

Director, Center for Sight Enhancement and Clinical Professor, University of Houston

Dr. Carman is the Director of the Center for Sight Enhancement, University Eye Institute at the University of Houston and is a professor at the university teaching Essentials of Leadership. Dr. Carman is a graduate of the University of South Florida and the Southern College of Optometry. She has over 35 years' experience with an emphasis in legal blindness/visual impairment and brain injury. She is the founder of the Low Vision Centers of Texas, Advanced Low Vision and the Polytrauma Vision Clinics at the Dallas Department of Veterans Affairs (VA) Medical Center and the Baylor Vision Rehabilitation Clinic in Dallas. Her research experience includes operating Alcon's Off-Site Clinical Research Center with involvement as a principal investigator and sub-investigator, and she has also served on an Institutional Review Board (IRB) for many years. Dr. Carman is a national and international lecturer, is past Chair of the Texas Optometry Board, and presently serves on the State of Texas Medical Advisory Board.

Patrick M. Dougherty, Ph.D.

H.E.B. Professor in Cancer Research, Department of Pain Medicine at The University of Texas MD Anderson Cancer Center, Houston, TX and Adjunct Professor, Department of Neurobiology & Anatomy, The University of Texas Health Science Center, Houston, TX

Dr. Dougherty specializes in anesthesiology and pain medicine. His research is primarily focused on gaining a multidisciplinary understanding of the neurochemical and physiological consequences of peripheral injury and inflammation on neural activity in the CNS. He has been a Principal Investigator on National Institutes of Health (NIH)/National Cancer Institute (NCI)-funded research for the past 30 years and is a member of the M.D. Anderson Cancer Survivorship Task Force. Dr. Dougherty has received numerous awards for both his research and teaching activities.

Maria A. Langas, Pharm.D.

Medical Affairs Communications Manager, Cardiovascular and Metabolism Medical Science Liaison (MSL) Strategy and Operations Team, Janssen Pharmaceuticals, Ewing, NJ

Maria supports the development of resources and scientific training across Cardiovascular and Metabolism areas for the MSL Team. Maria holds a Doctor of Pharmacy degree from the University of Pittsburgh School of Pharmacy and a Bachelor of Science degree in Pharmaceutical Science from the University of Pittsburgh.

Srini Potluri, M.D.

Medical Director, Cardiac Catheterization Laboratory, The Heart Hospital Baylor Plano, TX

Dr. Potluri is a cardiologist who is affiliated with multiple hospitals in the Plano, TX, area, including Baylor Scott and White Medical Center–Frisco and Baylor Scott and White Medical Center–Plano. He received his cardiology training at Ochsner Clinic, New Orleans and has been in practice since 2006.

Susan Strong

Director of Patient Engagement, Heart Valve Voice US

Susan Strong is the founding President and current Director of Patient Engagement for Heart Valve Voice US, the only patient-lead organization in the country that exclusively focuses on improving the diagnosis, treatment and management of heart valve disease. A champion of patient advocacy and engagement, Susan serves as an AHA Heart Valve Ambassador, a member of the National Quality Forum Cardiology Standing Committee, and as a PCORI Ambassador. She has presented the patient perspective on panels at numerous professional conferences including the American College of Cardiology, Transcatheter Cardiovascular Therapeutics, Transcatheter Valve Therapies, Society for Medical Decision Making, and NIH. With a keen focus on meaningful inclusion of patient stakeholders, Ms. Strong is passionate about improving patient experience and outcomes through collaborative partnerships in research, healthcare systems and industry. With an extensive network of patient advocates and non-profit organizations, she collaborates with a wide range of stakeholders to help improve processes and policies that impact patient care.

Jeff Todd

President and CEO, Prevent Blindness

Jeff Todd is the President and CEO of Prevent Blindness. Todd joined Prevent Blindness in 2003 as Director of Public Health and later served as Chief Operating Officer where he oversaw the mission-based work of Prevent Blindness, focusing on program outreach, education, public health, and policy. Additionally, Todd serves as Chair of Vision 2020 USA, is a member of the

Advisory Board to Jonas Children's Vision Care at Columbia University Medical Center, and is a past chair of the Vision Care Section of the American Public Health Association.

Talk NERDY to Me Training Staff

Sara Collina, J.D.

Blueberry Hill Strategies

Sara has worked in the field of breast cancer advocacy for almost 20 years, training breast cancer advocates to get involved in breast cancer research as partners in the research process. More recently, she worked with PCORI to create a training program for patients serving as reviewers of PCORI applications. Sara currently runs a small consulting firm that provides legal and science education to advocacy organizations and teaches in the Women's and Gender Studies Department at Georgetown University.

Sarah DiGiovine

Director of Development, Alliance for Aging Research

Sarah serves as the Director of Development at the Alliance for Aging Research. Her current role includes oversight of corporate sponsorship and grant outreach, implementation of the Salesforce donor database, and building a sustained individual giving program. Sarah has been at the Alliance for over five years and previously served as the Development Manager and Development & Meetings Coordinator. In these roles, Sarah managed the organization's health education and public policy events and supported the broader goals of the development department.

Beth Mathews-Bradshaw

Senior Program, Policy, and Regulatory Affairs Analyst, Leidos

Beth has been an employee of Leidos, formerly SAIC, for 18 years. Her work has encompassed a variety of clients from the NIH and Department of Defense to non-profit organizations. She has expertise in regulatory (clinical trials) work for drugs and biologics, science writing, and program management in strategic planning and evaluation for health programs. She has been a caretaker for family members with significant health challenges as well as a volunteer for an adult dementia program.

Lauren Smith Dyer

Vice President of Communications, Alliance for Aging Research

Lauren brings to the Alliance more than 15 years of experience in traditional and digital health advocacy communications, having counseled government agencies, nonprofit organizations and biopharmaceutical companies through complex business and communications challenges related to critically-important health, research, and regulatory issues. Lauren was most recently at the U.S. Food and Drug Administration (FDA), where she served as a spokesperson for the agency on the Commissioner's senior media affairs team. Lauren was responsible for planning and executing media engagement strategies in support of FDA actions and accomplishments, specifically those related to the agency's patient-focused drug development program, human research subject protection and oversight, clinical trials, and pharmaceutical quality. Before joining the FDA, Lauren led the public relations, branding and reputation management efforts for the Melanoma Research Foundation.

Susan "Sue" Peschin, M.H.S.

President and CEO, Alliance for Aging Research

Sue is President and CEO of the Alliance for Aging Research, a Washington, D.C.-based national nonprofit organization dedicated to accelerating the pace of scientific discoveries and

their applications to vastly improve the universal human experience of aging and health. As a thought leader on aging-related issues, Sue has led the Alliance in efforts to boost older adult immunization rates; increase funding for Alzheimer's disease and aging research at the NIH; raise awareness of geriatric cardiac issues; and co-organize a first-ever NIH geroscience summit. Sue was fortunate to grow up with both sets of grandparents and two of her great-grandmothers, all of whom taught her about the importance of family connection, health, and well-being.

Talk NERDY to Me Training Participants

Joni Armstrong Rocklin, CA

Joni was born and raised in Pleasanton, California. She has neck and shoulder issues and hip, back, and sciatica issues. Her father has back issues and a condition called Dish. She signed up for the training to learn more about how she can become a good caretaker for her father. She has advocacy experience working with ACPA, but no direct research experience. She is a very passionate person about pain, wants to learn to help others, and has been involved with patient-centered outcomes research (PCOR) in terms of finding people to fill-in for PCORI related programs.

Patricia "Patty" Baumiller Fairfax Station, VA

Patty has Atrial Fibrillation. She wants to learn more and help educate others about this chronic condition. She is retired and has time to give her all to something meaningful. She does not have previous advocacy, research or PCOR experience. She has had three ablations for her a-fib and it is currently under "control" with medications.

Marion Cunic Denville, NJ

Marion was born and raised in Brockton, Massachusetts. She has A-Fib and Type 2 Diabetes. She was a long-distance caregiver for her mother, who had dementia. She has always believed in "giving back" and feels that her experiences will be of help to another person. She has done advocacy work as a Legal Clinic coordinator, Women's Center of Morris County; Visitor, Mended Hearts; Denville Township Local Assistance Board; Past Member, Crisis Assistance, Parsippany Police Department; and Chairman of the Denville Municipal Alliance Committee. She is a mother of four children, two boys and two girls.

Joseph Davidson Williams Dunwoody, GA

Joseph has dry macular degeneration and epiretinal membrane. A very good friend, who heads a Georgia non-profit that advocates for eye health, suggested the training as something he might enjoy. For 8 years, Joseph was a volunteer with Score, which advocates for small business development through the Small Business Administration. He has not yet participated in research or PCOR. His personal fact: "I like outdoor activities."

Joan Durnell Powell Laguna Niguel, CA

Joan has myelodysplastic syndrome (MDS). She is participating in the training as a Patient Advocate and is inspired to engage and participate as a partner in medical advocacy and scientific research. She has been involved with the following organizations as an advocate: the Myelodysplastic Syndromes Foundation, Aplastic Anemia and Myelodysplastic Syndromes

International Foundation, PCORI, Leukemia and Lymphoma Society, National Organization of Rare Diseases, National Patient Advocate Foundation, Personalized Medicine Coalition, Congressionally Directed Medical Research Programs (Department of Defense), and Patient Access Network Foundation. She has been a Patient Consumer Reviewer and a Bone Marrow Failure Consumer Reviewer. She participated in research with Adelphi Values (Adelphi Mill, Bollington, Cheshire, SK10 5JB, UK) in June 2018 and was selected to be on an international study for her MDS disease. Additionally, Patty was a Patient Peer Reviewer for a research proposal in 2017 for “Practical Guidance for Involving Stakeholders in Health Research,” which was accepted in 2018 for publication in the *Journal of General Internal Medicine*. She also was selected as a Patient Advocate Speaker for the EPharma Conference in March 2019 in New York City.

**John Hall
Philadelphia, PA**

John is living with age-related macular degeneration and is a caregiver for his father-in-law who is experiencing cognitive decline. He is interested in the important role of patients in research design. John has no experience with PCOR, but has been involved with the National Breast Cancer Coalition Agewell Collaboratory at Drexel University and has submitted several proposals to university IRBs. He does not have any experience with PCOR. John is an avid cyclist.

**Denisha Hobbs, B.S., Gerontology
Baltimore, MD**

Denisha is from Philadelphia, Pennsylvania. She signed up for this workshop because she wants to be a better advocate for older adults. Denisha is very interested in the medical research she learned about through working with older adults, as well as in her academic career. She has been an advocate for the Baltimore County Department of Aging (LTC) Ombudsman Program and was an (LTC) Ombudsman. She has not participated in research or PCOR. Ms. Hobbs is currently in an MPA program specializing in Healthcare Policy and Administration at the University of Baltimore.

**Vera Howard
Ohio**

Vera has A-fib and helped provide care for her mother-in-law with Alzheimer's. She signed up for the training to learn how to help advocate for senior care. In the past, she has advocated on behalf of a summer food service program to provide lunch for low-income children during the summer when school was out of session. She has not participated in research or PCOR previously. She believes the type of advocacy this agency provides is of utmost importance.

**Diane Kinsella
Cincinnati, OH**

Diane has osteoarthritis, for which she has had surgeries on both feet, both hands, and a spinal fusion on August 1, 2019. She stated that, “My mother is in great health at 85, works out with a personal trainer (she can bench press 185 lbs.), but has ankylosing spondylosis, which means she cannot stand up straight to save her life. She also has osteoarthritis.” Diane was asked to participate by Joni Armstrong at the ACPA and has not yet participated in research, advocacy, or PCOR. Her personal fact: “I've been happily married to John for 34 years and during that time we've had a tornado, fire, and flood. We are resilient, if nothing else!”

Marlene Klein
Commack, Long Island, NY

Marlene has wet macular degeneration in both eyes. She signed up to participate because her condition is a result of smoking and she wants to be part of panels so that doctors and researchers can see through her eyes. She did commercials for the Centers for Disease Control and Prevention for their tip campaigns to help stop smoking. She does not have previous research or PCOR experience. She believes our eyes are very important to us and recommends having them checked yearly if not more often. She sees her retina specialist every month for eye injections.

Mary-Beth Lindenmuth
Raleigh, NC

Mary-Beth is originally from Pittsburgh, Pennsylvania but she has lived in Miami, Laguna Beach, Phoenix, Las Vegas, and Memphis. She lives with a-fib/mitral valve prolapse and has been a caretaker and advocate for her mother with Alzheimer's disease. She believes there is much to do to ensure that patient-centered outcomes are achieved in greater numbers and are not confused with bottom lines and conflicts of interest for doctors and those with possible different agendas. She has worked with the Interfaith Food Shuttle to advocate and provide food, in classroom and grocery store education, and on urban farms to the feed the underserved hungry of the Raleigh, Durham, and Chapel Hill, NC area. She also volunteers at the Conservators Center, where she provides education and conservation for carnivores (lions, tigers, leopards, wolves, and smaller wild felines) through public tours on weekends. She has not been involved in research or PCOR previously. Her mother's brain was donated to the Harvard Brain Tissue Resource center for autopsy related to Alzheimer's disease.

Jay MacIntosh
Chicago, IL

Jay had a failed back surgery and has degenerate disc disease, osteoarthritis, and severe nerve damage. He signed up for the training to enhance his knowledge of caretaking, not only for himself but for the group he facilitates at the ACPA. He has not participated in research or PCOR. Jay is trying to do a small part in letting people know it is hard living with pain, and it is okay to ask for help. He is also a huge comedy nerd!

Angel Mason
Chicago, IL

Angel is originally from south Bronx, New York. She has had Type 1 diabetes for over 40 years and has diastolic heart failure; advanced peripheral neuropathy; large and small cells in her feet, legs, and hands; blindness (no light perception); diabetic Charcot joints in both feet; and partial paralysis in both legs (from the thigh down on her left leg and below the knee on her right leg). She walks with a stance control computerized brace. She believes that, when it comes to working with people with chronic pain, any additional training is helpful in our pursuit. Ms. Mason has worked with the ACPA, the Foundation for Peripheral Neuropathy, the Depression and Bipolar Support Alliance, National Alliance on Mental Illness, and Deborah's Place as an advocate for housing, working directly with local, state, and federal politicians. She also participates in Speak up Illinois, where she advocates for better research, medication, and resources (including training) for people with mental health issues. After her health failing and losing her eyesight completely, Angel has discovered that living in the service of others gives her the strength to keep fighting.

Vivian Agnes Morgan Hicks, Ph.D.
North Central, TX

Vivian lives in an active senior facility in north central Texas. She has had double macular degeneration for 6 years. She would like to learn more about her condition and, perhaps, be an inspiration to others suffering from the disease. She does not have previous advocacy experience. She has been involved in research in her professional role in the past and is a member of the control group for exercise-related physical fitness at the University of Texas at Tyler. In addition to age-related macular degeneration in both eyes, she recently had a brain stem-generated stroke that affected her physical mobility.

Tom Olsen
Georgetown, Texas

suffers from A-fib, A-flutter, sleep apnea, high blood pressure, high cholesterol, and obesity. He signed up for the training to be a part of the solution, or at least a voice in the discussion. He volunteers at Pet Partners, a national organization for therapy dogs and handlers, advocating for improvement in the human-animal bond and the usefulness of therapy animals in various settings. He does not have previous experience in research or PCOR. His favorite retirement activity, other than taking trips, is working with his therapy dogs because it is so much fun to see the positive effect they have on the people they meet.

Patty Peterson
Minneapolis, MN

Patty is a survivor of aortic dissection in 2007. She was invited to consider participating in the training by Heart Valve Voice as a result of speaking to the FDA on "life with Valve replacement." She has spoken at numerous Aortic Symposiums with Amy Yasbeck and believes in a mission to proceed with additional awareness opportunities. She has participated in the AHA Go Red for Women Ambassador Thoracic Aortic Disease Coalition aortic awareness on many social media sites, including aortic valve replacement. She has not yet participated in research or PCOR. She is grateful to be alive and celebrates life everyday through song, speaking, and loving her family. Raising awareness is a passion so that others may not have to go through what she did; she believes that raising awareness saves lives.

Suzanne Proctor
Bristow, VA

Suzanne has A-Fib, Type 2 diabetes, sleep apnea, hypotension, asthma, hypoxia, arthritis, bursitis, mobility issues, deafness, and is visually impaired. She would like to make people aware that, even at a younger age, these health issues can affect your life. She does not have previous advocacy, research, or PCOR experience. Suzanne has been married for 18 years and is a grandmother of 10 wonderful grandchildren. She apparently has had A-fib for almost 10 years, so she was in her early 40s when it first started.

James Weil
Fremont, CA

James was born in Los Angeles and suffers from chronic migraine; peripheral neuropathy; peripheral edema (controlled), high blood pressure and cholesterol, and sleep apnea. He was invited by the ACPA to participate in the training and was asked to provide education to the ACPA group he facilitates and to the City of Fremont, where he volunteers as a Senior Peer Counselor. James has also participated on the Study Advisory Committee of Empower and is a Life Member of the Institute of Electrical and Electronics Engineers.

**Mary Worstell
Washington, DC**

Mary is a caregiver for her 96-year-old mother who suffers from moderate chronic kidney disease and heart failure and is nearly blind and nearly deaf. She signed up for the training because she sees a need to advocate for the aging and their sensory disabilities (vision and hearing loss), which are often severe and, in the case of her mother, have led to a loss of independence, personal pleasures (her mother read constantly and cherishes her books), and socialization, causing depression. She worked for 40+ years in public health, domestically and internationally, directing community health needs assessments, project design and implementation, and health policy advocacy on multiple health topics. She directed a national education/research and advocacy organization for 15 years, was founding director of a national Medicare patient advocate coalition, and served for decades in federal service in the NIH Secretary's office, promoting health policy and services for all Americans. She was member of the National Heart, Lung, and Blood Institute Asthma and Education Policy and Program Coordinating Council, establishing patient care guidelines and research priorities. She developed a new initiative for bench research with the National Institute of Allergy and Infectious Diseases, established a program of allied health research to improve patient care, and has reviewed non-bench research proposals for national funding. Mary was also a member of a patient-centered policy board advising national patient outcomes measurement guidelines for asthma.

**Teresa Wright-Johnson
Easton, PA**

Teresa is originally from Hillside, New Jersey. She lives with congenital heart disease and multiple sclerosis. She is also a caregiver to her mother. She signed up for this workshop to learn how she can participate in improving the quality of life for patients, caregivers, and aging parents, as well as to share her experiences from both perspectives. She is a board member and advocate for her local AHA, a board member for Heart Valve Voice-US, an engagement committee member for iConquerMS, and is on the Heart Valve Voice Advisory Board. She would like others to know that she is passionate about diversity and inclusion and seeks to bridge the gap between racial, socioeconomic, and gender barriers.

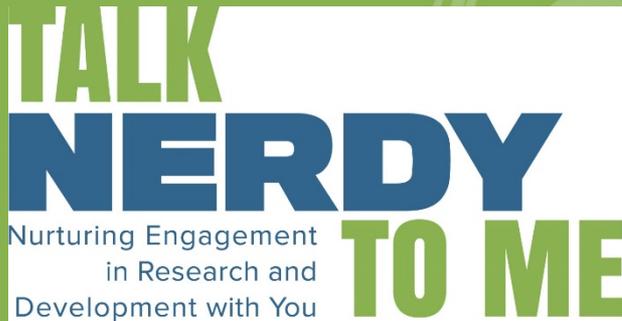
**Brenda Wyatt
Vancouver, WA**

Brenda has hypothyroidism, A-fib, gastrointestinal reflux disease, and chronic ankle pain. She is a volunteer Ombudsman for the State of Washington. Several of her close relatives have suffered and died from Alzheimer's disease. Now that she is retired, she has the time to volunteer to help find ways to manage/cure this dreadful condition. She believes in a holistic lifestyle and approach to healing, such as exercise, good diet, and stress management, along with modern science.

**Fred Young, Ph.D.
Medford, OR**

Fred lives in a rural community in southern Oregon. He has a transcatheter aortic valve replacement (TAVR), diabetes, slight hypertension, neuropathy, and arthritis. He takes care of his wife, who had a stroke and has a retina condition; difficulties in walking, focusing, and remembering things; and has just had back surgery. He signed up for the training because he thought it would help with his wife and to get involved with senior or disabled people who could use his help. His previous advocacy work has centered around TAVR people and helping with their lobbying efforts to help prevent new Medicare guidelines from restricting this kind of surgery. He has been on the human subjects ethics committee at the University of Dayton, been

a reviewer for the Bowling Green State University Social Policy Center, and has been on the faculty committee responding to new federal guidelines for universities getting federal subsidies. Fred has been an ethics philosopher who helped write some of the articles that led to the transition from doctor to patient-oriented guidelines for professional conduct. He practices martial arts.



Webinar One

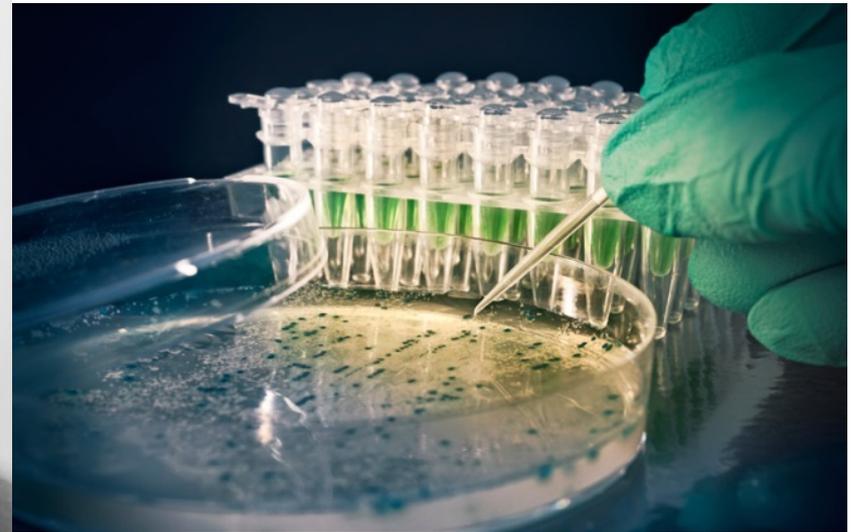
Senior Patient & Family Caregiver Network Patient-Centered Outcomes Research

Sue Peschin, President and CEO, Alliance for Aging Research
Sara Collina, Curriculum Developer, LEIDOS

Participants will:



- ✓ Understand what to expect at the upcoming Training
- ✓ Learn what *Research Advocacy* is and why it matters
- ✓ Explore the key elements of *Patient-Centered Outcomes Research*



WHO WE ARE

The Alliance for Aging Research is the leading non-profit organization dedicated to accelerating the pace of scientific discoveries and their application in order to vastly improve the universal human experience of aging and health. WWW.AGINGRESEARCH.ORG

Catalyzing Innovation for Healthy Aging

Senior Patient and Family Caregiver Network



 **PATIENT-CENTERED
OUTCOMES RESEARCH**

 2 years of funding received from PCORI to launch the first ever Senior Patient & Family Caregiver Network



Catalyzing Innovation for Healthy Aging

Senior Patient and Family Caregiver Network



- Organized by the Alliance for Aging Research
- Funded by the Patient-Centered Outcomes Research Institute (PCORI)
 - Comparative Effectiveness Research
 - Patient and Family Caregiver Engagement Projects, such as the Senior Patient and Family Caregiver Network!

New for 2019

- New name: Talk N.E.R.D.Y. to Me
- Two new clinical areas: heart valve disease and age-related macular degeneration.
 - Keeping older adults with Alzheimer's disease, atrial fibrillation (AFib), chronic pain/disability, and/or sarcopenia; and, family caregivers.
- Different levels of knowledge and experience—let's all learn from each other

Staying the same in 2019

- Different levels of knowledge and experience—let's all learn from each other
- Participate in two webinars in October 2019 prior to the workshop—this is the first one!
- Watch the 6-minute Alliance for Aging Research video, *Pay it Forward: Volunteering for a Clinical Trial*, between now and the second webinar—we will send everyone the link

Staying the same in 2019

- Webinar #2 on Understanding Clinical Trials: Tuesday, October 15 at 2:00 pm Eastern
- Complete the *Progress for Patients Video Training*. A link to the training will be sent to you via e-mail after the next webinar

Talk N.E.R.D.Y. to Me

- Participate in the in-person workshop from Tuesday evening November 19 to Thursday mid-day on November 21 in Dallas, TX
 - Dress is casual—wear what is comfortable
 - Bring a sweater in case it's cold in the room
- Participate in a post-workshop interview to refine the curriculum in December 2019
- Provide feedback on a revised curriculum (December 2019 – February 2020)
- Stay in touch with online network

How will you use this training?

- Volunteer opportunities to provide input into the medical research process from the patient/caregiver perspective at the national or local level
- Participants will receive:
 - Covered travel, lodging, and a stipend of \$400 for full participation
 - A Certificate of Completion for participating in the training

Research Advocacy

- What is *research advocacy*?
- What is the purpose of *research advocacy training*?

Patient-Centered Outcomes Research

- What is medical (or health care) research?
- What is outcomes research?
- What makes outcomes research patient-centered?

Patient-Centered Outcomes Research

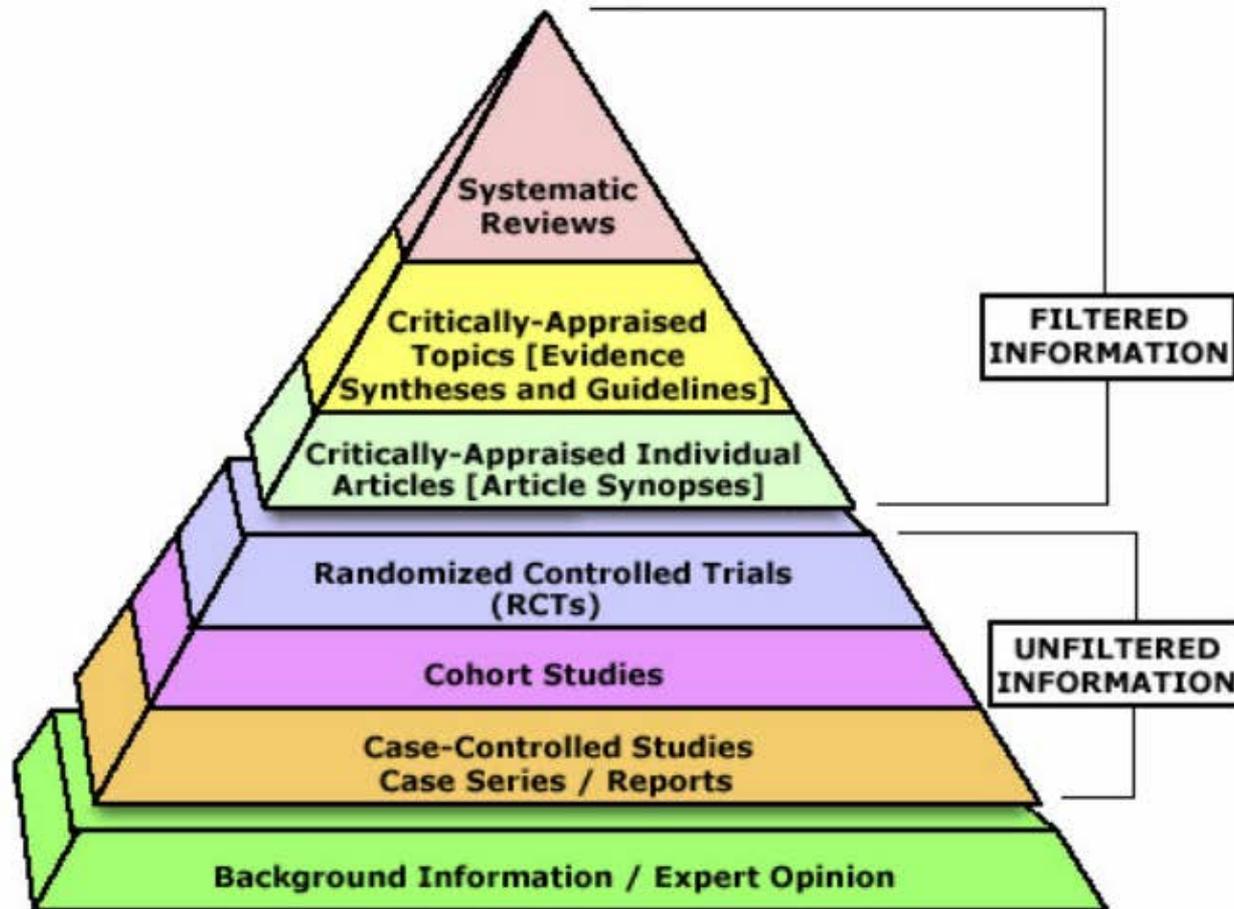
What is medical (or health care) research?

The key ingredient for...

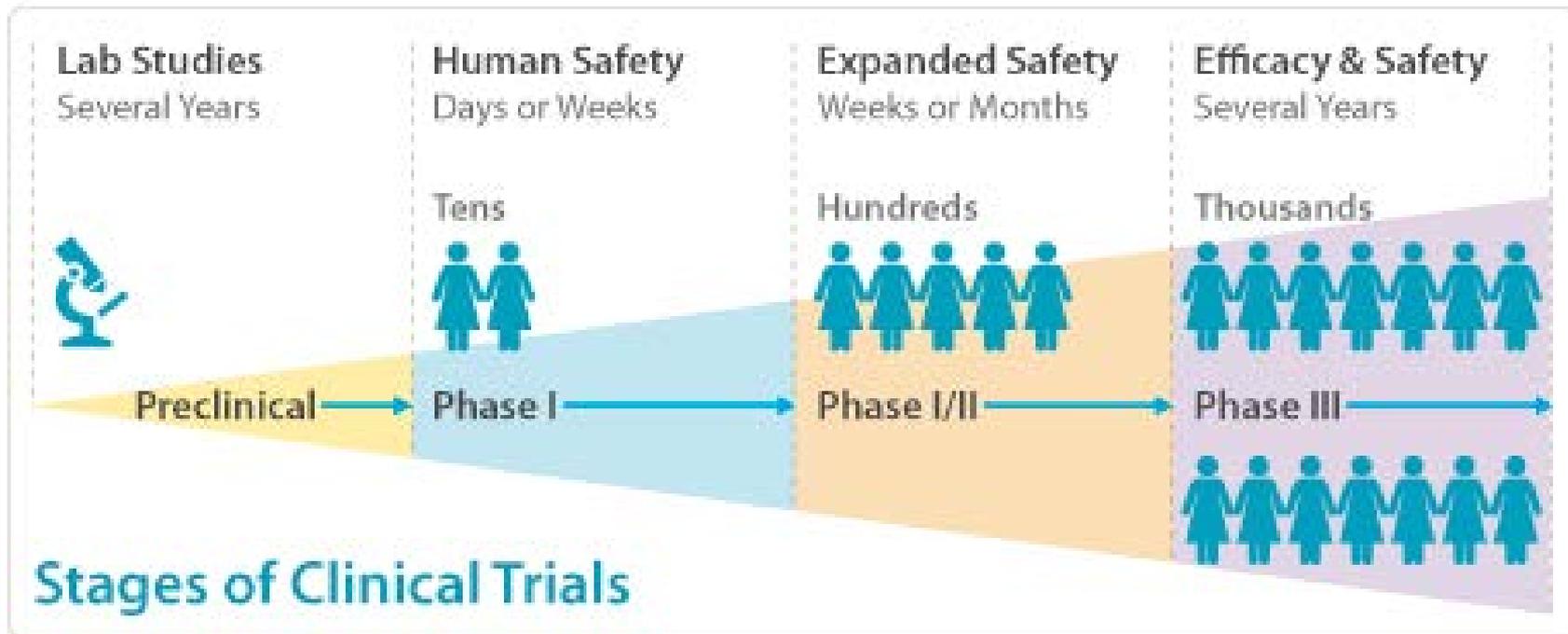
Wait, aren't ALL health care decisions based on at least some evidence?

Evidenced-Based Health Care

Kinds of Research



Clinical Trials



Patient-Centered Outcomes Research



What is outcomes research? Focus is on end result.

Prospective studies: researchers follow participants into the future to record when and how they developed a particular outcome

Retrospective studies: researchers jump back in time to look at records of patients and follow their histories to determine when, why, and how they developed a particular outcome

Patient-Centered Outcomes Research



There are two types of data used to measure outcomes:

Data that is **quantitative**

Can be expressed as a number

Data that is **qualitative**

Cannot be expressed as a number

Who Funds Medical Research?

- Federal, State, and Local Governments
- Universities and Colleges
- Foundations
- Medical Research Organizations
- Diseased-Focused Organizations
- Industry (Pharmaceutical, Biotechnology, etc.)

Who Funds Medical Research?

Foundations

Medical Research Organizations

Diseased-Focused Organizations

Universities and Colleges

State and Local Governments

Federal Government

Industry

Patient-Centered Outcomes Research

Comparative Effectiveness Research (CER)

The direct comparison of two or more treatments to determine what works best for which patients.

Patient-Centered Outcomes Research (PCOR)

A kind of comparative effectiveness research that specifically answers patient-centered questions.

What is Patient-Centered Outcomes Research?

Given my personal characteristics, conditions, and preferences, what should I expect will happen to me?

What are my options, and what are the potential benefits and harms of those options?

How can clinicians and the care delivery systems they work in help me make the best decisions about my health and health care?

What is Patient Centered Outcomes Research?

Some Terms to Know

Efficacy Trials

Could it work in ideal settings?

Effectiveness or Pragmatic Trials

Does it work in the real world?

What is Patient-Centered Outcomes Research?

A Few More Terms to Know

Patient **Reported** Outcomes

Any report about a patient's health condition that comes directly from the patient, without interpretation by a clinician or anyone else.

Patient **Engagement**

Including patients in the research process itself.

Patient-**Centered** Outcomes

A health result (event or nonevent) that actually matters to patients.



Patient Centered Outcomes Research Institute (PCORI)

Catalyzing Innovation for Healthy Aging

What makes PCOR different?

- Patients can participate in **PLANNING** the research.
- Patients can participate in **CONDUCTING** the research.
- Patients can participate in **DISSEMINATING** the research.

Translating our Concerns into Research Questions

THE PEOPLE

Who are the people that should be studied? This is the population of interest.

THE OPTIONS

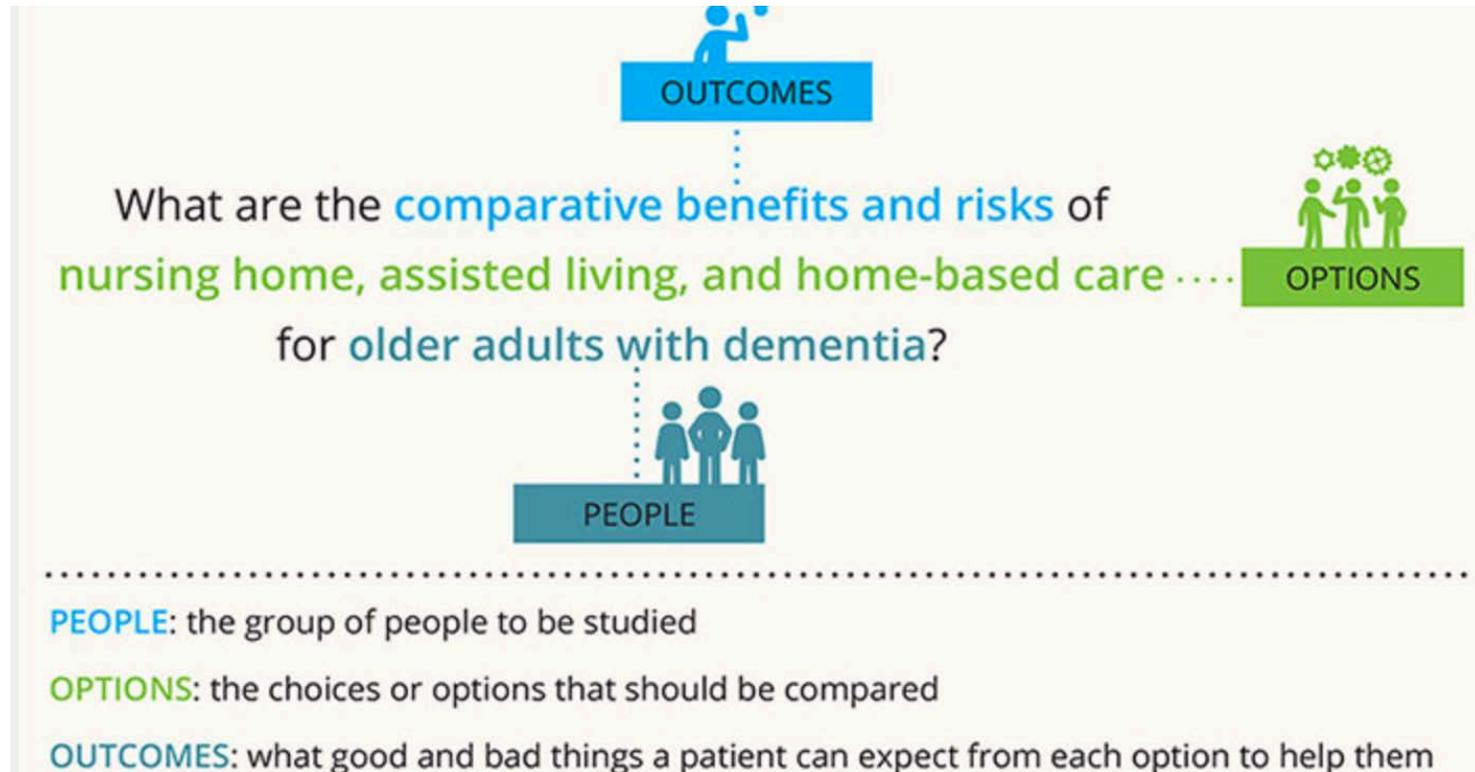
INTERVENTION and
COMPARATOR

What options should be compared? These are the decisions the research is intended to inform.

THE OUTCOMES

How can people make informed choices between options? These are the factors that people will consider when making a decision between/among options.

Translating our concerns into research questions



Did we succeed?

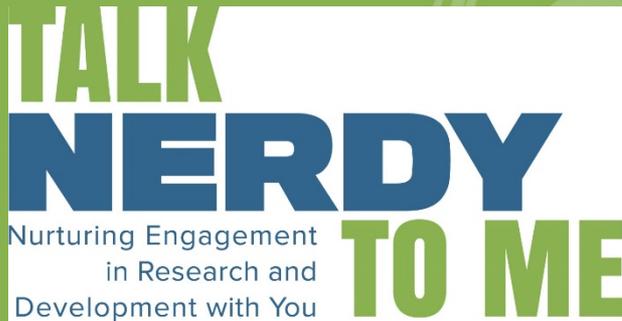


- ✓ Understand what to expect at the upcoming Training
- ✓ Learn what Research Advocacy is and why it matters
- ✓ Explore the key elements of Patient-Centered Outcomes Research



If you have questions after the webinar,
please email Sue Peschin at
speschin@agingresearch.org, or call me
at 202-688-1246

Thank you!



Webinar Two

Clinical Trial Research

How do clinical trials work?

Jack M. Guralnik, M.D., Ph.D.

Department of Epidemiology and Public Health
University of Maryland School of Medicine

Participants will be able to:

- ✓ Explain key elements of clinical trial design
- ✓ Extract key information from a scientific abstract



Randomized Controlled Trials

Background

- The randomized trial is considered the ideal design for evaluating both the effectiveness and the side effects of new forms of intervention¹
- The randomized controlled trial is at present the unchallenged source of the highest standard of evidence used to guide clinical decision making²

1. Gordis L. Epidemiology. 4th edition. Philadelphia: Saunders (Elsevier); 2009.
2. Lavori PW, Kelsey J. Introduction and overview. Epidemiol Rev. 2002;24:1-3.

Randomized Controlled Trials

- Treated and untreated participants are followed over time to determine whether they experience the outcome
- Assignment to treatment or non-treatment is by randomization



Randomization

- Process by which all participants have equal probability of being assigned to the treated group or the untreated group
- Removes the potential for conscious or unconscious bias in the allocation of subjects to the treatment groups

Timing of RCTs

- Must have preliminary evidence of treatment's efficacy and safety
- Must know enough about treatment to know which outcomes to assess
- Before treatment becomes part of standard medical practice



Equipoise

- A state of genuine uncertainty about the benefits or harms that may result from different exposures or interventions. A state of equipoise is an indication for a randomized controlled trial, because there are no ethical concerns about one regimen being better for a particular patient.

Porta M (ed.). A dictionary of epidemiology. 5th ed. New York: Oxford University Press; 2008

Avoidance of Bias in RCTs

- Generation of truly random allocation sequence
- Concealment of allocation sequence
- Blinded outcome assessment



Blinding of Outcome Assessment

- Knowledge of participant's group allocation could bias outcome assessment
- Blinding:
 - Participants
 - Research staff who are assessing the outcome
 - Health care professionals caring for the patient
 - Data analysts

Blinding of Outcome Assessment

- Blinding may not always be possible
 - Effectiveness of an exercise intervention in patients after myocardial infarction
- Side effects may affect ability to maintain blinding
 - Nausea, hair loss

Treatment of Controls

- No treatment
- Placebo
- Standard treatment



Placebo Effect

- Placebo:
 - A treatment that appears identical to the study treatment but that lacks the active component(s)
- Placebo effect:
 - Apparently beneficial effect of a treatment resulting solely from administration of the treatment

Purpose of Placebo Group

- To maintain blinding
- To strengthen bond between participant and study
- To control for placebo effect

Intention-to-Treat Approach

- Study participants who do not adhere to treatment protocol or who switch groups are analyzed according to original group assignment
- Answers the question, “How does the treatment work in the people to whom it is targeted?”
- Simulates the “real world”

Generalizability

- Study population
 - Systematic differences between study and target populations (eligibility criteria)
 - Volunteerism
- Trial conditions
 - Difference between trial conditions and “real world” conditions

Ethical Issues

- Is it ethical to randomize people
 - to receive the experimental treatment?
 - to not receive the experimental treatment?
- Is the sample size too small?
- Is the sample size too big?
- Informed consent
- Interim analyses, stopping rules

Strengths of RCTs

- Study design with the greatest ability to provide valid results
- Randomization prevents bias that may occur when allocating participants to groups
- Randomization usually results in groups that are comparable to each other in regard to known and unknown confounding variables

Limitations of RCTs

- Only useful for studying potentially beneficial factors
- Potential participants may be reluctant to agree to randomization
- Generalizability
- Timing/equipoise
- Expense

Five Concepts that Really Matter in Clinical Trial Design with Examples from the EAFT Trial

European Atrial Fibrillation Trial

Secondary prevention in non-rheumatic atrial fibrillation after transient ischaemic attack or minor stroke, EAFT (European Atrial Fibrillation Trial) Study Group, *The Lancet*, Volume 342, Issue 8882, 1993, Pages 1255-1262

European Atrial Fibrillation Trial - Abstract

Several studies have established the value of anticoagulation for primary prevention of thromboembolic events in patients with non-rheumatic atrial fibrillation (NRAF). However, in patients with a recent transient ischaemic attack (TIA) or minor ischaemic stroke the preventive benefit of anticoagulation or aspirin remains unclear. Physicians in 108 centres from 13 countries collaborated to study this question.

European Atrial Fibrillation Trial - Abstract

1007 NRAF patients with a recent TIA or minor ischaemic stroke were randomised to open anticoagulation or double-blind treatment with either 300 mg aspirin per day or placebo (group 1, 669). Patients with contraindications to anticoagulation were randomised to receive aspirin or placebo (group 2, 338). The measure of outcome was death from vascular disease, any stroke, myocardial infarction, or systemic embolism.

European Atrial Fibrillation Trial - Abstract

During mean follow-up of 2.3 years, the annual rate of outcome events was 8% in patients assigned to anticoagulants vs 17% in placebo-treated patients in group 1 (hazard ratio [HR] 0.53; 95% confidence interval [CI] 0.36-0.79). The risk of stroke alone was reduced from 12% to 4% per year (HR 0.34; 95% CI 0.20-0.57). Among all patients assigned to aspirin (groups 1 and 2), the annual incidence of outcome events was 15%, against 19% in those on placebo (HR 0.83; 95% CI 0.65-1.05). Anticoagulation was significantly more effective than aspirin (HR 0.60; 95% CI 0.41-0.87). The incidence of major bleeding events was low, both on anticoagulation (2.8% per year) and on aspirin (0.9% per year). No intracranial bleeds were identified in patients assigned to anticoagulation.

European Atrial Fibrillation Trial - Abstract

We conclude that anticoagulation is effective in reducing the risk of recurrent vascular events in NRAF patients with a recent TIA or minor ischaemic stroke. In absolute terms: 90 vascular events (mainly strokes) are prevented if 1000 patients are treated with anticoagulation for one year. Aspirin is a safe, though less effective, alternative when anticoagulation is contraindicated; it prevents 40 vascular events each year for every 1000 treated patients.

1. Bias/Randomization

Bias/Randomization

- Observational (Real World) vs. Experimental / Interventional
- Superiority vs. Noninferiority vs. Equivalence
- Prospective vs. Retrospective
- Randomization
- Blinding

2. Protocol/Reproducibility

Protocol/Reproducibility

- Eligible population
 - Selection Criteria – change selection criteria, will get a different answer to the same question
 - Eligible pool – is the recruitment population a subset of all affected patients
 - Patients in a particular country
 - Patients in a particular health care system



Protocol/Reproducibility (cont.)

- Baseline Characteristics
 - Age
 - Gender
 - Disease States
- Confounders
 - Differences in laboratory testing in different countries (Anticoagulation, Troponin)

3. Endpoints/Outcomes

Endpoints/Outcomes

Primary safety endpoint	Major and nonmajor clinically relevant bleeding	Major bleeding (ISTH criteria)	Major bleeding	Major bleeding (modified ISTH criteria)
Major bleeding definition	<ul style="list-style-type: none"> Clinically overt bleeding associated with fatal outcome, involving a critical site, or clinically overt bleeding associated with a fall in Hb ≥ 2.0 g/dL or leading to transfusion of ≥ 2 units of packed red blood cells or whole blood 	<ul style="list-style-type: none"> Acute or subacute clinically overt bleeding accompanied by a Hb reduction ≥ 2 g/dL over a 24-hour period or transfusion of ≥ 2 units of packed red blood cells, occurring at a critical site, or resulting in death 	<ul style="list-style-type: none"> Hb reduction ≥ 2 g/dL, blood transfusion ≥ 2 units of blood, or symptomatic bleeding in a critical site or fatal outcome 	<ul style="list-style-type: none"> Clinically overt bleeding meeting one of the following criteria: fatal bleeding; symptomatic bleeding in a critical site; clinically overt bleeding event that caused a fall in Hb ≥ 2.0 g/dL (or a fall in Hct of $\geq 6.0\%$ in the absence of Hb data), when adjusted for transfusions

Outcomes

- Definitions
- Relative Risk reduction (RRR)
- Absolute Risk reduction (ARR)
- Number needed to treat
- Scientists are just as capable of “spin” as politicians:
 - In some cases, will use RRR to overemphasize benefit
 - 25% reduction in events (from 12% to 9%)
 - And then use ARR to minimize risk
 - Only a 1% increase in bleeding (from 1% to 2%)

4. Selection Criteria (Inclusion/Exclusion)

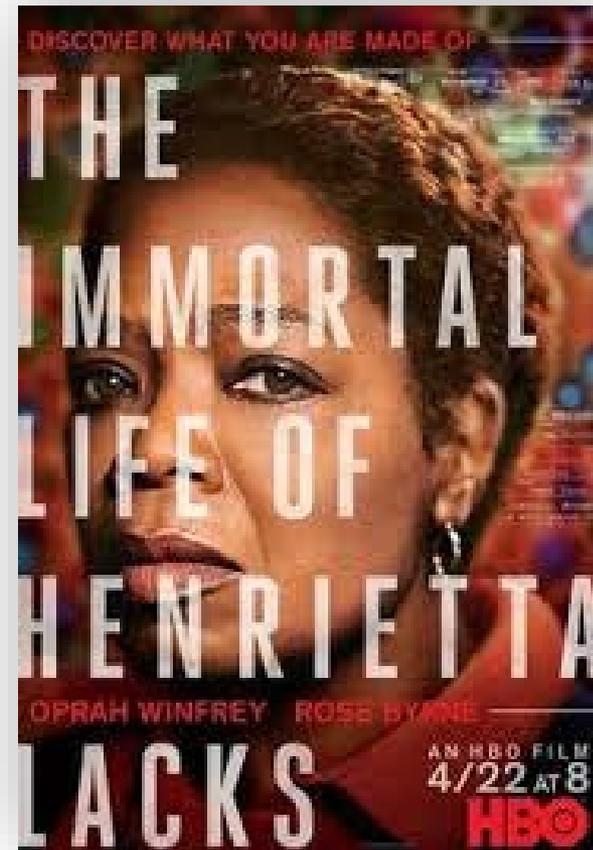
Selection Criteria

- How Specific?
 - Differential Diagnosis for Stroke vs. TIA
 - High blood pressure – treated, untreated, for how long
 - Smoking – total pack years exposure, how long since quit
 - Congestive heart failure – multiple subcategories and severity
 - Diabetes – great variations in level of control

5. Informed Consent

Informed Consent

- Tuskegee Airmen
- Declaration of Helsinki
- Common Rule
- HIPPA
- Third World vaccine trials





If you have questions after the webinar,
please email Sue Peschin at
speschin@agingresearch.org, or call me
at 202-688-1246

Thank you!

**SENIOR PATIENT AND FAMILY CAREGIVER NETWORK
ADVOCATE TRAINING
NOVEMBER 19-21, 2019
Glossary**

Absolute Risk Reduction (ARR): Absolute risk of a disease is the risk of developing the disease over a time period. Absolute Risk Reduction (ARR) is the change in the risk of an outcome in relation to a comparison treatment or activity.

Anticoagulant: Medicines that help prevent blood clots

Accuracy: The closeness of agreement between a data value and the true value.

Association: A connection or relationship between things.

Adverse Event (AE): An undesirable experience associated with the use of a medical product in a patient. An event is considered a Serious Adverse Event (SAE) when the patient outcome is death; life-threatening hospitalization; disability; congenital anomaly/birth defect; or rapid intervention is required to prevent permanent impairment.

Bias: A systematic error in sampling or testing that encourages one outcome or answer over others.

Biologic: A therapeutic agent derived from living things.

Biologics License Application (BLA): A form submitted to the Food and Drug Administration (FDA) after a Phase III trial that requests permission to label and market a biological product.

Blinding: The process of keeping secret the assignment of participants to study groups from researchers, participants, or both. This is done to minimize bias.

Causation: When changes in one variable directly cause changes in the other. In the clinical trial context, cause and effect can only be effectively studied through randomization. An association between two items does not necessarily mean that one caused the other.

Clinical Trial Phases: Clinical trials are conducted in a series of steps, called phases, to answer a separate research question.

- **Phase I:** Researchers test a new drug or treatment in a small group of people for the first time to evaluate its safety, determine a safe dosage range, and identify side effects.
- **Phase II:** The drug or treatment is given to a larger group of people to see if it is effective and to further evaluate its safety.
- **Phase III:** The drug or treatment is given to large groups of people to confirm its effectiveness, monitor side effects, compare it to commonly used treatments, and collect information that will allow the drug or treatment to be used safely.
- **Phase IV:** Studies are done after the drug or treatment has been marketed to gather information on the drug's effect in various populations and any side effects associated with long-term use.

Cohort: A group of individuals who share a common exposure, experience, or characteristic. For example, a study may choose to follow a group, or cohort, of individuals who were exposed to contaminated water.

Collection Methods: The process of gathering and measuring information on variables of interest in an established, systematic fashion that enables one to answer stated research questions, test hypotheses, and evaluate outcomes.

Community-based participatory research (CBPR): A research approach that engages community partners in each stage of the process. CBPR differs from patient-centered outcomes research (PCOR) in that it is always steeped in community engagement, nurtures partnerships to realize shared outcomes over the long term, and often occurs outside of the clinical setting. PCOR can use a CBPR approach.

Comparative effectiveness research: Research focusing on building and evaluating evidence that assesses the benefits and risks of two or more methods that are designed to address the prevention, diagnosis, treatment, or monitoring of a clinical condition, or to improve health care delivery.

Control group: A group in an experimental study that serves as a comparison group. The experimental treatment, procedure, or program is not given to those in the control group; instead, this group

receives either the usual available care, or an alternative such as a placebo.

Demographics: Personal information collected about an individual such as name, country of origin, birth date, race/ethnicity, occupation, education level, and income level.

Descriptive Research: A study in which information is collected without changing the environment (that is, nothing is manipulated).

Drug: A substance recognized by an official pharmacopeia or formulary intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease.

Efficacy: The performance of an intervention under ideal and controlled circumstances.

Effectiveness: The performance of an intervention under “real-world” conditions.

Endpoint: A direct measure of something substantial such as improved survival, improvement in systems or functional capacity, or decrease in the chance of developing a disease complication.

Equipoise: Genuine uncertainty as to the balance of benefits and harms that may result from two or more interventions; this genuine uncertainty makes randomization in clinical trials ethical.

Equivalence Trails: Aim to show the new drug/treatment is no better and no worse than a standard treatment.

Exclusion Criteria: Factors that are used to exclude people from participating in a clinical trial.

Experimental Research: A research design that uses manipulation and controlled testing to understand causality.

Generalizable: Extending research results or patterns found in a sample population to the wider population (which the sample represents).

Hypothesis: A prediction or explanation about future data based on previously collected data.

Inclusion Criteria: Factors that allow someone to participate in a clinical trial.

Informed Consent: The continuous process of ensuring that participation in research is voluntary. The process includes informing participants about the purpose of the research and the risks involved in participating.

Institutional Review Board (IRB): An independent group that reviews, approves, and monitors research plans and conduct to ensure that the safety and interests of research participants are protected.

Intention to Treat (ITT): A comparison of the treatment groups that includes all patients as originally allocated after randomization. ITT ignores noncompliance, protocol deviations, withdrawal, and anything that happens after randomization. This is the recommended method in superiority trials to avoid bias.

Intervention: A treatment or action taken to prevent or treat disease, or improve health in other ways.

Investigational New Drug Application (IND): A form submitted to the Food and Drug Administration (FDA) requesting permission to study a drug in humans for the first time. In limited circumstances, an IND Exemption can be requested.

Investigator's Brochure: A summary of the clinical and nonclinical data of an investigational product (IP).

Ischemic: Describes restriction in blood supply to tissues

Mean/Medium: The **mean** is the "average," the sum of all the numbers divided by the number of numbers. The **median** is the "middle" value in the list of numbers.

Meta-Analysis: A scientific, statistical method for combining data from several studies to gain more precise evidence of a treatment's effects.

Myocardial infarction: Heart attack

New Drug Application (NDA): A form submitted to the Food and Drug Administration (FDA) after a Phase III trial that requests permission to label and market a drug.

Noninferiority Trials: Aim to show that a new drug/treatment is no worse than standard treatment.

Number Needed to Treat: The average number of patients who need to be treated to prevent one additional bad outcome.

Observational Research: Studies that observe and measure variables of interest without assigning treatments to the subjects. Data can be collected prospectively (defining the question first) or retrospectively (answering a question using historical data).

On-Treatment Analysis: Also called per-protocol analysis, this is a comparison of treatment groups that includes only patients who adhered perfectly to the clinical trial instructions (completed the treatment)

Patient engagement: The inclusion of patients in the research process, from topic selection through study design and conduct, to dissemination of findings.

Patient-reported outcomes (PRO): A health outcome directly reported by the patient who experiences it.

Placebo: An inactive drug that may be used in research.

Placebo Effect: A beneficial effect that cannot be attributed to the properties of the placebo itself, and must therefore be due to the patient's belief in that treatment. When an inactive drug or treatment worsens symptoms this is called a **Nocebo Effect**.

Principal Investigator (PI): The lead researcher responsible for all aspects of a research study.

Pragmatic Trials: A kind of research that take place in a real-world environment, as opposed to a research setting. Pragmatic trials tend to exclude fewer people, and minimize the burden on trial participants so that the patient experience of those enrolled in the study is similar to the experience of patients who are not enrolled in the study.

Protocol: A detailed plan developed by a research team that must be followed when carrying out the study.

Randomized Controlled Clinical Trial: A study design that randomly assigns participants to receive one of two (or more) approaches to treatment. Randomization helps to minimize bias.

Relative Risk Reduction (RRR): Relative risk compares the risk in two different groups of people. Relative Risk Reduction (RRR) is the *ratio* of the probability of an event occurring in an exposed group to the probability of the event occurring in a comparison (non-exposed) group.

Reliability: The degree to which the result of a measurement, calculation, or specification can be depended on to be accurate.

Reproducibility: The ability of another researcher or group to accurately reproduce the results of a research study, using either the same or very similar data.

Risk-Benefit Analysis: A comparison of the risks and inconveniences on individuals with the anticipated benefit(s) of the study. The anticipated benefits of a trial must outweigh the potential risks.

Sample Size: The number of people who are enrolled in a study, often expressed as “n.” n=250 means 250 people were enrolled.

Standards of Care: A process that a clinician should follow to diagnose and treat a certain type of patient, illness, or clinical circumstance.

Superiority Trials: Aim to show that one treatment/drug is superior to another than a standard treatment.

Temporal Association: Two or more events that occur around the same time but may be unrelated, chance occurrences.

Variables: An attribute or property of a person, event, or object that is known to vary in a given study.



Authorization for Use of Photographs, Video, Audio and Interviews

This agreement authorizes the Alliance for Aging Research, its affiliates and/or any outside entity it designates to take and/or use photographs, video, audio recordings and/or any other media content of me for use in Alliance-related materials, including print, video, social media and/or any other related media. I understand that this agreement also extends to any interviews I may record with outside media entities designated by the Alliance for Aging Research. I understand that I am not entitled to any form of compensation from the Alliance for Aging Research for the use of photographs, video, audio recordings or any other media content of me. I understand that this material will be publicly disseminated. I understand by signing this agreement, I release all copyright interest in any photographs, video, audio recordings or any other media content of me. I understand by signing this agreement, I am legally bound and hereby release the Alliance for Aging Research, its affiliates and/or any outside entity it designates from any liability arising from, or in connection with, the use of such photographs, video, audio recordings and/or any other media content in whatever form this use takes.

Name _____

Address _____

Phone number _____

Email Address _____

Signature _____

Date _____

TRAVEL REIMBURSEMENT POLICY

Print Name: _____ Date: _____

Signature: _____

By signing, you acknowledge that you have received, read, and understood the terms of this policy

The Alliance for Aging Research (Alliance) will reimburse meeting participants for reasonable business travel expenses incurred while traveling to/from the meeting. This includes the actual costs of travel, meals not provided by the meeting, lodging, and other expenses directly related to accomplishing meeting travel objectives.

- All business travel must be coordinated through the Alliance.
- Meeting participants are expected to limit expenses to reasonable amounts.

Please ask for, and keep track of, itemized receipts for all reimbursable expenses, unless otherwise noted below.

As a 501(c)(3) nonprofit organization, the Alliance is governed by federal and state rules and regulations regarding allowable and non-allowable costs; which are outlined below:

TRAVEL

- Once we book your travel, please let us know within 24 hours if any changes are needed. If changes are made to your travel after 24 hours, you may be responsible for any additional costs.

Air/Train

- Air and train travel in coach or economic class (or the lowest available fare) will be arranged and covered for you by the Alliance for Aging Research based on your travel needs.
- First class travel is not reimbursable.

Personal Car

- Travel in a personal car will be reimbursed by mileage at the prevailing current rate. Gas is not reimbursable if mileage is reimbursed.
- Side trips are not reimbursable.

Taxi/Bus Service/Public Transportation

- Taxi (including Uber and Lyft) fares, fares for shuttle or airport bus service, and public transportation for ground travel to and from the meeting, are reimbursable.
- Tips up to 20% of the total cost of a taxi fare are reimbursable.

HOTEL

- Room and tax for hotel stay at the meeting hotel will be covered by the Alliance Meeting Master Account. A personal credit card will still be required upon check-in for incidentals charged to your room during your stay.
- Charges for telephone calls, fax, Internet, and similar services required for your symposium purposes, are reimbursable.
- Entertainment costs—including amusements, social activities, and related incidental costs—are not reimbursable.

MEALS

- Cost of meals not already provided for you will be reimbursed at the federal per diem rate - **\$17 breakfast, \$18 lunch, \$34 dinner.**
- The cost of alcoholic beverages will not be reimbursed and must be deducted from meal receipts prior to submitting for reimbursement.
- Tips up to 20% of the total cost of a meal are reimbursable.

CASH TIPS

- Cash tips, up to \$5 each, without a receipt will be reimbursed. Maximum # of tips per day: 2.

WHEN TRAVEL IS COMPLETED

- Meeting participants should submit the provided Alliance reimbursement form within 30 days.
- Itemized receipts must be submitted for all expenses to be fully reimbursed. Expenses without receipts, except for cash tips (up to \$5 each), cannot be reimbursed.
- To appeal for reimbursement of an expense with a missing receipt, please contact the Alliance and request to fill out a Missing Receipt Affidavit. See the Missing Receipt Affidavit policy for more details.

EXPENSE REIMBURSEMENT REPORT



Alliance for Aging Research
 1700 K Street, NW
 Suite 740
 Washington, DC 20006
 (202) 293-2856

Full Name:	
Date:	
Address (if you'd like your reimbursement check to be mailed to you):	

Please submit either the original copy, or a scanned copy, of your receipt for each line item.

Fill out the appropriate fields below, with one expense per line:									INTERNAL USE ONLY			Totals
Receipt #	Date (MM/DD/YY)	Travel from/to and business purpose	Airfare	Hotel	Meals	Taxi/Parking	Miles driven	Mileage Reimb.*	Expense Code	Project	Project Task	
Ex.	01/01/19	Uber from Dulles Airport to Hotel				\$ 10.00		\$ -				\$ 10.00
1								\$ -				\$ -
2								\$ -				\$ -
3								\$ -				\$ -
4								\$ -				\$ -
5								\$ -				\$ -
6								\$ -				\$ -
7								\$ -				\$ -
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12								\$ -				\$ -
13								\$ -				\$ -
14								\$ -				\$ -
15								\$ -				\$ -
TOTALS			\$ -	\$ -	\$ -	\$ -	0.00	\$ -	GRAND TOTAL:			\$ -

PLEASE NOTE: The Alliance for Aging Research will reimburse meeting participants for reasonable business travel expenses incurred while traveling to/from the meeting. Please refer to the Alliance for Aging Research's Travel Reimbursement Policy for more information.

**Mileage will be reimbursed at the prevailing current rate: 58 cents per mile (as of January 1, 2019).*

EXPENSE REIMBURSEMENT REPORT

INTERNAL USE ONLY

Submitted By: _____

Approved By: _____

Date: _____