Inflammatory aspects in the creation and rupture of brain AVMs

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Helen Kim, MPH, PhD
Associate Professor
Center for Cerebrovascular Research
Departments of Anesthesia, Epidemiology & Biostatistics
University of California, San Francisco
helen.kim2@ucsf.edu

Outline

- Introduce brain AVMs
- Working hypothesis
- Evidence for inflammatory involvement in bAVM
  - Genetic studies
  - Tissue studies
  - Animal studies
- Markers of inflammation
- Therapeutic studies of inflammation

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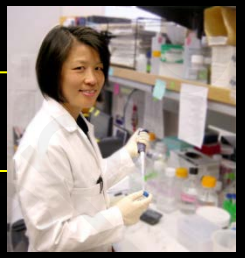
BAVM: *Research Approaches*

Human

Laboratory



Epidemiology

Genetics

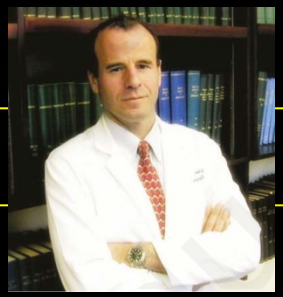
Cell Culture
Primary and cell line,
siRNA gene knockdown,
gene overexpression,
growth factor stimulation
functional analysis

Mouse Model
KO, conditional KO,
transgenic mice,
viral vector mediated gene
KO and overexpression

Risk Stratification

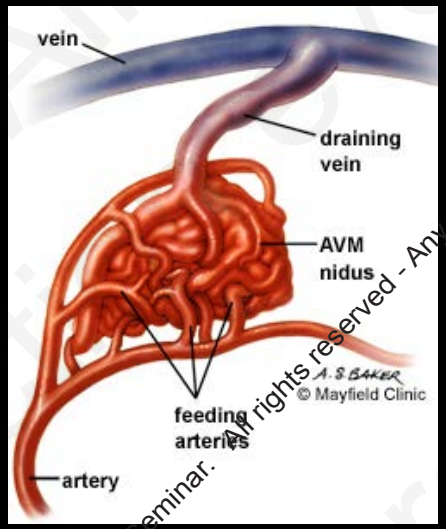
Etiopathogenesis

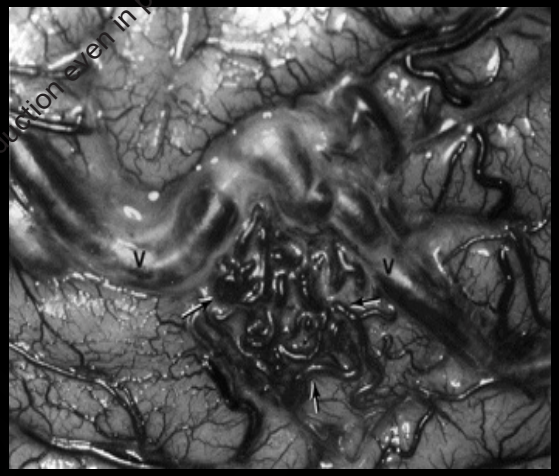
Therapy Development

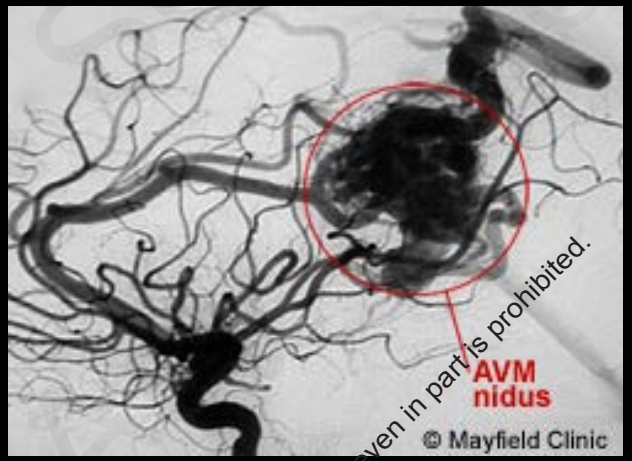


CCR UCSF *center for cerebrovascular research*

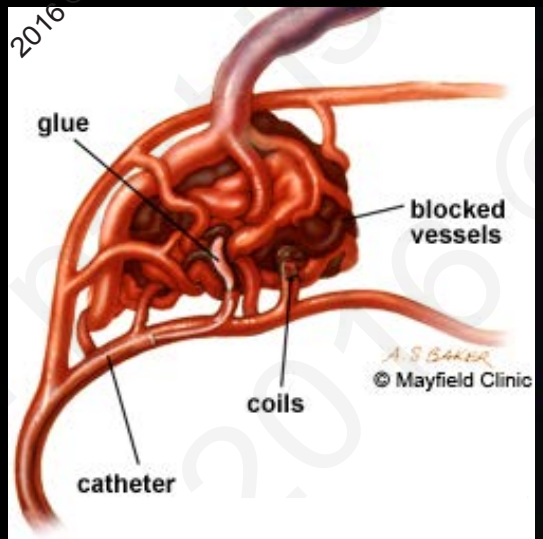
Brain Arteriovenous Malformation

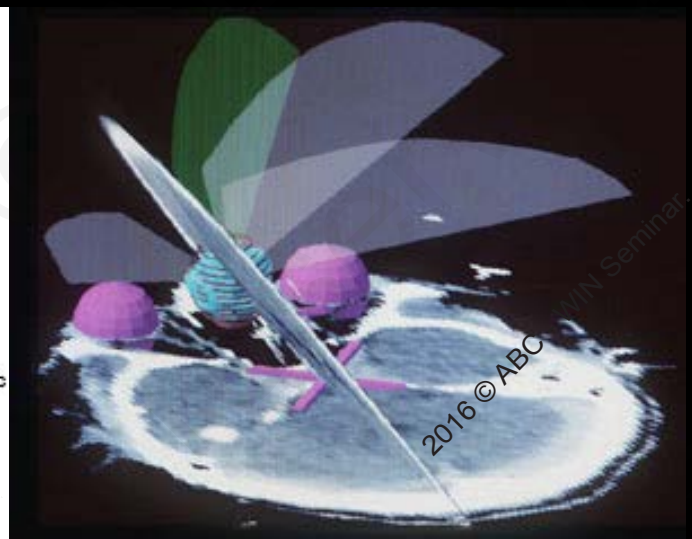


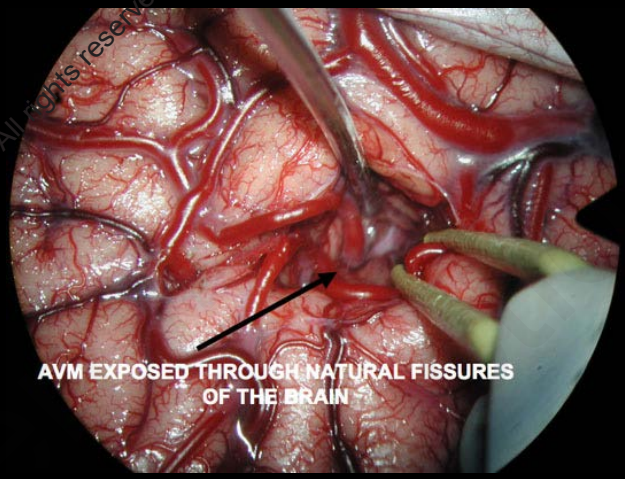




Procedural Treatment Options







Epidemiology of BAVM

- Rare disease affecting both genders
  - Prevalence: 10 - 18 / 100,000
  - Incidence: 1.3 / 100,000 P-Y (Gabriel, *Stroke* 2010)
- Mean age of detection between 20-40 yrs
- Associated with neurological morbidity/mortality
  - 30-50% morbidity
  - 10-30% mortality, 1-3% mortality rate per year after ICH
- Primary presenting symptom is ICH (50%)
  - Seizure (~25%), headache (~10%), neurological deficit
  - Incidental 1/2000 (0.05%) (Morris, *BMJ* 2009)

Accurate predictors are needed to weigh risks-benefits

Treatment risk

Procedural complications
Morbidity and death

Natural history risk

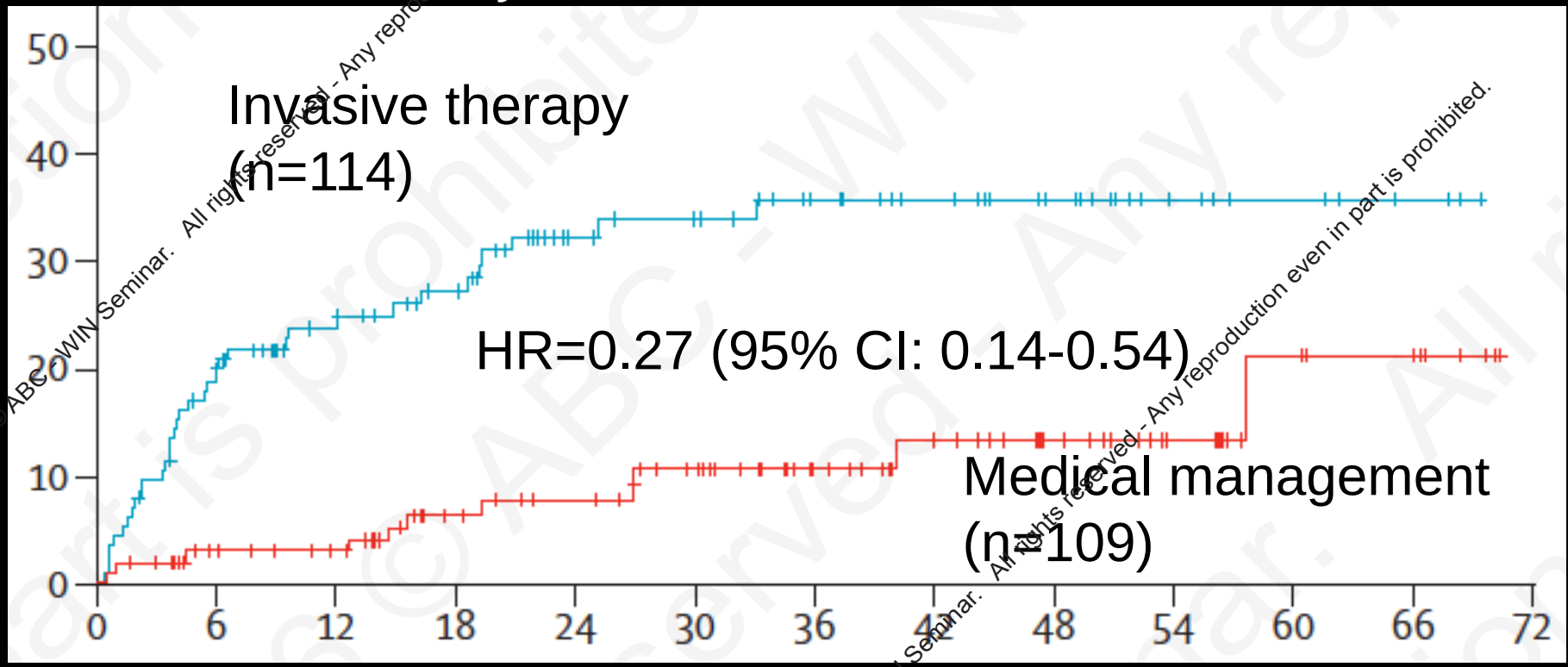
Intracranial hemorrhage
Seizures, deficits

ARUBA: A Randomized Trial of Unruptured Brain AVM
(Mohr JP et al., *Lancet* 2014)

Scottish Audit of Intracranial Vascular Malformations
(Al-Shahi Salman R et al., *JAMA* 2014)

ARUBA: A Randomized Trial of Unruptured Brain AVMs

Intent-to-treat analysis



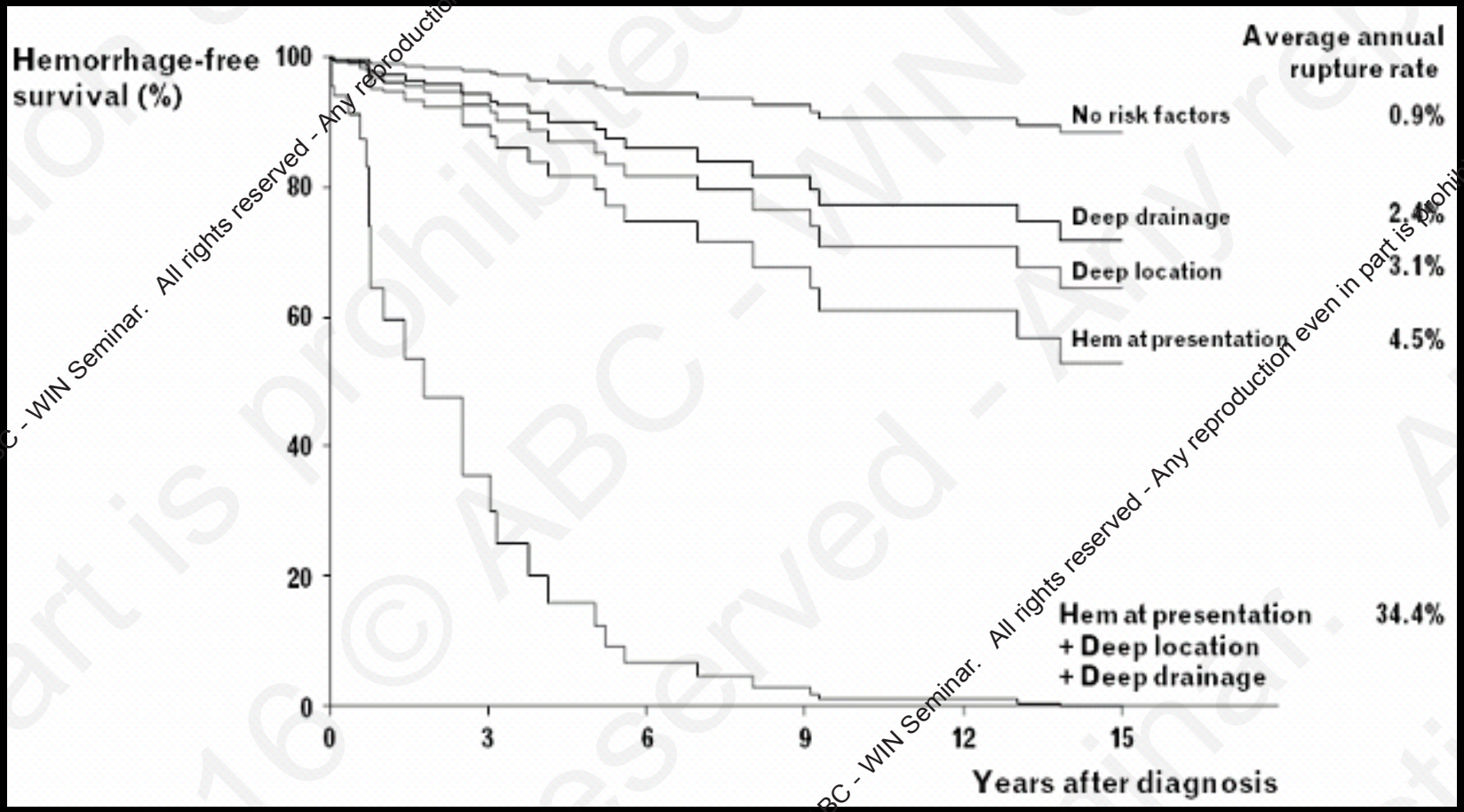
ICH: 2.2% (0.9-4.5)

Mohr JP et al., *Lancet* 2014

Risk factors for AVM hemorrhage

- Previous hemorrhage
- Demographic factors
  - Female gender
  - Increasing age
  - Hispanic race/ethnicity
- Angioarchictectural
  - Deep venous drainage
  - Deep location
  - Infratentorial
  - AVM size
  - Associated aneurysms
- Genetic factors?
  - Inflammatory genes (IL1B, TNFA, APOE)
- Silent hemorrhages?
  - Microhemorrhage in tissue and MRI

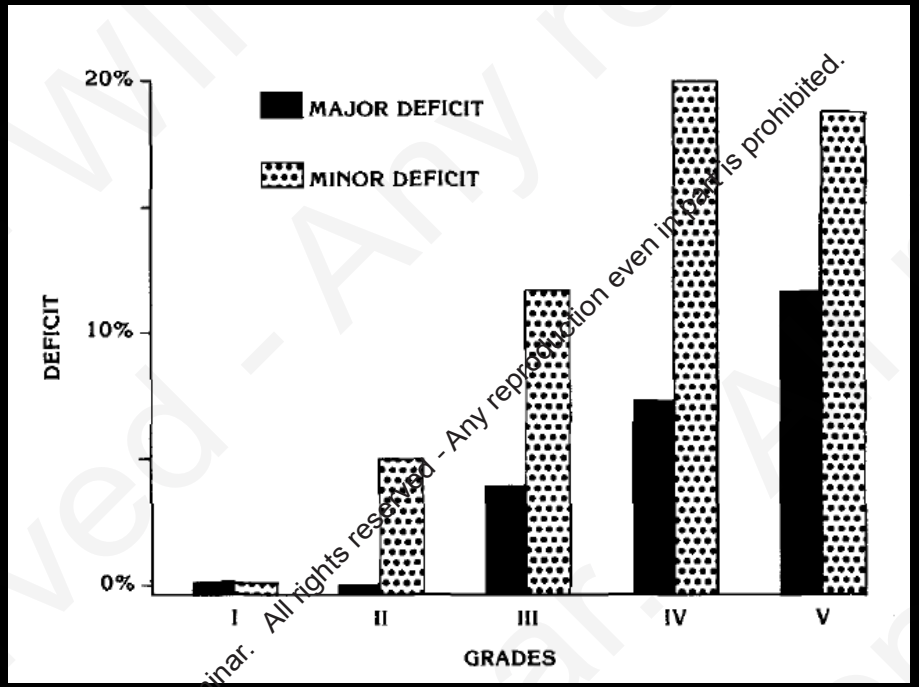
Risk of AVM hemorrhage in the natural course varies by risk factors



Stapf C et al. *Curr Opin Neurol* 2006

Higher Spetzler-Martin (SM) and SM-Supplemented scores correspond to greater surgical deficits

SM Grade	SM-Supplemented	Points Assigned
AVM size	Age	
small (<3cm)	<20yr	1
medium (3-6cm)	20-40yr	2
large (>6cm)	>40yr	3
Eloquence of adjacent brain	Diffuse border	
no	no	0
yes	yes	1
Venous drainage pattern	Unruptured AVM	
superficial only	no	0
deep	yes	1
TOTAL (1-5)	TOTAL (1-5)	COMBINED (2-10)



Spetzler and Martin, *J Neurosurg*. 1986
Lawton MT et al., *Neurosurg* 2010
Kim et al., *Stroke* 2012

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What causes AVM development?

- Presumed static congenital lesions
- AVMs can grow or regress (Du R, *J Neurosurg* 2007)
- AVMs can appear de novo (Mahajan A, *Neurosurg Rev* 2010)
- AVMs can reappear after treatment (Kader A, J Neurosurg 1996)

1987: 5 yo presents with ICH, resection of small AVF in posterior superficial sylvian fissure

1994: 12 yo presents with seizures, 3 cm AVM supplied by anterior choroidal

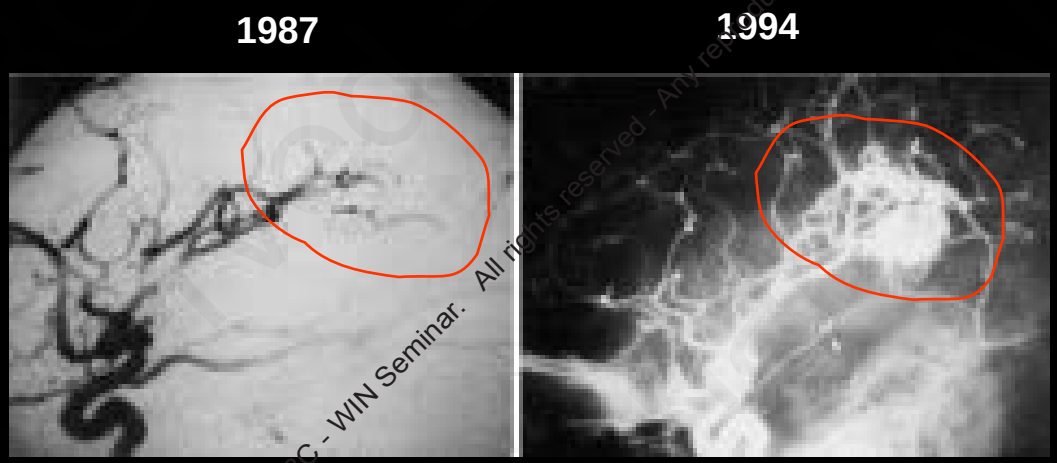


Fig 4. from Kader, *J Neurosurg* 1996

Familial AVM cases

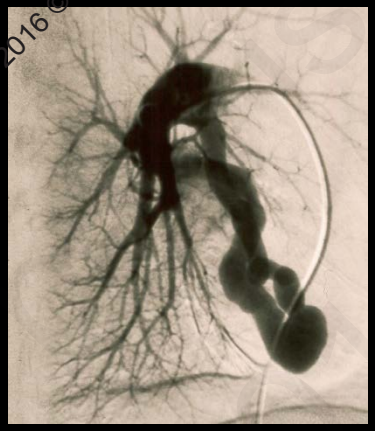
- Hereditary Hemorrhagic Telangiectasias
  - ENG, ALK1, SMAD4
  - ↓TGF-B activation, ↑VEGF production

- Capillary malformation-AVM syndrome
  - RASA1
  - p120 RasGAP, is a Ras GTPase–activating protein

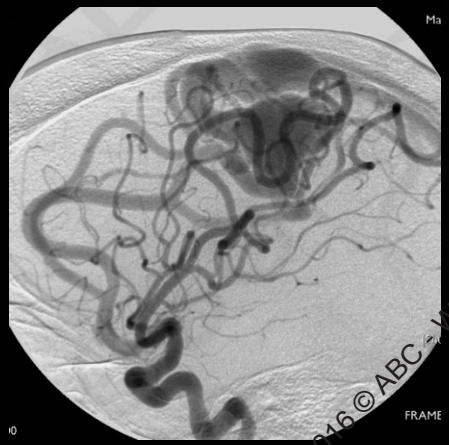
Hereditary Hemorrhagic Telangiectasia (HHT)
Rendu-Osler-Weber Syndrome

- Autosomal dominant disorder
- Mucocutaneous telangiectasia and visceral organ AVMs
- 80% of cases have functional haploinsufficiency of Endoglin (HHT1) or ALK1 (HHT2)

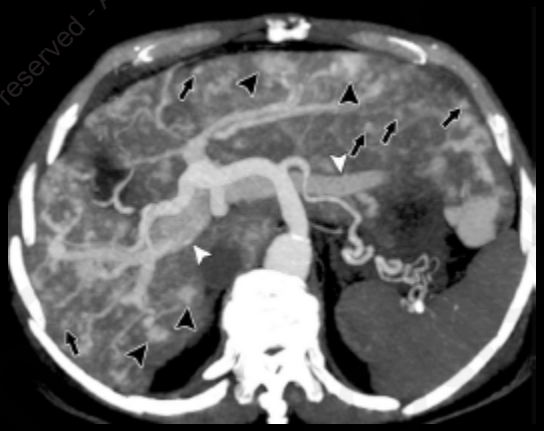
Lung AVM



Brain AVMs



Liver AVM



Working Hypothesis

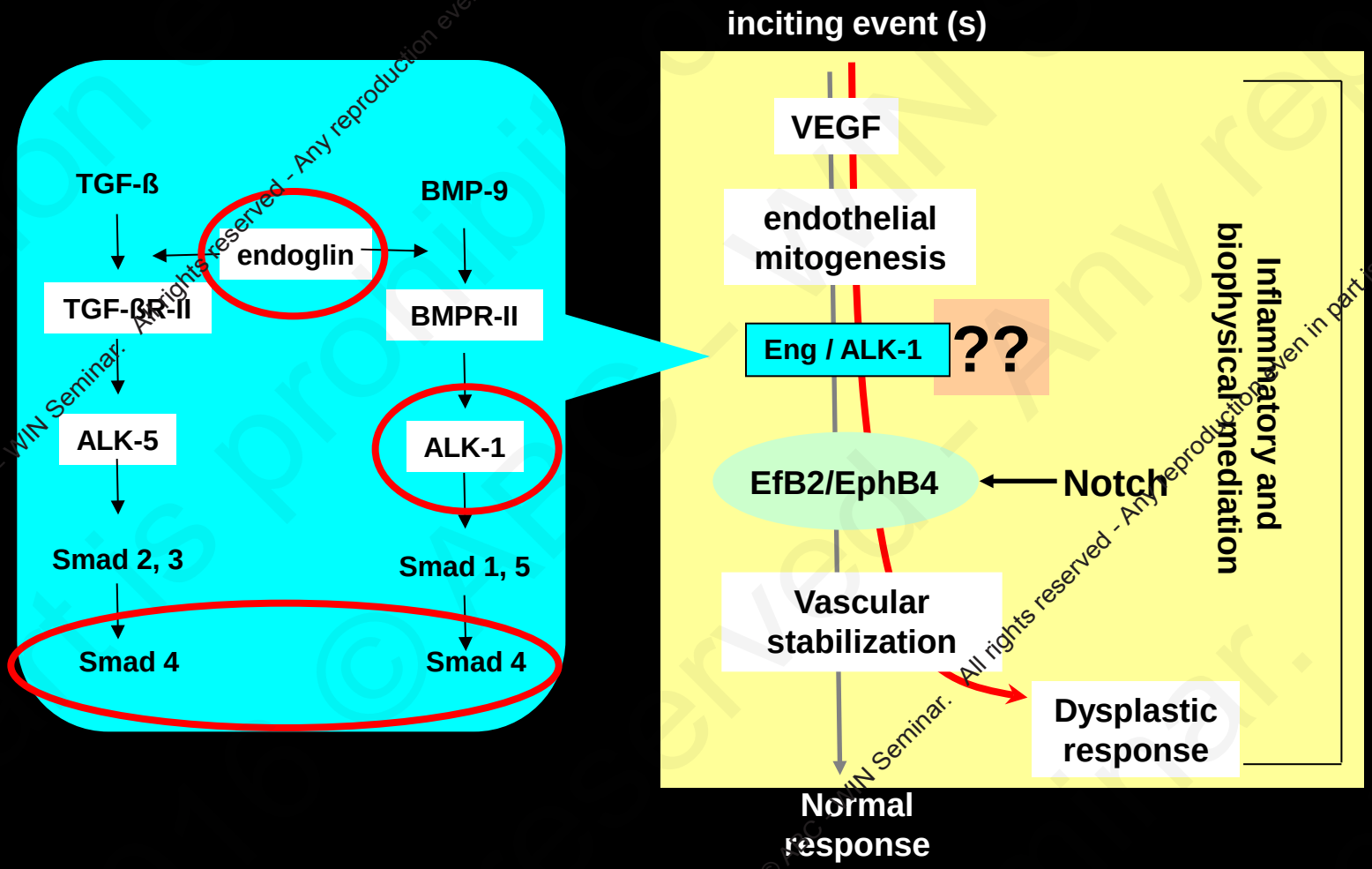
What is the "injury"?

— any pro-angiogenic or inflammatory stimulus

What determines disordered response to injury?

— genetic variation- necessary but not sufficient

BAVM Pathogenesis: "Response-to-Injury" Paradigm



Kim H et al, *Acta Neurochir* 2011

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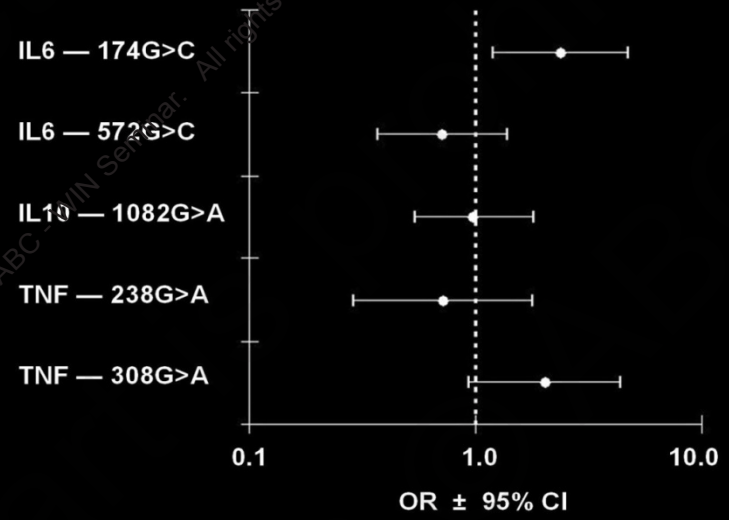
Genetic variants play a major role on the magnitude of inflammatory response

Polymorphism	Overexpressed Mediators	Outcome	Magnitude of Outcome	Reference
TNFα -238 AG	IL6	Increased ICH risk	4x increase	Achal, Stroke 2006
IL6 -174 GG	IL6, IL1, TNFα, IL8, MMP3, MMP9, MMP12	Increased ICH risk	3x increase	Pawlikowska, Stroke 2004
IL1A -889 CT or TT	IL1α	Increased AVM susceptibility	2.5x increase	Fontanella, Neurosurg 2012
IL1RN Allele 1	IL1RN	Increased AVM susceptibility	2x increase	Fontanella, Neurosurg 2012
IL1B -31 CC	IL1β	Both	2.7x increase	Kim, Cerebrovasc Dis 2009
IL1B -511 TT	IL1β	Both	2.6x increase	Kim, Cerebrovasc Dis 2009

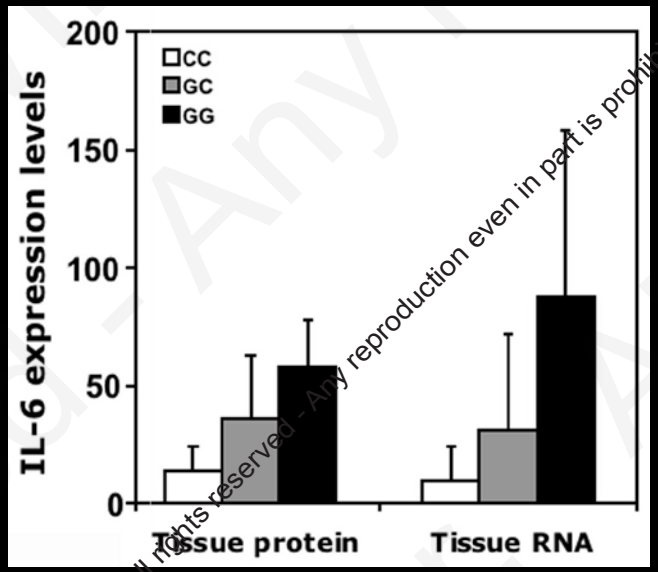
Mouchtouris et al., JCBFM 2015

Proinflammatory Interleukin-6 is associated with ICH and increased in BAVM

IL-6 genotype associated with ICH presentation

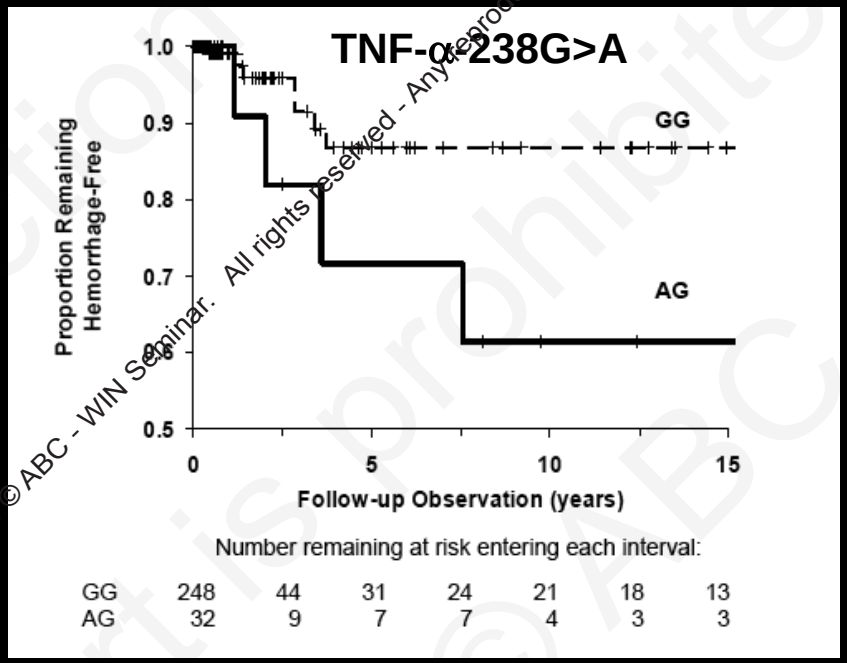


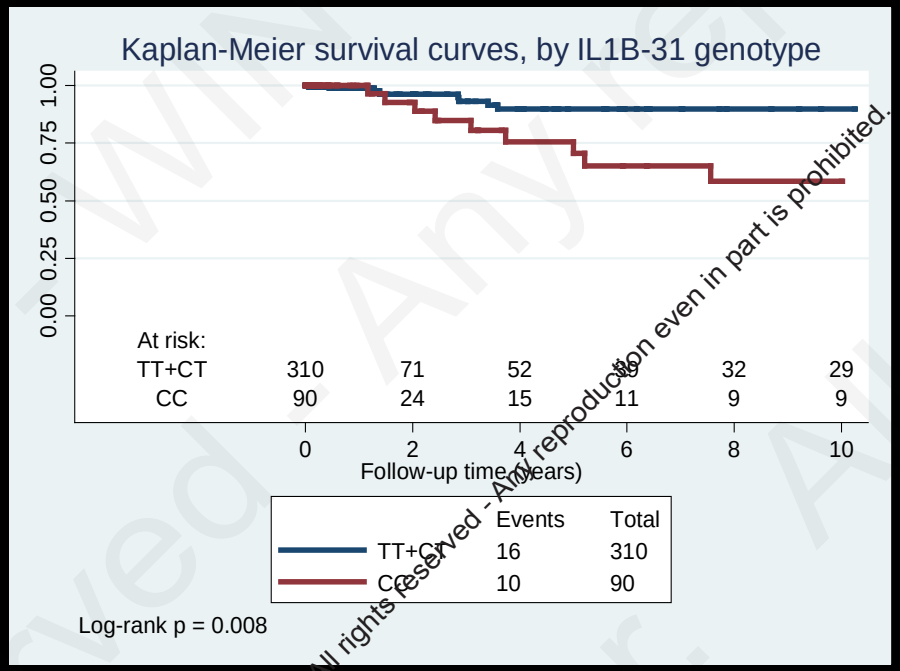
Pawlikowska L et al. *Stroke* 2004



Chen Y et al. *Ann Neurol* 2006

Polymorphisms in inflammatory genes predict AVM rupture in untreated course





HR=4.0 (95% CI=1.3-12.3)

HR = 2.7 (95% CI=1.1-6.6)

Achrol A et al., *Stroke* 2006

Kim H et al., Cerebrovasc Dis 2009

Gene Expression Profiling of BAVM

Tissue sample, 6 BAVM vs. 5 controls
35 mRNA up-regulation
37 mRNA down-regulation
Fold-change (FC) > 3, P<0.05

Tissue sample, 11 BAVM vs. 2 controls
49 mRNA up-regulation
25 mRNA down-regulation
FC > 300

Blood sample, 20 BAVM vs. 20 controls
33 mRNAs down-regulation
FC ≥ 1.2, FDR<0.05

Examples: PTX3, IL8, IL6, CXCL10, GBP1, CHRM3, CXCL1, IL1R2, CCL18, CCL13

Takagi Y et al., *Neurol Med Chir* 2014
Weinsheimer S et al., *Transl Stroke* 2011
Hashimoto T et al., *Neurosurg* 2004

Hashimoto. Genes expression 6 BAVM vs Control, Tissue

TABLE 3. Ranking of genes that were significantly up-regulated in brain arteriovenous malformations[a]

Accession no.	Gene (function)	Fold change	P value
D50918	Septin 6 (involved in cytokinesis [potential])	967.50	0.006
D11139	Tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)	376.76	
M55210	Laminin, γ1 (formerly LAMB2)	163.42	0.005
U88964	Interferon-stimulated gene (20 kD)	109.15	0.003
J03464	Collagen, Type I, α2 (Type I collagen is a member of Group I collagen [fibrillar-forming collagen])	102.90	0.005
AF013570	Myosin, heavy polypeptide 11, smooth muscle (muscle contraction)	87.99	0.005

Takagi. Genes expression 11 BAVM vs Control, Tissue

Gene symbol	Description	Category	Regulation
IL6	Homo sapiens interleukin 6 (interferon, beta 2) (IL6), mRNA	A, I	up
MMP9	Homo sapiens matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) (MMP9), mRNA	D(death-related)	up
LIF	Homo sapiens leukemia inhibitory factor (cholinergic differentiation factor) (LIF), Mrna	D(death-related)	up
SOD2	Homo sapiens superoxide dismutase 2, mitochondrial, mRNA (cDNA clone MGC: 21350 IMAGE: 4184203), complete cds	D(death-related)	up
BCL2A1	Homo sapiens BCL2-related protein A1 (BCL2A1), mRNA	D(death-related)	up

Weinsheimer. Genes expression 20 BAVM vs Control, Blood

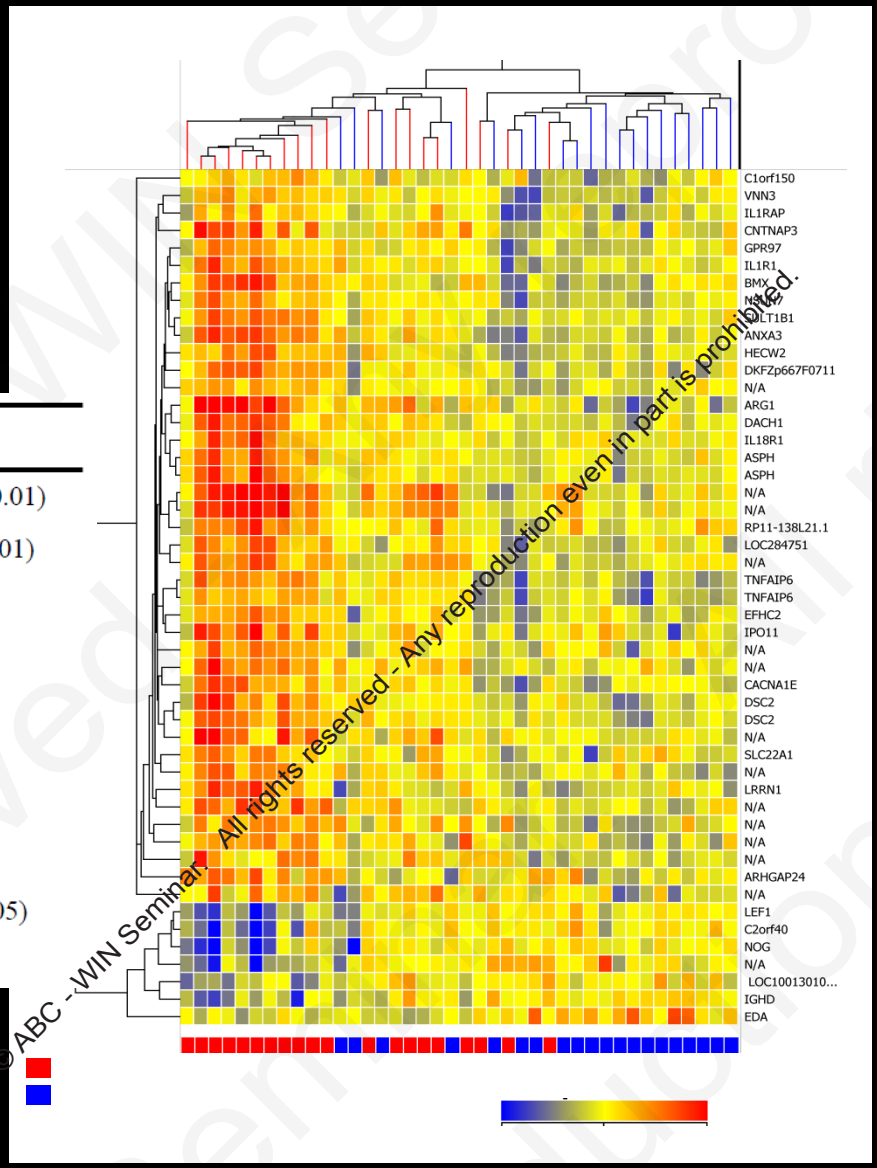
Gene symbol	Description	Fold change((FC) values control vs. unruptured	Category	Regulation
ARPC5	actin related protein 2/3 complex, subunit 5, 16kDa	1.28		Down
C1orf58	CDNA FLJ37597 fis, clone BRCOC2008225	1.37		Down
C9orf4	chromosome 9 open reading frame 4	1.27		Down
CBS	cystathionine-beta-synthase	1.75		Down
CHST7	carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 7	1.49		Down
COL2A1	collagen, type II, alpha 1	1.28		Down
EPHB1	EPH receptor B1	1.6		Down
ERGIC1	endoplasmic reticulum-golgi intermediate compartment (ERGIC) 1	1.36		Down
H3F3A	H3 histone, family 3A	1.24		Down
HECW1	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1	1.23		Down
HIST1H2BC	histone cluster 1, H2bc	1.89		Down
IDS	iduronate 2-sulfatase	1.34		Down
IL1RAP	interleukin 1 receptor accessory protein	2.12		Down

Gene Expression Profiling of BAVM

20 ruptured vs. 20 unruptured BAVM
896 mRNAs |FC| ≥ 1.2, FDR<0.05
49 mRNAs |FC| ≥ 2.0, FDR<0.05

Examples: TNFAIP6, IL1R1, IL1RAP

KEGG Biological[a] Pathways	Gene Count[b]	p[c]
Natural killer cell mediated cytotoxicity	23 (8)	< 0.001 (0.01)
T cell receptor signaling pathway	18 (8)	0.004 (0.01)
MAPK signaling pathway	40	0.004
Fc epsilon RI signaling pathway	14	0.01
VEGF signaling pathway	12	0.02
Antigen processing and presentation	(5)	(0.02)
B cell receptor signaling pathway	11	0.04
GnRH signaling pathway	15	0.04
Wnt signaling pathway	19 (8)	0.05 (0.05)
Notch signaling pathway	6	0.06



Weinsheimer S et al., *Transl Stroke* 2011

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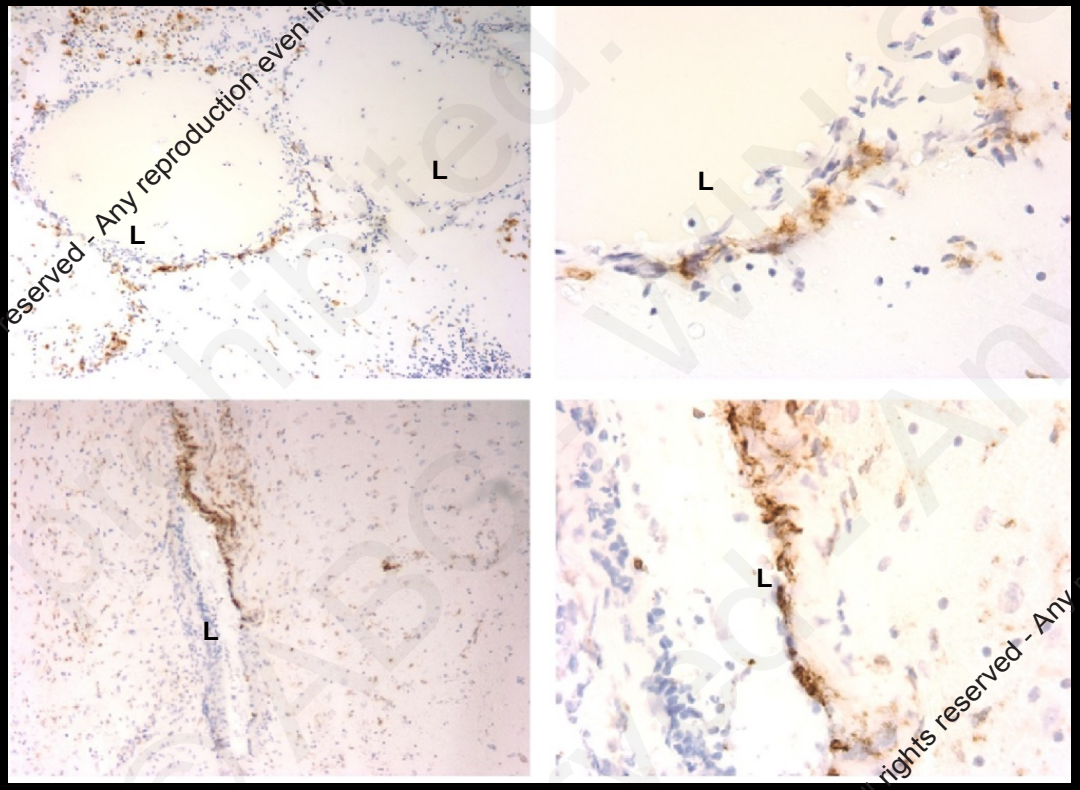
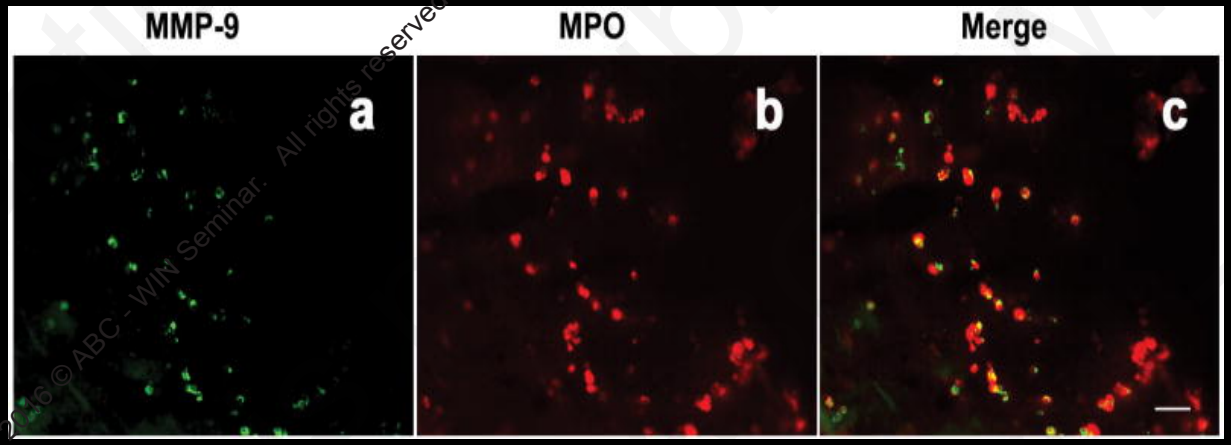
Inflammatory cells present in BAVM nidus

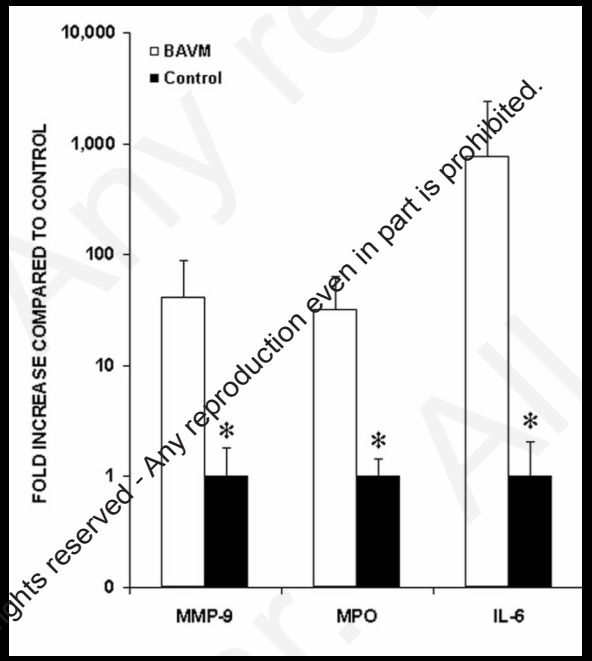


Macrophage (CD 68) in unruptured, non-embolized AVM specimens

Chen Y et al., *Neurosurgery* 2008
Chen Y et al., *Ann Neurol* 2006
Chen Y et al., *Front Biosci* , 2006

Neutrophils are a major source of MMP9 in BAVM tissue





Chen Y et al., *Neurosurg* 2008

Distinct distribution of T-lymphocytes in unruptured, non-embolized BAVM

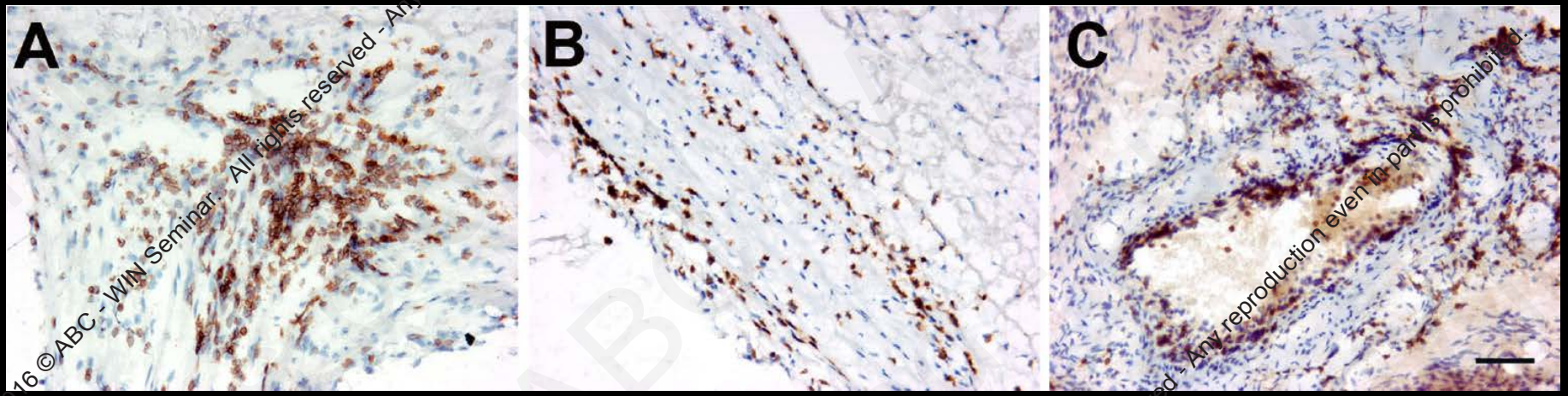
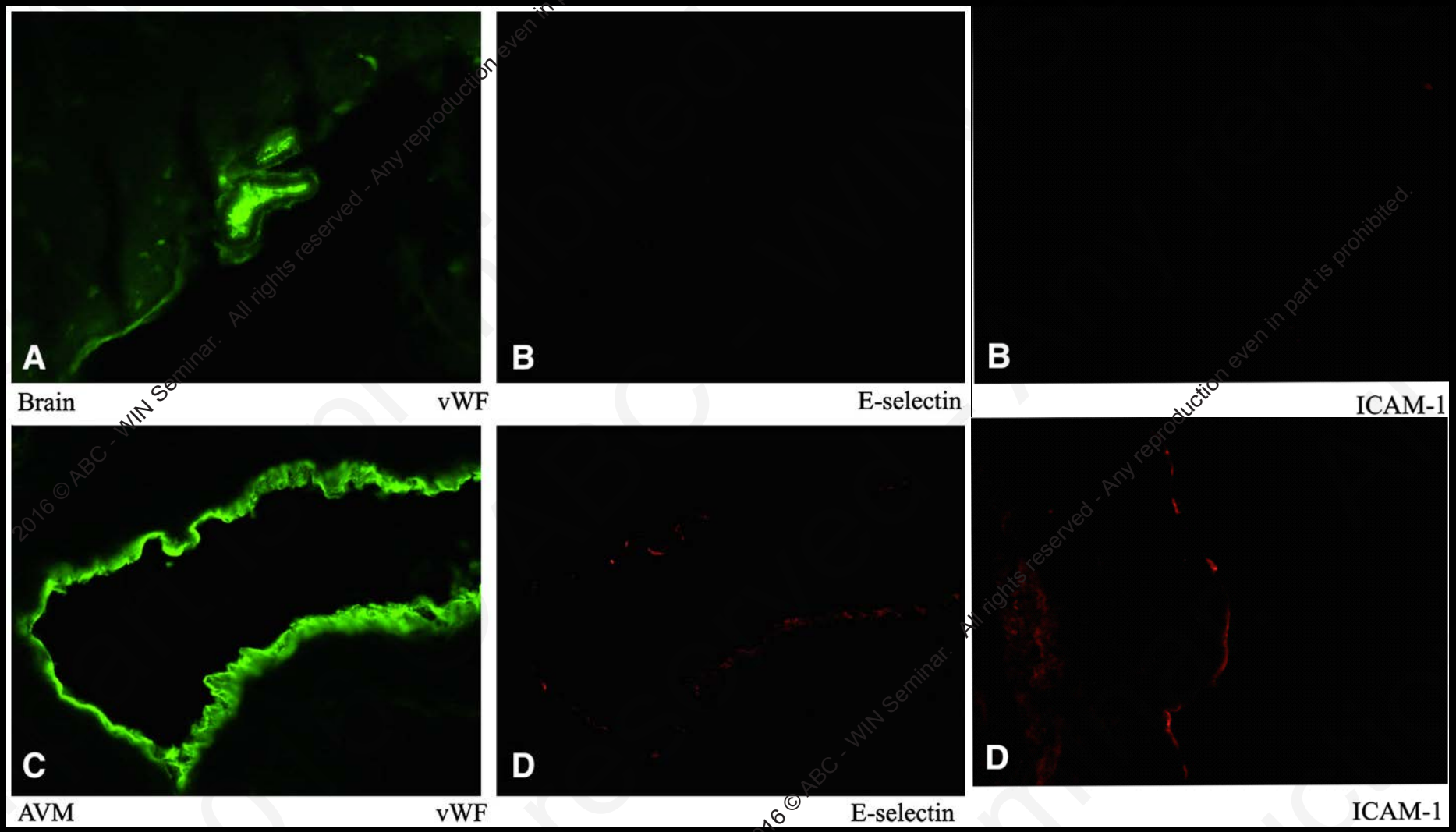


Figure 3.
Location of $CD3^+$ T-lymphocytes. T-lymphocytes were distributed in the perivascular region (**A**), in the vessel wall (**B**), and on the surface of the endothelial lining (**C**). Scale bar: 50μm.

Guo Y et al., *Neuroimmunol Neuroinflamm* 2014.

Upregulation of E-selectin and ICAM-1 in BAVM tissue



Storer KP, J Clin Neurosci 2008

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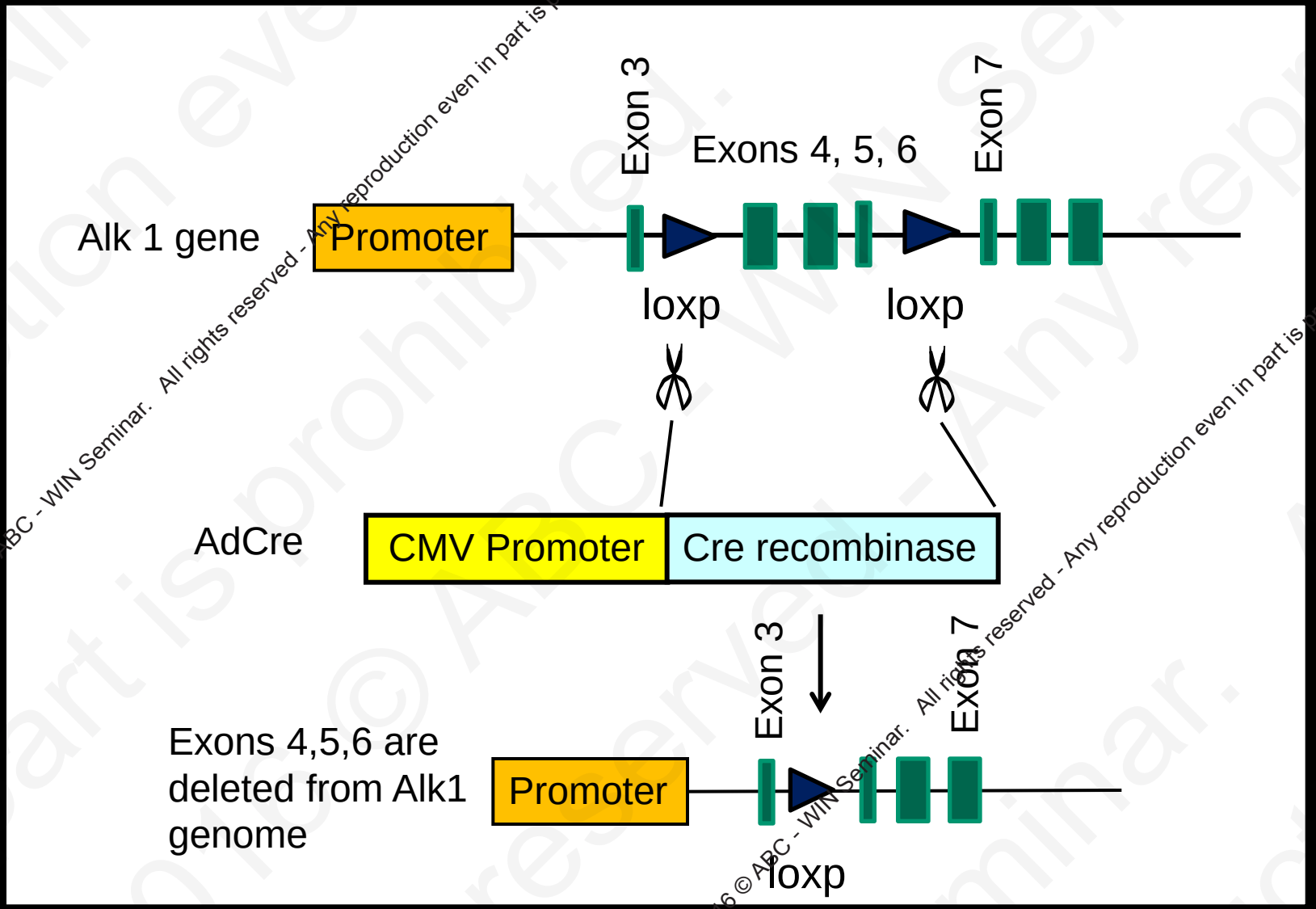
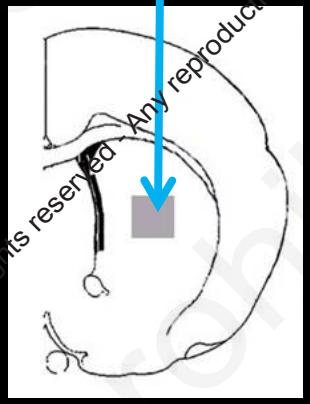
AdCre – Regional Conditional Deletion of *Alk1*



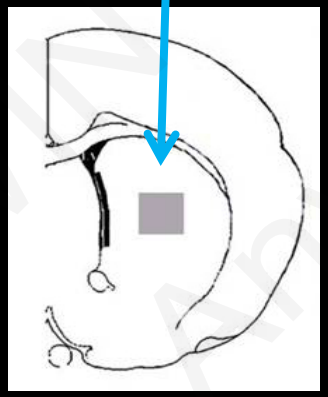
Alk1 Regional Conditional Deletion Plus VEGF Stimulation Results in Brain AVM

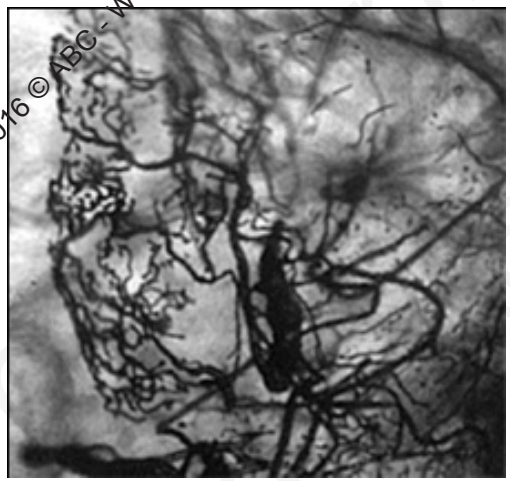
AdCre + AAV-VEGF

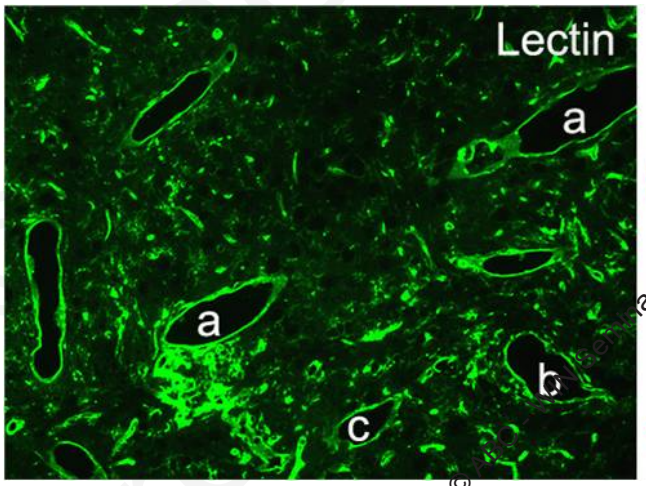


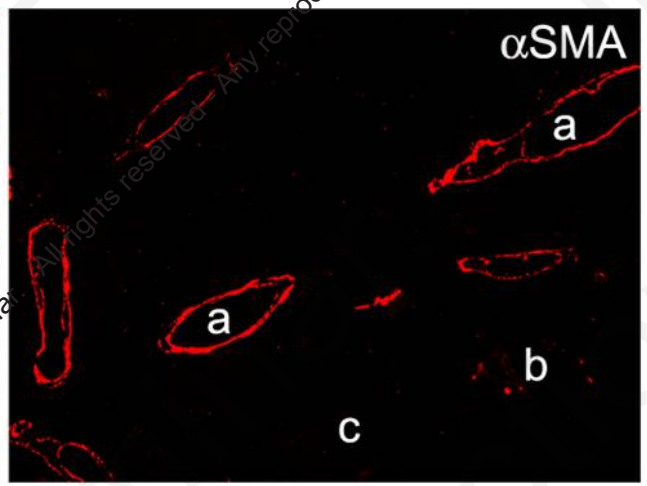
8 wks

Alk1$^{-/-}$ Angiogenesis



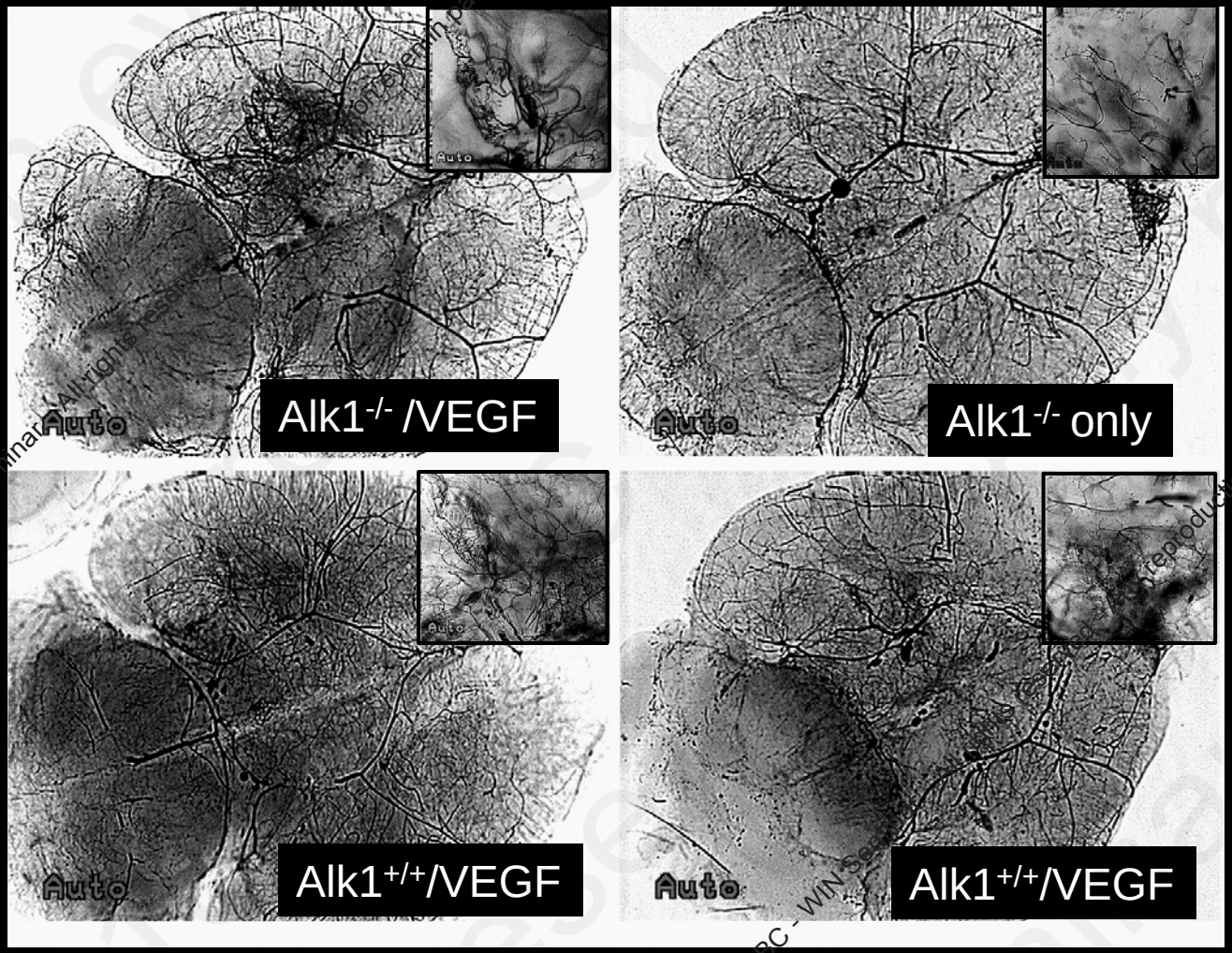






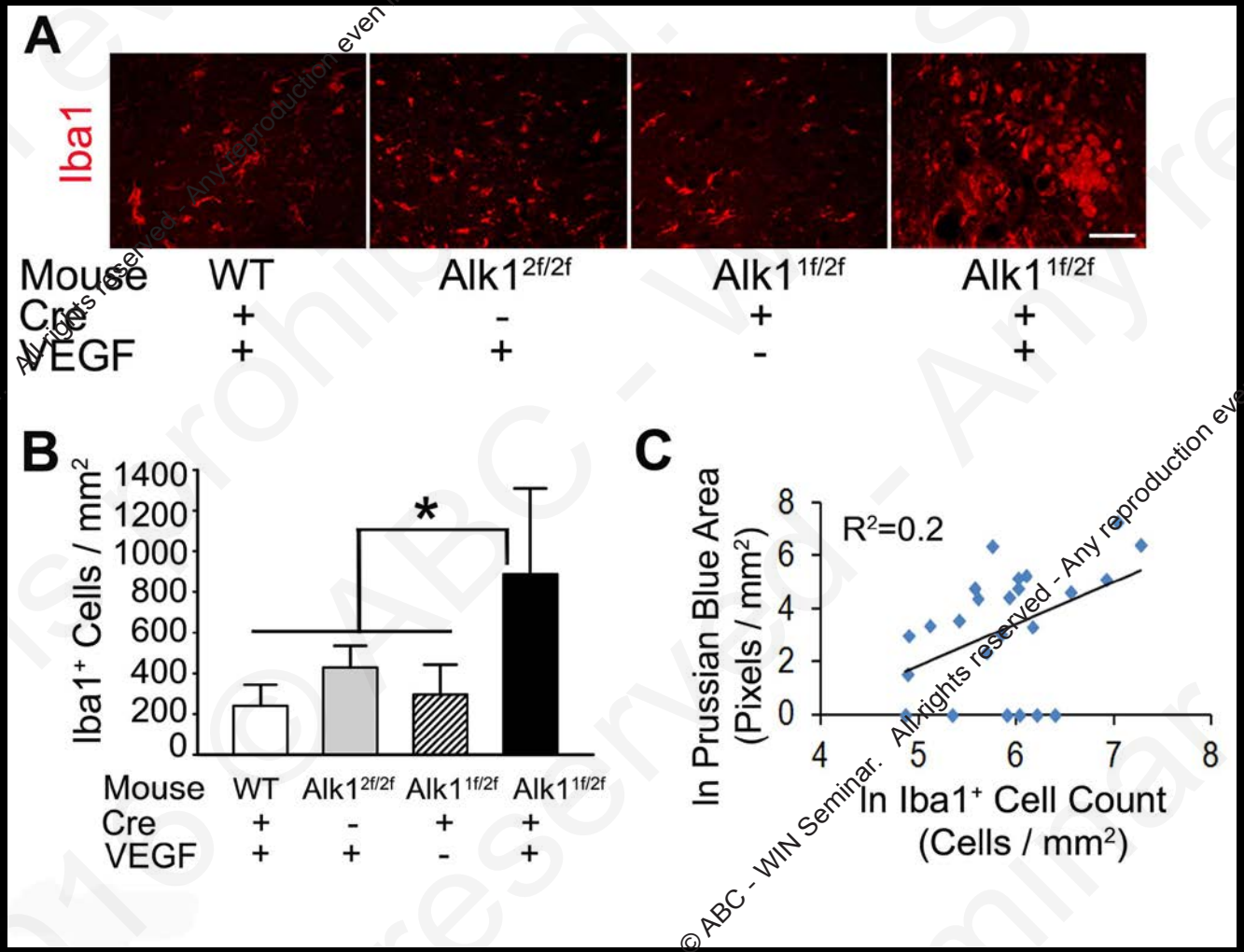
Walker et al. *Ann Neurology*, 2011

VEGF Stimulation is Necessary for Brain AVM Formation



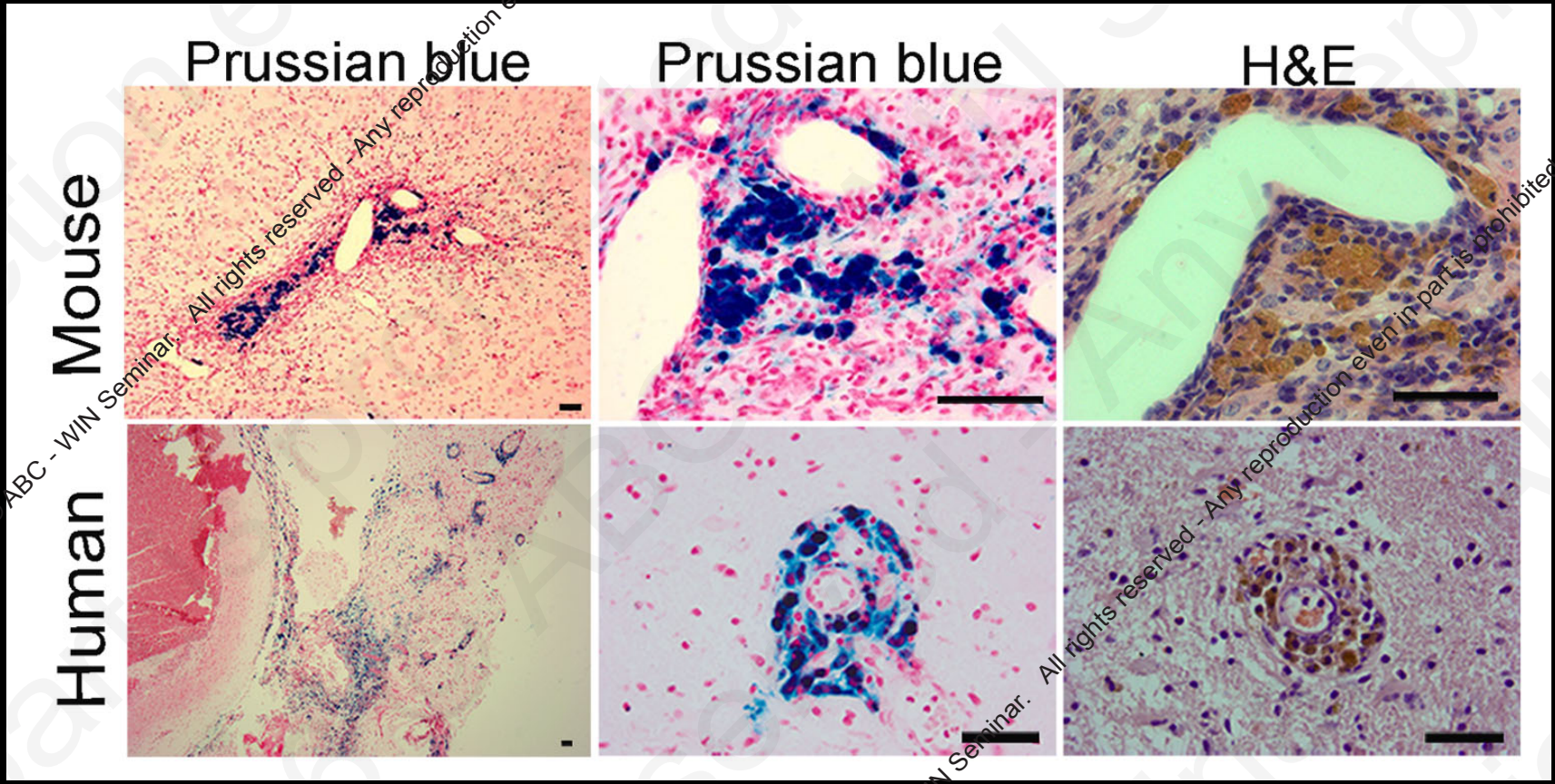
Walker et al. Ann Neurology, 2011

Increased macrophage/microglia infiltration in Alk1-deficient mouse brain



Chen W et al. ATVB, 2013

Microhemorrhage detected around dysplastic vessels in both mouse and human BAVM



Chen et al. ATVB, 2013

Outline

- Introduce brain AVMs
- Working hypothesis
- Evidence for inflammatory involvement in bAVM
  - Genetic studies
  - Tissue/blood studies
  - Animal studies
- Markers of inflammation
- Therapeutic studies of inflammation

Hemosiderin positivity is associated with index hemorrhage

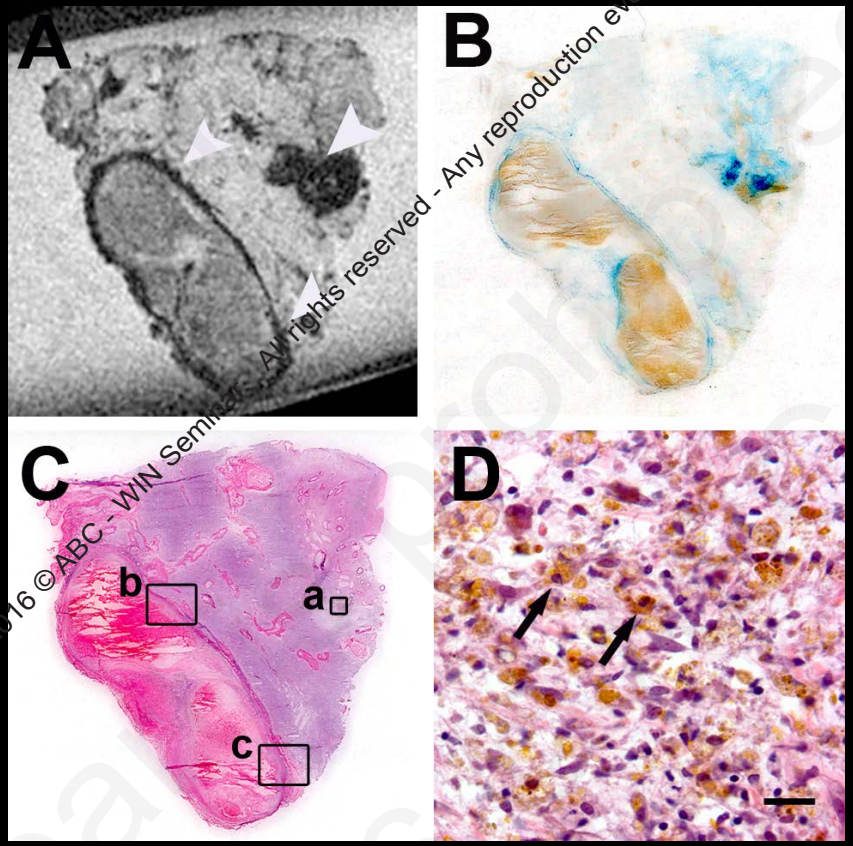


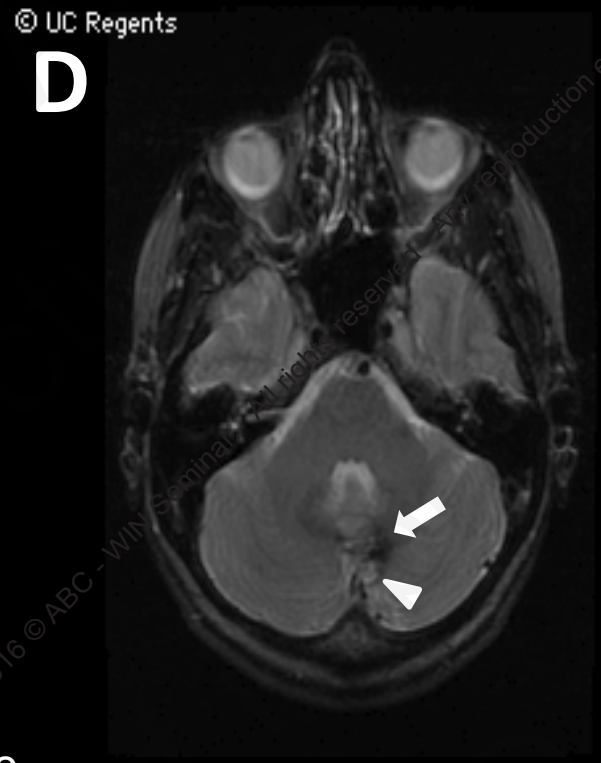
Table 3. Multivariable Associations for Association of Hemosiderin Positivity With Index ICH

	OR	Lower	Upper	*P*
Hemosiderin positive	3.64	1.11	12.00	0.034
Age at diagnosis (decade)	0.58	0.42	0.79	0.001
Sex, male	0.66	0.22	2.02	0.470
Deep only venous drainage	8.09	1.29	50.91	0.026
Maximal AVM size, cm	0.98	0.62	1.56	0.942
Deep location	0.36	0.05	2.75	0.326
Associated arterial aneurysm	3.25	1.24	18.90	0.023

Guo Y et al., *Stroke* 2012

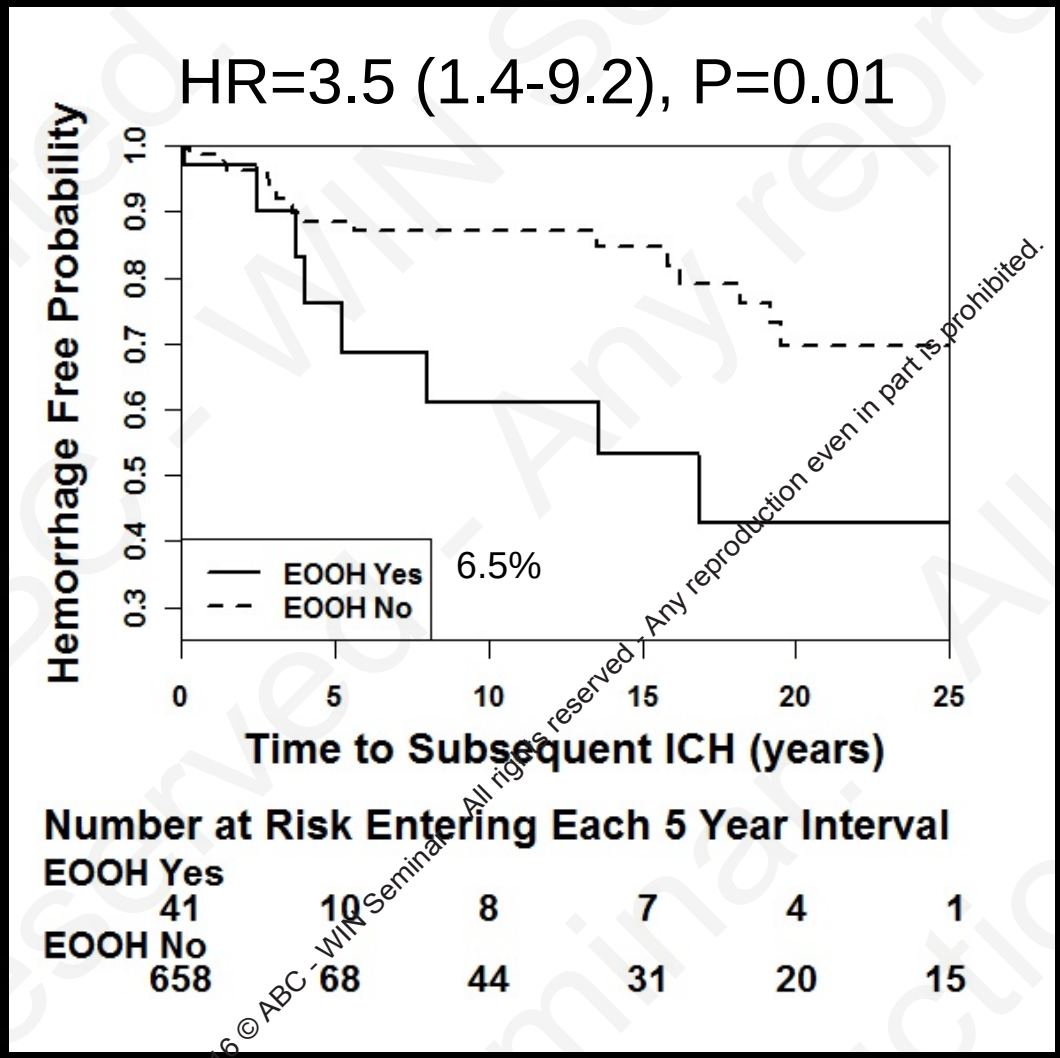
N=79, prevalence=36% (47% ruptured, 30% unruptured)
N=86, prevalence=48% (58% ruptured, 34% unruptured)

Neuroradiological Evidence of Old Hemorrhage (EOOH) predicts new ICH



0=none
1=probably none
2=not sure
3=probably yes
4=definitely yes

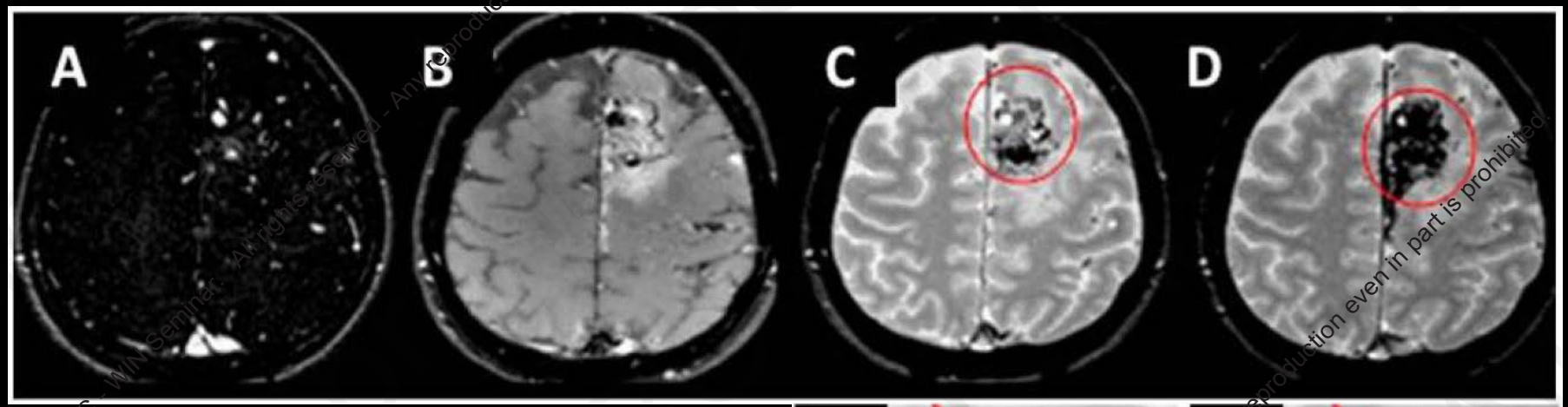
IRR=82.5%
kappa=0.62
P<0.001



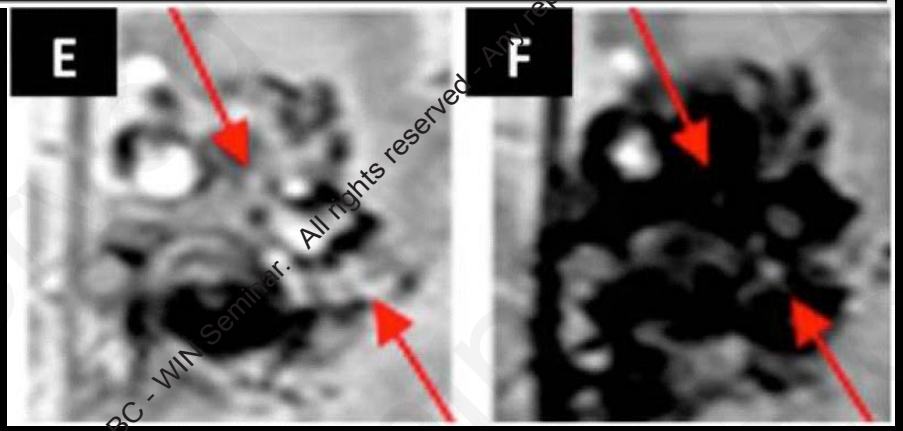
Guo Y et al., *Stroke* 2012

Ferumoxytol imaging: a novel imaging agent for inflammation

- Baseline MRI -> 5 mg/kg ferumoxytol -> 5d post-ferumoxytol MRI



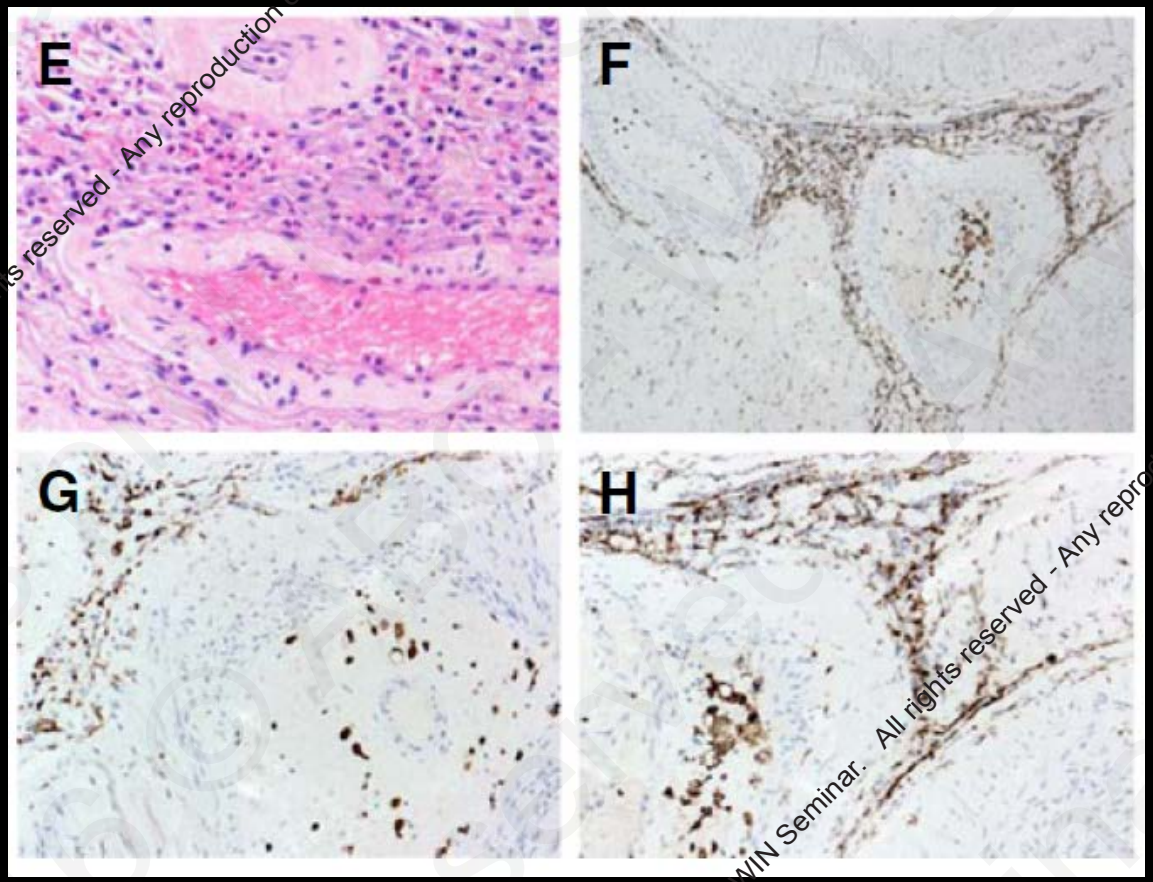
- Left frontal AVM dx in 2002 (A, angiogram; B, T1-weighted)
- Underwent GK in 2002 & 2007 and resection in 2010 (C, T2*GE)
- 5 day post-ferumoxytol imaging (D)
- Signal loss and blooming in nidus consistent with uptake of ferumoxytol (E and D)



CCR UCSF *center for cerebrovascular research*

Hasan et al., *TSR* 2012

Macrophage infiltration in BAVM wall



Hasan DM et al, *Transl Stroke Res* 2012

PET imaging for neuroinflammation

- Measures expression of translocated protein (TSPO) on activated microglia in brain
  - TSPO expression markedly upregulated in response to brain injury; normally low expression
- In vivo imaging tool for patterns of neuroinflammation in both preclinical and clinical studies of neurological diseases
- Limitations of older tracers include low brain permeability and high non-specific and plasma protein binding resulting in low signal-to-noise and lack of specificity

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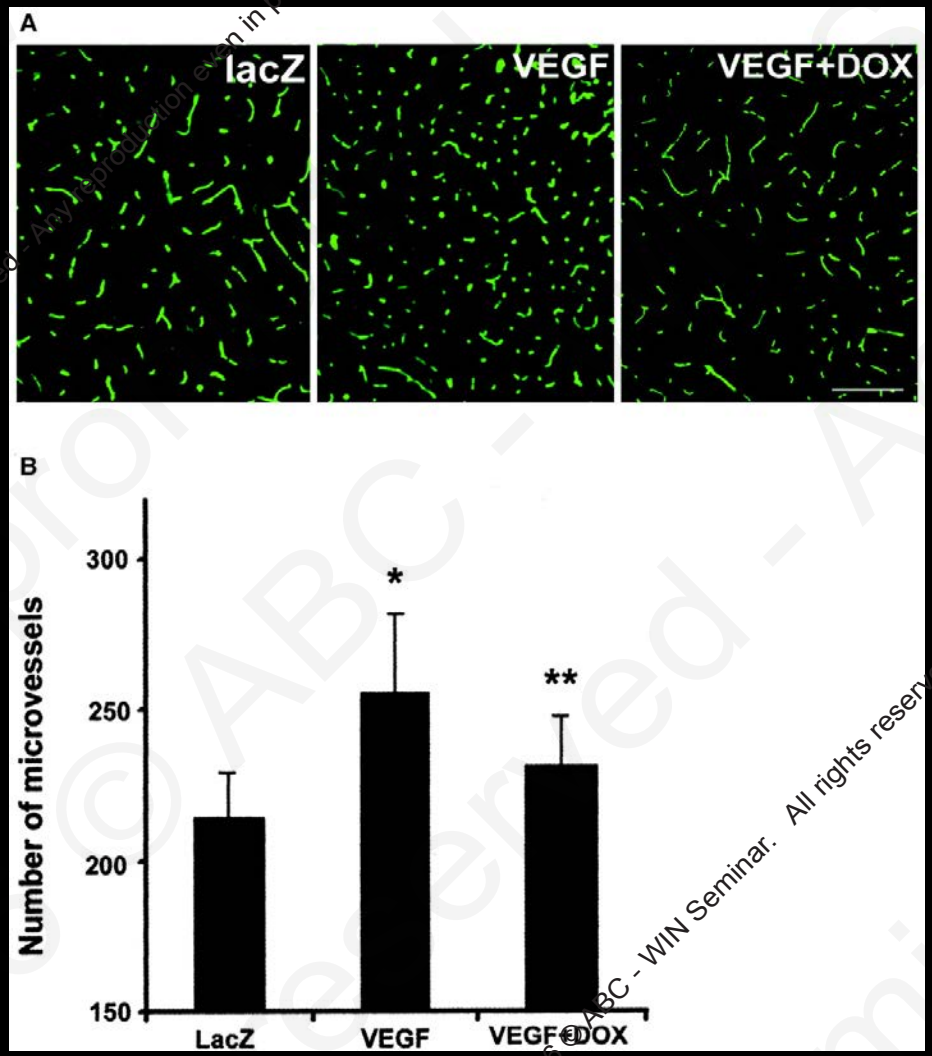
Therapy approaches in AVM

- Adjuvant to treatment options
  - Shrink AVM making treatment options easier
  - Speed up the obliteration process
  - Improve the overall obliteration rate
  - Achieve same obliteration rate at lower doses
- Stabilize vessel wall to reduce ICH (vasculostatic approach)

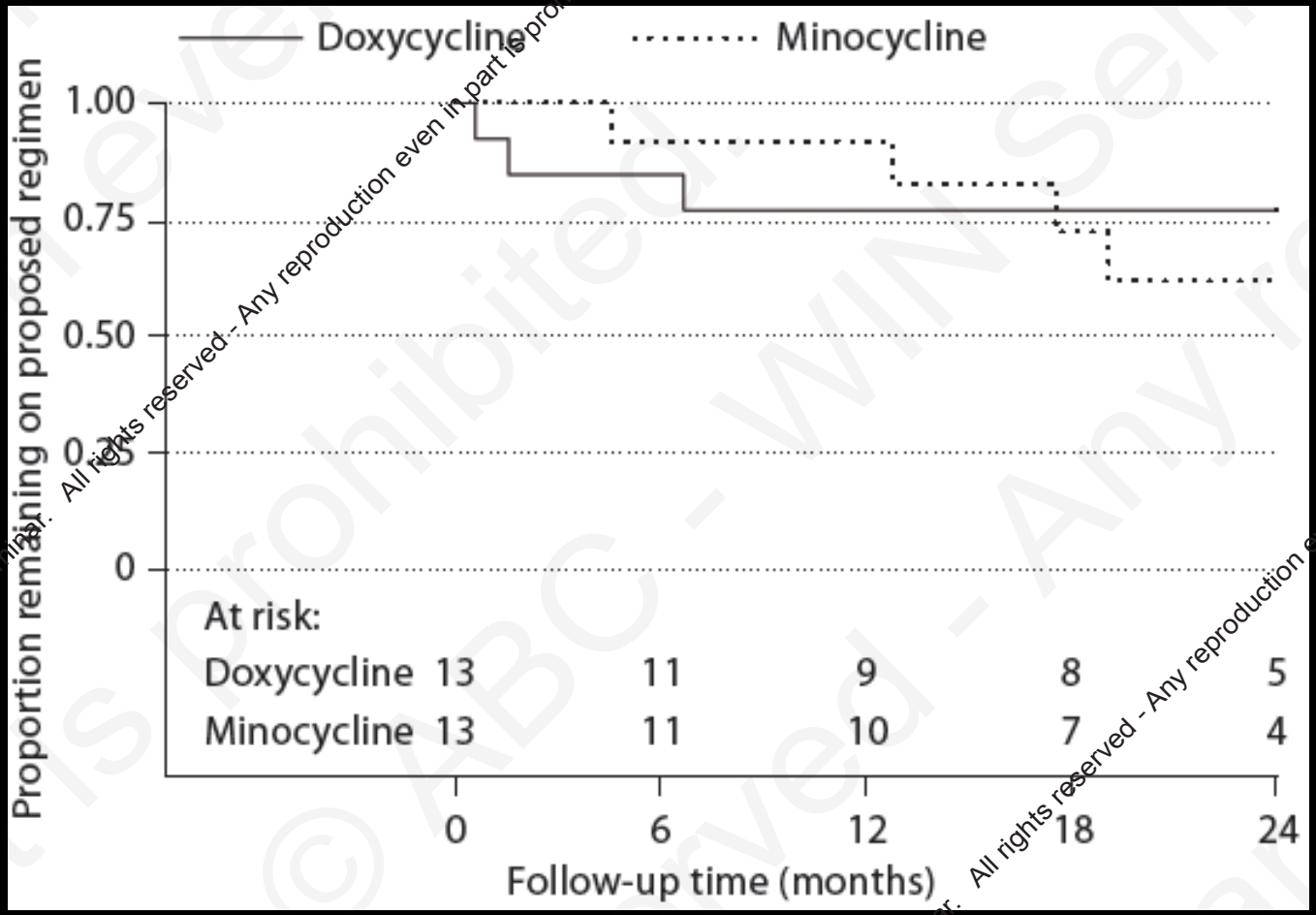
Anti-Inflammation

Doxycycline Treatment Reduces Angiogenesis in VEGF Treated Mouse Brain



Lee, C. Z. et al. *Stroke* 2004

Long-term tetracycline use is safe in AVM



- Photosensitivity (23%), vertigo (12%), yeast infection (8%), GI symptoms (8%), hyperpigmentation (4%), and allergic reaction (4%)
- 1 hemorrhage event in AVM (5.6%/yr) and 1 in aneurysm (5.8%/yr)

Frenzel T et al, *Cerebrovasc Dis* 2008

Summary

1. Invasive therapies are associated with considerable risks.
2. No specific medical therapy is available.
3. Inflammation plays an important role in BAVM pathogenesis in both formation and rupture.
4. Novel imaging and therapeutic approaches targeting inflammation exist.

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