Rottura dell'integrità della barriera: danni e vantaggi

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Disclosures of TERESA CALIMERI

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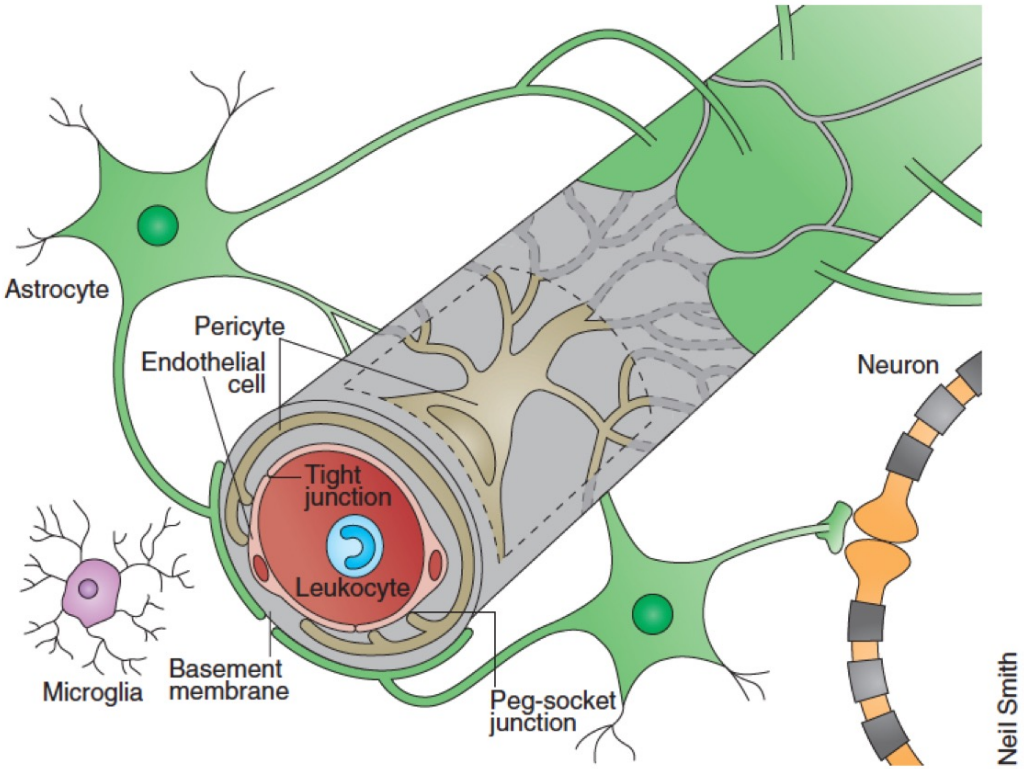
Sandoz	-	-	-	-	-	-	x
Gilead	-	-	-	-	-	-	x

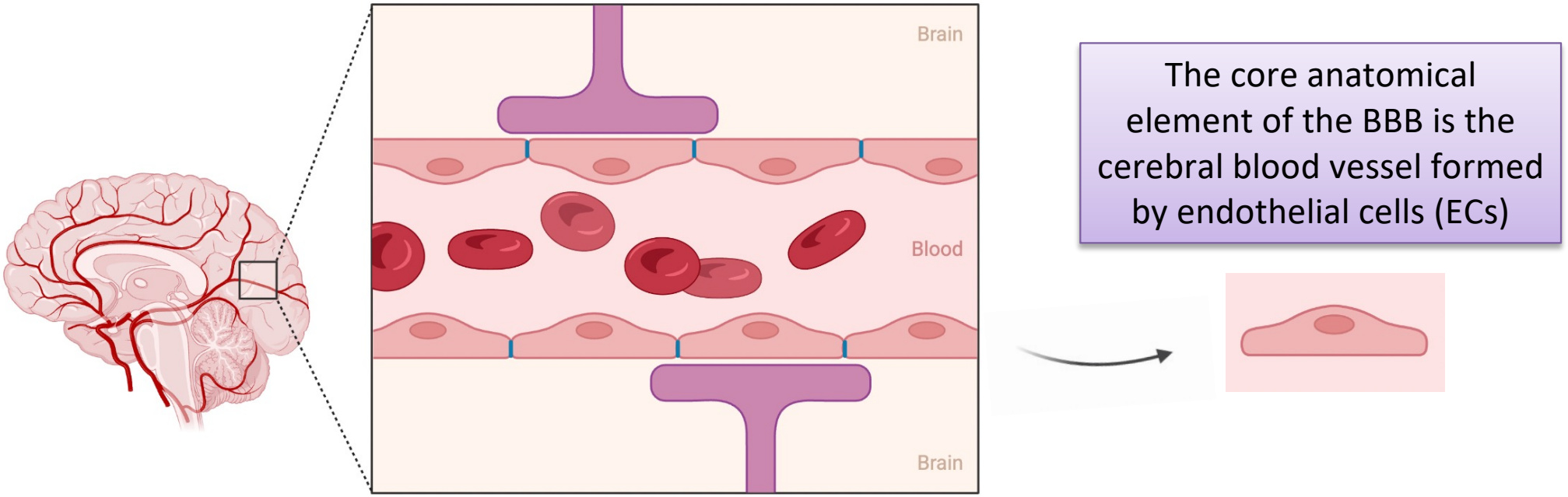
The Neurovascular Unit (NVU)



The BBB is part of the NVU, which represents an elaborate interplay of central and peripheral cells.

Obermeier B, Daneman R, Ransohoff RM. Nat Med. 2013

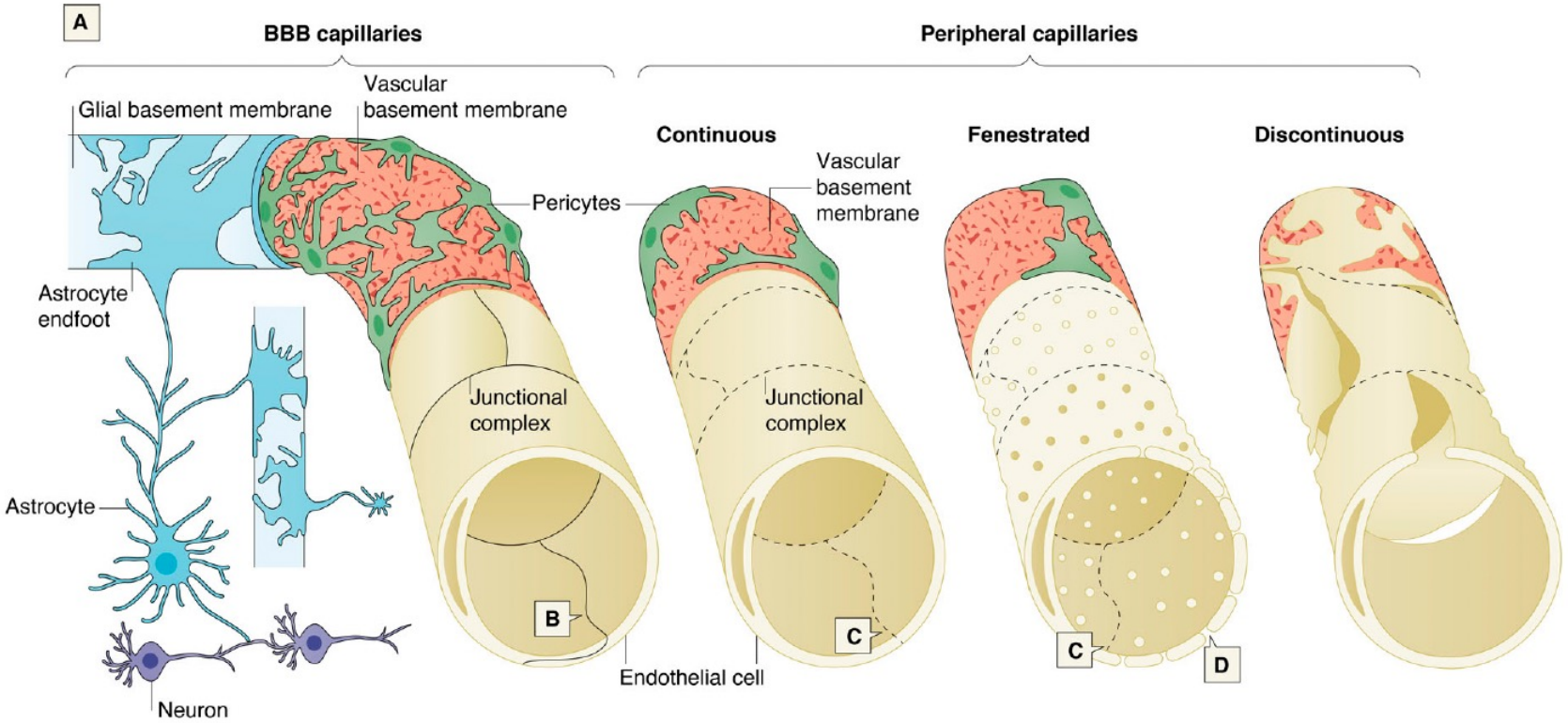
The Blood-Brain Barrier (BBB)



The core anatomical element of the BBB is the cerebral blood vessel formed by endothelial cells (ECs)

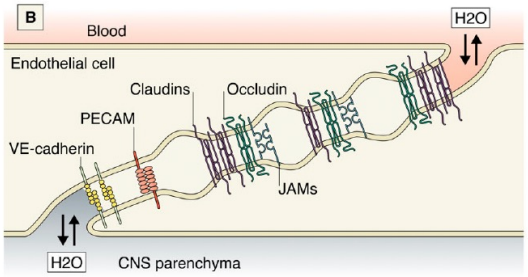
The BBB is a multicellular vascular structure that separates the CNS from the peripheral blood circulation

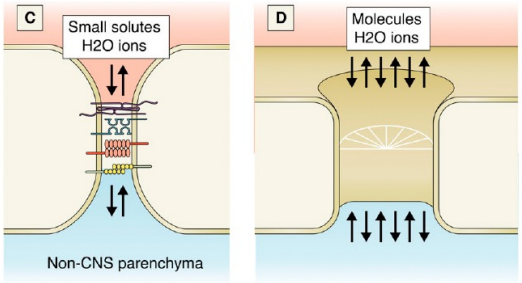
Obermeier B, Daneman R, Ransohoff RM. Nat Med. 2013



Profaci CP et al. J Exp Med. 2020

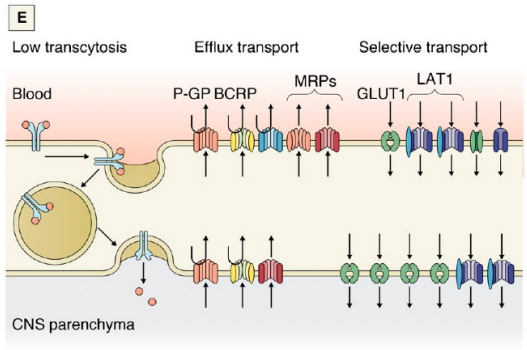
Junctional complexes of BBB ECs

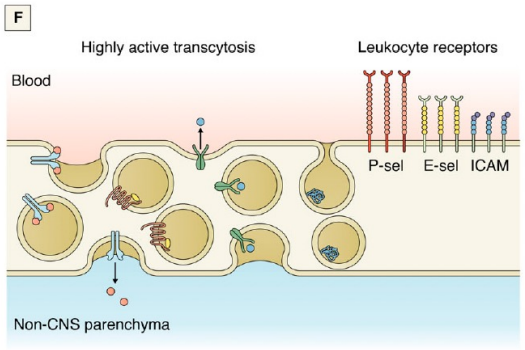




Junctional complexes of ECs in peripheral organs and peripheral endothelial fenestra

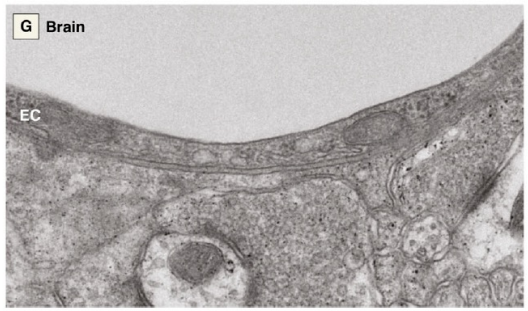
Transport mechanisms in CNS ECs

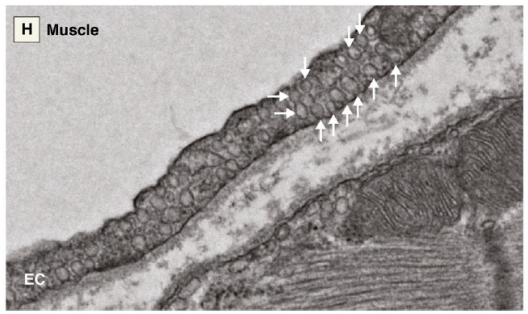




Transport mechanisms in peripheral ECs

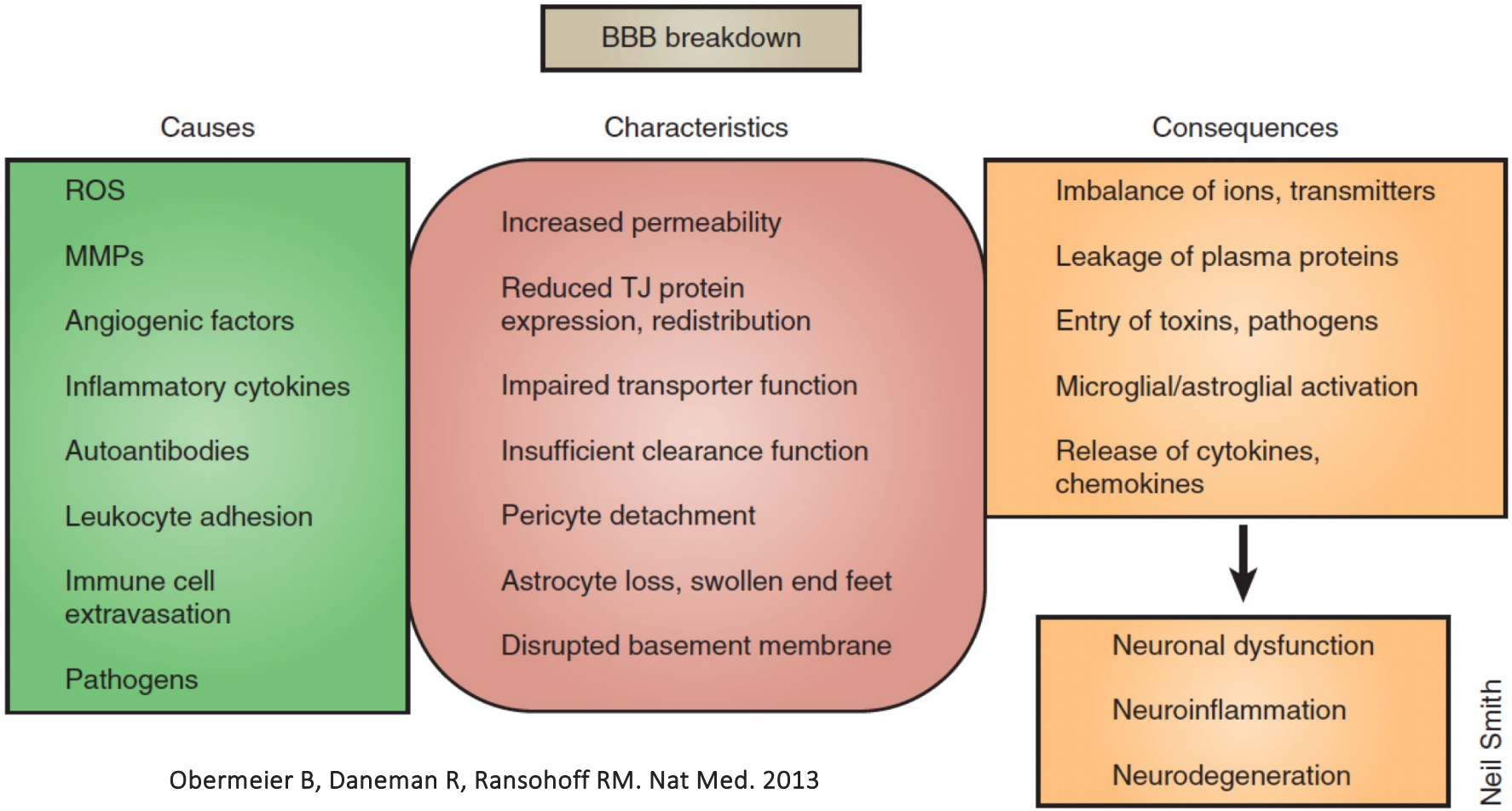
Electron micrographs of a mouse brain EC





Electron micrographs of a mouse muscle EC, which is densely packed with vesicle

Profaci CP et al. J Exp Med. 2020



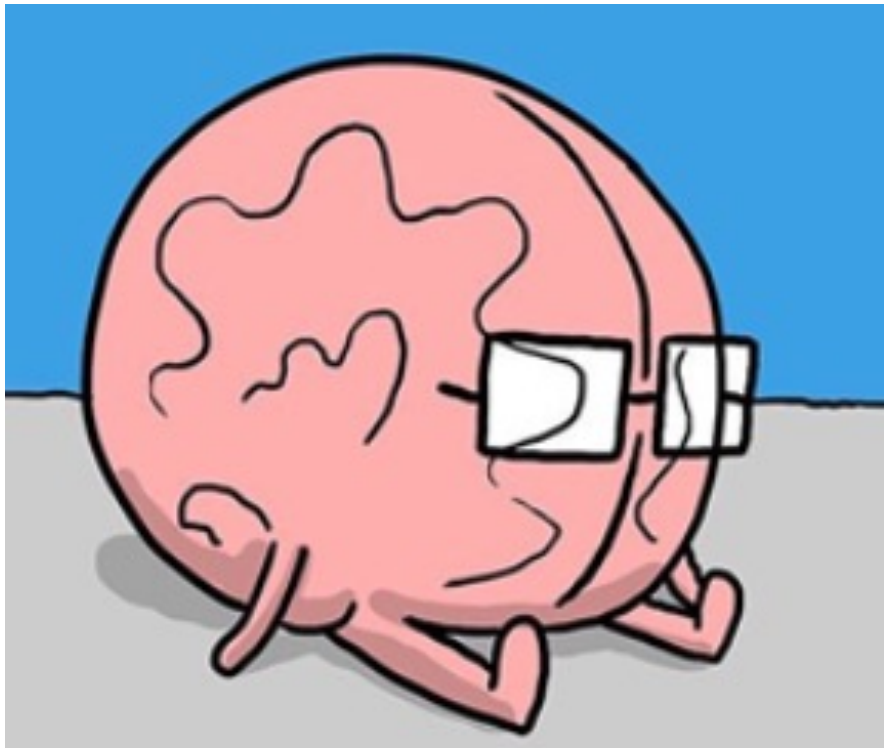
Obermeier B, Daneman R, Ransohoff RM. Nat Med. 2013

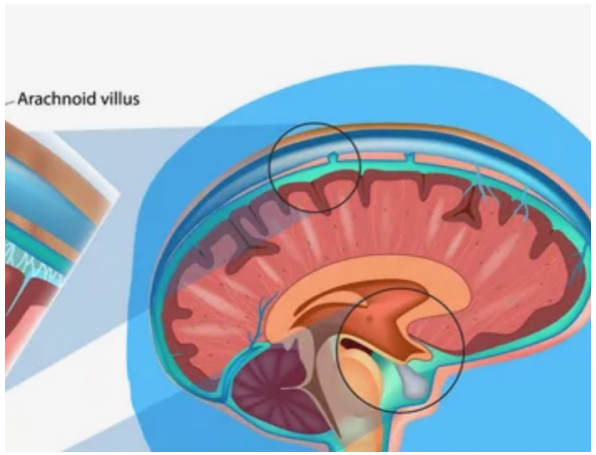
Table 2 Diseases linked to BBB dysfunction

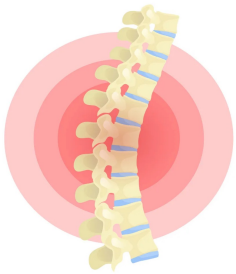
Disease	Level of BBB effect[a]	Comment	Refs.
Stroke	Primary	Microvascular injury induced by oxidative stress during ischemia-reperfusion	160
Epilepsy	Primary	Systemic inflammation can disturb brain homeostasis by allowing entry of ions and epileptogenic substances across the BBB	161,162
	Secondary	Seizures reduce BBB integrity, which enables entry of plasma proteins into the brain that sustain the epileptogenic state	
AD	Primary	BBB dysfunction, including defective amyloid-β clearance from brain and congophilic angiopathy	163,164
Familial ALS	Primary	Loss of BBB integrity at an ultrastructural level associated with expression of mutant SOD1 in brain capillary endothelial cells	164,165
PD	Secondary	Increased BBB permeability and decreased transport activity across the BBB, including inefficient efflux of toxic molecules via P-glycoprotein	166,167
MS	Secondary	Extravasation of autoreactive T cells and monocytes across a compromised BBB	168
Natalizumab-PML with IRIS	Secondary	Infiltration of T cells in perivascular space and parenchyma after discontinuation of natalizumab in context of PML	169
NMO	Primary	BBB breakdown including loss of AQP4 and of astrocytes caused by AQP4-specific IgG	170
Primary CNS vasculitis	Primary	Inflammation of cerebral vessels without systemic disorder	171,172
Secondary CNS vasculitis	Primary	Inflammation of cerebral vessels associated with systemic inflammatory illness	171
VZV vasculopathy	Primary	Viral infection (primary or upon reactivation) of cerebral arteries	173
Cerebral malaria	Primary	Sequestration of parasitized red blood cells in lumen of cerebral microvasculature	174
Primary CNS lymphoma	Secondary	Leaky angiogenic vessels in malignant tissue	175
Glioblastoma	Secondary	Leaky neoangiogenic vessels and loss of BBB integrity in preexisting vessels (by subcellular mislocalization of astroglial AQP4) in malignant tissue	176
PRES	Primary	Vascular injury by systemic influence, such as disorders of clotting or bleeding, and chemotherapy agents (particularly those which inhibit VEGFR kinase)	177
TBI	Secondary	Mechanical disruption of BBB followed by post-traumatic BBB dysfunction	178
Migraine	Secondary	Cortical spreading depression with subsequent vascular reaction	179
Diabetes	Secondary	Increased BBB permeability, possibly leading to cognitive impairment	180

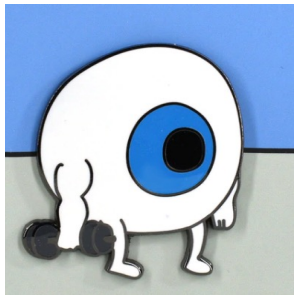
[a]Primary level of BBB effect indicates that the cerebrovasculature is probably compromised upstream from CNS pathogenesis, whereas secondary level of BBB effect is interpreted as happening downstream from the initial insult and aggravating disease. AD, Alzheimer's disease; PD, Parkinson's disease; PML, progressive multifocal leukoencephalopathy; IRIS, immune reconstitution inflammatory syndrome; VZV, varicella zoster virus; PRES, posterior reversible encephalophathy syndrome; TBI, traumatic brain injury.

Primary Central Nervous System Lymphoma (PCNSL)









1-2% of brain tumors
4-6% of Extranodal non-Hodgkin Lymphoma (NHL)

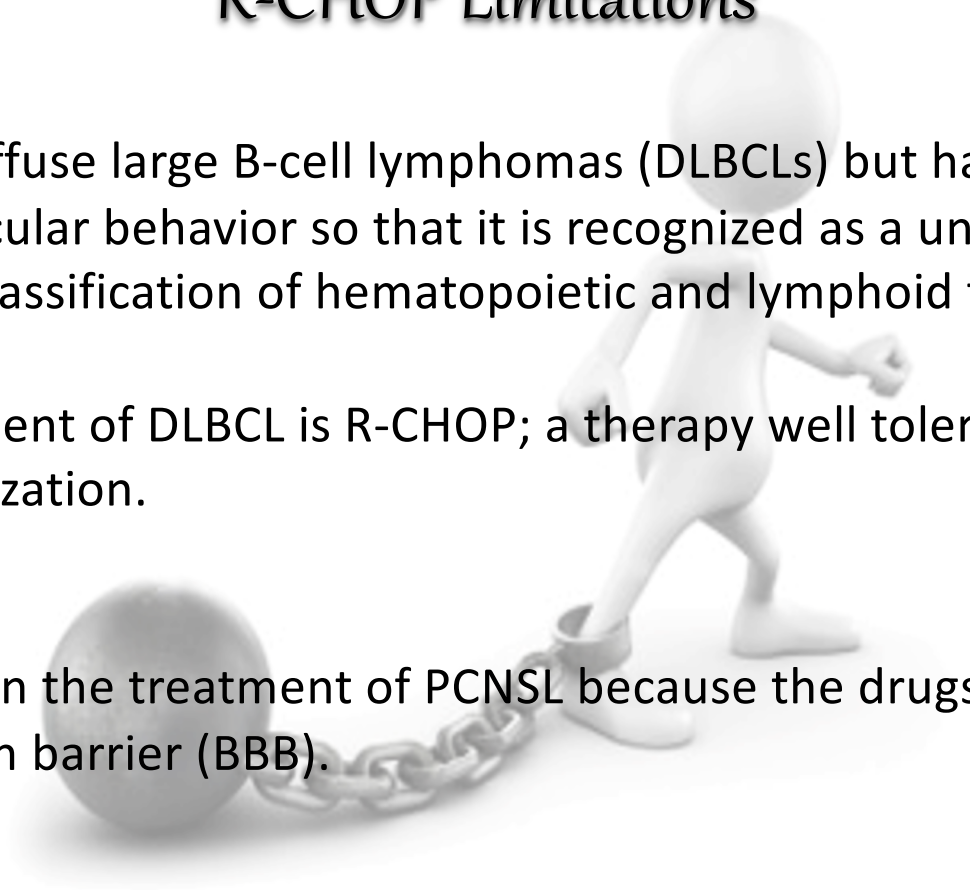
It is an aggressive disease confined to the brain, leptomeninges, eyes, or spinal cord, without involvement of other organs at initial diagnosis and with anecdotal cases of systemic dissemination at relapse

Ferreri AJM Am Soc Hematol Educ Program. 2017
Shields M.S. et al. BJH 2016

R-CHOP Limitations

PCNSL belongs to diffuse large B-cell lymphomas (DLBCLs) but has a peculiar biological and molecular behavior so that it is recognized as a unique biological entity in the WHO classification of hematopoietic and lymphoid tumors

The standard treatment of DLBCL is R-CHOP; a therapy well tolerated and that does not require hospitalization.



R-CHOP is not used in the treatment of PCNSL because the drugs used are unable to cross the blood-brain barrier (BBB).

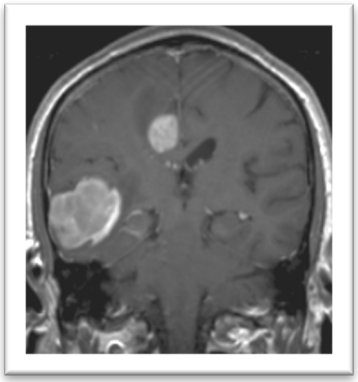
Chemotherapy

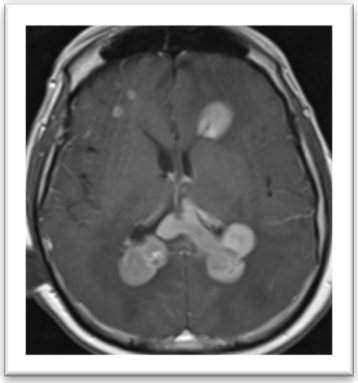
Its efficacy is limited by several factors including the biology and microenvironment of this malignancy, which is "protected" by the BBB

BBB penetration	Doses	CNS availability	Examples
Good	conventional	good	steroids, alkylating ag.
Low to moderate	high	good	MTX, araC
Poor	conventional (-limiting tox)	low	anthracyclines, vinca-alkaloids

HD-MTX doses of at least 3 g/m² with a rapid infusion time of 2-4 hours preceded by a fast bolus are recommended to achieve sufficient drug levels in the CNS

Modern Approach





- Age & PS
- Comorbidity
- Prognostic score
- Histotype (DLBCL)

→

- HD-MTX poly
- WBRT
- Others (HD-ITX)

- Induction
- Response
- Quality response
- Toxicity

→

- Observation
- WBRT
- HDC/ASCT
- Non-myeloablative
- Maintenance

Ferreri AJM et al. Am Soc Clin Oncol Educ Book. 2019
Ferreri AJM Am Soc Hematol Educ Program. 2017

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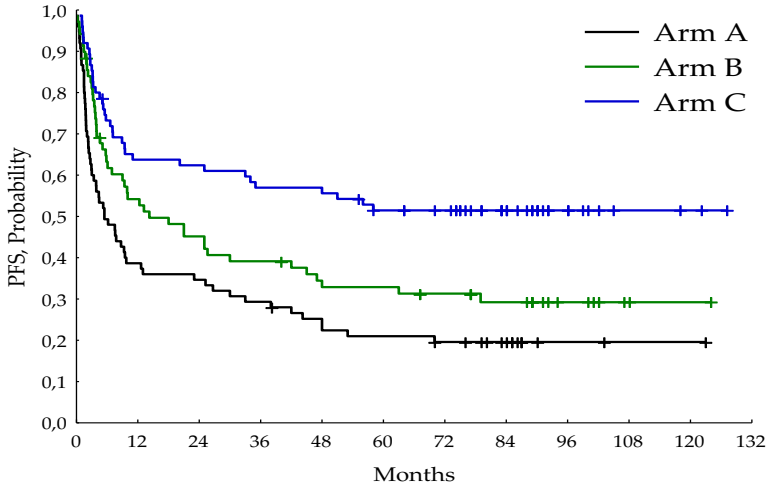
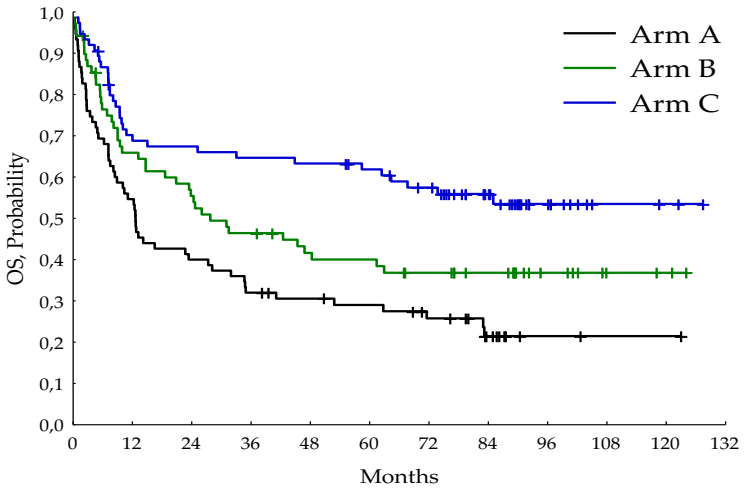


	HR	95%CI	p
A vs. B	0·66	0·43 - 1·02	0·06
A vs. C	0·41	0·21 - 0·61	0·00001
B vs. C	0·63	0·42 - 0·92	0·01



	HR	95%CI	p
A vs. B	0·64	0·41 - 0·99	0·049
A vs. C	0·42	0·24 - 0·64	0·00005
B vs. C	0·66	0·44 - 0·98	0·044

MEDIAN FOLLOW-UP:
88 MONTHS (IQR 77-99)

Ferreri AJM et al. Leukemia 2022

PCNSL and Salvage Therapies

Taking into account pts who did not respond to first line induction HD-MTX based (≈30%) and pts who relapse after consolidation (≈20%) salvage therapies represent an important unmet clinical need

Treatment of refractory and relapsed PCNCL has largely been based on:

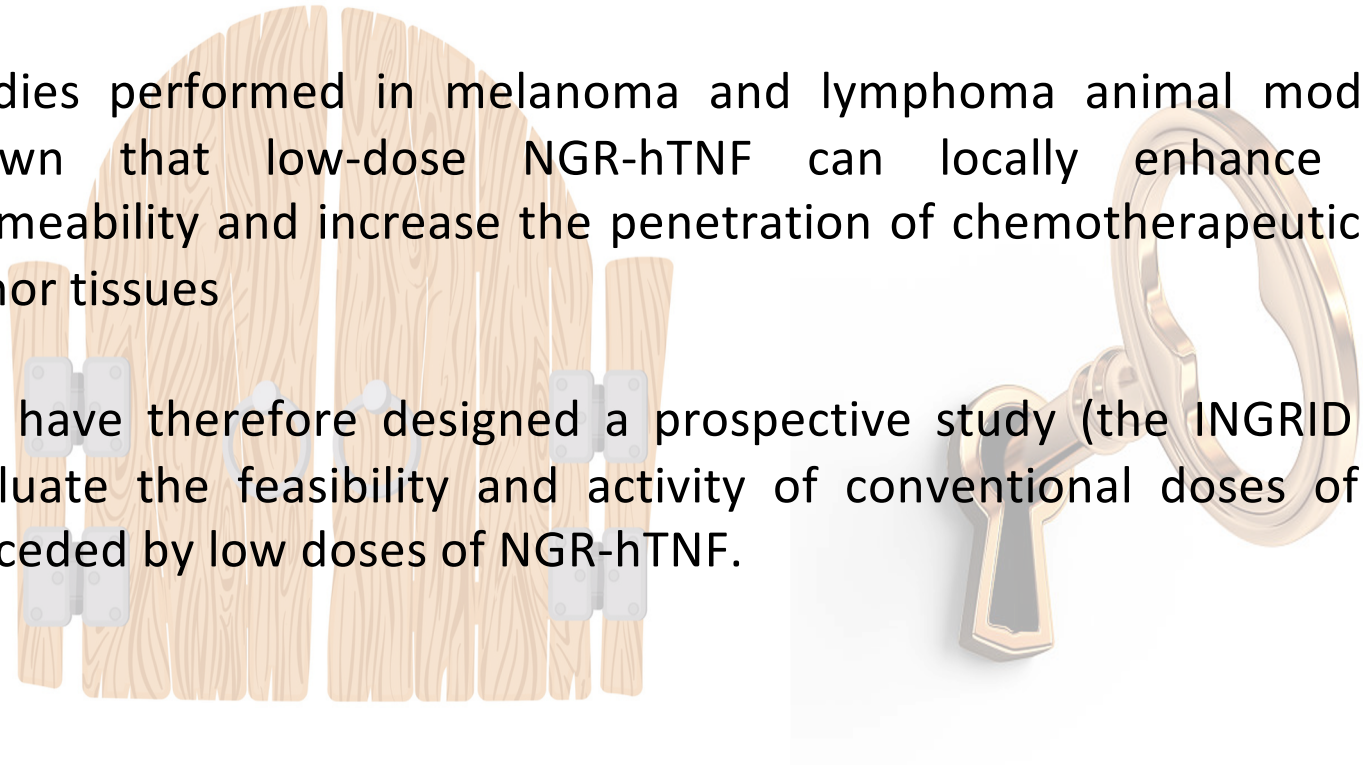
- small retrospective studies
- small, prospective clinical trials on single agents (pemetrexed, topotecan, temozolomide, and rituximab …) with modest response rates (31-55%) and overall limited median PFS (1.6-5.7 months)
- no randomized trials

An optimal salvage regimen has not been established for relapsed or refractory PCNSL patients

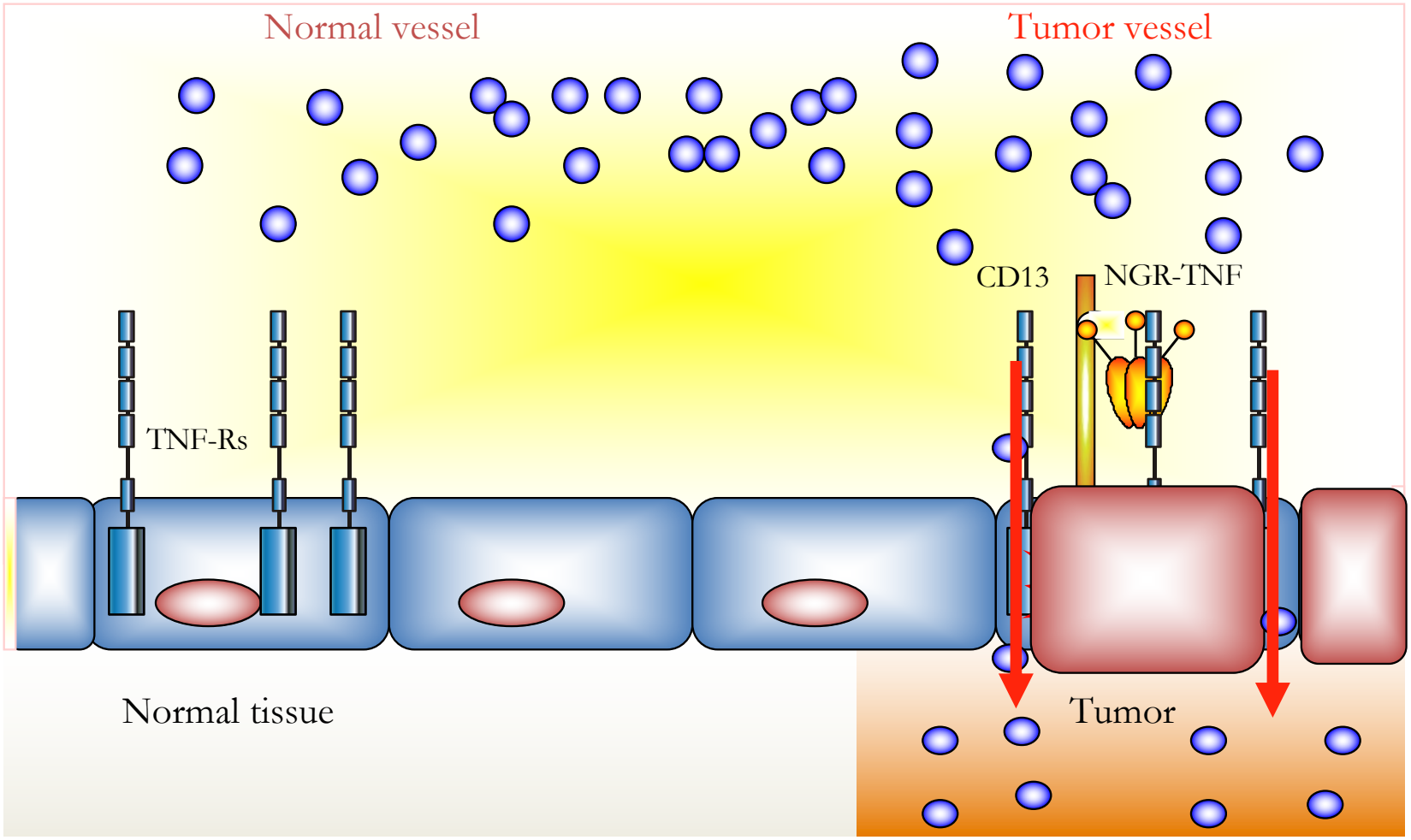
NGR-hTNF: the Key to open the Gate

Studies performed in melanoma and lymphoma animal models have shown that low-dose NGR-hTNF can locally enhance vascular permeability and increase the penetration of chemotherapeutic drugs in tumor tissues

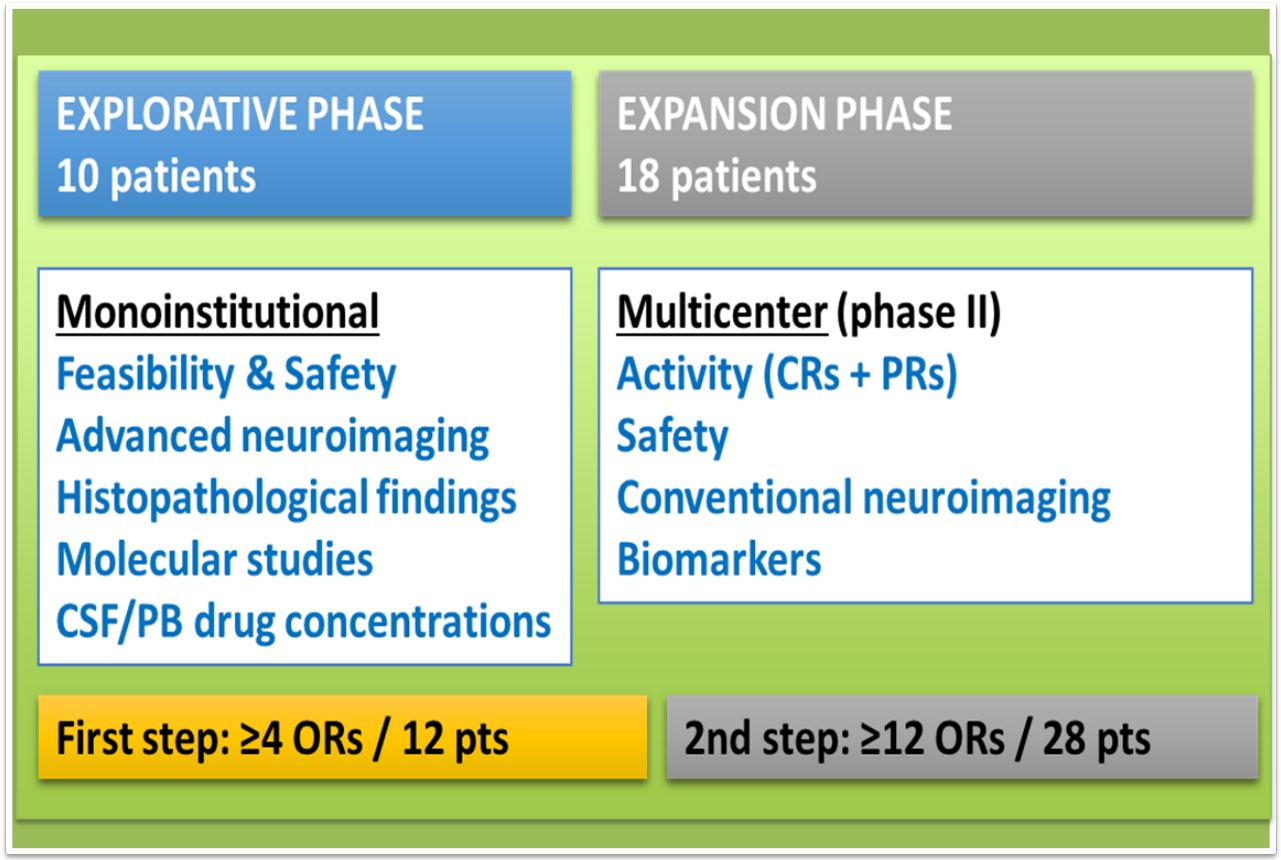
We have therefore designed a prospective study (the INGRID Trial) to evaluate the feasibility and activity of conventional doses of R-CHOP preceded by low doses of NGR-hTNF.



Corti, Curnis et al
Nature Biotechnol 2000
J Clin Invest 2002



INGRID - Trial Design



INGRID - Methods

Primary objective: **ORR (CRs + PRs) assessed by contrast-enhanced whole-brain MRI after 2nd, 4th & 6th courses.** The two-stage Simon Minimax design was used; sample size estimated to demonstrate an improvement from 30% ORR (P0) to 50% (P1) (one-sided test; α 10%; β 90%) was 28 pts. NGR-hTNF/RCHOP would be declared active if ≥12 responses were recorded.

Secondary objectives:

Changes in BBB permeability assessed by **cerebral dynamic contrast-enhanced (DCE) MRI** performed before and after the 1st, 2nd & 6th cycles. Permeability was assessed in enhanced lesions and perilesional areas; results were expressed as Ktrans values normalized using contralateral white matter.

Changes in BBB permeability assessed by **^{99m}Tc-DTPA brain scintigraphy (SPECT)** performed before (baseline) and after the 3rd course. For semiquantitative analysis, ROI were drawn manually, and by an automatic isocontour method (30% of maximum activity).

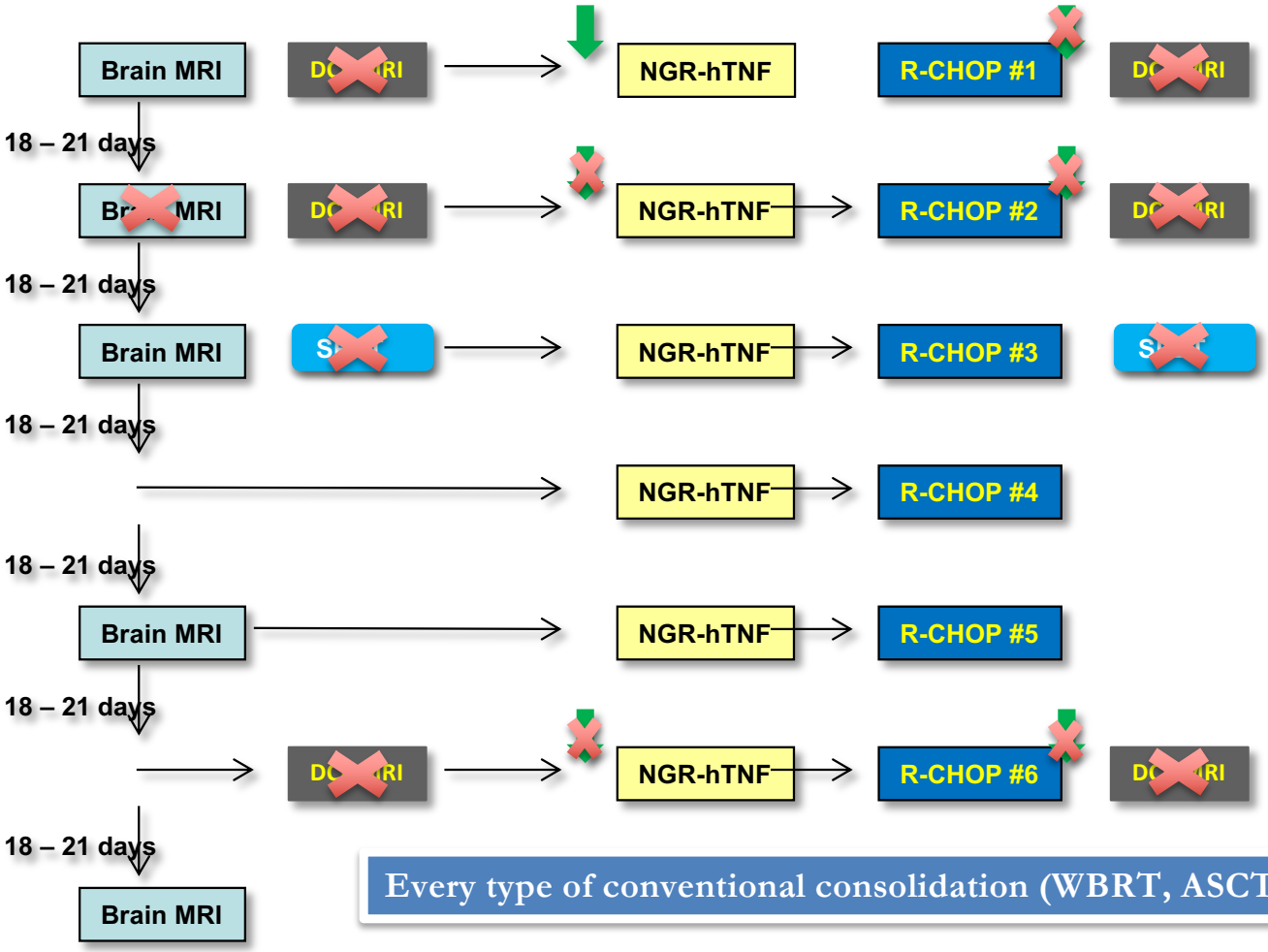
Changes in CTX, DOXO & rituximab concentrations in matched CSF/plasma samples were assessed by ELISA.

Expression of CD13 assessed in tissue samples by IHC using SP187 Mab (Ventana-Roche, Ultrabenchmark).

INGRID – Inclusion Criteria

- Histological or cytological diagnosis of DLBCL
- Disease exclusively localized into the CNS (brain, meninges, cranial nerves, eyes and/or spinal cord) both at first diagnosis and failure
- Progressive or recurrent disease
- Previous treatment with HD-MTX-based chemo ± WBRT
- At least one target lesion, bidimensionally measurable
- Age 18 - 80 years
- ECOG performance status 0-3
- HIV negative

CD13 expression by IHC & IFC.



Variations in the BBBp at the tumor and perilesional areas.
Results expressed as Ktrans.

^{99m}Tc-DTPA penetrate the disrupted BBB; the tracer's uptake increases proportionally to the BBBp.
Area of interest: 30% uptake

Specificity of BBBp:
Concentrations of rituximab, CTX and DOXO in matched CSF and plasma samples.

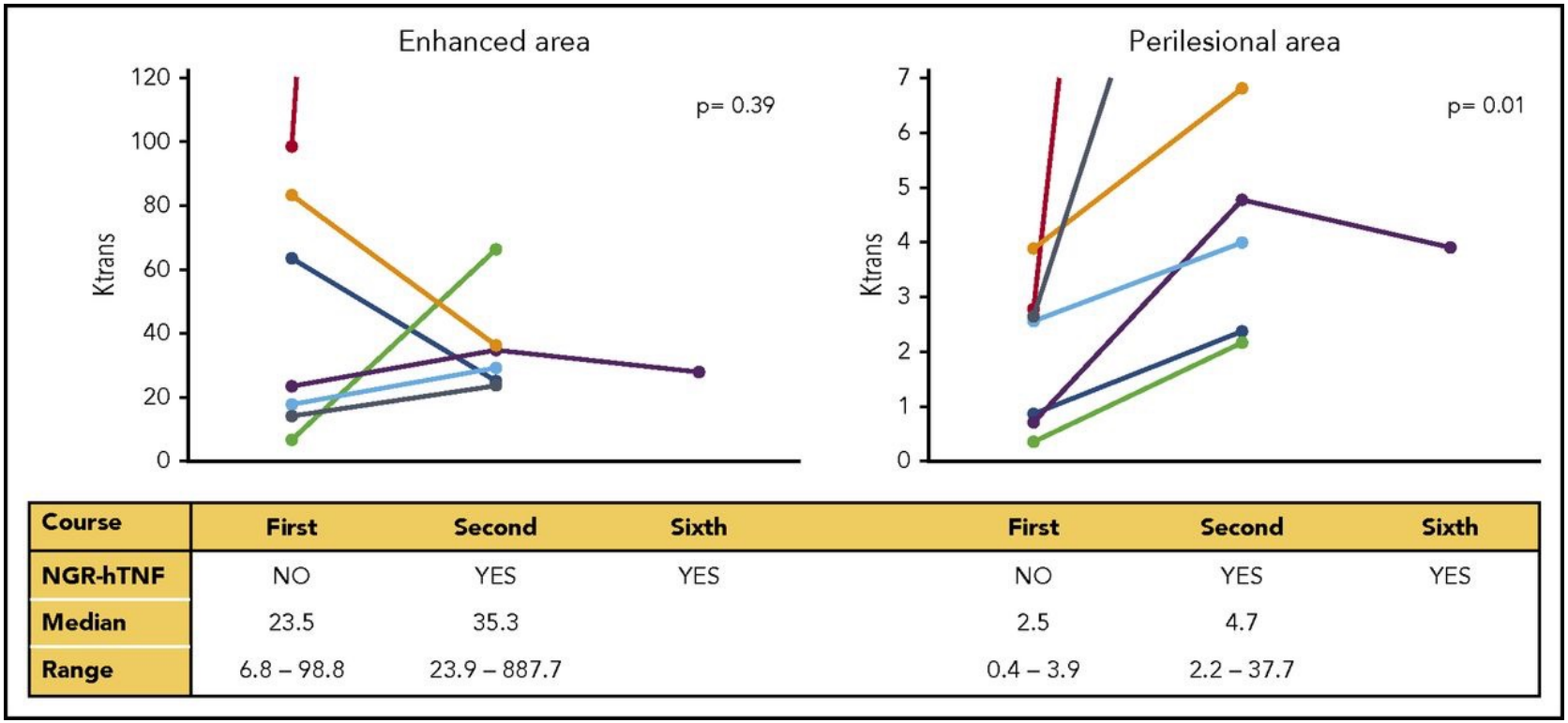
Response defined by changes in the size of target lesions (post-contrast T1 MRI).
Response on two consecutive MRIs

Every type of conventional consolidation (WBRT, ASCT, maintenance) is allowed

INGRID Trial: Study Population

Median Age	58 (range 26 - 78)
Male:Female	1
ECOG – Performance Status >1	15 (53%)
High lactic dehydrogenase serum level	11 (40%)
High cerebrospinal-fluid protein concentration	11/22 (50%)
Involvement of deep areas	12 (43%)
IELSG risk score	
Low	5 (18%)
Intermediate	19 (68%)
High	4 (14%)
Intraocular disease	3 (11%)
Meningeal dissemination	0 (0%)
Prior lines	
Prior lines ≥2	10 (36%)
Prior Autologous Stem Cell transplantation (ASCT)	7 (25%)
Prior Whole-Brain Irradiation (WBRT)	6 (21%)
Both ASCT + WBRT	4 (14%)
Refractory to prior lines	15 (54%)

INGRID TRIAL - BBB Permeabilization (DSE-MRI)



Course	First	Second	Sixth	First	Second	Sixth
NGR-hTNF	NO	YES	YES	NO	YES	YES
Median	23.5	35.3		2.5	4.7	
Range	6.8 – 98.8	23.9 – 887.7		0.4 – 3.9	2.2 – 37.7	

Ferreri AJM et al. Blood 2019

Permeability was increased after NGR-hTNF delivery. This effect was more evident in perilesional areas

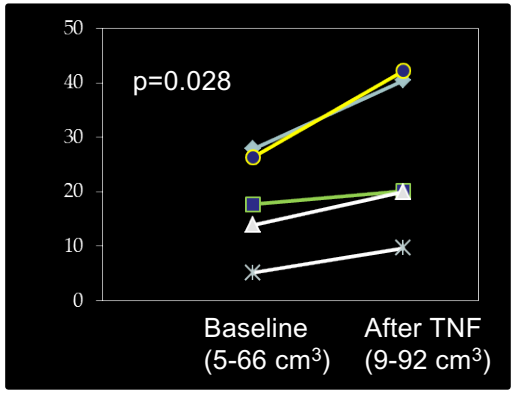
INGRID TRIAL - BBB Permeabilization (SPECT)

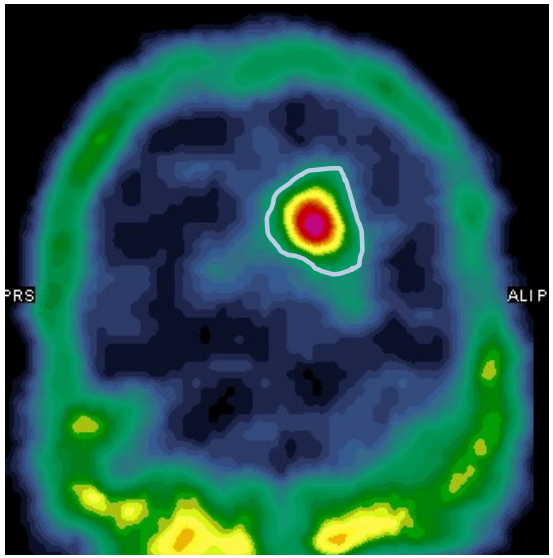
Before treatment
(22 cm^3)

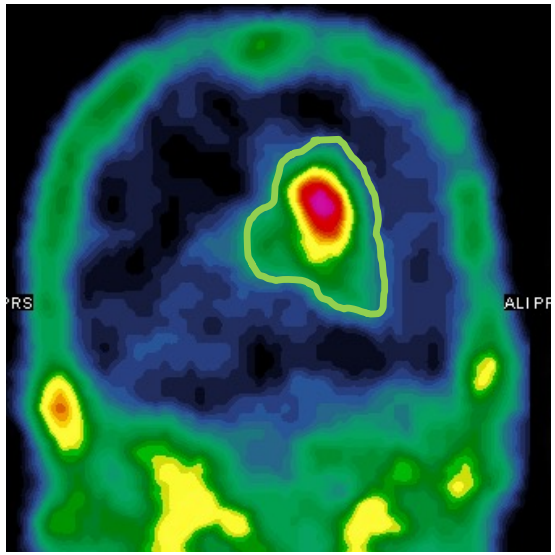
1h after treatment
(40 cm^3)

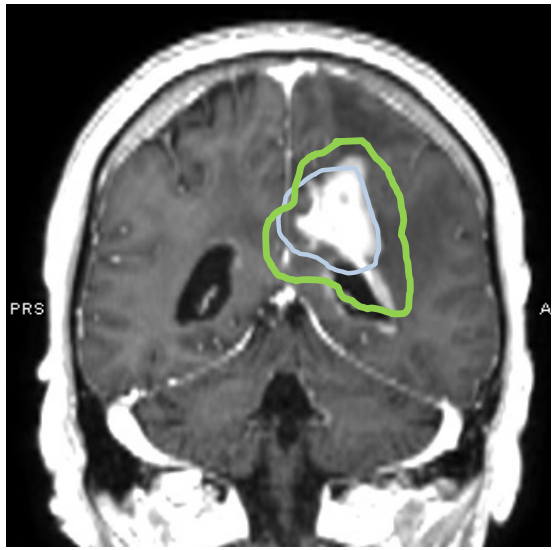
Volume increase
Median: 45%
Range: 14% - 87%

Ferreri AJM et al. Blood 2019









blood

INGRID TRIAL - Plasma and CSF Drug Concentration

The biological effect of NGR-hTNF was specific on the tumor area and did not change pharmacokinetics of investigated drugs

Ferreri AJM et al. Blood 2019

Table 3. Concentrations of doxorubicin, cyclophosphamide, and rituximab in plasma and CSF samples

Drug concentrations	Without NGR-hTNF*	After NGR-hTNF†	*P*
Plasma			
Doxorubicin, ng/mL	29.6 ± 7.4	26.0 ± 6.7	.43
Cyclophosphamide, mg/L	26.3 ± 7.7	27.8 ± 7.9	.17
Rituximab, ng/mL	45.4 ± 17.0	69.1 ± 13.4	.04
CSF			
Doxorubicin, ng/mL	<2.5 (all samples)	<2.5 (all samples)	
Cyclophosphamide, mg/L	14.1 ± 3.5	15.5 ± 4.8	.27
Rituximab, ng/mL	<1.0 (all samples)	<1.0 (all samples)	
CSF/plasma ratio‡			
Cyclophosphamide, %	60 ± 20	62 ± 19	.73

*Samples collected after the first course of treatment (ie, after R-CHOP without NGR-hTNF).

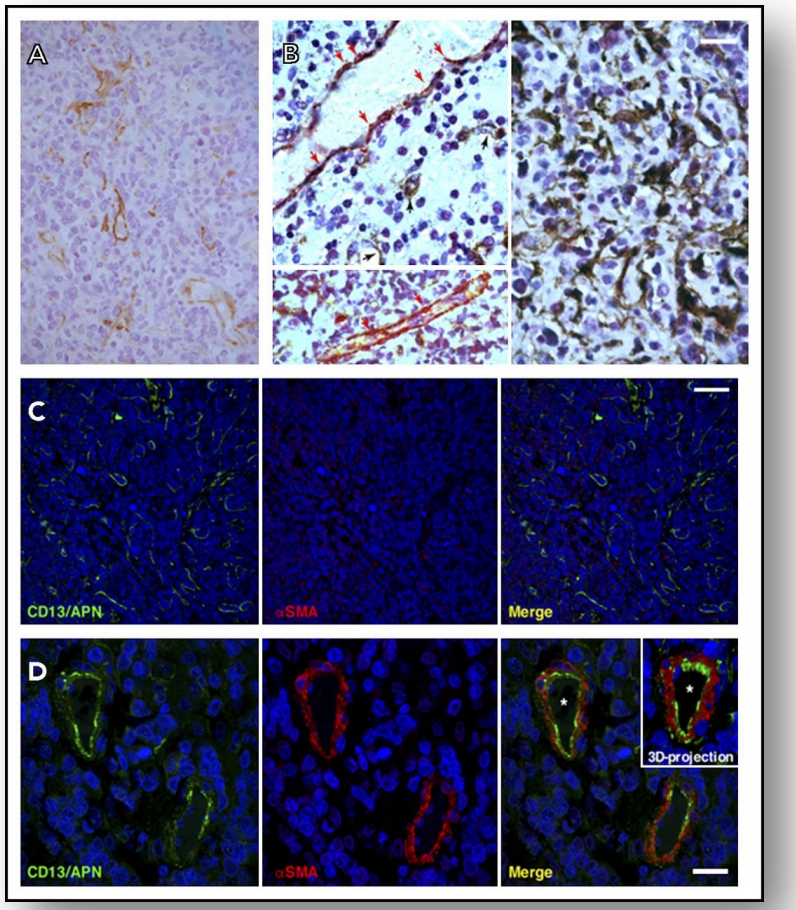
†Samples collected after the second course of treatment (ie, after NGR-hTNF followed by R-CHOP).

‡The ratio was not estimated for doxorubicin and rituximab because CSF concentrations resulted below the lower limit of quantification.

INGRID TRIAL - CD13 Expression

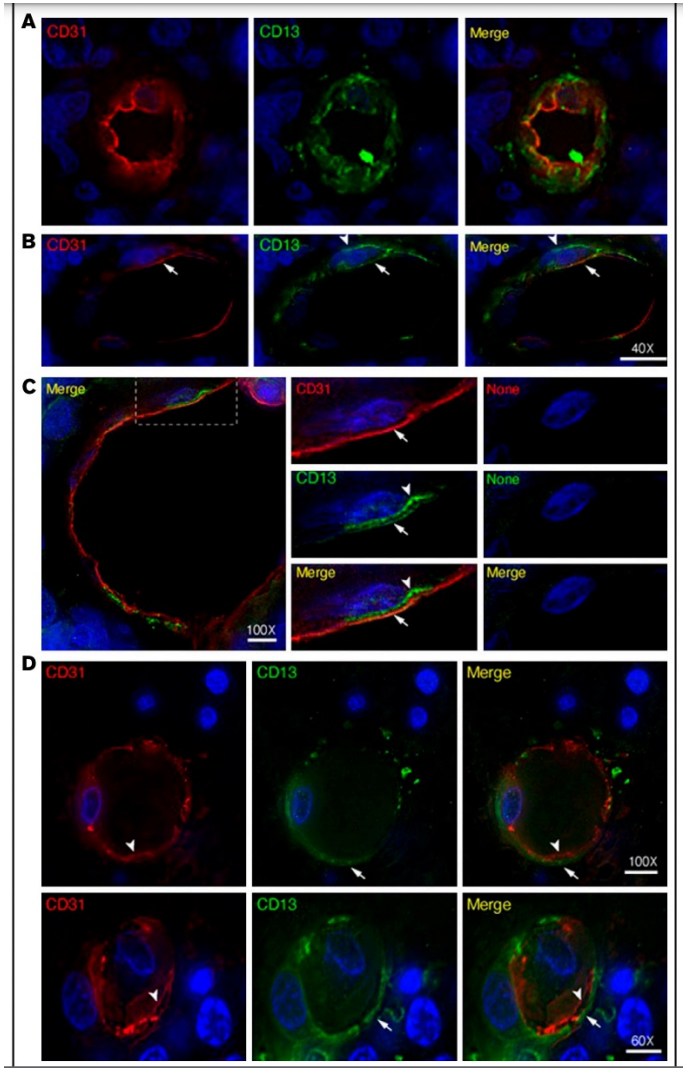
Immunostaining with anti-CD13 mAb of diagnostic brain biopsies of enrolled pts highlighted a proportion of vessels both in the intratumoral and peritumoral area

Confocal immunofluorescence analysis of a tissue section stained with a polyclonal anti-CD13 antibody (green) and with an antibody against aSMA, a marker of pericytes, (red) (400x, bar: 50 μm)

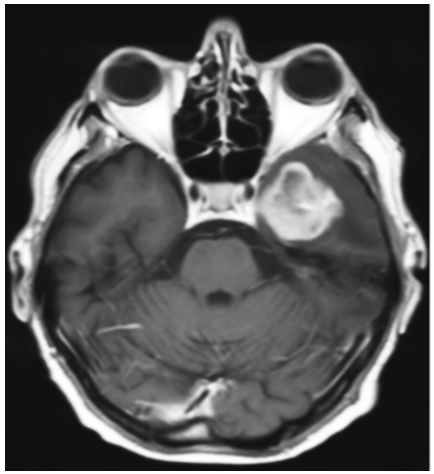


INGRID TRIAL - CD13 Expression

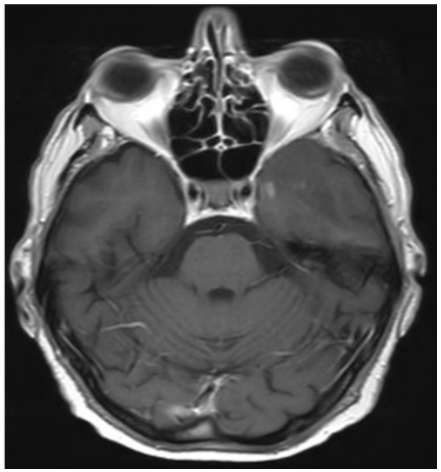
A deeper characterization of CD13 in PCNSL vessels demonstrates that this protein is expressed by endothelial cells and pericytes, which contrasts with the fact that CD13 is expressed only in pericytes in the normal brain vessels



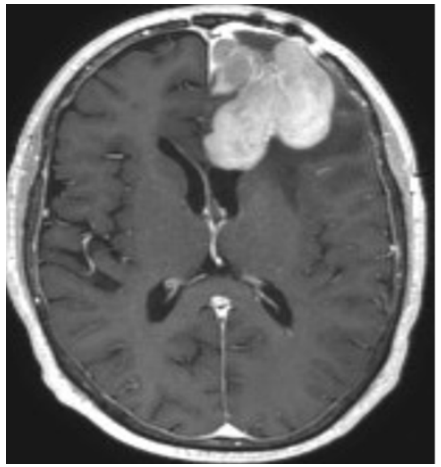
Overall Response Rate (ORR) ✧ Complete responses (CR) ✧ Partial Responses (PR)	**21 (75%)** 11 (39%) 10 (36%)	**95% CI: 64-86%** 95% CI: 21-57%
Progressive Disease (PD)	7 (25%)	



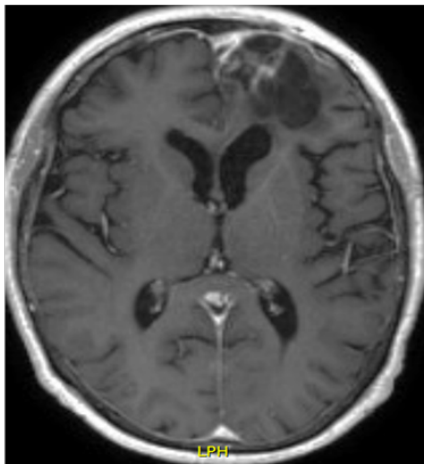
Baseline



After 2nd course



Baseline



After 2nd course

INGRID Trial: Activity (n=28)

Consolidation/Maintenance	# of pts
ASCT	5
WBRT	7
Lenalidomide	1
Combinations	4

Subgroups analysis did not show differences in responses based different variables such as IELSG risk categories, site and number of lesions, prior therapies, and refractoriness

Response lasted more than 6 months in all complete responders (median 10 months; range 6-19)

At a median follow-up of 52 months (range 45-58), five patients remain relapse-free and six patients are alive.

INGRID Trial: Toxicities (121 courses)

	Grade 1-2	Grade 3	Grade 4	Grade 5
Neutropenia	9 (7%)	17 (14%)	57 (47%)	-
Thrombocytopenia	34 (28%)	25 (21%)	26 (21%)	-
Anemia	86 (71%)	12 (10%)	2 (2%)	-
Febrile Neutropenia	-	5 (4%)	*1 (1%)	-
Hepatotoxicity	27 (22%)	4 (3%)	1 (1%)	-
Oral Mucositis	1 (1%)	3 (2%)	-	-
Infections	-	*5 (4%)	-	-
Seizures	*3 (2%)	-	-	-
Deep Vein Thrombosis	*2 (2%)	-	-	-
Syncope	-	*2 (2%)	-	-
LVEF reduction	*1 (1%)	-	-	-
Probable IPA	-	*1 (1%)	-	-
Nausea and vomiting	4 (3%)	-	-	-
Constipation	2 (2%)	*1 (1%)	-	-
TNF Infusion reaction§	9 (7%)	-	-	-

All toxic events other than alopecia are reported.

Denominator is the total number of delivered courses (n= 121).

LVEF= left ventricular ejection function;

IPA = Invasive Pulmonary Aspergillosis

§Fever (n=3), fever+arterial hypotension (n=1), chills (n=4), arterial hypertension (n=1)

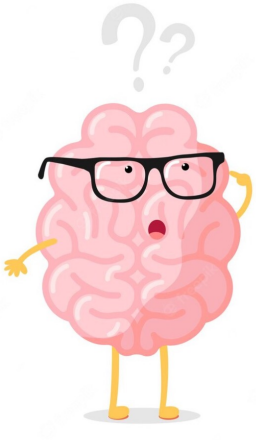
*** = SAEs**

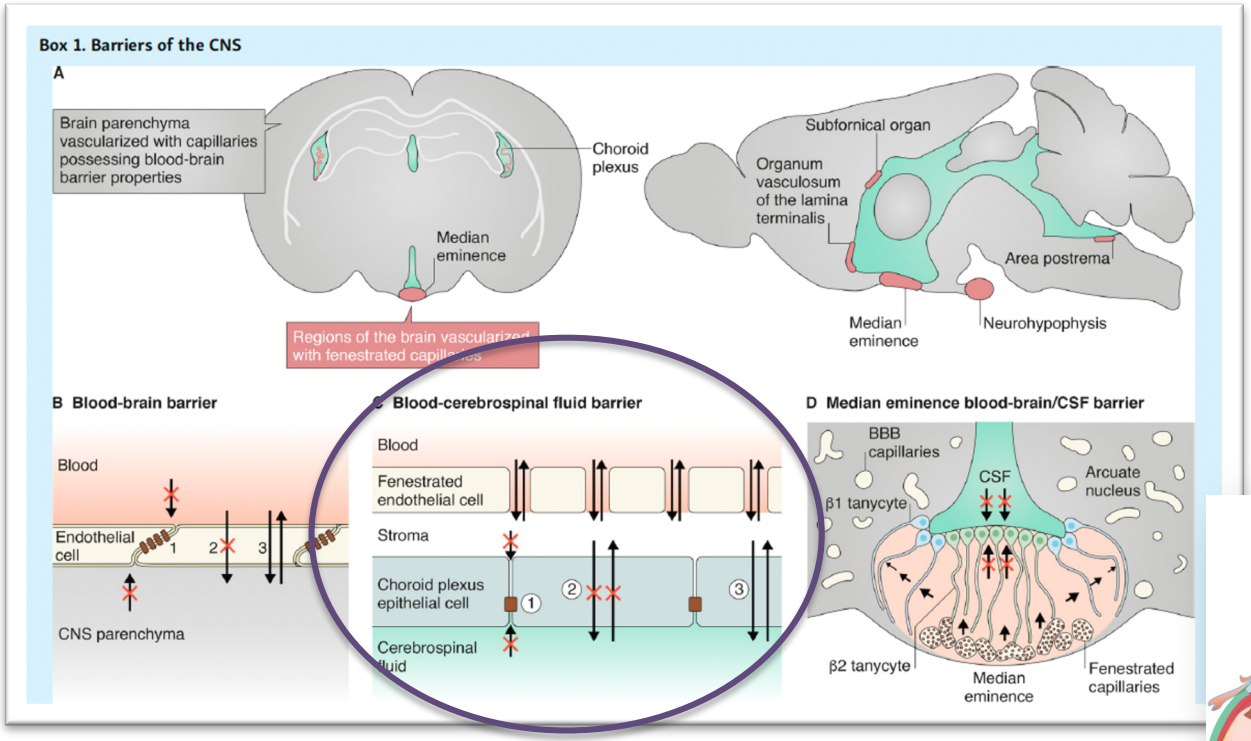
Ingrid - Conclusions

- ✓ NGR-hTNF/RCHOP is active and safe in heavily pretreated pts with r/rPCNSL.
- ✓ Most responsive pts received consolidative treatment.
- ✓ CD13, the target of TNF, was expressed in tumor tissue.
- ✓ Consistently, both DCE-MRI and SPECT data suggest that NGR-hTNF enhances vascular permeability specifically in tumor and perilesional areas.
- ✓ NGR-hTNF does not interfer with drugs pharmacokinetics.
- ✓ This innovative approach deserves to be addressed as first-line treatment in PCNSL pts.

Open questions

- ✓ Effects of NGR-TNF on the blood–retina barrier and the blood-CSF barrier ?
- ✓ Effects of NGR-TNF on microscopic foci ?
- ✓ Long term activity



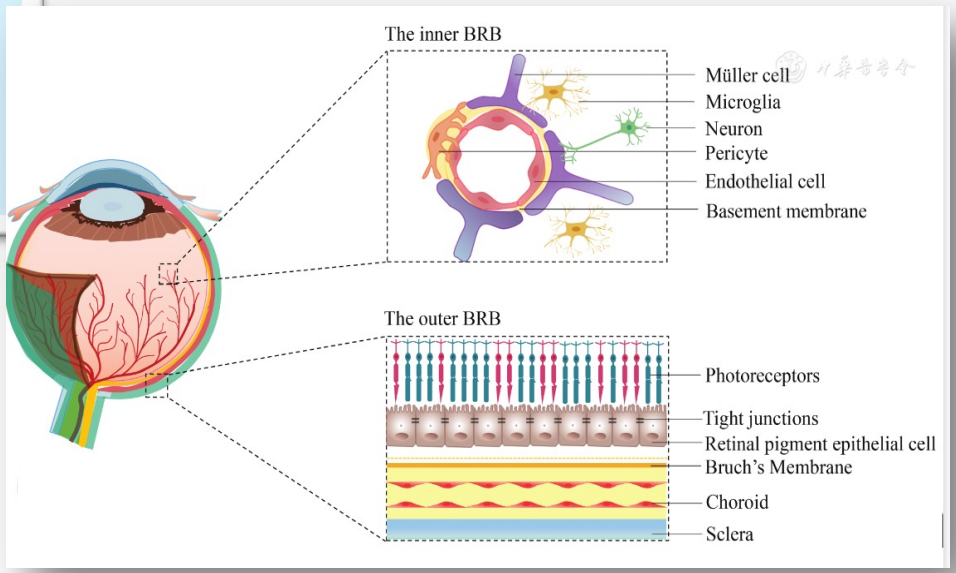


Profaci CP et al. J Exp Med. 2020

Barriers of the CNS

The Blood-cerebrospinal Fluid Barrier

The Blood-Retinal Barrier



Effects of NGR-TNF on the blood–retina barrier and the blood-CSF barrier ?

Patterns of relapse

Effects of NGR-TNF on microscopic foci ?

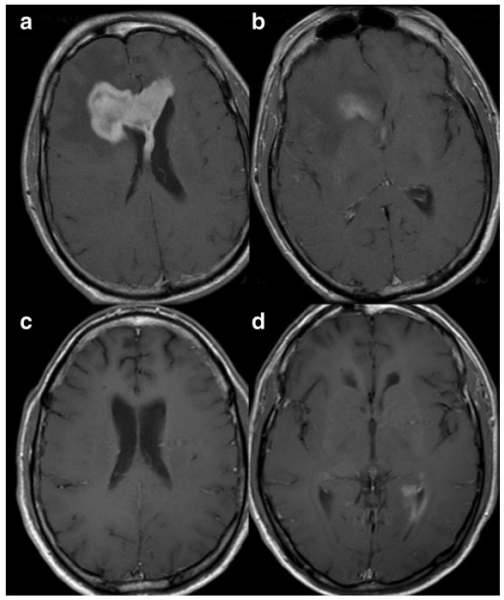


Fig. 2 Case 2; Axial T1-weighted post contrast imaging at initial presentation (**a**, **b**) and relapse (**c**, **d**). Initial presentation shows a localized large enhancing lesion centered in the genus of the corpus callosum (CC) with extension mainly into the right frontal lobe (**a**, **b**). After 10 months of HD-MTX treatment there is relapse in the ependymal surface of the left lateral ventricle (**d**) with complete response in the CC lesion (**c**)

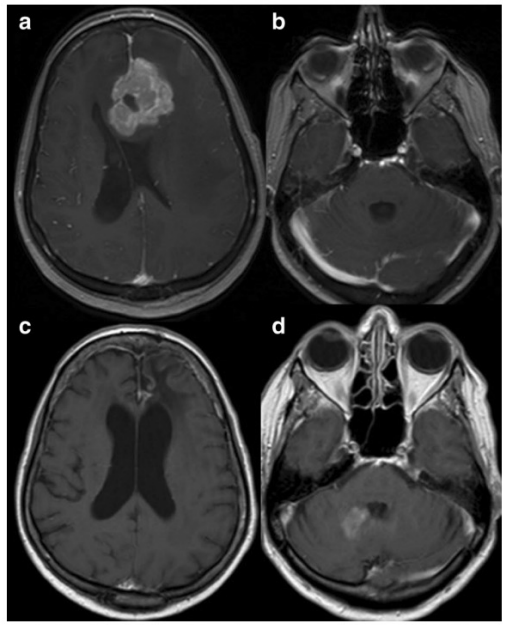


Fig. 3 Case 3; Axial T1-weighted post contrast imaging at initial presentation (**a**, **b**), after completing therapy with HD-MTX (**c**) and relapse (**d**). Initial presentation shows a large enhancing lesion in the left frontal lobe (**a**) and normal cerebellum (**b**). Patient was in complete response after completing 1 year of HD-MTX treatment (**c**) with radiographic relapse in the right cerebellum, 6 years after the initial diagnosis (**d**)

Role of consolidation/maintenance

INGRID Trial: Activity (n=28)

Consolidation/Maintenance	# of pts
ASCT	5
WBRT	7
Lenalidomide	1
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LONG TERM ACTIVITY

IBRUTINIB

Better outcomes in patients aged < 60 years with systemic DLBCL treated with IBR plus R-CHOP [1]

Permeabilizing effect on blood-brain barrier to cytostatics in pre-clinical models [2]

R-CHOP combined with NGR-hTNF obtained high ORR in rrPCNSL [3]

IBR was combined with R-CHOP or "CHOP-like" (I-RCHOP) regimen, based on safety data from systemic DLBCL[1]

NEW DRUGS

Multicentric nationwide retrospective study to evalutate safety and feasibility of IBR alone or within I-RCHOP in a "real life" setting of rrPCNSL

[1] Younes A et al. JCO, 2019 ;
[2] Meeks C et al. Neuro Oncol, 2019;
[3] Ferreri AJM et al. Blood, 2019

IBRUTINIB and R-CHOP

Disease response (IPCG 2005 criteria)*	Treated patients n=25 (100%)
Overall Response Rate ORR)	**15 (60%)**
Complete Response (CR)	7 (28%)
Partial Response (PR)	8 (32%)
Stable Disease (SD)	3 (12%)
Progressive disease (PD)	4 (16%)
Not evaluable	3 (12%)

Study	N° of patients	Treatment	ORR %	CR %	PR %
Soussain et al. [1]	38	IBR monotherapy	37%	21%	16 %
Grommes et al.[2]	15	IBR-Rituximab-HDMTX	80%	53%	27%
Lionakis et al.[3]	18	Da-TEDDi-R	94 %	67%	23%

Treatment group	N° of patients	ORR %	CR %	PR %
I-RCHOP	18	67%	**39%**	28%
IBR single agent	7	43%	**0%**	43%

* International PCNSL Collaborative Group 2005 criteria

[1]Soussain C et al. Eur J Cancer, 2019;
[2]Grommes C et al. Blood, 2019;
[3]Lionakis MS et al. Cancer Cell. 2017

Table 1 Strategies to disrupt the BBB

Methods and drugs	Study type	Results and outcome	Study conclusions	Notes	Reference
IA osmotic solution (warmed 25% mannitol) + IA MTX + i.v. CT^	Large series (149 untreated PCNSL) of patients treated over a 23-year period (Feb 1982 to Dec 2005)	ORR 82% (CR 58%, PR 24%); mPFS 1.8 years, mOS 3.1 years	BBBD/IA methotrexate-based chemotherapy results in successful and durable tumor control and outcomes	Treatment delivery regimen is complex and should be undertaken only by trained teams thus limiting its applicability to selected centers and its diffusion on a worldwide scale	(40)
MTX-liposome-coupled microbubbles + (FUS)	Preclinical	Significantly higher brain MTX concentration than controls[§]	MTX-liposome-coupled microbubbles may hold great promise as new and effective therapies for PCNSL and other central nervous system malignancies	No apparent brain tissue damage This strategy should be investigated in clinical trial	(41)
R-CHOP preceded by low dose of NGR-TNF	Phase II (28 r/r PCNSL)	ORR 75% (21/28); 5/21 patients are relapse free at 15+ months; 6/21 are alive at 15+ months	The combination is active and safe in patients with R/R PCNSL, and its antitumor activity is in line with the expression of CD13 in tumor vessels. This strategy needs to be addressed as first-line treatment in PCNSL patients	No data on blood-CSF and blood-retina barrier Impact on outcome masked by consolidation-maintenance	(42)

Calimeri T et al. Ann Lymphoma 2021

Invasive and non-invasive methods for overcoming the BBB

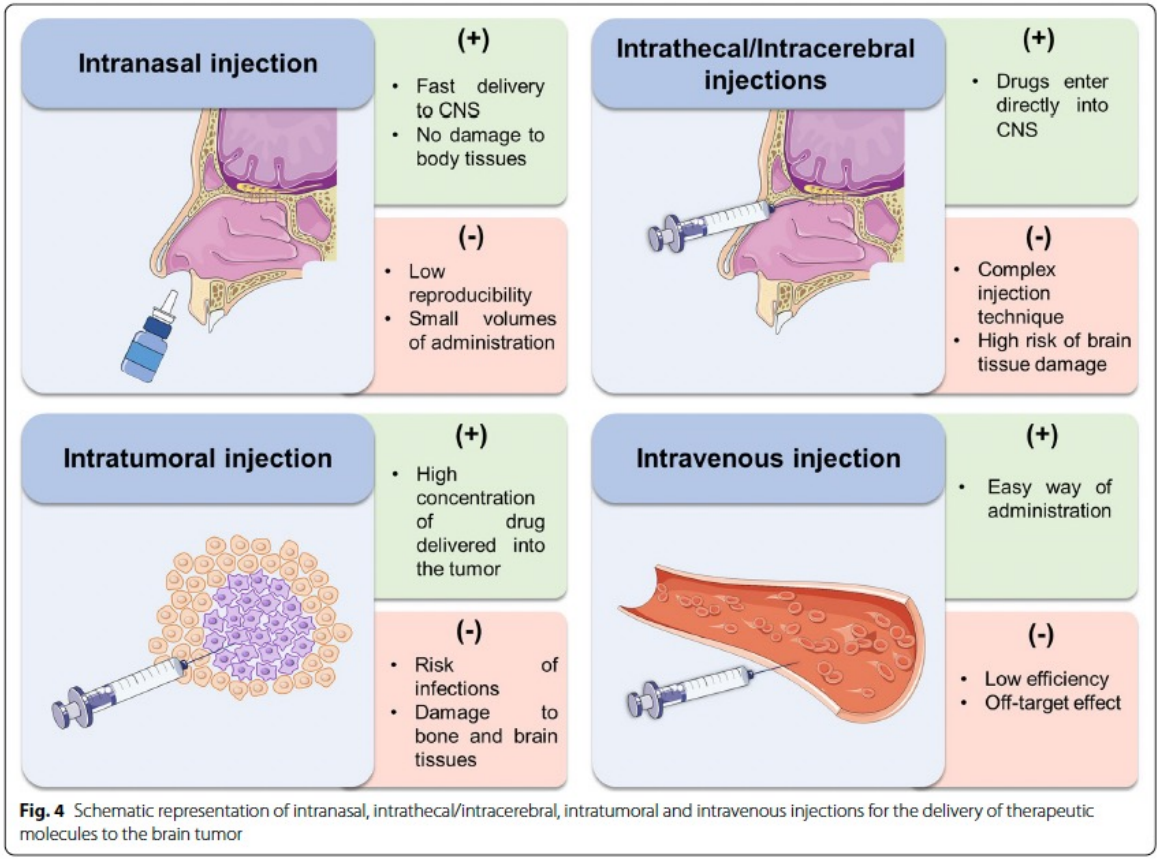


Fig. 4 Schematic representation of intranasal, intratheca/intracerebral, intratumoral and intravenous injections for the delivery of therapeutic molecules to the brain tumor

Mitusova et al. Journal of Nanobiotechnology 2022

Types of nanocarriers and their hybrids

Unfortunately, no crucial clinical translations were observed to date. In particular, chemotherapy and surgery remain the main methods for the therapy of brain tumors

Mitusova et al. Journal of Nanobiotechnology 2022

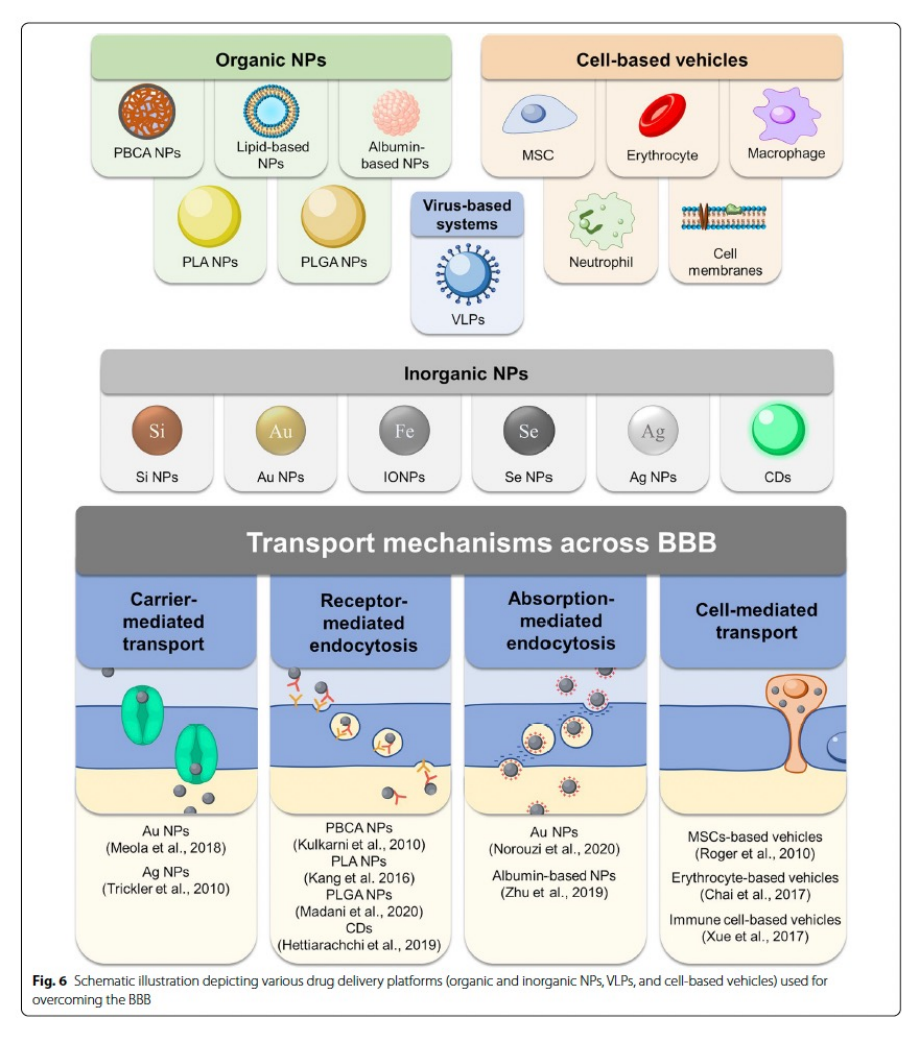
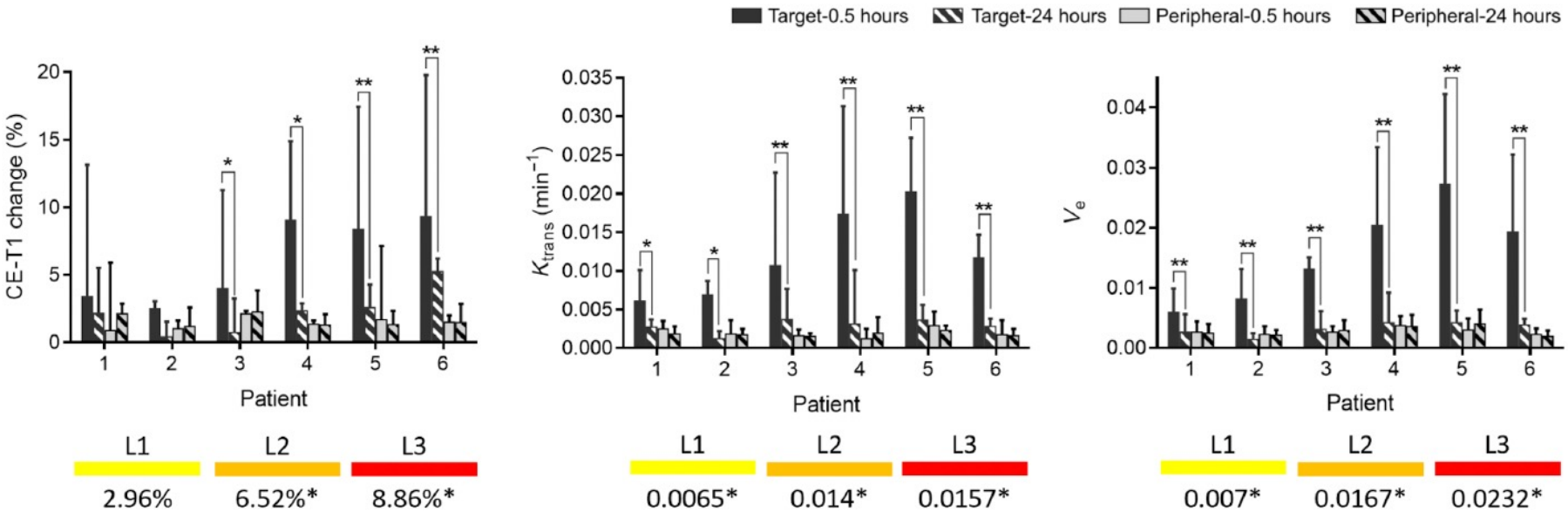


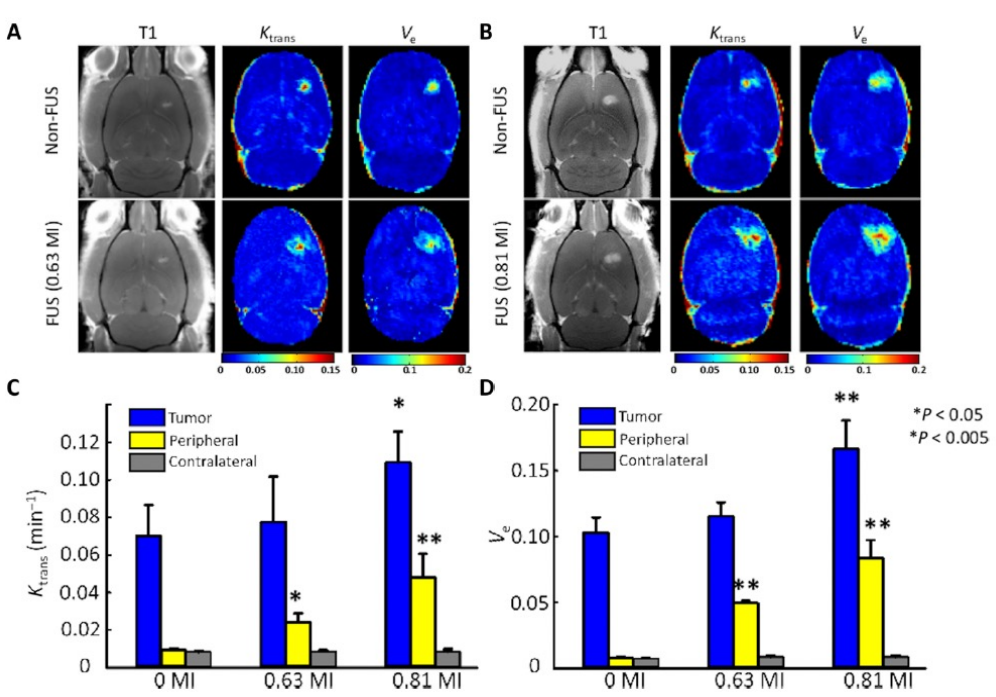
Fig. 6 Schematic illustration depicting various drug delivery platforms (organic and inorganic NPs, VLPs, and cell-based vehicles) used for overcoming the BBB

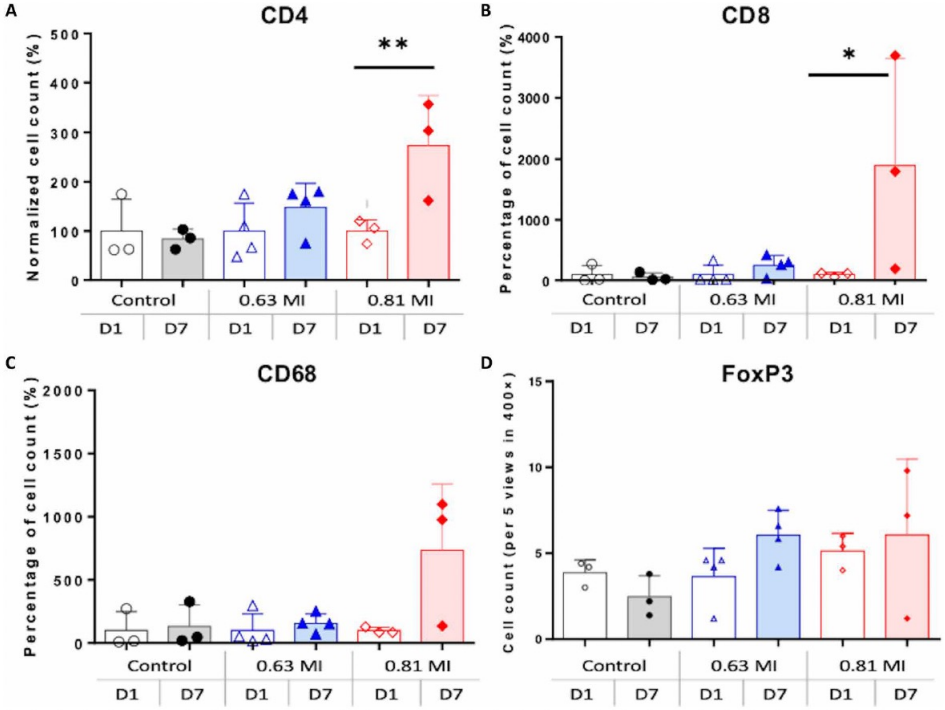
Neuronavigation-guided focused ultrasound for transcranial blood-brain barrier opening and immunostimulation in brain tumors



Chen et al. Sci. Adv. 2021

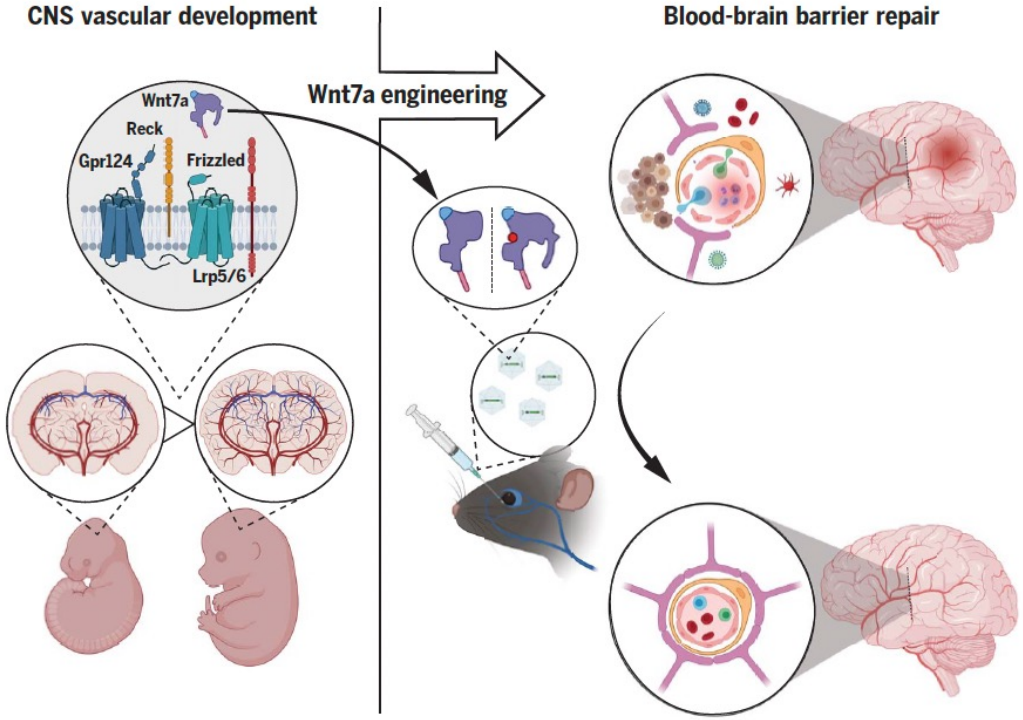
Neuronavigation-guided focused ultrasound for transcranial blood-brain barrier opening and immunostimulation in brain tumors





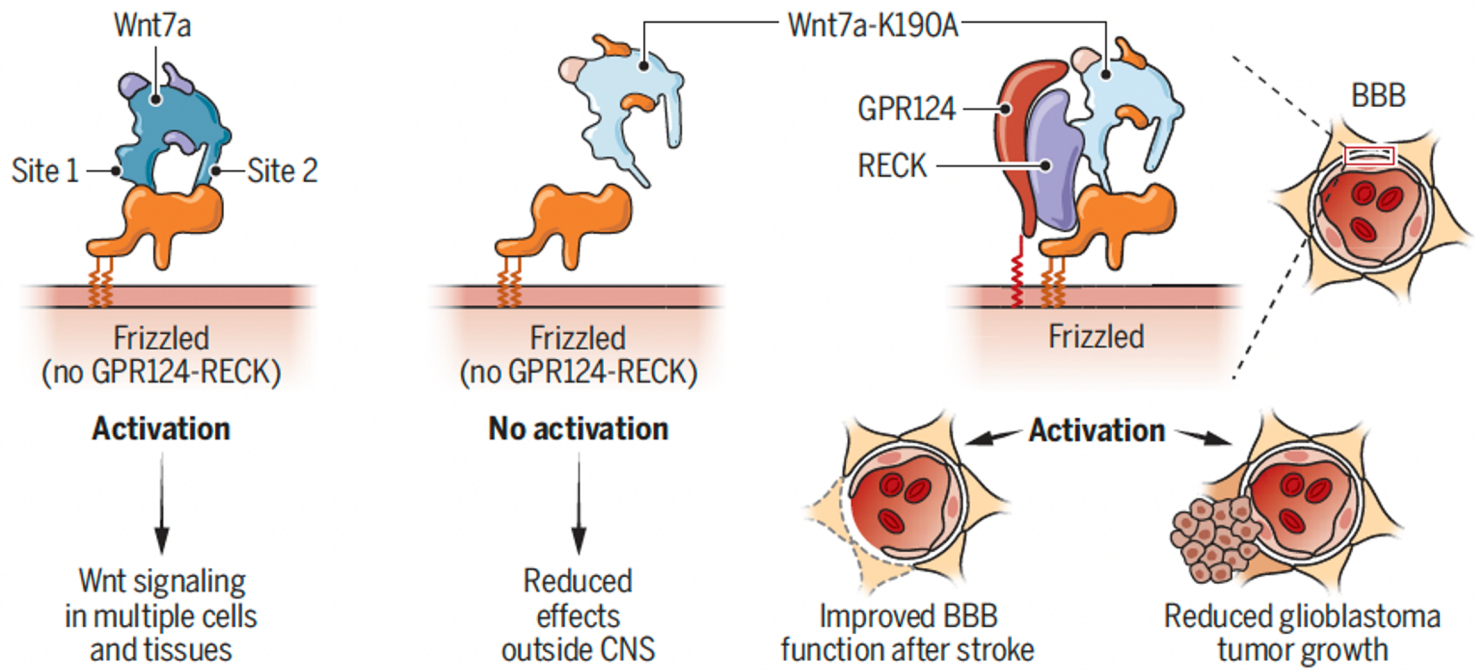
Chen et al. Sci. Adv. 2021

Therapeutic approaches to restore BBB functions



Repurposing Wnt7a ligands into BBB therapeutics

Martin M. et al. Science 2022
McMahon AP, Ichida JK. Science 2022



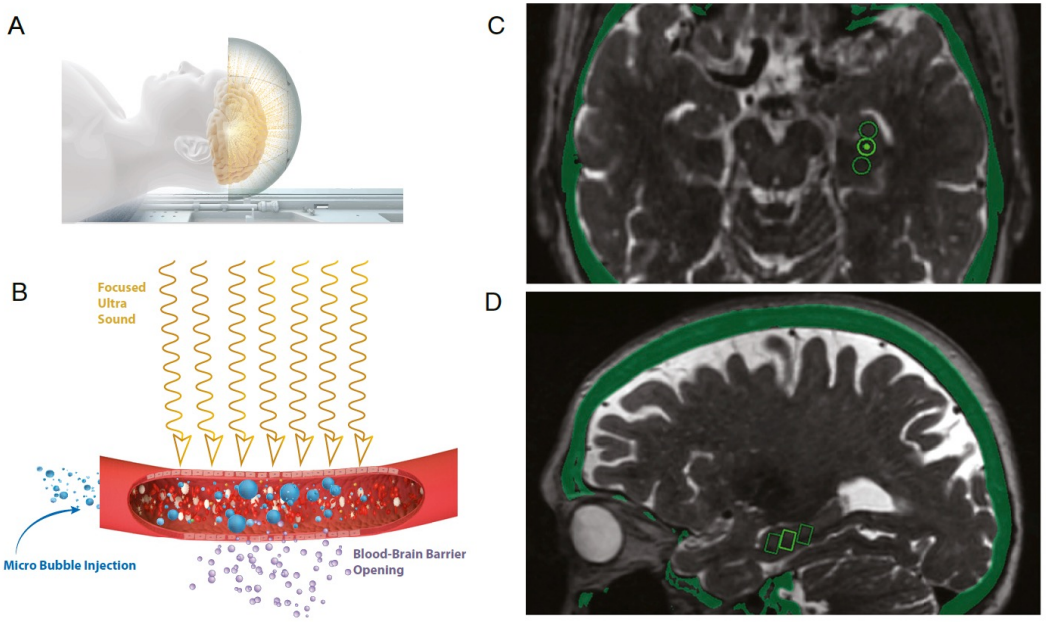
Martin M. et al. Science 2022
McMahon AP, Ichida JK. Science 2022

Acknowledgments

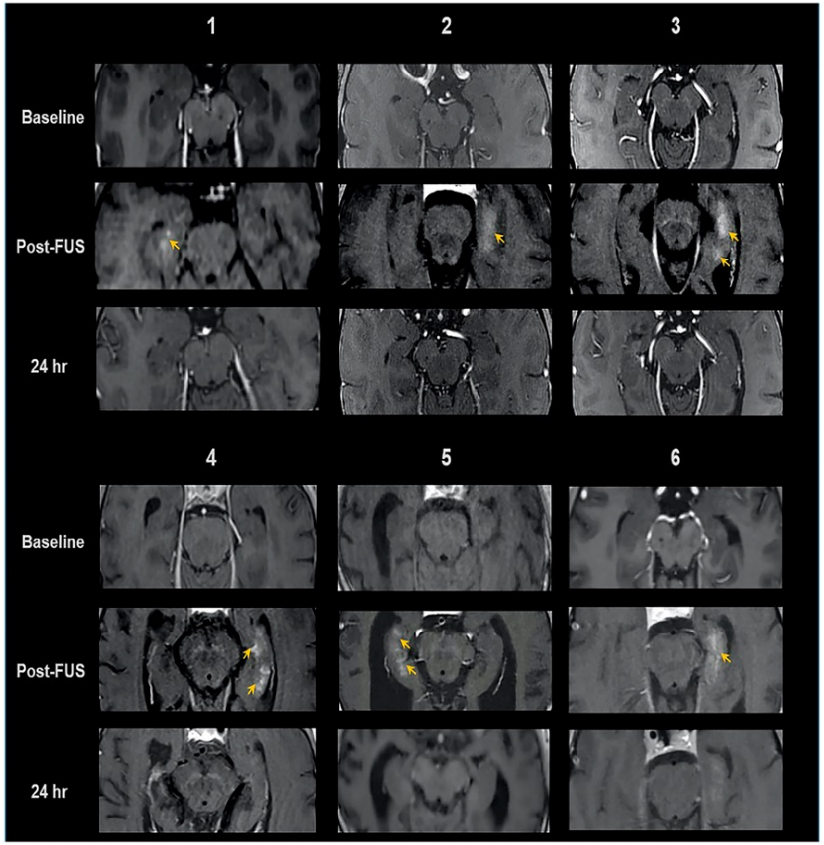
Our patients, their families and caregivers

Hematologists, oncologists, neuro-radiologists, radiation oncologist, ophthalmologist, pathologists and infectiologists

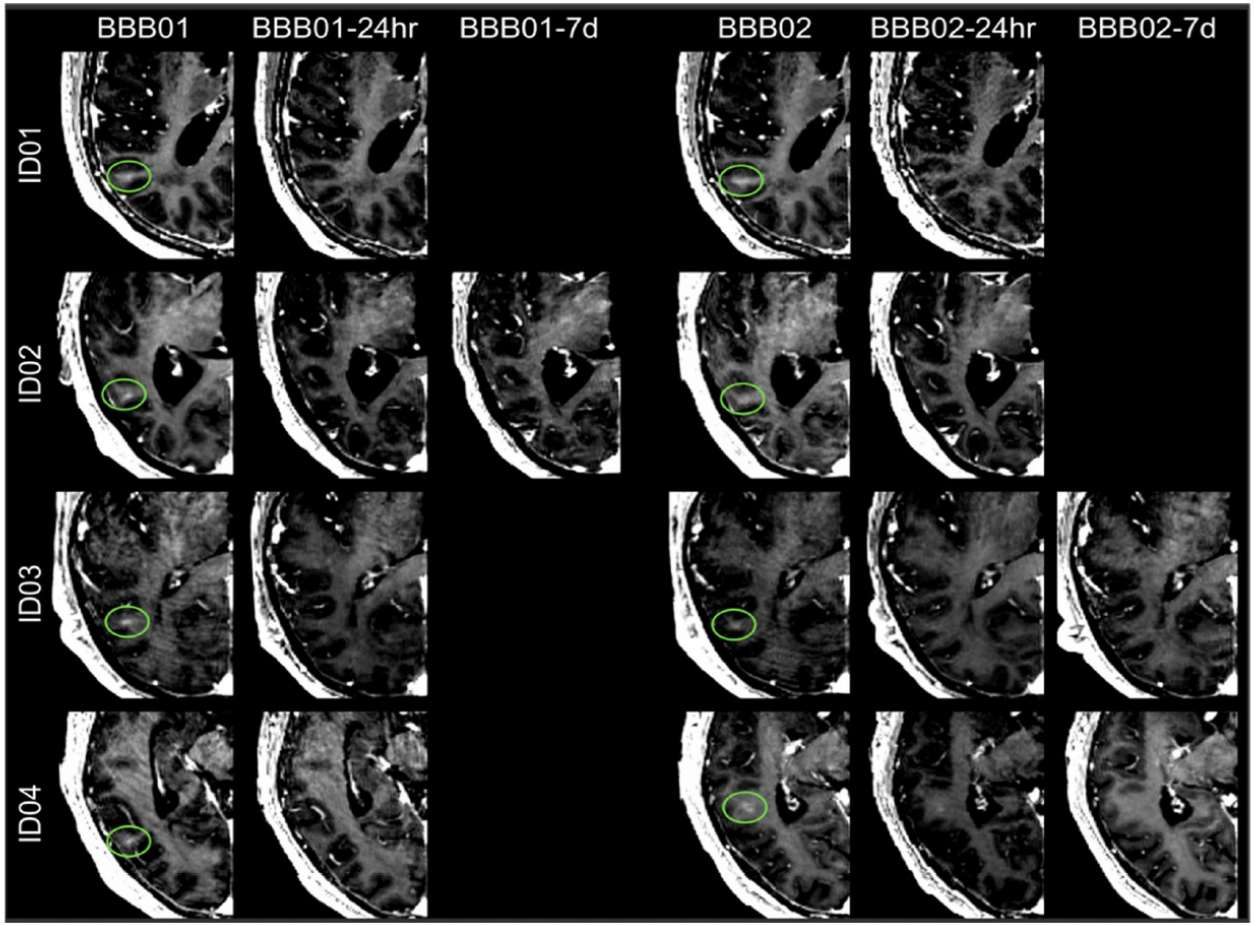
Noninvasive hippocampal blood–brain barrier opening in Alzheimer's disease with focused ultrasound



Rezai A.L. et al. PNAS 2020

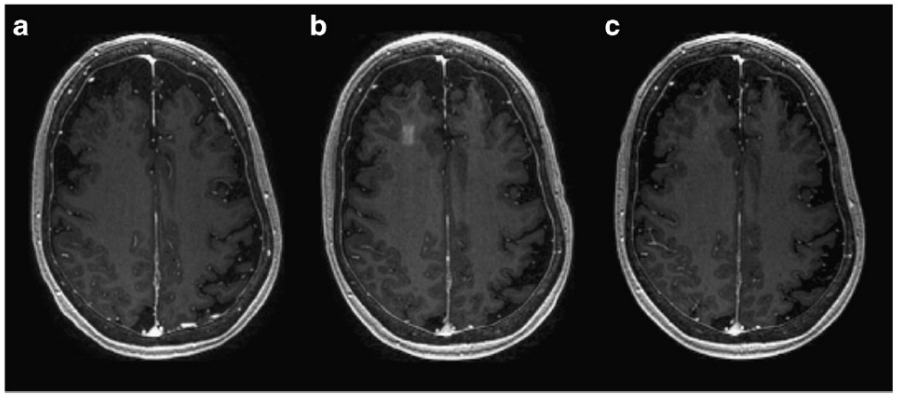


Blood-brain barrier opening with FUS in Parkinson's disease dementia



Gasca-Salas C. et al. Nat Commun. 2021

Blood–brain barrier opening in Alzheimer's disease using MR-guided focused ultrasound



Lipsman N. et al. Nat Commun. 2021