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PERSPECTIVE

Understanding recent advances in non-amyloid/non-tau (NANT) biomarkers and therapeutic targets in Alzheimer's disease

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Abstract

The Alzheimer's disease (AD) research community continues to make great strides in expanding approaches for early detection and treatment of the disease, including recent advances in our understanding of fundamental AD pathophysiology beyond the classical targets: beta-amyloid and tau. Recent clinical trial readouts implicate a variety of non-amyloid/non-tau (NANT) approaches that show promise in slowing cognitive decline for people with AD. The Alzheimer's Association Research Roundtable (AARR) meeting held on December 13–14, 2022, reviewed the current state of NANT targets on underlying AD pathophysiology and their contribution to cognitive decline, the current data on a diverse range of NANT biomarkers and therapeutic targets, and the integration of NANT concepts in clinical trial designs. Participants also discussed the current definition of therapies that target underlying AD pathophysiology, what endpoints best define what is considered a meaningful change beyond the current approved definition for clinical efficacy, and how the recent NANT findings should inform the development of future guidelines for AD classification and personalized treatment strategies.

KEYWORDS
Alzheimer's disease, beta-amyloid, biomarkers, cognitive decline, non-amyloid non-tau (NANT), tau

Highlights

- The Alzheimer's Association Research Roundtable (AARR) convened leaders from industry, academia, and government to review the current state of non-beta amyloid and non-tau (NANT) targets on underlying Alzheimer's disease (AD) pathophysiology.

- The totality of scientific and clinical evidence supports the hypothesis that emerging NANT targets play a role in cognitive decline and neurodegeneration in AD.
- New biomarkers based on NANT targets must be globally developed and implemented with specific consideration of fluid biomarkers as a cost-effective clinical option, to ensure better, more equitable treatment options for AD.

1 | INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with a complex underlying pathophysiology that shifts throughout the clinical course from cognitively normal to end-stage dementia. Decades of scientific and clinical research have built the framework for defining pathologies of AD based on amyloid plaques and neurofibrillary tau tangles.[1] Recent clinical trials of therapeutics have led to United States Food and Drug Administration (FDA) approval of treatments targeting beta-amyloid and thus supporting the amyloid hypothesis as a part of pathogenesis. Beta-amyloid is posited to trigger a cascade of events and different processes including inflammatory responses that result in disease progression and cognitive and functional decline.[2–6] Emerging evidence suggests non-amyloid/non-tau (NANT) targets may not only contribute to cognitive decline but may have additional clinical implications in AD. Given the emerging data on the relevance of NANT targets and biomarkers to AD pathophysiology and therapeutics, the Alzheimer's Association Research Roundtable (AARR) chose this topic for its December 2022 meeting. The December 2022 AARR convened leaders from industry and academia, as well as patients, clinicians, and government and regulatory agency scientists to discuss the topic, "Modifying the Underlying Biology of AD; Biomarkers and Therapeutic Targets in Non-Amyloid/Non-Tau Trials." Collectively, all stakeholders agreed that the totality of scientific and clinical evidence supports the thesis that NANT targets play a role in cognitive decline and neurodegeneration in AD, and have the potential to inform treatment strategies, alongside beta-amyloid and tau. Here, we report the proceedings of the AARR meeting that addressed three fundamental questions: (1) what is the evidence regarding the relationship between AD pathophysiology and NANT targets? (2) what evidence supports the clinical utility of NANT targeted therapies and biomarkers? and (3) how does this evidence impact the framework for defining and treating AD?

2 | ATN (β-amyloid [Aβ], tau, neurodegeneration) AND EMERGING BIOMARKERS REPRESENTING THE UNDERLYING PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

2.1 | Evolution of classification systems for AD biomarkers

Over the past decade, biomarkers have become increasingly important in understanding the relevant pathophysiology of AD, with biomarkers being used to assess diagnostic criteria across the AD clinical spectrum.[7] The first disease-specific biomarkers for AD, such as Aβ42/40, were cerebrospinal fluid (CSF) based,[8] followed by the development of imaging-based biomarkers to measure amyloid and tau burden and neurodegeneration.[9,10] The dominant thinking was that the downstream pathology and the symptoms predicted by the amyloid cascade hypothesis would be closely correlated with the onset, topography, and severity of amyloid plaques.[11] Additional efforts to expand AD biomarkers led to the identification and development of a number of CSF and imaging-based biomarkers for tau pathology, including various phospho-tau species in CSF (e.g., phospho-tau181, p-tau181, p-tau 231) and tau-positron emission tomography (PET). Based on an evolving understanding of the role of tau in neurodegeneration as well as biomarker data from clinical trials and observational cohorts, the National Institute on Aging and Alzheimer's Association (NIA-AA) Research Framework was developed in 2018[12] to provide a definition and classification system for AD to enable more accurate characterization. The Research Framework defined AD based on fluid-based and imaging biomarkers grouped into those that measure amyloid deposition, tau pathology, and neurodegeneration [AT(N)], but was designed to incorporate new biomarker groups beyond AT(N), including emerging NANT biomarkers, as they became available. A recent update to the 2018 Research Framework[13] provides pathology-based criteria for diagnosis and staging of AD, incorporating recent advances in fluid and imaging biomarkers. The revised criteria categorize biomarkers as Core Biomarkers (Aβ and tau pathology [AT]), Biomarkers of non-specific processes involved in AD pathophysiology (neuronal [N] and inflammation [I]), and Biomarkers of non-AD co-pathologies such as vascular injury [V] and α-synuclein pathology [S]). Thus, the new diagnostic framework proposes an integrated biological and clinical staging scheme, with an overall goal of serving as a bridge between research and clinical care. Whether and how new NANT targets and biomarkers will be incorporated into a comprehensive AD diagnosis and staging system are key areas for future exploration and development, and the 2024 diagnostic framework allows for such additions to be made in the future.

2.2 | Fluid biomarkers to measure NANT pathophysiology

One of the key hallmarks underlying the pathophysiological processes that contribute to cognitive decline in AD is neurodegeneration, which can be measured independently of amyloid and tau, with several fluid and imaging biomarkers available to measure this distinct pathology.

Fluid biomarkers have become increasingly prevalent in AD research as they are cheaper, minimally invasive, allow for the collection of repeated samples and the measurement of multiple biomarkers from a single sample. For example, neurofilament light chain (NfL), a neuron-specific cytoskeletal protein, is one of the most widely used NANT biomarkers for assessing neurodegeneration and neuronal damage. During axonal injury, NfL is released into the extracellular space, followed by the CSF, and ultimately into plasma at progressively lower concentrations.[14] Elevated CSF and plasma levels of NfL have been associated with neurodegenerative diseases such as frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). Recent clinical data have demonstrated that plasma NfL increases during both aging as well as AD progression and can predict cognitive decline.[15,16] Notably, this association between plasma NfL and cognitive impairment is independent of the presence of beta-amyloid. Conversely, neurogranin, another neuronal-specific protein that plays a key role in synaptic plasticity and is a promising CSF biomarker for neurodegeneration, can predict cognitive decline and brain atrophy but only in amyloid-positive individuals.[17] Large-scale mass spectrometry studies of fluid biomarkers have revealed several additional synaptic biomarkers of neurodegeneration, such as plasma beta-synuclein, that show promise for monitoring AD clinical progression.

2.3 | NANT Biomarkers: Insights from anti-amyloid and anti-tau clinical trials

Recent clinical trials of therapeutics that target beta-amyloid have proven effective in slowing cognitive decline in AD.[2,4–6,18] Due to their large study populations and collection of samples throughout the course of each clinical trial, these studies have provided an opportunity to identify and help validate NANT biomarkers. Among these, NfL has been the most widely examined NANT biomarker in anti-amyloid and anti-tau clinical trials. Participants treated with aducanumab, the first anti-amyloid monoclonal antibody to receive FDA approval,[2] showed elevated plasma NfL in amyloid-PET positive individuals at baseline, but with greater divergence at younger ages. Further, longitudinal assessment of NfL revealed smaller changes that were highly variable, indicating that NfL was not able to inform on treatment response. Trials of lecanemab, an anti-amyloid and anti-protofibril antibody, and tilavonemab, an anti-tau antibody, reported similar results with NfL remaining unchanged from baseline to follow-up.[3,19] However, the TRAILBLAZER-ALZ 4 clinical trial, comparing aducanumab to donanemab, revealed an increase in plasma NfL from baseline to follow-up in both treatment groups. Treatment effects on other NANT biomarkers, including GFAP, neurogranin, and volumetric magnetic resonance imaging (MRI), have also varied among anti-amyloid and anti-tau therapy trials, likely as a result of trial differences such as study design, study duration, baseline, and participant characteristics, as well as underlying heterogeneity of AD. Moving forward, inclusion of a robust biomarker strategy could provide a powerful dataset to not only support clinical efficacy for anti-amyloid and anti-tau therapies, but could also pave the way for emerging NANT biomarkers.

3 | BIOMARKERS OF INTEREST FOR NANT TARGETS

3.1 | NANT biomarkers for AD co-pathologies

Given the observed variability of both plasma and CSF/NfL to inform on clinical progression and treatment efficacy in AD, other NANT biomarkers of additional AD-related pathologies have recently emerged. For example, mutations in Progranulin (*GRN*), which are commonly observed in other neurodegenerative disease such as FTD, have also been reported in AD and are associated with TDP-43 pathology, wherein accumulation of TDP-43 protein aggregates promotes neuronal loss and brain atrophy.[20] However, reliable biomarker assays to quantify TDP-43 in the CSF are still under development.[21] Similarly, α-synuclein, a major component of Lewy bodies in Parkinson's disease and dementia with Lewy bodies, has also been implicated in the pathophysiology of AD, with biomarker assays demonstrating the potential to differentiate CSF α-synuclein in AD from other related dementias.[22] Additionally, cerebrovascular pathologies commonly coexist alongside AD, particularly in older adults with cognitive impairment. NANT blood biomarkers for cerebrovascular co-pathologies, such as ceramides,[23] have the potential to offer a non-invasive and low-cost solution, as a supplement to imaging biomarkers. However, blood biomarkers for cerebrovascular pathologies have yet to be evaluated in AD. Thus, while NANT biomarkers based on co-pathologies show great promise, differentiating AD-specific neurodegeneration or vascular changes from other related-dementias or cerebrovascular pathologies remains challenging.

3.2 | Neuroinflammatory biomarkers for monitoring treatment effectiveness

Genome-wide association studies (GWAS) of both human AD brain tissue and mouse models of AD have highlighted the role of neuroinflammation in AD pathophysiology, with many of the AD-risk genes being involved in innate immune responses[24] and being highly expressed in microglia.[25] Currently, many NANT biomarkers for neuroinflammation, such as glial fibrillary acidic protein (GFAP), Chitinase-3-like protein 1 (YKL-40)(β-amyloid [Aβ], tau, neurodegeneration), and triggering receptor expressed on myeloid cells 2 (TREM2, can be readily identified using mass spectrometry-based proteomics approaches. GFAP can be assessed in both plasma and CSF, whereas the others are reliant on detection in CSF. Circulating inflammatory biomarkers may be influenced by peripheral inflammation. Indeed, CSF proteomics analyses have demonstrated that neuroinflammatory biomarkers can classify distinct pathological subtypes of AD in two independent patient cohorts.[26] These large-scale studies have served as the foundation for developing specific immunoassay CSF biomarker panels, consisting of multiple neuroinflammatory targets, that can distinguish between AD versus non-AD controls.[27] Additionally, a Phase 2 clinical trial of pepinemab, a monoclonal antibody that selectively targets SEMA4D-induced neuroinflammation, reduced

neuroinflammation and cognitive decline in Huntington's disease,[28] and is currently being evaluated as a therapeutic strategy in patients with early AD. Lastly, neuroinflammatory biomarkers shift during early to late-stage[29] and thus offer a potential tool to monitor AD clinical progression, but present a challenge for selecting a therapeutic window for neuroinflammation targets. Thus, developing therapeutic strategies to target neuroinflammation in AD requires better understanding of the dynamic nature of individual neuroinflammatory biomarkers through the clinical course of AD.

3.3 | Synaptic plasticity and neuroprotection in Alzheimer's disease

In addition to amyloid plaque and tau tangle burden, another hallmark pathophysiological change that occurs in AD is loss of synaptic plasticity and ultimately synaptic failure, with synaptic density loss having a stronger association with cognitive decline than amyloid or tau.[30,31] One of the attractive features of synaptic dysfunction as a potential NANT target is that it occurs early in disease progression. Therefore, preserving synaptic plasticity may represent a new therapeutic approach that could be independent of amyloid or tau status. For example, it has been reported[32] that cognitively unimpaired individuals with AD pathology, either amyloid or tauopathy, can have normal synaptic density similar to age-matched controls; this has led to identification of candidate "resilience" genes that may play a role in preserving synaptic function in AD. One such candidate is the p75 neurotrophin receptor which serves to regulate synaptic pruning during synapse development.[33] Preclinical studies have shown that modulation of p75 inhibits amyloid- and tau-mediated synaptic loss in mouse models of AD,[34,35] supporting the potential utility of synaptic function as a viable NANT target. Recent results from a Phase 2a trial testing a p75 modulator in patients with mild to moderate AD suggest that this approach warrants further investigation in larger trials.[36] Moreover, several CSF NANT biomarkers for synaptic integrity, including synaptotagmin, SNAP-25, and neurogranin, have been identified from exploratory Phase 2 clinical trials.[37] In addition to CSF biomarkers, the ability to track synaptic loss via measuring synaptic density in vivo offers a new approach that could be used to monitor disease-modifying treatments for AD. As such, imaging-based techniques including ^{18}F-FDG PET and SV2A-PET can also serve as biomarkers for synaptic density.[38,39]

3.4 | Apolipoprotein E (ApoE) variants

ApoE variants are one of the most well-known genetic risk factors for developing AD, and ApoE has been studied for decades as an NANT target and biomarker. Epidemiological data have shown that the ApoE4 variant increases one's lifetime risk of developing AD, with carriers having an earlier age of onset and faster clinical progression after disease onset in a gene-dose proportional manner. Conversely, ApoE2 carriers have the opposite phenotype, with ApoE2 being associated with a later age of onset and milder clinical presentation.[40] Notably, biomarker studies evaluating total ApoE levels in the CSF, rather than specific variants, showed that total ApoE is strongly associated with CSF Aβ, but only in women.[41] As an NANT target, preclinical studies have revealed that reduction of ApoE4, either via inhibiting expression of the variant or gene transfer, can inhibit amyloid accumulation and reduce tauopathy in AD mouse models.[42,43] This suggests that gene therapy may be a viable strategy by inducing ApoE2 expression in the brain of ApoE4 carriers, with the goal of transferring the neuroprotective benefits of ApoE2. Indeed, viral-mediated expression of ApoE2 in the brain reduces amyloid burden, prevents synapse loss, and reduces neuroinflammation in mice with amyloid deposition, even in the presence of ApoE4. These observations have laid the foundation for early clinical studies that have demonstrated ApoE2 induction via gene therapy can reduce amyloid and tau CSF biomarkers in small number of AD patients. Interesting, a mutation of APOE, the R136S Christchurch mutation, confers resistance against AD cognitive decline and tau pathology, even in the presence of high amyloid burden.[44–48]

4 | NANT CLINICAL TRIALS: STUDY DESIGN, BIOMARKERS, AND TREATMENT INTERVENTIONS

4.1 | Mechanistic diversity in the pipeline and implications for clinical trials

Despite anti-amyloid therapies making significant strides in the clinic, most recent AD clinical trials have shifted focus to NANT targeted therapies, many of which can be classified based on their targets: neurotransmitter receptors, neuroinflammation, synaptic plasticity, and/or metabolism.[49] The potential to select a target that converges on multiple pathways or shares a common mechanism for disease modification represents a powerful approach for selecting NANT targets for clinical trials. Indeed, the advent of rapid and inexpensive genotyping has led to identification of numerous AD risk variants that implicate a common target. For example, studies have shown that microglial activity, which involves multiple NANT mechanisms, can be modulated through targeting TREM2, a transmembrane receptor selectively expressed on the surface of microglia.[50] Recently, it was demonstrated that elevated soluble TREM2 in CSF is associated with reduced cognitive decline in AD,[51] highlighting that TREM2 activation may represent a viable NANT treatment strategy to impact multiple underlying microglia-driven pathologies. Engineered TREM2 receptor-binding antibodies can increase soluble TREM2 in the CSF and activate diseased microglia, as well as impact immune activity and metabolism, in mouse models of AD. As targeting TREM2 activity in vivo has demonstrated preclinical efficacy, several clinical trials evaluating the safety and efficacy of TREM2-targeted therapies in AD are currently underway.

4.2 | Clinical data suggest modification of NANT targets affects cognition and function

Additional current and ongoing clinical trials that aim to evaluate safety and efficacy of NANT targeted therapies in AD are also based on preclinical evidence modulating several underlying mechanisms of

AD pathophysiology described above or based on therapeutic benefit observed in other neurodegenerative diseases. For instance, an exploratory Phase 2 clinical trial of Fosgonimeton, a compound that can positively modulate the hepatocyte growth factor/mesenchymal epithelial transition factor receptor (HGF/MET) pathway activity, has shown promise in individuals with mild to moderate AD dementia. The HGF/MET pathway is a neuroprotective system that activates signaling pathways to protect neurons from oxidative stress, excitotoxicity, and apoptosis.[52] In preclinical models, Fosgonimeton has been demonstrated to repair and protect neuronal networks, as well as reduce neuroinflammation. These findings were consistent with Phase 2 clinical outcomes, showing reduced neuroinflammatory (GFAP and YKL-40) and NfL plasma biomarkers, decreased amyloid burden (Aβ42/40) and p-tau181, and improved cognition in participants without donepezil add-on therapy.[53] Together, these results have informed the larger Phase 2/3 trial ongoing in parallel, powered to demonstrate a meaningful effect size in cognition and function.

Another Phase 2 study testing nilotinib, a tyrosine kinase inhibitor that selectively inhibits discoidin domain receptor 1 (DDR1), has shown potential clinical efficacy in amyloid positive AD. During the Phase 2 nilotinib study, DDR1 in the CSF was found to be longitudinally activated in mild to moderate AD dementia, with nilotinib treatment significantly decreasing DDR1 activity.[54] Importantly, nilotinib's inhibition of DDR1 activity can trigger clearance of toxic protein aggregates including amyloid, reduce inflammation, and offers a potential option to improve cerebrovascular damage. Currently, the NILEAD Phase 2/3 study to evaluate nilotinib as a treatment option for amyloid positive individuals with AD is underway.

Semaglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist that has been widely used for the treatment of type 2 diabetes, has recently gained traction as a potential NANT targeted therapy for AD. The GLP-1 receptor is highly expressed in the brain, and GLP-1 has been shown to play a key role in the pathophysiology of AD via multiple neuroinflammatory, vascular, and other pathophysiological processes involved in AD.[55–57] Thus, GLP-1 receptor agonists may represent promising therapeutic candidates to slow AD progression through multifaceted mechanisms of action that modulate neuroinflammation[58] and potentially reduce neurodegeneration.[59] Additionally, the potential clinical efficacy of semaglutide in AD is highlighted by the findings that patients with type 2 diabetes taking semaglutide are half as likely to develop dementia throughout their lifetime.[60] Given that safety of semaglutide has already been shown in clinical trials of older individuals with type 2 diabetes, Phase 3 trials in patients with early AD are underway. These include both the Evoke and Evoke+ Phase 3 trials of semaglutide treatment in early AD, which include sub-studies for exploratory biomarker analyses, and a randomized trial assessing single cell transcriptomics in both CSF and plasma.

Collectively, all the NANT targeted therapies presented at the AARR meeting consistently demonstrate: (1) the potential to target multiple underlying disease mechanisms, (2) measurable changes in relevant AD biomarkers, and (3) evidence to suggest potential impact on the defining core pathologies amyloid and tau, and/or on slowing of clinical decline.

5 | CONCLUSIONS

During the December 2022 AARR, participants aimed to answer three fundamental questions: (1) what is the role of NANT targets in AD? (2) does the current evidence support a clinical benefit of NANT biomarkers and targeted therapies? and (3) how does this evidence impact the framework for defining and treating AD? At the conclusion of the 2-day meeting, presenters, panelists, and the AARR membership in attendance came to several conclusions based on the totality of scientific evidence presented and discussed. First, the scientific and clinical data support the hypothesis that NANT biomarkers and targets can help us better understand the underlying pathophysiology of AD. Second, NANT biomarkers and targets show great promise in preclinical and early clinical studies, but there is still work to be done to fully explore their clinical potential. Last, as new NANT biomarkers for disease classification and clinical progression monitoring emerge, as well as new NANT targets for disease modification, guidelines must be established to ensure their proper implementation within the current research framework.

In addition to highlighting the current evidence to date of the clinical potential of NANT biomarkers, participants also discussed the need to define what questions should NANT biomarkers aim to answer about the underlying pathophysiology of the disease. Collectively, NANT biomarkers have the potential to be used diagnostically, alongside the current classification systems, to inform on clinical efficacy, and/or to be used for safety monitoring, for instance predicting and tracking amyloid-related imaging abnormalities (ARIA) or other adverse events. Cross-sectional and longitudinal data will be key for elucidating the utility of each NANT biomarker or panel of NANT biomarkers. Other considerations include where individual NANT biomarkers fall within the disease progression continuum, and whether they can act as a surrogate for measuring underlying shifts in neurodegeneration. Further, even in homogeneous populations, NANT biomarkers can be inherently variable, and may require large sample sizes and longer trial durations for validation. Last, not all NANT biomarkers are also ideal therapeutic targets, and may only serve as an up- or downstream readout of the underlying disease modification; thus, additional in vivo studies may be required to understand these discrepancies.

Participants also discussed important considerations for developing NANT targeted therapies. Given that many NANT targets aim to modify an underlying pathophysiology that may shift throughout the clinical progression of AD, there is a need to better understand stage-specific treatment strategies. Additionally, preliminary trials with shorter intervention intervals may provide insights into optimal therapeutic windows for NANT targets based on pharmacodynamic studies. Another key consideration for evaluating specific NANT targets is whether the target will offer a symptomatic or disease modifying treatment option. For example, some NANT targets may be better suited for use as a combination therapy with other disease modifying treatments, with potential for a synergistic effect. Finally, as more NANT targeted therapies advance in clinical trials, consideration will need to be given to redefining efficacy and inclusion criteria beyond amyloid and tau.

AARR participants collectively agreed that the totality of scientific and clinical evidence supports the hypothesis that emerging NANT targets play a role in cognitive decline and neurodegeneration in AD. However, more work is needed to understand their intersection and positioning within the recently published revised criteria for diagnosis and staging of Alzheimer's disease.[13] In addition, new biomarkers based on NANT targets need to be developed that can be easily implemented on a global scale, with an emphasis on incorporating more fluid biomarkers as a cost-effective clinical option, to ensure better, more equitable treatment options for AD. Future directions that will continue to move the field forward include creating a biomarker system that allows for subdividing patient populations and providing personalized treatment strategies.

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CONFLICT OF INTEREST STATEMENT

L.V.E. is the director of the Sanders-Brown Center on Aging at University of Kentucky. E.S. is a full-time employee of Acumen, and has received consulting fees from Cogstate Ltd., Cortexyme Inc., Partner Therapeutics Inc., Vaccinex Inc., Gates Ventures LLC, Hoffman La Roche Ltd., US Green Valley Pharmaceuticals Inc., has participated on a DMSB for Hoffman La Roche Ltd., has been an unpaid consultant and unpaid board of directors member for the Alzheimer's Association and Bright Focus Foundation, respectively, and is a stockholder for Acumen and Eli Lilly. E.C. is a full-time employee of Eli Lilly & Co. M.G. is a full-time employee of Compass Pathways (was a full-time employee of AbbVie at the time of the Fall 2022 AARR meeting). D.H. is a full-time employee of Janssen Pharmaceutical Companies of Johnson & Johnson. P.J. is a full-time employee of Novo Nordisk. HJM is a full-time employee of Athira Pharma. M.S. is a full-time employee of Biogen. J.Z. is a full-time employee of Eisai, Inc. M.C.C. and C.J.W are full-time employees of the Alzheimer's Association. Author disclosures are available in the supporting information.

CONSENT STATEMENT

Consent (i.e., all human subjects provided informed consent) was not applicable.

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SUPPORTING INFORMATION

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

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