

Featured Articles

## Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials: Recommendations from the Alzheimer's Association Research Roundtable Workgroup

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### Abstract

Amyloid imaging related abnormalities (ARIA) have now been reported in clinical trials with multiple therapeutic avenues to lower amyloid- $\beta$  burden in Alzheimer's disease (AD). In response to concerns raised by the Food and Drug Administration, the Alzheimer's Association Research Roundtable convened a working group to review the publicly available trial data, attempts at developing animal models, and the literature on the natural history and pathology of related conditions. The

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spectrum of ARIA includes signal hyperintensities on fluid attenuation inversion recovery sequences thought to represent “vasogenic edema” and/or sulcal effusion (ARIA-E), as well as signal hypointensities on GRE/T2\* thought to represent hemosiderin deposits (ARIA-H), including microhemorrhage and superficial siderosis. The etiology of ARIA remains unclear but the prevailing data support vascular amyloid as a common pathophysiological mechanism leading to increased vascular permeability. The workgroup proposes recommendations for the detection and monitoring of ARIA in ongoing AD clinical trials, as well as directions for future research.

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**Keywords:** MRI; Amyloid; Therapeutic trials; Vasogenic edema; Microhemorrhage

## 1. Introduction

Over the past decade since amyloid-modifying therapeutic agents have entered Alzheimer's disease (AD) clinical trials, the occurrence of magnetic resonance imaging (MRI) abnormalities has required careful consideration by academic investigators, pharmaceutical companies, and regulatory authorities. MRI signal changes, thought to represent “vasogenic edema” (VE) and cerebral microhemorrhage (mH), were first observed in trials of a monoclonal antibody against amyloid- $\beta$  (A $\beta$ ) [1–3], and have since been associated with other amyloid-modifying therapies [4]. In response to guidance issued by the U.S. Food and Drug Administration (FDA) to various sponsors on the conduct of clinical trials of amyloid-modifying agents for the treatment of AD, the Alzheimer's Association Research Roundtable convened a Workgroup in July 2010.

The Workgroup was composed of academic and industry representatives identified on the basis of their expertise and interest in this area, and was tasked with the objective of providing expert advice regarding the FDA's concerns related to MRI abnormalities, including signal changes thought to represent VE and mH and the relationship of these MR abnormalities to experimental treatment with amyloid-modifying therapies. Because VE and mH are typically detected on different MRI sequences, and seem to represent a spectrum of image abnormalities that may share some common underlying pathophysiological mechanisms, both in the natural history of AD and in the setting of amyloid-modifying therapeutic approaches, the Workgroup suggests referring to this spectrum as amyloid-related imaging abnormalities (ARIA). Despite the likelihood of shared underlying mechanisms, there may be instances in which it is useful to describe specific phenomena, thus the Workgroup further refined the terminology: ARIA-edema/effusions (ARIA-E) refers to the MR signal alterations thought to represent VE and related extravasated fluid phenomena. ARIA-hemosiderin deposition (ARIA-H) refers to the MR signal alterations attributable to mH and hemosiderosis.

The Workgroup reviewed the relevant publicly available information, including natural history studies and spontaneous occurrence of these imaging abnormalities in aging and AD populations, the occurrence of ARIA in occurrence in the setting of trials of amyloid-lowering agents for AD, similar clinical conditions from which parallels might be

drawn, and existing animal models that may elucidate the underlying mechanisms. The Workgroup sought to develop specific recommendations regarding the conduct of AD clinical trials in the setting of ARIA, including inclusion/exclusion criteria, safety monitoring, and potential areas of research that might help increase our understanding of these events.

## 2. The phenomenon formerly known as “VE”: ARIA-E alterations

Although early animal work had reported evidence of mH with anti-amyloid immunotherapy [5], an unexpected type of MRI signal alteration was first observed in the single-dose ascending phase I trial of a monoclonal antibody against A $\beta$ . Three of 10 patients in only the highest dose group (5 mg/kg) developed transient signal abnormalities on T2-weighted/fluid attenuation inversion recovery (FLAIR) sequences, approximately 4 to 6 weeks after a single dose of bapineuzumab [1]. Additional cases were reported in the phase II study [2,3]. Initially, the appearance and transience of the MRI abnormalities were thought to be reminiscent of posterior reversible encephalopathy syndrome (PRES) observed in hypertensive patients and in those with preeclampsia, but as additional cases in AD clinical trials became apparent, this new entity was initially described as VE, based on the MRI characteristics.

The term *vasogenic edema* for this entity evolved from the observation that the increased MR signal on FLAIR sequences was usually transient, and was not associated with evidence of restricted diffusion, tissue necrosis, or other sequelae associated with cytotoxic edema. At a cellular level, VE is thought to represent an increase in extracellular fluid volume because of increased permeability of brain capillary endothelial cells to serum proteins, as opposed to cytotoxic edema, which is increased intracellular fluid, thought to be related to high intracellular osmolality from cellular damage (clinically most often seen in acute infarction, where the mechanism is thought to be failure to maintain a homeostatic sodium/potassium gradient across the cell membrane). There is very limited histopathological evidence available to determine whether the signal changes observed on MRI are in fact related to underlying VE. The term “VE” has also sometimes been applied to other MR alterations

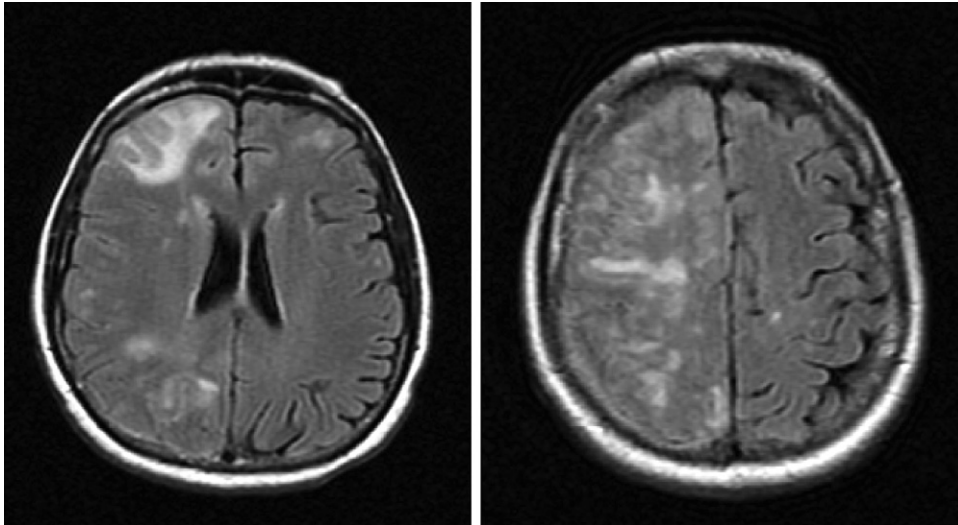


Fig. 1. ARIA-E which occurred during a monoclonal antibody trial, as seen on fluid attenuation inversion recovery (FLAIR) magnetic resonance images demonstrating increased signal in multiple regions of the right hemisphere affecting both gray and white matter.

observed in the setting of amyloid-modifying therapy that vary in signal characteristics on different MR sequences and in location within the intracranial space. In particular, increased signal intensity on FLAIR images has been reported in the leptomeningeal or sulcal space with anti-amyloid treatment, and may represent leakage or effusion of proteinaceous fluid from meningeal vessels. Thus, we refer to the signal hyperintensities seen in the parenchyma and leptomeninges more specifically as “ARIA-E” to cover the MRI alterations thought to represent edema in the gray and white matter, and effusion or extravasated fluid in the sulcal space (Figs. 1 and 2).

### 3. Characteristics of ARIA-E observed in amyloid-modifying therapeutic trials

ARIA-E most commonly manifests as increased MR signal intensity on FLAIR or other T2-weighted sequences in the parenchyma and/or leptomeninges in the parietal, occipital, and frontal lobes, but has also been observed in the cerebellum and brainstem [3]. It is not yet clear whether the edema begins in gray matter in some cases, as associated gyral swelling is sometimes apparent, with edema tracking into the underlying white matter, or whether there may be separate processes that affect gray and white matter. The

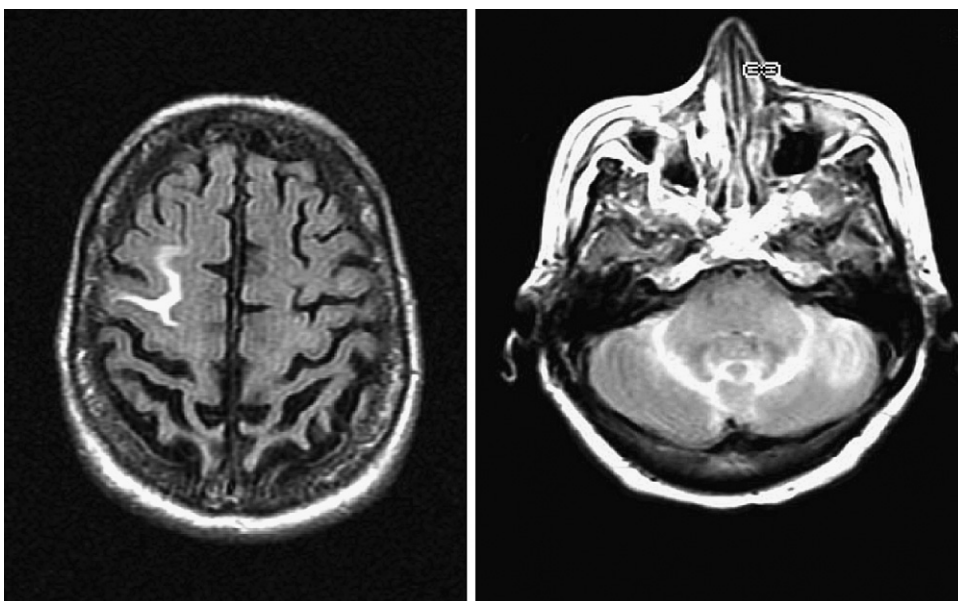


Fig. 2. ARIA-E detected on FLAIR images from a monoclonal antibody trial study demonstrating increased MR signal in sulci, thought to represent proteinaceous fluid tracking in the leptomeninges and sulcal spaces.

parenchymal signal abnormalities can be subtle in a single region, multifocal, or nearly panhemispheric (Fig. 1).

In some cases, the increased MR signal is primarily seen in what appears to be the leptomeningeal space (Fig. 2). This leptomeningeal involvement may be seen in isolation or near associated gray matter alterations. This focal increased signal has on occasion been misinterpreted as subarachnoid hemorrhage, but neither cerebrospinal fluid (CSF) studies nor susceptibility-weighted imaging (SWI) has revealed evidence of blood products in these cases. It remains unknown whether this increased signal in leptomeningeal compartments might represent other proteinaceous collections of fluid.

Cerebral amyloid angiopathy (CAA) with associated vascular or perivascular inflammatory infiltrates [6,7] is another condition that has clinical and neuroimaging features that appear similar to ARIA associated with amyloid-modifying therapy. This spontaneously occurring syndrome presents on neuroimaging with a range of involvement of leptomeninges, gray matter, and white matter [8,9], similar to those reported with amyloid therapy-associated VE. CAA-related inflammation appears pathologically to be driven by a spontaneous inflammatory response to the vascular amyloid deposits. The CSF pattern in inflammatory CAA (elevated protein, mostly without elevated white blood cells) is similar to the CSF pattern seen in some treatment-related VE with bapineuzumab [3]. This supports the assertion that focal CAA inflammation need not be associated with elevated CSF cell count. After its resolution, either spontaneously or with steroids, inflammatory CAA findings also reverse. In addition, both CAA-related inflammation and “VE” with amyloid therapy have a shared association with apolipoprotein E  $\epsilon$ 4 (*APOE*  $\epsilon$ 4) [9]. There has been at least one report of a similar pattern of leptomeningeal involvement in CAA [10]. A potential connection between inflammatory CAA and immunotherapy-associated ARIA was most recently suggested by identification of anti-A $\beta$  autoantibodies in the CSF of a patient with the spontaneously occurring syndrome [11]. A similar spectrum of MR abnormalities, as described in ARIA, involving both gray and white matter, has been reported in PRES syndrome [12,13], although prominent leptomeningeal signal hyperintensities are not common.

#### 4. Issues of ascertainment

Patients identified with CAA-related ARIA or PRES typically present with symptoms, whereas the majority of treatment-associated ARIA-E cases reported in previously published studies have been asymptomatic and identified on “per-protocol” safety MRIs. A small number of ARIA-E cases in the bapineuzumab trials were detected on off-protocol MRI, prompted by change in clinical status or symptoms. These symptomatic cases generally occurred within 4 to 8 weeks after infusion, suggesting that ARIA-E is not occurring early (at peak of antibody levels in blood). The timing

of ARIA-E may provide a clue regarding potential pathophysiological mechanisms in bolus-dose immunotherapy, but it remains unclear whether there will be a temporal relationship between dosing and ARIA-E that may occur in other amyloid-lowering therapeutic approaches.

To our knowledge, there are no reports in previously published studies regarding the incidence of spontaneous ARIA-E in community-based samples, but it is likely that there has not been systematic surveillance outside of clinical trials. The preliminary reports of rare cases of ARIA-like phenomenon being detected in large cohorts being screened for clinical trials suggest that spontaneous ARIA-E may occur rarely in the natural history of AD, and perhaps, more commonly, in patients with genetic risk factors for high vascular amyloid burden and/or presumed CAA [4,14]. The low incidence of spontaneous ARIA-E observed in clinical trial screening might reflect a bias toward recruiting subjects who have less vascular disease and vascular risk factors. A substantial proportion of patients with clinical AD harbor some evidence of CAA changes at autopsy, particularly in *APOE*  $\epsilon$ 4 carriers [15].

#### 5. Clinical course associated with ARIA-E

There are currently very limited publicly available data regarding the clinical course associated with ARIA-E occurring in the setting of clinical trials of amyloid-modifying therapies. Therefore, the Workgroup reviewed the data from bapineuzumab trials, but it is unknown whether ARIA seen in other amyloid-modifying therapies will have a similar clinical course. In the phase I bapineuzumab study, two of three ARIA cases were asymptomatic at time of detection on per-protocol MRI. In retrospect, one of these patients had a reported period of transient confusion a few weeks before ARIA-E. The third patient had acute drop in Mini-Mental State Examination and confusion prompting an off-protocol MRI 4 weeks after the infusion, revealing ARIA-E. This patient improved after several weeks without treatment [1].

In the first reports of the double-blind phase II multiple-dose study, 10 of 12 ARIA-E cases were detected on per-protocol MRI [2,3]. Six among the 10 were asymptomatic even after retrospective review of all adverse events in the 30 days before or after detection of ARIA-E. Four of 10 were found to have reported transient symptoms of headache, confusion, and visual disturbance in the 30 days before or after detection of ARIA-E. The remaining two cases were symptomatic, including confusion, headache, and gait difficulties, prompting off-protocol MRI. One of these subjects underwent treatment with intravenous steroids with resolution of symptoms.

There are very limited data on the long-term clinical course of individuals with asymptomatic ARIA-E, but preliminary analyses of the asymptomatic cases of ARIA-E from the phase II study did not reveal significant impact on the Mini-Mental State Examination compared with the non-ARIA-E-treated

group [3]. Additional analyses on neuropsychological and functional outcomes in asymptomatic cases of ARIA-E are ongoing, as well as a more systematic central review of all MRI scans from the phase II bapineuzumab studies.

## 6. Risk factors for ARIA-E

The pathophysiological mechanisms underlying VE remain to be elucidated; however, the bapineuzumab phase II data have provided some insight into the risk factors associated with the appearance of ARIA-E [3]. The most significant risk factor was dose of bapineuzumab, with 11 of 12 cases occurring in the 1- or 2-mg/kg dose groups. The phase III bapineuzumab noncarrier study terminated the highest dose arm because of the number of ARIA-E cases observed in this dose cohort.

The presence of *APOE*  $\epsilon$ 4 allele was also found to be a significant risk factor for the development of ARIA-E, with 6 of 18 (33%)  $\epsilon$ 4/4 homozygotes; 4 of 56 (7.1%)  $\epsilon$ 4 heterozygotes; and only 2 of 47 (4.3%) noncarriers developing ARIA-E in the phase II double-blind study. These findings led to the plans for separate  $\epsilon$ 4 carrier and noncarrier phase III protocols with different dose levels of bapineuzumab. Preliminary data from ARIA-E seen in trials with other amyloid-modifying therapies also suggest that *APOE*  $\epsilon$ 4 carrier status may be a risk factor [16]. Similarly, the preliminary reports of the rare cases of ARIA detected in patients with AD being screened for clinical trials have been in *APOE*  $\epsilon$ 4 carriers, suggesting vascular A $\beta$  pathology, such as CAA, as a common underlying mechanism [14].

## 7. ARIA-H: MRI findings thought to represent hemosiderin deposits and mHs

Even the first reports of the ARIA cases raised the possibility of a relationship between FLAIR abnormalities, thought to represent ARIA-E, and the appearance of alterations on long echo time, gradient refocused echo (T2\*-GRE) sequences, thought to represent hemosiderin deposits, including mHs and superficial siderosis [1]. mH typically manifests as a focal, round, very low-intensity (relative to adjacent brain tissue) lesion in the brain parenchyma, detected on an appropriately weighted (T2 or T2\*) MRI sequence, such as GRE sequences. Additional SWI may be imparted by postprocessing to improve mH visualization. mHs are small deposits of iron in tissue in the form of hemosiderin and are thought to represent residua of a small leakage of blood from a vessel into adjacent tissue parenchyma. Size criteria have been recommended, with mH defined by a cutoff of  $\leq 10$  mm in diameter in some studies and  $\leq 5$  mm in diameter in other studies. However, using size criteria to define mH is problematic without specifying the technical features of image acquisition linked to the size criteria because the apparent size of the low-intensity lesion depends on features of image acquisition, as outlined later in the text [17]. In addition, the apparent size of an mH on MRI

is generally greater than the size of the histologically defined area of hemosiderin deposited in tissue. This is a well-established phenomenon in MRI referred to as the “*blooming effect*”, which occurs when the microscopic field gradient causing signal loss extends spatially beyond the histologically defined hemosiderin deposit.

Curvilinear low intensities on an appropriately weighted (T2 or T2\*) sequence that lie adjacent to the brain surface are referred to as superficial siderosis. Similar to mH, siderosis is attributed to deposition of iron in the form of hemosiderin and is thought to represent residua of a blood leakage from a vessel into the adjacent subarachnoid space or the periaventricular compartment (as opposed to leakage into parenchyma to form an mH) [18].

## 8. Technical issues of image acquisition for ARIA-H

The conspicuity of mH and superficial siderosis can be enhanced or diminished by specific attributes of the image acquisition. MRI sequences that enhance signal loss because of microgradients in tissue are generally used for detection of mH and superficial siderosis. Two general approaches have been used, T2\*-weighted GRE sequences and SWI.

### 8.1. T2\*-gradient echo sequences

T2 represents the loss of signal because of the intrinsic dephasing of spins moving randomly in the local tissue environment. T2' represents signal loss resulting from the dephasing of spins that is attributable to eddy currents, macroscopic susceptibility effects, or focal microscopic magnetic susceptibility effects that would result from an mH or other sources of iron or mineralization. T2\* represents the sum of T2 and T2' effects. T2-weighted sequences use a spin echo, whereas T2\* use a gradient recalled echo. T2\*-GRE images are gradient echo sequences with a long echo time (TE), which enhances spin dephasing and hence focal loss of signal in the immediate vicinity of an mH. T2\*-GRE sequences can be executed in either two-dimensional or three-dimensional (3D) mode. An advantage of 3D is generally thinner slices and hence reduced partial volume averaging effects [19]. A major disadvantage, however, is that manufacturer-available product 3D T2\*-GRE sequences are a purchasable option that is not available on all MRI systems. The majority of MRI systems in a typical clinical trial based on community recruitment centers would not have access to this technology at this time.

### 8.2. Susceptibility-weighted imaging

SWI is essentially a T2\* gradient echo sequence that has added susceptibility weighting. This is accomplished by forming both a magnitude and a phase image, enhancing the phase image, and then multiplying the magnitude image by the enhanced phase image [20]. SWI was originally developed as a method to improve visualization of cortical veins, but it is also a more sensitive technique for detection of mH than T2\*-GRE images [21] (Fig. 3).

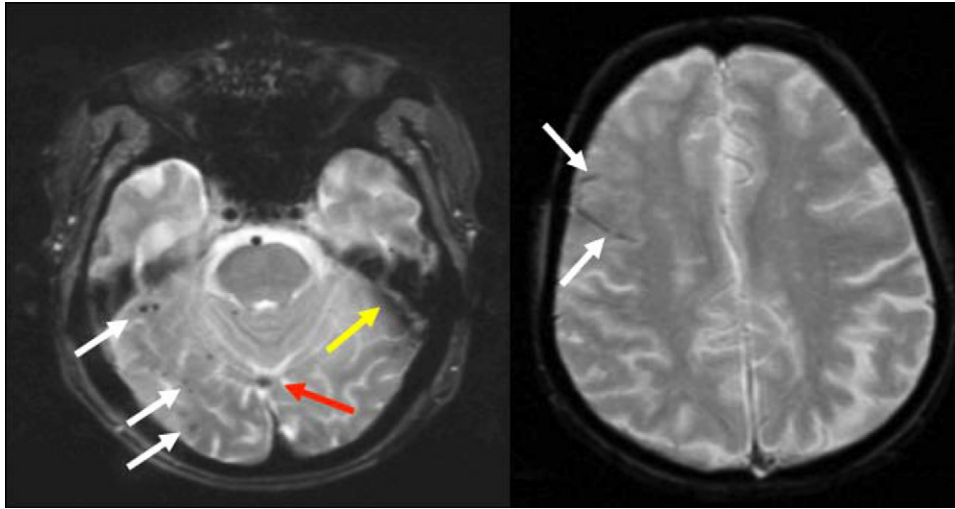


Fig. 3. Microhemorrhage (mH) and superficial siderosis. Left, White arrows indicate multiple 1- to 3-mm dark foci in the right inferior temporal and occipital lobes, typical of the appearance of mH. Red arrow indicates inferior sagittal sinus, and yellow arrow indicates susceptibility artifact, because vascular structures and artifacts can sometimes mimic the appearance of mH and siderosis. Right, White arrows indicate curvilinear dark sulci in the right frontal lobe, typical of the appearance of superficial siderosis. Both images were acquired at 1.5 T with a two-dimensional long TE (30 ms) GRE sequence.

The conspicuity of mH, and hence sensitivity to detection, is also increased by higher field strength [22], longer TE, lower readout bandwidth, and asymmetric centering of the echo with respect to the readout gradient. Small voxel sizes (i.e., higher resolution) will decrease partial volume averaging of small mHs, thus increasing conspicuity; however, all else being equal, smaller voxels produce a proportional reduction in image signal-to-noise ratio (SNR). Low SNR images incur a penalty in diagnostic accuracy. The image geometry parameter that varies most from protocol to protocol in determining voxel size is slice thickness; in-plane resolution typically is not greatly different across different imaging protocols. Thinner slices increase resolution but decrease SNR, and all else being equal, systems with superior SNR performance will have greater diagnostic sensitivity. The two major hardware features that improve SNR are field strength (SNR scales linearly with field strength), and receiver radiofrequency coil type (multichannel arrays have greater SNR than single-channel volume coils).

## 9. Ascertainment

To our knowledge, reliable automated algorithms do not exist for identification of mH or superficial siderosis from medical images. Ascertainment by visual reading of scans by trained experts is the only way, but counting is not as exact as desired because it is dependent on several things: the level of training of a reader; where a particular reader falls on the receiver-operating characteristic curve (i.e., is he/she an undercaller or an overcaller); features of image acquisition discussed previously; and artifacts in the image itself. Despite this, inter- and intraobserver agreement has been satisfactory, with reported kappas for interobserver agreement ranging from 0.33 to 0.78 [17].

Common interpretative difficulties are as follows: (1) motion artifacts, (2) bulk susceptibility effects that produce regional signal loss (e.g., skull base, sinuses, and metallic foreign bodies like dentures), (3) partial volume effects, (4) distinguishing true mH from vessel flow voids that also appear as punctuate low-intensity areas when the vessel transects the imaging plane of section perpendicularly, (5) distinguishing true mH from physiological mineralization in the basal ganglia, and (6) mH number, for example, the tendency exists to exclude a dubious mH in a patient without other mHs, in contrast to a similar lesion in the context of many mHs that will tend to receive the benefit of the doubt. On the other end of the spectrum, when mHs are very numerous, difficulties with counting may arise as well, especially when lesions become confluent. Each of these may contribute to false-positive or false-negative diagnostic readings.

Many mHs cannot be ruled in or out with absolute confidence on a single scan. In some individuals, areas of the brain exist where mH can never be detected, for example, bulk susceptibility artifact in areas next to paranasal sinuses or at the base of the brain in subjects with dentures, and it can sometimes be difficult to differentiate mH from vascular structures in a single plane (Fig. 4). There are instances when artifacts on a baseline MRI scan preclude identification of an mH that becomes apparent only when (side-by-side) comparisons are made to follow-up MRI examinations.

## 10. Etiology and clinical significance of mH

mHs are generally attributed to one of two etiologies: small-vessel angiopathy and CAA. In the stroke literature, mHs, attributed to small-vessel angiopathy, increase in

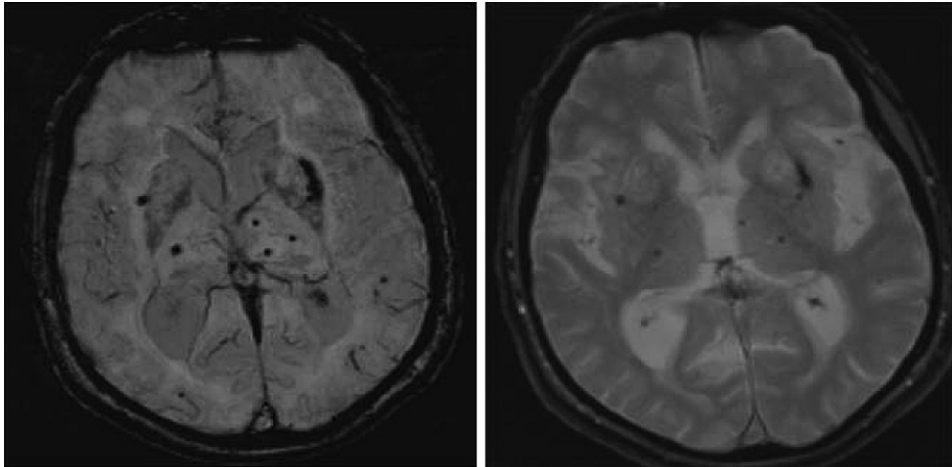


Fig. 4. Conspicuity depends on technique. Susceptibility-weighted imaging (SWI) versus T2\*-GRE (gradient refocused echo): SWI image (left) compared with T2\* image of same patient on same day on the right. Note, there are four more MBs detected on the SWI image.

prevalence with age and vascular risk factors, especially hypertension [23]. mH also occurs in patients who undergo cardiac bypass surgery and is attributed to microemboli [24]. In AD, mH and superficial siderosis are attributed to blood leakage from CAA vessels [25]. CAA is known to weaken the vessel wall, increasing the risk of microleaks of blood into adjacent brain tissue, forming mH. Some data suggest that the locations of mHs are related to etiology, with lobar mH more often being attributed to CAA, whereas deep central gray and brainstem mHs are more often attributed to hypertensive angiopathy [26].

mH is, in part, an age-related phenomenon that occurs even in neurologically healthy individuals (Table 1). Prevalence increases with age [19,30,32]. Additionally, a greater number of mHs at baseline confers greater risk of subsequent incident mH [42]. The prevalence of mH is significantly increased in elderly individuals with cardiovascular risk factors and/or evidence of a previous cerebrovascular event [27]. The incidence of new mHs in a longitudinal study of ischemic stroke patients over a mean 5.5-year follow-up period was 23%. In patients with cerebrovascular disease, the presence of mH has been linked to worse cognitive outcomes; however, it is uncertain whether mHs themselves cause impairment or are simply a marker of cerebrovascular disease [33,43]. Indeed, although the point prevalence of mH in a healthy (with no cerebrovascular disease) elderly population is ~6%, this value rises to ~50% to 80% in elderly individuals with cerebrovascular disease [44]. Thus, in a patient population with a high prevalence of cardiovascular risk factors, an association with a significant proportion of individuals with mH would be expected. In the setting of AD and presumed CAA, it is not established that mH itself causes neurological symptoms [31]. A recent study from a memory disorders clinic noted that 12% of the patient population showed incident mHs during an average follow-up period of 1.9 years [27] with no clinically apparent symptoms attributable to these incident mHs. However,

mHs are thought to be indicators of CAA and hence identify individuals at risk for a more serious complication of CAA—lobar hemorrhage. In individuals who present with an initial lobar hemorrhage, the number of mHs increases risk of a subsequent lobar hemorrhage [45,46]. The prevalence of superficial siderosis is much less common than mH (only 0.7% in the Rotterdam population-based study), and also is thought to be an indicator of CAA [18,47].

#### 11. Association of ARIA with pre-existing white matter disease or other abnormalities

The cross-sectional association between mH and other MRI markers of small-vessel disease has been repeatedly reported. For example, one cross-sectional study of mild-moderate probable AD subjects, compared with normal control subjects, reported that patients with mH had higher white matter disease scores using a standardized rating scale (the age-related white matter changes score) [48], particularly in the frontal and parietal-occipital regions [31]. Also, the number of mHs correlated significantly with the age-related white matter changes score, particularly in the parieto-occipital regions of the subjects with AD, and in the occipital region of control subjects [31].

Confluent periventricular white matter hyperintensities may relate to leakage from the deep medullary veins, because of venous collagenosis, a veno-occlusive disease of aging, exacerbated by vascular risk factors [49]. This may interfere with cerebral interstitial fluid circulation and drainage of protein solutes from the brain, and could encourage amyloid deposition in the perivascular spaces of the penetrating arterioles of the cerebral cortex. Clearance of amyloid from the brain may thus be reduced, possibly exacerbating amyloid angiopathy and amyloid deposition [50]. Further studies are required to better understand this apparent association between white matter disease, mHs, and amyloid clearance.

**Table 1**  
mH prevalence and incidence rates in referred clinic samples, epidemiology surveys, clinical trials

Citation	Description of the population	Sample size	Baseline prevalence	Incidence	Technical features
Goos et al, 2010 [27]	Memory clinic—includes demented and nondemented individuals	254	Overall: 19%	Controls: 11% MCI: 21% AD: 12% Overall: 12% over average 1.9 years	2D T2*-GRE; 1.0-T; 5-mm skip, 1-mm slices; TE = 22 ms
Goos, in press [28]	Memory clinic Comparing GRE vs. SWI on the same 1.5-T MRI	141	Overall: 23% GRE, 40% SWI AD: 20% GRE, 39% SWI	2D T2*-GRE; 1.5-T; 5-mm skip, 1.5-mm slices; TE = 25 ms	3D SWI; 1.5-T; 2-mm skip, 2-mm slices; TE = 40 ms
Cordonnier et al, 2006 [29]	Memory clinic	772	VasD: 65% AD: 18% MCI: 20% SubMm: 10% Overall: 17%		2D T2*-GRE; 1.0-T; 5-mm skip, 1.5-mm slices; TE = 22 ms
Vernooij et al, 2008 [19]	Population-based sample	1062	60–69 years old: 17.8% 70–79 years old: 31.3% >80 years old: 38.3% 4.7%		3D T2*-GRE; 1.5-T; 1.6-mm skip, 0-mm slices; TE = 31 ms
Jeerakathil et al, 2004 [30]	Healthy community-based sample (Framingham)	472			2D T2*-GRE; 1.0-T; 5-mm skip, 0.5-mm slices; TE = 26 ms
Pettersen et al, 2008 [31]	Memory clinic	105	AD: 29%; healthy controls: 12%		2D T2*-GRE; 1.5-T; 6-mm skip, 2-mm slices; TE = 33 ms
Sveinbjornsdottir et al, 2008 [32]	Healthy community-based sample (AGES)	1962	11.1%		2D GRE-EPI; 1.5-T; 3-mm skip, 0-mm slices; TE = 50 ms
Werring et al, 2004 [33]	Suspected stroke or transient ischemic attack	214	13.6		T2*; 1.5-T; TE = 40 ms
Lee et al, 2004 [34]	Hypertensive population	129	55.8		T2*-GRE; 1.5-T; 5-mm skip, 2-mm slices; TE = 15 ms
Yakushiji et al, 2008 [35]	No history of neurological disorder	518	6.8%		T2*-GRE; 1.5-T; 7-mm skip, 1.4-mm slices; TE = 20 ms
Igase et al, 2009 [36]	Neurologically healthy population	377	5.6%		T2*-GRE; 3.0-T; mH definition <5 mm. Note: 20/21 patients with mH had one mH, with one patient having three mHs
Hanyu et al, 2003 [37]	AD (mean MMSE, 17.9); controls without neurological deficits	59 (AD), 55 (no neurological deficit)	32.2% (AD), 7.3% (no neurological deficit)		T2*-GRE; 1.5-T; 5-mm skip, 1.5-mm slices; TE = 26 ms
Roob et al, 1999 [38]	Population-based sample	280	6.4%		2D T2*-GRE; 1.5-T; 5-mm with 10% gap; TE = 16–20 ms
Tsushima et al, 2002 [39]	Population-based sample	450	3.1%		2D T2*-GRE; 1.0-T; 5-mm skip, 2.5-mm slices; TE = 30 ms
Bednar et al, 2010 [40]	Mild-moderate AD (MMSE, 16–26); screen data for therapeutic trial	231	21.6%		2D T2*-GRE; 1.5-T; 5-mm skip, 1-mm slices; TE = 20 ms
Novartis data on file	Mild AD (MMSE, 20–26) patients recruited for a multinational active immunotherapy trial	137	Any mb: 38% >1 mb: 21% >2 mb: 12%	N/A	2D T2*-GRE; 1.5-T; TE = 23 ms; 5-mm slices, no gap
Poels et al, 2010 [41]; follow-up to Vernooij et al, 2008 [19]	Community-dwelling people aged 45 years and older	3979	Range from 6.5% in 45–50-year-olds to 35.7% in ≥80-year-olds (15.3% overall had at least 1 mH)	N/A	3D T2*-GRE; 1.5-T; 1.6-mm skip, 0-mm slices; TE = 31 ms

Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; 2D, two-dimensional; T2\*-GRE, long echo time, gradient refocused echo; TE, echo time; SWI, susceptibility-weighted imaging; MRI, magnetic resonance imaging; 3D, three-dimensional; VasD, vascular dementia; SubMm, subjective memory complaints; GRE-EPI, gradient echo-echo planar imaging; AGES, Age, Gene, Environment Susceptibility study; mH, microhemorrhage; MMSE, Mini-Mental State Examination; mb, microbleed.

Moreover, a large prospective population-based study associated mHs in deep locations (basal ganglia, thalami, and infratentorial) with arterial hypertension, white matter hyperintensities, and lacunar infarcts [19]. This is in line with the cross-sectional and temporal relation reported between new nonlobar mHs and progression of white matter hyperintensities and lacunes. In the article by Goos et al in 2010 [27], no association was found between lobar incident mHs and progression of white matter hyperintensities. In

line with these findings, a former longitudinal study in patients with CAA did not find a relation between incident lobar mHs and progression of white matter hyperintensities [51]. These findings might further support the notion that etiology of mHs may differ according to mH location, with deep mH caused by hypertensive vasculopathy, more closely associated with vascular risk factors and other markers of small-vessel disease, and lobar mHs caused by CAA and potentially related to *APOE*  $\epsilon$ 4 allelic status.

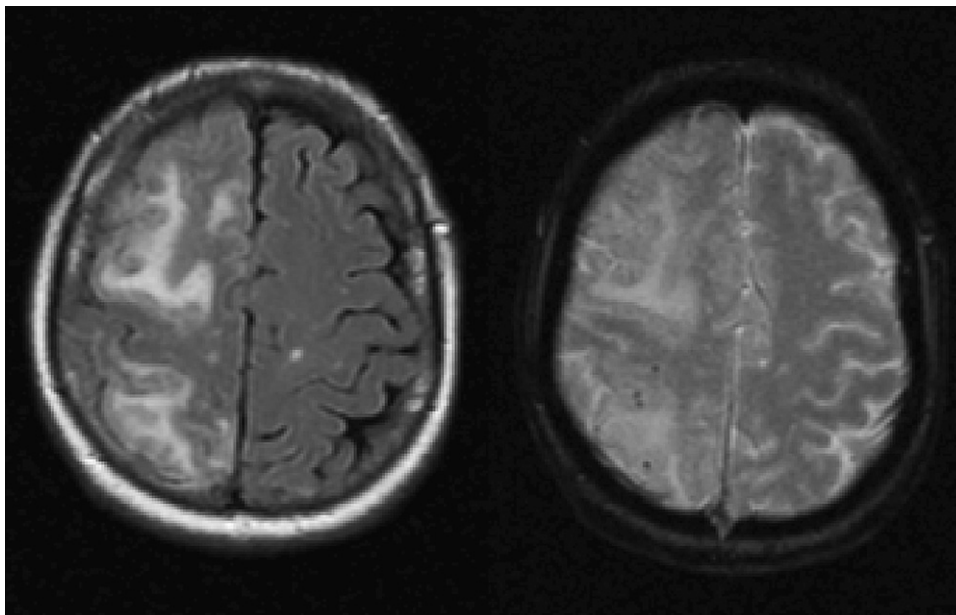


Fig. 5. Relationship between ARIA-E and ARIA-H. Left, FLAIR image demonstrating ARIA-E with increased signal in white and gray matter and sulcal effacement in right frontal and parietal regions. Right, GRE image showing mH (ARIA-H) in right parietal region only.

## 12. Association between components of ARIA

There are very limited data available to date regarding the relationship between mH and/or hemosiderosis detected on FLAIR images (ARIA-H) and “VE” and/or sulcal effusions/exudates detected on GRE/T2\* images (ARIA-E). Preliminary analyses of the phase II bapineuzumab trial initially noted baseline mH as a risk factor for developing ARIA-E (baseline mH, 6/17 [35%] vs. no baseline mH, 6/107 [6%]) [3]. This finding may be confounded by the overlapping risk due to *APOE*  $\epsilon$ 4 carrier status, because all of the ARIA-E cases with baseline mH were also  $\epsilon$ 4 carriers.

Similarly, there are limited publicly available data on incident mH in the setting of ARIA-E associated with amyloid-modifying therapies. One of three ARIA-E cases in phase I bapineuzumab [1] and 4 of 12 ARIA-E cases in one phase II study (double blind) were reported to have incident mH [2,3]. It also seems that ARIA-H can occur in some regions with ARIA-E and not others (Fig. 5). It will be important to study the relationship between these two phenomena in the setting of amyloid-lowering clinical trials, and in particular, to understand whether the co-occurrence entities is

related to any change in clinical outcome or response to therapy.

## 13. Common pathophysiological processes underlying ARIA

The limited findings reported to date suggest several potential pathophysiological mechanisms that might underlie and tie together the various components of ARIA. The relationship to dose level in the bapineuzumab studies suggests that ARIA may be related to increased clearance of parenchymal plaque with transient increase in vascular amyloid. This hypothesis is supported by the published autopsy results from the AN-1792 (active immunization) trial [52,53]. It remains unclear whether rapid movement of amyloid from parenchymal plaques into perivascular space might result in a “drainage backup” leading to excess fluid shifts. It is also possible that movement of amyloid into cerebral vessel walls might result in increased vascular friability and increased permeability. This mechanism might also relate to increased incident mH, if the vessel wall integrity is sufficiently impaired to permit small amounts of red blood cell passage.

Table 2  
Categorization of ARIA components

Nature of leakage products	Primary imaging sequence	Location of increased vascular permeability	
		Parenchyma	Leptomeninges
Proteinaceous fluid	FLAIR for ARIA-E	“Vasogenic edema”	Sulcal effusion/exudate
Heme products	T2*-GRE/SWI for ARIA-H	microhemorrhage	Superficial hemosiderosis

Abbreviations: ARIA, amyloid-related imaging abnormalities; FLAIR, fluid attenuation inversion recovery; ARIA-E, amyloid-related imaging abnormalities-edema/effusions; ARIA-H, amyloid-related imaging abnormalities-hemosiderin deposits.

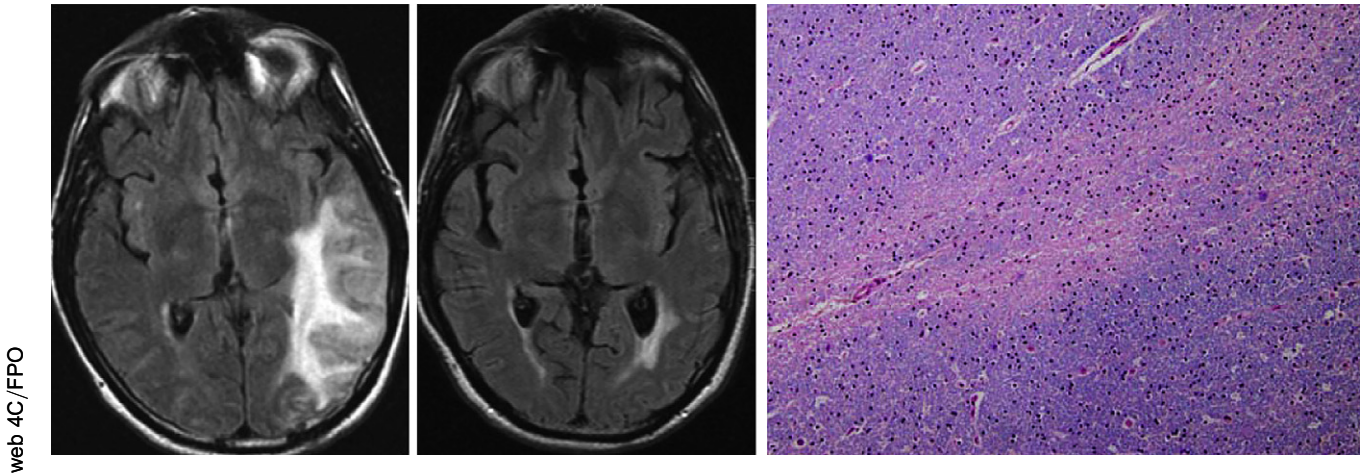


Fig. 6. Spontaneous cerebral amyloid angiopathy-related inflammation. MR images show vasogenic edema-like appearance (left) with resolution following course of corticosteroids (center). Pathology image (right) shows white matter rarefaction without necrosis.

Indeed, the various FLAIR and T2\*-GRE abnormalities observed in ARIA might be conceptualized as related to altered vascular permeability according to the location of the vessels and the nature of the material allowed to leak through the vessels (Table 2).

The increased risk of ARIA in *APOE*  $\epsilon 4$  carriers in the bapineuzumab trials and the reports of spontaneous ARIA in CAA are also suggestive of vascular amyloid burden as a common pathophysiologic mechanism underlying these phenomena [8,9,54]. *APOE*  $\epsilon 4$  carriers are known to have higher vascular amyloid burden than noncarriers [15,55]. Positron emission tomography amyloid imaging studies in patients with CAA have suggested that mHs are more likely to occur in regions with high amyloid burden, as assessed by 11C-Pittsburgh compound B [56,57].

It is possible that direct removal of amyloid from the vessel wall would be associated with compromise in the vascular integrity. Alternatively, there may be amyloid-related endothelial cell dysfunction resulting in increased vascular permeability, which might explain the similarity to increased permeability, as described previously, and as seen in PRES. It is also possible that there is a focal inflammatory component that would result in both ARIA-E and ARIA-H, as suggested by the pathology reports from patients with CAA. The CSF results from a small number of participants identified with ARIA in the phase II bapineuzumab studies are not consistent with a widespread inflammatory process. Normal CSF has also been reported in inflammatory CAA, and it is possible that focal amyloid-related vascular inflammation may play a role in some cases of ARIA. Thus far, it does not seem that ARIA cases show evidence of meningoencephalitis, as was reported in the early trials of active immunization with AN-1792 [58]. It also remains unknown whether different forms of immunotherapy or specific antibodies are more or less likely to be associated with ARIA [59].

Because there are very limited data regarding ARIA in nonimmunotherapeutic approaches for lowering A $\beta$ , it is difficult to speculate on more general additional mechanisms

that might be associated with ARIA. Preliminary reports of ARIA occurrence in therapeutic strategies aimed at decreasing production of specific A $\beta$  peptides suggest that decreasing A $\beta_{1-42}$  or altering the ratio of various A $\beta$  species might change the dynamics of amyloid production and clearance, resulting in ARIA through similar mechanisms discussed previously [14,60]. The preliminary reports of spontaneous ARIA in patients with AD detected at screening, or occurring rarely during treatment with placebo, suggest that ARIA may represent the by-product of the natural A $\beta$  clearance processes in the aging brain [14]. Similar to the CAA cases described in previously published reports, it is likely that specific genetic factors, such as the presence of *APOE*  $\epsilon 4$  alleles, influence the likelihood of spontaneous ARIA occurrence. Perhaps, the common link with treatment-associated ARIA across several therapeutic approaches and the rare spontaneous ARIA cases may be that we are tipping the balance of “Mother Nature’s” clearance mechanisms, but additional data are clearly needed to elucidate the underlying pathophysiology.

To further elucidate the potential pathophysiological mechanisms underlying the appearance of ARIA in both amyloid-modifying trials and in the natural history of AD, the working group also researched the limited literature on the histopathology of various aspects of ARIA and potentially relevant conditions.

#### 14. Histopathological correlates of ARIA

To date, no neuropathologic descriptions of ARIA-E have been reported, perhaps because of the transient nature of ARIA-E. Our working understanding of the pathology and pathophysiology underlying this condition is therefore based on extrapolation from conditions with similar clinical and neuroimaging features.

As described previously, ARIA shares some features with PRES disorders, which are also characterized by subacute clinical symptoms and reversible T2 hyperintensities [61,62].

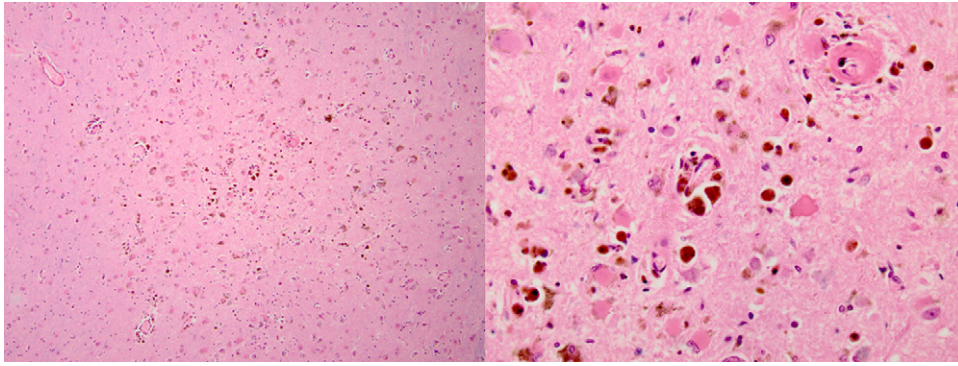


Fig. 7. Cerebral microhemorrhages seen in association with advanced cerebral amyloid angiopathy.

Neuropathologic descriptions of PRES [63,64] are sparse and somewhat difficult to interpret because of the heterogeneity of the underlying processes that can trigger PRES, including hypertension, preeclampsia, and medications. From the limited available data, PRES-associated T2 hyperintensities seem to be generated by rarefaction and vacuolation of the white matter, without reported tissue infarction.

CAA-related inflammation, a second condition that shares some of the neuroimaging features of ARIA (Fig. 6), similarities to VE [6,8], as well as a shared association with *APOE*  $\epsilon$ 4 [2,9], demonstrates similar white matter pathology to that reported in PRES. CAA-associated inflammation seems to be driven by a spontaneous inflammatory response to the vascular amyloid deposits, with accumulations of leukocytes surrounding amyloid-laden vessel segments and multinucleated giant cells containing amyloid-immunoreactive material. White matter rarefaction is again the likely neuropathologic correlate of the observed T2 hyperintensities [6]. After resolution of CAA-related inflammation, the underlying inflammatory pathology also seems to reverse [6,65].

The cases of ARIA in amyloid-modifying therapies do not seem to share the clinical or CSF findings of the meningoencephalitis reaction reported in clinical trial participants receiving active vaccination with the AN-1792 formulation of  $A\beta_{42}$  [58]; however, there are limited histopathological reports that may be relevant to understanding the pathophysiology. The postmortem examination of two meningoencephalitis cases demonstrated advanced CAA [52,66], suggesting that active amyloid immunotherapy might trigger a response similar to that observed in spontaneous CAA-related inflammation. Examination of the white matter in the meningoencephalitis cases again disclosed vacuolation and rarefaction without infarction [52].

There is a much more extensive histopathology literature regarding ARIA-H. Cerebral mHs are focal deposits of hemosiderin in the brain, typically associated with localized cell loss and gliosis. The hemosiderin can be found in macrophages, astrocytes, and microglia. Although most lesions are imaged or observed pathologically in the remote state (i.e., having occurred in the past), acute lesions are occasion-

ally found, with extravasation of red blood cells and associated reactive changes.

Cerebral mHs are typically seen in association with diseases of the small arteries and arterioles, in particular, hypertensive vasculopathy (often in the white matter) and CAA (restricted to the gray matter) [23,67–69]. A recent study [57] found that among patients with probable CAA, cerebral mHs are associated with increased local amyloid deposits, as measured by Pittsburgh compound B, a finding that highlights the spatial association between small-vessel pathology and mH location.

Two features regarding the neuropathology of cerebral mH are worth highlighting. First, the lesions are small, generally 1 to 2 mm in diameter [69] (“blooming” to a larger size on T2\*-weighted MRI sequences, as described in section 1). Although there is associated gliosis and some neuronal loss at mH loci (Fig. 7), there is little or no evident abnormality in the surrounding brain tissue.

A second point is that, aside from brains with florid vascular disease, mHs are a rare neuropathologic finding. Systematic sampling of brain sections from both nondemented and demented individuals [70,71] identifies many clinically silent ischemic microinfarctions, but only rare mH. The apparent discrepancy between the high prevalence of mH reported in MRI-based studies versus their low prevalence in neuropathologic series likely reflects the ability of MRI (but not neuropathologic examination) to sample the entire brain with high sensitivity. Based on the well-characterized mathematical relationship between the abundance and size of mH versus the neuropathological “slicing” required to accurately sample them [72], the standard sectioning and sampling of the brain at autopsy is predicted to be insensitive to the presence of a small number of lesions of size similar to mH. Thus, the one, two, or three mHs detected by MRI in a given individual may indeed represent close to that individual’s total lesion burden.

As evident from the aforementioned discussion, CAA may be a neuropathologic common denominator for both the treatment-related ARIA and the rare spontaneous cases reported in screening cohorts [4]. CAA—present at the time of

study entry or perhaps exacerbated by amyloid immunotherapy [53,73]—might thus be the underlying factor predisposing individuals to adverse treatment responses. It might be wrong to conclude, however, that anti-amyloid immunotherapy is necessarily deleterious for CAA. In a postmortem study of nine patients immunized with AN-1792, two of the longest treated participants demonstrated essentially no CAA [53]. This and data from anti-amyloid antibody-treated transgenic mice [74,75] suggest that amyloid can be successfully cleared from vessels without triggering substantial bleeding or vascular dysfunction. If achievable, clearance of CAA might reduce an individual's risk both for future adverse treatment responses as well as for other manifestations of CAA, such as intracerebral hemorrhage.

### 15. Insights from animal models

Given the paucity of human histopathological data in ARIA, the Workgroup also explored relevant reports from animal models of AD and CAA, particularly those exposed to amyloid-modifying therapies. It is now roughly 15 years since the first successful transgenic mouse models of AD were developed; over the ensuing interval, there have been additional models generated, yet there remain a relatively small number of models that are widely used by investigators [76–83]. The development of these models drew on the identification of mutations in APP that are associated with familial forms of AD—those adjacent to the BACE cleavage site (amyloid precursor protein-Swedish mutation [APP-sw]), those which are just past the  $\gamma$ -secretase site, those internal to the A $\beta$  peptide sequence, or elaborate combinations of these mutations. Transgenes were expressed under the control of active, heterologous promoters (prion promoter [PRNP], platelet derived growth factor-beta [PDGF- $\beta$ ] chain, Thy1) at high levels to accelerate the accumulation of A $\beta$  in the brains of animals [76,78,82,83].

These models were then advanced by the inclusion of mutated forms of presenilin-1 (PS-1) to preferentially shift the A $\beta$  toward the 42-amino acid form; these combinations were generated either by crossing individual strains or by the co-injection of constructs during the derivation of the mouse lines [77,79,80]. The endpoint for determination of an “acceptable” or “useful” mouse model was the presence of A $\beta$  deposition in the brain, often accompanied by vascular wall deposits in the form of CAA. Many of these models were subsequently shown to have some of the other alterations associated with affected brain regions in AD, including gliosis and microglial activation.

Importantly, neither significant neuronal loss on par with the changes observed in AD nor the presence of tangles followed the overexpression of the APP and PS1 transgenes even in the face of significant A $\beta$  deposition. Attempts have been made by the use of additional transgene constructs to provide a model in which both plaques and tangles appear,

but to develop tangles, it was necessary to use a mutant form of tau that is associated with frontotemporal lobar degeneration but not with AD [81].

It remains important to recognize that these mouse models of A $\beta$  deposition capture only some aspects of the pathologic changes observed in brains of individuals with AD. In addition to being incomplete models, it is also likely that they do not replicate all of the anatomic findings observed in AD.

### 16. mHs in animal models of treatment of AD/CAA

In addition to being useful models of AD for experimental analysis of disease mechanism and pathogenesis, these transgenic mice provide a fruitful testing arena for potential therapeutic interventions to slow, reverse, or prevent the pathologic changes of AD. Again, it is necessary to emphasize that these models were engineered to develop elevated brain A $\beta$  levels that progress to plaque formation. Additionally, the models have gradually been enhanced to accelerate the process because a major determining factor of the cost of animal experiments is the age of the animals under study (i.e., a study that can be performed with 4-month-old mice will have roughly 25% the animal housing costs of a study that requires 16-month-old mice).

There is no widely agreed on individual animal model that consistently develops mH, and little evidence for consistent or prominent edema, either at baseline or after various therapeutic challenges. Rather, there has been a suggestion that, in mouse models, there are effects of mode of delivery of immunotherapeutics that may influence the development of mH, as well as an apparent dependence on the presence of CAA. There is not clear evidence from animal studies that there is an increased incidence of mH with nonimmunologic approaches to alterations of brain A $\beta$  levels.

Although not a feature of the earliest studies demonstrating the potential of anti-A $\beta$  antibody-based approaches to clearance of A $\beta$  from the brains of transgenic mice [84], there have been subsequent studies (using a variety of mouse models, types of immunization, distinct antibodies/epitopes, duration and age of animals tests) in which mHs were detected [5,73,75,85–90]. As summarized in a series of recent reviews [91–93], these studies were mostly focused on clearance of existing deposits (which included both parenchymal and vascular A $\beta$ ). Because mHs are associated, by definition, with an alteration in vascular integrity, there has been specific interest on the changes in CAA burden that follow the treatment and accompany the development of mH. It is not possible to determine a priori whether an increase in CAA burden or a decrease in CAA burden would be more likely to be responsible for mH—or even if the pre-existing A $\beta$  burden on a vessel-by-vessel basis might play a role. Some of the studies that identified increased mH also found an increase in the vascular CAA burden, suggesting that increased vascular wall injury associated with “mobilization” of parenchymal A $\beta$  is at least

partially responsible [85–87]. Another study, which compared intraventricular versus systemic antibody delivery, observed that the approach associated with decreased CAA burden as well as parenchymal A $\beta$  clearance (intraventricular) was also associated with decreased mH incidence [94]. Despite the evidence from the studies cited previously regarding the association of mH with immunologically based therapeutic approaches, it should be stated that these are by no means an absolute finding across models, types of immunization approaches, and epitopes (reviewed in [91–93]).

When considering the translation of these findings on mH associated with therapeutic interventions that increase CAA A $\beta$  burden while decreasing parenchymal burden to human subjects, it is necessary to recognize the fact that there is far greater vessel-to-vessel A $\beta$  vascular burden heterogeneity in humans with AD than there is in mouse models. Given the current concept that A $\beta$  departs the central nervous system along perivascular spaces of arterioles [95–100], in a human with patchy CAA, there will be a significant number of perivascular pathways along which any deposited A $\beta$  would represent an initiation event for local CAA, whereas in a uniformly and heavily affected mouse model, a comparable level of deposition might well be shifting the vessel over the threshold from moderate to severe CAA, and thus increasing the risk of local hemorrhage. It is disappointing that there has not been a direct association between the level of local vascular A $\beta$  burden and the risk of mH in these mouse models of immunotherapies, because such studies might have provided relevant answers to the question of “how much CAA can you have before clearing A $\beta$  along perivascular spaces becomes a riskier proposition?”

In a recent article, imaging was used to track the appearance of mH in Tg2576 mice treated with a series of antibodies in a passive immunotherapy protocol for 12 or 18 weeks [101]. This study found that the antibodies used did increase the number of MR-detected mHs and that these correlated well with histologic observations at the endpoint of the experiment. Given the associated interest in ARIA-E, it is disappointing that there is no information regarding this other potential complicating feature of anti-A $\beta$  therapy in the article. Another recent article described the combination of passive immunization and adoptive transfer of activated T-cells [102]. The combination produced hemorrhage in animals with CAA, but neither of the two could produce the same effect by itself. This experiment highlights the possible complex interactions in immune responses that determine whether—and when—mH might occur in the setting of anti-amyloid therapies.

In humans, the presence of lobar-predominant mH is a strong predictor of the presence of CAA. In the mouse models, the presence and timing of CAA is a well-characterized component of each specific model, although it varies across models [103–105]. Moreover, even in the animal models, CAA is not always tightly aligned with mH

after therapeutics (which to date is focused almost exclusively on immunotherapy). The variability of detection of mHs in animal models may be, in part, because the condition does not occur uniformly in all animals, even within a specific age range of a specific transgenic background, and the likelihood of detecting mHs can vary substantially from experiment to experiment. Additionally, it may be influenced by the severity of CAA, which is more variable than plaque burden in some animal models.

## 17. Difficulty in modeling ARIA-E in transgenic animals

Other aspects of ARIA, in particular, the phenomenon known as “VE,” have not been reported in animal models; however, it is unclear whether systematic MR surveillance with sequences sensitive to ARIA-E has been performed longitudinally in a large number of animal models treated with amyloid-modifying therapies.

As mentioned previously, cerebral edema is the increase in water content of the extravascular but intraparenchymal compartment of the brain. It includes water that is present in the extracellular space as well as within cellular components of the brain. In general, cerebral edema is divided into two types: cytotoxic (associated with altered handling of water and ion transport across cellular membranes of neurons and glia) and vasogenic (associated with alterations in the blood–brain barrier either through direct actions on cerebrovascular endothelial cells or as mediated through the neurovascular unit).

In many disease processes, the etiology of cerebral edema is a combination of cytotoxic edema and VE. Mouse models of some human diseases, particularly the well-studied models of hypoxic/ischemic injury and brain tumors, include edema as a component of the pathologic process. This appropriately mimics the situation in human disease processes by involving both vasogenic and cytotoxic edema. VE, because it is dependent on the integrity of the blood–brain barrier, may be measured through the capacity of the cerebral vasculature to prevent the leakage into the brain of an agent typically retained within the intravascular compartment. Overall measurement of marked edema in model systems can also be performed through MRI methods, akin to clinically relevant studies in humans. Histologic methods are not well suited to the detection and quantification of edema, particularly when focal and toward the less severe end of the disease spectrum.

In the available imaging data from therapeutic trials for AD, there was evidence of transient imaging abnormalities in the white matter of some trial participants with the characteristics of ARIA-E in the absence of cytotoxic features. Despite this observation, there has been no evidence from studies of therapeutic candidates in mouse models of A $\beta$  deposition in the form of plaques and CAA for the development of edema (either cytotoxic or vasogenic). In general, these studies have been endpoint studies that would not be well suited to detect transient changes that might occur in

the early stages of treatment. Additionally, these studies have used a variety of endpoint measures, such as behavioral assays, histopathologic measurements (including plaque burden, inflammatory reactions, and mH), and biochemical determination of A $\beta$  in brain and CSF.

At present, no well-established protocols or specific models are available to consistently model A $\beta$  therapy-induced ARIA-E. ARIA-H is observed to various extents in different models and with different therapeutic interventions. The emphasis of investigators to date has been on the validation of specific therapeutic approaches (e.g., alteration of A $\beta$  generation, immunologic and nonimmunologic clearance of A $\beta$ ). There remains an opportunity for development and characterization of model systems in which therapeutic interventions aimed at modifying A $\beta$  levels in the brain can be consistently associated with relevant potential side effects, particularly those with activation of the immune system.

## 18. Challenges to development of an animal model for ARIA

Could one develop a standardized animal model of ARIA, ideally a model that would exhibit all components of ARIA observed in patients with AD? There are certain barriers to be considered: for ARIA-E, there is uncertainty about whether it exists in animal models at all. For example, there are no publications describing this, and unpublished studies of which the Workgroup is aware have been regularly negative. The absence of ARIA-E in mice might reflect a difference in animal model and human pathobiology, or technical difficulties in imaging timing and sequence optimization, but to date this component of ARIA has not been described in animal models.

Although we endorse the idea of careful screening for mH complications with any anti-amyloid therapy, certain barriers exist in this context as well. There is uncertainty about the predictive value of mH in a mouse regarding likelihood of developing ARIA-E in patients (or even ARIA-H in patients). Barriers to establishing a common and uniform animal model of mH are also substantial—access to specific models is not universal, the specific model appropriate for each therapeutic approach may differ (e.g., the presence or absence of a PS-1 mutation that accelerates pathology can affect age of animals; the specific APP mutation can affect the amount of CAA), and phenotypic drift may occur within models. These factors will also potentially significantly confound the interpretation of studies undertaken by sponsors with different experimental models because they follow the FDA's advice to conduct nonclinical studies investigating the potential for induction of ARIA.

## 19. Monitoring for ARIA in clinical trials

The Workgroup concluded that there is very limited information thus far regarding ARIA in the natural setting or pub-

licly available information from ongoing clinical trial programs to make conclusive recommendations for clinical trial policies. In particular, there are limited data regarding the relationship between ARIA-H and ARIA-E. Additional information regarding whether the presence of baseline ARIA-H acts as a potential risk factor for ARIA-E, and whether in turn ARIA-E acts as a risk factor for incident ARIA-H, is needed to provide guidance for the conduct of these studies. Most importantly, we need information about whether specific components of ARIA have an effect on clinical course and response to treatment.

The limited data available thus far suggest that there may be common risk factors for the components of ARIA, in particular, *APOE* genotype. This genetic risk factor, as well as limited reports from patients with CAA, suggests that vascular amyloid may be a pathophysiological mechanism for ARIA, but additional investigation is needed. Because the *APOE*  $\epsilon$ 4 allele is present in a large proportion of patients with AD, and some degree of vascular amyloid is present in nearly all patients with AD, it is critically important to gather additional information on the relationship of genotype to the incidence of ARIA in the setting of amyloid-lowering therapy.

From the limited data available thus far, it seems that amyloid-modifying treatment can be continued with careful monitoring and possible dose adjustment after the occurrence of ARIA. At this point, it seems premature to preclude continued therapy in the setting of incident ARIA, because trial participants with ARIA are currently being redosed in ongoing trials, without clear evidence of any untoward effects. If ARIA is related to successful amyloid clearance, it might introduce significant bias in ongoing trials to terminate all of these trial participants. It will be important to gather systematic data regarding the clinical course associated with ARIA in the setting of amyloid-modifying therapy. In addition, it is important to better understand the frequency of spontaneous ARIA, the risk factors associated with this phenomenon, and its clinical course. Given the evidence that incident mHs are relatively frequent in the course of AD, and the likelihood that spontaneous mHs share some common aspects of pathophysiology with CAA, it is likely that additional spontaneous cases of ARIA will be identified in ongoing research studies.

It will be critically important to continue to monitor for ARIA in ongoing studies, and especially to relate the imaging features of ARIA to the clinical course of these patients. To that end, the Workgroup developed a set of recommendations for the FDA and the industry to consider in the conduct of clinical trials.

### 19.1. Recommendations

The Workgroup recognizes important issues both in the technical aspects of MR image acquisition to detect ARIA as well as the interpretation of MRI findings suggestive of ARIA. In turn, the recommendations that are included in

this report provide guidance on how technical consistency and a uniform neuroradiological approach to ARIA might be accomplished. In addition, the Workgroup provides some recommendations regarding exclusion from participation in clinical trials based on baseline or incident ARIA and for ongoing monitoring during clinical trials of amyloid-modifying therapies.

#### 19.1.1. MRI protocols for detection of ARIA within amyloid therapy trials

##### 19.1.1.1. MRI protocol minimum standards

A protocol that can be implemented in a wide variety of settings of care is suggested, particularly one that can be undertaken in an average community-based setting. It might optimally include the following:

- a. Scanner field strength: Although high field strength scanners are likely to have greater sensitivity, the use of 1.5-T scanners is endorsed as a minimum standard, recognizing that the availability of higher field units is limited to certain centers. The implementation of more sensitive MRI measures to detect ARIA-H needs to be balanced by the clinical importance of such findings.
- b. Scan sequences: Two-dimensional T2\*-GRE, to identify ARIA-H, are presently available on any scanner worldwide and recommended.
- c. Slice thickness of 5 mm or less.
- d. TE = 20 ms or greater.
- e. T2 FLAIR sequence for identification of ARIA-E.

#### 19.1.2. Frequency and intensity of scanning protocols within clinical development

- a. **In phase I through early phase II, more frequent scanning to ascertain the rates of these abnormalities is warranted.** As new amyloid-lowering therapies are introduced into early clinical development, knowledge regarding the frequency and timing of ARIA occurrence is likely to be limited.
- b. **Discussions of the scanning requirements for phase III should be actively undertaken based on phase II results,** given the significant burden to patients and cost implications of frequent scanning. Additional monitoring for ARIA in the setting of clinical symptoms may be more appropriate than a priori fixed frequencies, given the transience of ARIA-E.
- c. **Pharmacodynamic effects are an important consideration in determining the optimal timing of MRI scanning.** The timing of MR scans in relation to dosing should be considered for bolus intravenous administration (i.e., x number of weeks postdosing) as well as the pharmacokinetic levels. However, duration of dosing (i.e., exposure) should also be considered.
- d. **Frequency of surveillance scans in amyloid-lowering trials to detect ARIA-E.** It is likely not feasible to monitor frequently enough to capture all ARIA-E. It is also not clear that “missing” transient ARIA-E

that is clinically asymptomatic will result in any untoward effects. In addition to scheduled surveillance scans, scans should also be prompted by onset of symptom clusters suggestive of ARIA-E. Taken together, surveillance and triggered scans should adequately characterize the incidence and behavior of ARIA. It is important to consider how scans will be read and tracked in these drug development programs.

- e. **A short interval rescans** provision should be built into each protocol to reassess subjects who develop ARIA during treatment, particularly if they are symptomatic.

#### 19.1.3. Reading and reporting standards

##### 19.1.3.1. Image interpretation/ascertainment

It should be recognized that variable levels of diagnostic certainty are inherent in any radiological diagnosis. ARIA may be definitely present or absent, but often uncertainty exists, and allowance for this should be made in their ratings as follows:

- a. A radiological reading of a “possible” mH (ARIA-H) should not be an exclusionary criterion. Inclusion/exclusion should only be based on readings labeled definite mH. Guidance should also recognize that distinction between “possible” and “definite” may require serial MRI scans, that is, that changes in diagnostic confidence will inevitably occur over the course of a series of scans, as is true in all diagnostic imaging. Change in categorization of an mH from “possible” to “definite” in the course of a series of scans that “move” a trial participant over an exclusionary threshold should not constitute a “retrospective” protocol violation.
- b. ARIA-E should be interpreted both for severity as well as relevance to clinical symptoms. ARIA-E can manifest as rather subtle alterations in MR signal, and can be missed. It is not clear, however, that missing subtle asymptomatic ARIA will have any clinical consequence. Additionally, fulminant ARIA-E can be misidentified as other processes (subarachnoid hemorrhage, venous infarction). Thus, it is important to consider how scans will be read and tracked in these drug development programs.

##### 19.1.3.2. Procedures for reading and reporting

There are pros and cons to “central reading” versus “local” reads. Central reading has the advantage of a small number of individuals with detailed knowledge of ARIA to monitor all scans, increasing both accuracy and inter-rater reliability. Most hospitals require a local read to be performed in any case for safety, and generally this can be accomplished quickly with rapid notification to the investigator.

- a. If monitoring for ARIA is to be done with local reads, it will be important to educate both the local radiologists and the investigators about the MR appearance of ARIA, particularly the various MR manifestations

associated with ARIA with multiple case examples. It may be useful to also provide a “central” resource for local radiologists to send possible ARIA cases for immediate consultation.

- b. A detailed reporting form (with checkboxes) covering the spectrum of findings should be provided to the local radiologist that specifically asks about presence or absence of signal abnormalities consistent with ARIA-E (presence of increased signal on FLAIR sequences consistent with parenchymal edema or effusions in the leptomeninges or sulcal space) and/or ARIA-H (presence of mH or hemosiderosis).
- c. It may also be useful to develop a more quantitative scoring or rating scale for ARIA, especially for use by central monitoring, to provide additional information about the potential relationship between ARIA-H and ARIA-E, as well as to clinical symptoms/outcome. This scoring system might include detailed information about anatomic location of ARIA-E and/or ARIA-H and a severity index.

#### 19.1.4. Thresholds for exclusion based on ARIA in clinical trials with amyloid-lowering therapies

In response to the original FDA guidance on exclusion of individuals with evidence of ARIA-H at baseline or incidence of ARIA in trials, the Workgroup discussed potential thresholds for number of mHs extensively. The Workgroup believed it was obviously important to protect clinical trial participants from potential adverse outcomes related to ARIA, but they thought that it was also important not to be unnecessarily stringent in excluding participants with ARIA, based on the current literature and publicly available information. It was thought to be important to elucidate the effects of treatment in the general AD clinic population, where patients with evidence of ARIA-H at baseline would be likely to receive treatment with considerably less monitoring than is possible in a clinical trial. Given the frequency of mHs and the incomplete knowledge of their clinical significance, an ascertainment of the risk of meaningful clinical complications during amyloid treatment is a recognized need. Stringent criteria would also limit the acquisition of knowledge needed to optimally manage patients with AD and underlying CAA and/or hypertensive small-vessel cerebrovascular disease. An additional consideration is that discontinuation of asymptomatic patients from ongoing treatment with incident ARIA would also preclude the evaluation of potential clinical benefits associated with vascular A $\beta$  clearance in the AD population. Thus, given the importance of allowing the field to advance the development of amyloid-modifying treatments for AD, the Workgroup developed recommendations for exclusion criteria based on the recognition that there are limited data available to justify strict exclusions on the basis of baseline ARIA-H, and that current MR methods and clinical reading procedures are imperfect in their detection and tracking of small numbers of mHs. It is likely that recommendations will continue to

evolve as additional data from large clinical trial databases become available.

##### 19.1.4.1. Exclusions for presence of baseline ARIA-H (mH or hemosiderosis)

There are only very limited data about the risks of amyloid-modifying treatment with amyloid-lowering therapy in patients with evidence of ARIA-H at baseline. It is recognized that substantial numbers of lobar mHs likely reflect the presence and severity of CAA, raising diagnostic and therapeutic considerations. Current prevalence estimates in mild to moderate AD are that 80% of patients with mH will have two or fewer mHs. Given the uncertainty of risk and concerns about CAA severity, the Workgroup supports the recommendation that the cutoff value of four mHs be used for exclusion in trials of amyloid-modifying therapies for AD. This threshold would allow the potential for imaging measurement variability to be taken into account and reflect the uncertainty regarding the clinical relevance of small numbers of mHs.

##### 19.1.4.2. Exclusionary criteria for incident ARIA-H (based, in part, on using the recommended technical MR characteristics noted previously)

Development of asymptomatic ARIA-H may result from A $\beta$  clearance associated with amyloid-lowering therapy. Additionally, because ARIA-H may also occur within the natural history of AD, we recommend that the appearance of incident mH or hemosiderosis not automatically disqualify a patient from further treatment. Rather, we suggest that discontinuation of trial participants with incident ARIA-H be reserved for those in whom these MRI findings are associated with significant clinical symptoms or evidence of precipitous clinical decline. Until there are more available data, clinical consideration may also be given to discontinuing specific patients who are still asymptomatic but with a large number of incident mHs. The Workgroup suggests that as more data become available, this recommendation would merit continued review.

## 20. Recommendations for further research into the mechanisms underlying ARIA

In parallel with close monitoring of patients in ongoing clinical trials of amyloid-modifying treatments, further research is clearly needed to elucidate the mechanisms underlying the phenomena observed in ARIA. In particular, it would be valuable to use animal models to determine whether amyloid-lowering therapies indeed are associated with increased vascular permeability, perhaps through fluorescent labeling of plasma proteins of different sizes. Additional understanding of the genetic and age factors that favor vascular clearance of amyloid might also be achieved through transgenic breeding, perhaps crossing transgenics containing human isoforms of *APOE* with *APP/PS-1* mutants.

In human studies, the combination of positron emission tomography amyloid imaging with frequent MRI

monitoring in *APOE*  $\epsilon$ 4 carriers receiving amyloid-lowering therapies may serve to clarify whether ARIA will occur preferentially in regions with high amyloid burden, and to demonstrate evidence of amyloid clearance proximate to ARIA. Most importantly, longitudinal natural history studies are needed to better understand the spontaneous occurrence of ARIA and the associated clinical course, as well as detailed analyses of the cognitive, behavioral, and functional outcomes of individuals who develop ARIA in the setting of amyloid treatment trials.

## References

- [1] Black RS, Sperling RA, Safirstein B, Motter RN, Pally A, Nichols A, et al. A single ascending dose study of bapineuzumab in patients with Alzheimer disease. *Alzheimer Dis Assoc Disord* 2010;24:198–203.
- [2] Salloway S, Sperling R, Gilman S, Fox NC, Blennow K, Raskind M, et al. A phase 2 multiple ascending dose trial of bapineuzumab in mild to moderate Alzheimer disease. *Neurology* 2009;73:2061–70.
- [3] Sperling R, Salloway S, Fox N, Barackos J, Morris K, Francis G, et al., eds. Risk Factors and clinical course associated with vasogenic edema in a phase II trial of bapineuzumab. Seattle, WA: American Academy of Neurology; 2009.
- [4] Sperling R, Bronen R, Greenberg S, Sorensen G, Salloway S, Gass A, et al. Three cases of apparent vasogenic edema (VE) from a Phase 2 clinical trial of the gamma secretase inhibitor BMS-708163 in patients with mild-to-moderate AD. *Alzheimer's Association International Conference on Alzheimer's disease*. Paris, France; 2011.
- [5] Pfeifer M, Boncristiano S, Bondolfi L, Stalder A, Deller T, Staufenbiel M, et al. Cerebral hemorrhage after passive anti-Abeta immunotherapy. *Science* 2002;298:1379.
- [6] Eng JA, Frosch MP, Choi K, Rebeck GW, Greenberg SM. Clinical manifestations of cerebral amyloid angiopathy-related inflammation. *Ann Neurol* 2004;55:250–6.
- [7] Scolding NJ, Joseph F, Kirby PA, Mazanti I, Gray F, Mikol J, et al. Abeta-related angitis: primary angitis of the central nervous system associated with cerebral amyloid angiopathy. *Brain* 2005;128(Pt 3):500–15.
- [8] Oh U, Gupta R, Krakauer JW, Khandji AG, Chin SS, Elkind MS. Reversible leukoencephalopathy associated with cerebral amyloid angiopathy. *Neurology* 2004;62:494–7.
- [9] Kinnecom C, Lev MH, Wendell L, Smith EE, Rosand J, Frosch MP, et al. Course of cerebral amyloid angiopathy-related inflammation. *Neurology* 2007;68:1411–6.
- [10] Greenberg SM, Parisi JE, Keegan BM. A 63-year-old man with headaches and behavioral deterioration. *Neurology* 2007;68:782–7.
- [11] DiFrancesco JC, Brioschi M, Brighina L, Ruffmann C, Saracchi E, Costantino G, et al. Anti-Abeta autoantibodies in the CSF of a patient with CAA-related inflammation: a case report. *Neurology* 2011;76:842–4.
- [12] Stott VL, Hurrell MA, Anderson TJ. Reversible posterior leukoencephalopathy syndrome: a misnomer reviewed. *Intern Med J* 2005;35:83–90.
- [13] Tungksaereerak C, Phanthumchinda K. Reversible posterior leukoencephalopathy syndrome: a retrospective study in King Chulalongkorn Memorial Hospital. *J Med Assoc Thai* 2008;91:427–32.
- [14] Carlson C, Estergard W, Oh J, Suhy J, Jack CR Jr, Siemers E, et al. Prevalence of asymptomatic vasogenic edema in pretreatment Alzheimer's disease study cohorts from phase 3 trials of semagacestat and solanezumab. *Alzheimers Dement* 2011;7:396–401.
- [15] Greenberg SM, Rebeck GW, Vonsattel JP, Gomez-Isla T, Hyman BT. Apolipoprotein E epsilon 4 and cerebral hemorrhage associated with amyloid angiopathy. *Ann Neurol* 1995;38:254–9.
- [16] Sperling R, Salloway S, Arrighi M, Morris K, Lu, Liu E, et al. Revised estimates of incidence and risk factors for Amyloid Related Imaging Abnormalities (ARIA) in the Phase 2 studies of bapineuzumab for mild to moderate Alzheimer's disease. *Alzheimer's Association International Conference on Alzheimer's disease*. Paris, France; 2011.
- [17] Cordonnier C, Potter GM, Jackson CA, Doubal F, Keir S, Sudlow CL, et al. Improving interrater agreement about brain microbleeds: development of the Brain Observer MicroBleed Scale (BOMBS). *Stroke* 2009;40:94–9.
- [18] Linn J, Halpin A, Demaerel P, Ruhland J, Giese AD, Dichgans M, et al. Prevalence of superficial siderosis in patients with cerebral amyloid angiopathy. *Neurology* 2010;74:1346–50.
- [19] Vernooij MW, van der Lugt A, Ikram MA, Wielopolski PA, Niessen WJ, Hofman A, et al. Prevalence and risk factors of cerebral microbleeds: the Rotterdam Scan Study. *Neurology* 2008;70:1208–14.
- [20] Haacke EM, Xu Y, Cheng YC, Reichenbach JR. Susceptibility weighted imaging (SWI). *Magn Reson Med* 2004;52:612–8.
- [21] Kirsch W, McAuley G, Holshouser B, Petersen F, Ayaz M, Vinters HV, et al. Serial susceptibility weighted MRI measures brain iron and microbleeds in dementia. *J Alzheimers Dis* 2009;17:599–609.
- [22] Stehling C, Wersching H, Kloska SP, Kirchhoff P, Ring J, Nassenstein I, et al. Detection of asymptomatic cerebral microbleeds: a comparative study at 1.5 and 3.0 T. *Acad Radiol* 2008;15:895–900.
- [23] Fazekas F, Kleinert R, Roob G, Kleinert G, Kapeller P, Schmidt R, et al. Histopathologic analysis of foci of signal loss on gradient-echo T2\*-weighted MR images in patients with spontaneous intracerebral hemorrhage: evidence of microangiopathy-related microbleeds. *Am J Neuroradiol* 1999;20:637–42.
- [24] Moody DM, Brown WR, Challa VR, Stump DA, Reboussin DM, Legault C. Brain microemboli associated with cardiopulmonary bypass: a histologic and magnetic resonance imaging study. *Ann Thorac Surg* 1995;59:1304–7.
- [25] Nakata-Kudo Y, Mizuno T, Yamada K, Shiga K, Yoshikawa K, Mori S, et al. Microbleeds in Alzheimer disease are more related to cerebral amyloid angiopathy than cerebrovascular disease. *Dement Geriatr Cogn Disord* 2006;22:8–14.
- [26] Lee SH, Kim SM, Kim N, Yoon BW, Roh JK. Cortico-subcortical distribution of microbleeds is different between hypertension and cerebral amyloid angiopathy. *J Neurol Sci* 2007;258:111–4.
- [27] Goos JD, Henneman WJ, Sluimer JD, Vrenken H, Sluimer IC, Barkhof F, et al. Incidence of cerebral microbleeds: a longitudinal study in a memory clinic population. *Neurology* 2010;74:1954–60.
- [28] Goos JD, van der Flier WM, Knol DL, Pouwels PJ, Scheltens P, Barkhof F, Wattjes MP. Clinical relevance of improved microbleed detection by susceptibility-weighted magnetic resonance imaging. *Stroke* 2011 May 12; [Epub ahead of print].
- [29] Cordonnier C, van der Flier WM, Sluimer JD, Leys D, Barkhof F, Scheltens P. Prevalence and severity of microbleeds in a memory clinic setting. *Neurology* 2006;66:1356–60.
- [30] Jeerakathil T, Wolf PA, Beiser A, Hald JK, Au R, Kase CS, et al. Cerebral microbleeds: prevalence and associations with cardiovascular risk factors in the Framingham Study. *Stroke* 2004;35:1831–5.
- [31] Pettersen JA, Sathiyamoorthy G, Gao FQ, Szilagyi G, Nadkarni NK, St George-Hyslop P, et al. Microbleed topography, leukoaraiosis, and cognition in probable Alzheimer disease from the Sunnybrook dementia study. *Arch Neurol* 2008;65:790–5.
- [32] Sveinbjornsdottir S, Sigurdsson S, Aspelund T, Kjartansson O, Eiriksdottir G, Valtysdottir B, et al. Cerebral microbleeds in the population based AGES-Reykjavik study: prevalence and location. *J Neurol Neurosurg Psychiatry* 2008;79:1002–6.
- [33] Werring DJ, Frazer DW, Coward LJ, Losseff NA, Watt H, Cipolotti L, et al. Cognitive dysfunction in patients with cerebral microbleeds on T2\*-weighted gradient-echo MRI. *Brain* 2004;127(Pt 10):2265–75.

- [34] Lee SH, Bae HJ, Ko SB, Kim H, Yoon BW, Roh JK. Comparative analysis of the spatial distribution and severity of cerebral microbleeds and old lacunes. *J Neurol Neurosurg Psychiatry* 2004; 75:423–7.
- [35] Yakushiji Y, Nishiyama M, Yakushiji S, Hirotsu T, Uchino A, Nakajima J, et al. Brain microbleeds and global cognitive function in adults without neurological disorder. *Stroke* 2008;39:3323–8.
- [36] Igase M, Tabara Y, Igase K, Nagai T, Ochi N, Kido T, et al. Asymptomatic cerebral microbleeds seen in healthy subjects have a strong association with asymptomatic lacunar infarction. *Circ J* 2009; 73:530–3.
- [37] Hanyu H, Tanaka Y, Shimizu S, Takasaki M, Abe K. Cerebral microbleeds in Alzheimer's disease. *J Neurol* 2003;250:1496–7.
- [38] Roob G, Schmidt R, Kapeller P, Lechner A, Hartung HP, Fazekas F. MRI evidence of past cerebral microbleeds in a healthy elderly population. *Neurology* 1999;52:991–4.
- [39] Tsuchida Y, Tanizaki Y, Aoki J, Endo K. MR detection of microhemorrhages in neurologically healthy adults. *Neuroradiology* 2002; 44:31–6.
- [40] Bednar M, Kantarci K, Kupiec J, Burstein A, Landen J, Reyes D, et al. The prevalence of brain microhemorrhages and infarcts in a cohort of patients with mild–moderate Alzheimer's disease. In: *International Conference on Alzheimer's Disease*; 2010; Honolulu, Hawaii. P2–372.
- [41] Poels MM, Vernooij MW, Ikram MA, Hofman A, Krestin GP, van der Lugt A, et al. Prevalence and risk factors of cerebral microbleeds: an update of the Rotterdam scan study. *Stroke* 2010;41(Suppl 10):S103–6.
- [42] Gregoire SM, Brown MM, Kallis C, Jager HR, Yousry TA, Werring DJ. MRI detection of new microbleeds in patients with ischemic stroke: five-year cohort follow-up study. *Stroke* 2010; 41:184–6.
- [43] Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, et al. Cerebral microbleeds: a guide to detection and interpretation. *Lancet Neurol* 2009;8:165–74.
- [44] Fiehler J. Cerebral microbleeds: old leaks and new haemorrhages. *Int J Stroke* 2006;1:122–30.
- [45] Jeon SB, Kang DW, Cho AH, Lee EM, Choi CG, Kwon SU, et al. Initial microbleeds at MR imaging can predict recurrent intracerebral hemorrhage. *J Neurol* 2007;254:508–12.
- [46] Greenberg SM, Eng JA, Ning M, Smith EE, Rosand J. Hemorrhage burden predicts recurrent intracerebral hemorrhage after lobar hemorrhage. *Stroke* 2004;35:1415–20.
- [47] Vernooij MW, Ikram MA, Hofman A, Krestin GP, Breteler MM, van der Lugt A. Superficial siderosis in the general population. *Neurology* 2009;73:202–5.
- [48] Wahlund LO, Barkhof F, Fazekas F, Bronge L, Augustin M, Sjogren M, et al. A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke* 2001;32:1318–22.
- [49] Black S, Gao F, Bilbao J. Understanding white matter disease: imaging-pathological correlations in vascular cognitive impairment. *Stroke* 2009;40(Suppl 3):S48–52.
- [50] Weller RO, Boche D, Nicoll JA. Microvasculature changes and cerebral amyloid angiopathy in Alzheimer's disease and their potential impact on therapy. *Acta Neuropathol* 2009;118:87–102.
- [51] Chen YW, Gurol ME, Rosand J, Viswanathan A, Rakich SM, Groover TR, et al. Progression of white matter lesions and hemorrhages in cerebral amyloid angiopathy. *Neurology* 2006;67:83–7.
- [52] Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med* 2003;9:448–52.
- [53] Boche D, Zotova E, Weller RO, Love S, Neal JW, Pickering RM, et al. Consequence of Abeta immunization on the vasculature of human Alzheimer's disease brain. *Brain* 2008;131(Pt 12):3299–310.
- [54] Lim SY, Wesley Thevathasan A, Gonzales M, Mitchell PJ, Evans A. Vasogenic oedema with no mass lesion. *J Clin Neurosci* 2008; 15(1048):1075–6.
- [55] Alonzo NC, Hyman BT, Rebeck GW, Greenberg SM. Progression of cerebral amyloid angiopathy: accumulation of amyloid-beta40 in affected vessels. *J Neuropathol Exp Neurol* 1998;57:353–9.
- [56] Johnson KA, Gregas M, Becker JA, Kinnecom C, Salat DH, Moran EK, et al. Imaging of amyloid burden and distribution in cerebral amyloid angiopathy. *Ann Neurol* 2007;62:229–34.
- [57] Dierksen GA, Skehan ME, Khan MA, Jeng J, Nandigam RN, Becker JA, et al. Spatial relation between microbleeds and amyloid deposits in amyloid angiopathy. *Ann Neurol* 2010;68:545–8.
- [58] Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, et al. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology* 2003;61:46–54.
- [59] Siemers E, Friedrich S, Dean R, Sethuraman G, Demattos R, Jennings D, et al. Safety, tolerability and biomarker effects of an Aβ monoclonal antibody administered to patients with Alzheimer's disease. *Alzheimers Dement* 2008;4(Suppl 2). T774.
- [60] XX3. ICAD.
- [61] Hinchey J, Chaves C, Appignani B, Breen J, Pao L, Wang A, et al. A reversible posterior leukoencephalopathy syndrome. *N Engl J Med* 1996;334:494–500.
- [62] Bartynski WS. Posterior reversible encephalopathy syndrome, part 2: controversies surrounding pathophysiology of vasogenic edema. *Am J Neuroradiol* 2008;29:1043–9.
- [63] Schiff D, Lopes MB. Neuropathological correlates of reversible posterior leukoencephalopathy. *Neurocrit Care* 2005;2:303–5.
- [64] Okeda R, Kawamoto T, Tanaka E, Shimizu H. An autopsy case of drug-induced diffuse cerebral axonopathic leukoencephalopathy: the pathogenesis in relation to reversible posterior leukoencephalopathy syndrome. *Neuropathology* 27:364–370.
- [65] Greenberg SM, Rapalino O, Frosch MP. Case records of the Massachusetts General Hospital. Case 22-2010. An 87-year-old woman with dementia and a seizure. *N Engl J Med* 2010;363:373–81.
- [66] Ferrer I, Boada Rovira M, Sanchez Guerra ML, Rey MJ, Costa-Jussa F. Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. *Brain Pathol* 2004;14:11–20.
- [67] Tanaka A, Ueno Y, Nakayama Y, Takano K, Takebayashi S. Small chronic hemorrhages and ischemic lesions in association with spontaneous intracerebral hematomas. *Stroke* 1999;30:1637–42.
- [68] Tatsumi S, Shinohara M, Yamamoto T. Direct comparison of histology of microbleeds with postmortem MR images: a case report. *Cerebrovasc Dis* 2008;26:142–6.
- [69] Schrag M, McAuley G, Pomakian J, Jiffry A, Tung S, Mueller C, et al. Correlation of hypointensities in susceptibility-weighted images to tissue histology in dementia patients with cerebral amyloid angiopathy: a postmortem MRI study. *Acta Neuropathol* (in press).
- [70] Petrovitch H, Ross GW, Steinhorn SC, Abbott RD, Markesbery W, Davis D, et al. AD lesions and infarcts in demented and nondemented Japanese-American men. *Ann Neurol* 2005;57:98–103.
- [71] Schneider JA, Arvanitakis Z, Leurgans SE, Bennett DA. The neuropathology of probable Alzheimer disease and mild cognitive impairment. *Ann Neurol* 2009;66:200–8.
- [72] Weiss L, Harlos JP. The validity of negative necropsy reports for metastases in solid organs. *J Pathol* 1986;148:203–6.
- [73] Wilcock DM, Jantzen PT, Li Q, Morgan D, Gordon MN. Amyloid-beta vaccination, but not nitro-nosteroidal anti-inflammatory drug treatment, increases vascular amyloid and microhemorrhage while both reduce parenchymal amyloid. *Neuroscience* 2007;144:950–60.
- [74] Prada CM, Garcia-Alloza M, Betensky RA, Zhang-Nunes SX, Greenberg SM, Bacskai BJ, et al. Antibody-mediated clearance of amyloid-beta peptide from cerebral amyloid angiopathy revealed by quantitative in vivo imaging. *J Neurosci* 2007;27:1973–80.

- [75] Schroeter S, Khan K, Barbour R, Doan M, Chen M, Guido T, et al. Immunotherapy reduces vascular amyloid-beta in PDAPP mice. *J Neurosci* 2008;28:6787–93.
- [76] Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell C, et al. Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature* 1995;373:523–7.
- [77] Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen P, et al. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med* 1998;4:97–100.
- [78] Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, et al. Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* 1996;274:99–102.
- [79] Jankowsky JL, Fadale DJ, Anderson J, Xu GM, Gonzales V, Jenkins NA, et al. Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. *Hum Mol Genet* 2004;13:159–70.
- [80] Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, et al. Intra-neuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci* 2006;26:10129–40.
- [81] Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kaye R, et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Aβ and synaptic dysfunction. *Neuron* 2003;39:409–21.
- [82] Sturchler-Pierrat C, Abramowski D, Duke M, Wiederhold KH, Mistl C, Rothacher S, et al. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc Natl Acad Sci U S A* 1997;94:13287–92.
- [83] Davis J, Xu F, Deane R, Romanov G, Previti ML, Zeigler K, et al. Early-onset and robust cerebral microvascular accumulation of amyloid beta-protein in transgenic mice expressing low levels of a vasculotropic Dutch/Iowa mutant form of amyloid beta-protein precursor. *J Biol Chem* 2004;279:20296–306.
- [84] Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 1999;400:173–7.
- [85] Wilcock DM, Alamed J, Gottschall PE, Grimm J, Rosenthal A, Pons J, et al. Deglycosylated anti-amyloid-beta antibodies eliminate cognitive deficits and reduce parenchymal amyloid with minimal vascular consequences in aged amyloid precursor protein transgenic mice. *J Neurosci* 2006;26:5340–6.
- [86] Wilcock DM, Rojiani A, Rosenthal A, Subbarao S, Freeman MJ, Gordon MN, et al. Passive immunotherapy against Aβ in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage. *J Neuroinflammation* 2004;1:24.
- [87] Karlinski RA, Rosenthal A, Alamed J, Ronan V, Gordon MN, Gottschall PE, et al. Deglycosylated anti-Aβ antibody dose-response effects on pathology and memory in APP transgenic mice. *J Neuroimmune Pharmacol* 2008;3:187–97.
- [88] Racke MM, Boone LI, Hepburn DL, Parsadainian M, Bryan MT, Ness DK, et al. Exacerbation of cerebral amyloid angiopathy-associated microhemorrhage in amyloid precursor protein transgenic mice by immunotherapy is dependent on antibody recognition of deposited forms of amyloid beta. *J Neurosci* 2005;25:629–36.
- [89] Burbach GJ, Vlachos A, Ghebremedhin E, Del Turco D, Coomaraswamy J, Staufenbiel M, et al. Vessel ultrastructure in APP23 transgenic mice after passive anti-Aβ immunotherapy and subsequent intracerebral hemorrhage. *Neurobiol Aging* 2007;28:202–12.
- [90] Petrushina I, Ghochikyan A, Mkrtichyan M, Mamikonyan G, Movsesyan N, Ajdari R, et al. Mannan-Aβ28 conjugate prevents Aβ-plaque deposition, but increases microhemorrhages in the brains of vaccinated Tg2576 (APPsw) mice. *J Neuroinflammation* 2008;5:42.
- [91] Wilcock DM, Colton CA. Immunotherapy, vascular pathology, and microhemorrhages in transgenic mice. *CNS Neurol Disord Drug Targets* 2009;8:50–64.
- [92] Wisniewski T, Boutajangout A. Immunotherapeutic approaches for Alzheimer's disease in transgenic mouse models. *Brain Struct Funct* 2010;214:201–18.
- [93] Tabira T. Immunization therapy for Alzheimer disease: a comprehensive review of active immunization strategies. *Tohoku J Exp Med* 2010;220:95–106.
- [94] Thakker DR, Weatherspoon MR, Harrison J, Keene TE, Lane DS, Kaemmerer WF, et al. Intracerebroventricular amyloid-beta antibodies reduce cerebral amyloid angiopathy and associated microhemorrhages in aged Tg2576 mice. *Proc Natl Acad Sci U S A* 2009;106:4501–6.
- [95] Weller RO, Boche D, Nicoll JA. Microvasculature changes and cerebral amyloid angiopathy in Alzheimer's disease and their potential impact on therapy. *Acta Neuropathol* 2009;118:87–102.
- [96] Weller RO, Subash M, Preston SD, Mazanti I, Carare RO. Perivascular drainage of amyloid-beta peptides from the brain and its failure in cerebral amyloid angiopathy and Alzheimer's disease. *Brain Pathol* 2008;18:253–66.
- [97] Carare RO, Bernardes-Silva M, Newman TA, Page AM, Nicoll JA, Perry VH, et al. Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroimmunology. *Neuropathol Appl Neurobiol* 2008;34:131–44.
- [98] Schley D, Carare-Nnadi R, Please CP, Perry VH, Weller RO. Mechanisms to explain the reverse perivascular transport of solutes out of the brain. *J Theor Biol* 2006;238:962–74.
- [99] Weller RO, Massey A, Kuo YM, Roher AE. Cerebral amyloid angiopathy: accumulation of Aβ in interstitial fluid drainage pathways in Alzheimer's disease. *Ann N Y Acad Sci* 2000;903:110–7.
- [100] Weller RO, Massey A, Newman TA, Hutchings M, Kuo YM, Roher AE. Cerebral amyloid angiopathy: amyloid beta accumulates in putative interstitial fluid drainage pathways in Alzheimer's disease. *Am J Pathol* 1998;153:725–33.
- [101] Luo F, Rustay NR, Seifert T, Roesner B, Hradil V, Hillen H, et al. Magnetic resonance imaging detection and time course of cerebral microhemorrhages during passive immunotherapy in living amyloid precursor protein transgenic mice. *J Pharmacol Exp Ther* 2010;335:580–8.
- [102] Meyer-Luehmann M, Mora JR, Mielke M, Spires-Jones TL, de Calignon A, von Andrian UH, et al. T cell mediated cerebral hemorrhages and microhemorrhages during passive Aβ immunization in APPPS1 transgenic mice. *Mol Neurodegener* 2011;6:22.
- [103] Domnitz SB, Robbins EM, Hoang AW, Garcia-Alloza M, Hyman BT, Rebeck GW, et al. Progression of cerebral amyloid angiopathy in transgenic mouse models of Alzheimer disease. *J Neuropathol Exp Neurol* 2005;64:588–94.
- [104] Garcia-Alloza M, Robbins EM, Zhang-Nunes SX, Purcell SM, Betensky RA, Raju S, et al. Characterization of amyloid deposition in the APPsw/PS1dE9 mouse model of Alzheimer disease. *Neurobiol Dis* 2006;24:516–24.
- [105] Robbins EM, Betensky RA, Domnitz SB, Purcell SM, Garcia-Alloza M, Greenberg C, et al. Kinetics of cerebral amyloid angiopathy progression in a transgenic mouse model of Alzheimer disease. *J Neurosci* 2006;26:365–71.