UNIVERSITÀ DEL PIEMONTE ORIENTALE

Blood-brain and blood-tumour barrier: from biology to diagnosis and therapy

Gianluca Gaidano, M.D., Ph.D.

Division of Hematology
Department of Translational Medicine
Università del Piemonte Orientale
Novara, Italy

Disclosures of Gianluca Gaidano

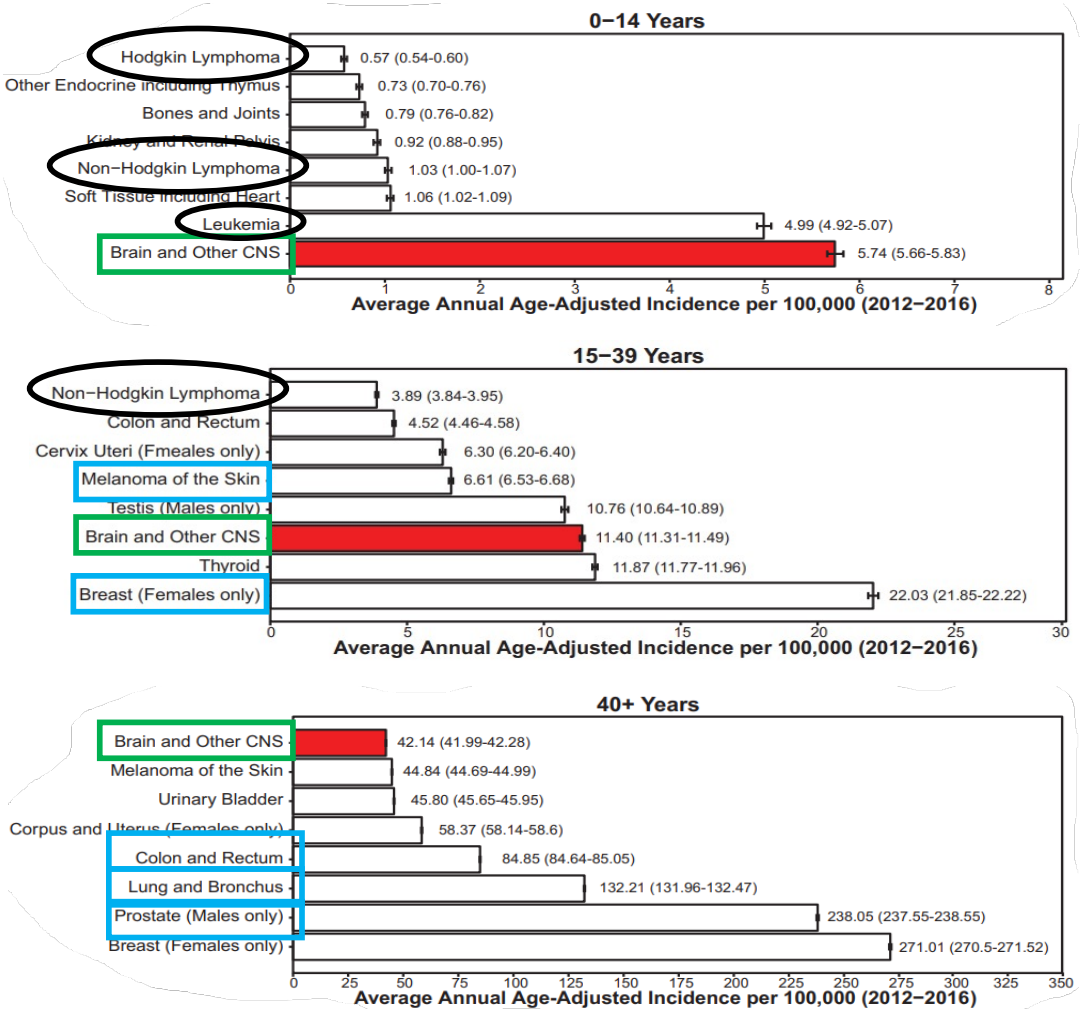
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Abbvie					√		
Astra Zeneca						√	
BeiGene						√	
Incyte						√	
Janssen					√	√	
Roche						√	

Agenda

- **Blood-brain barrier (BBB): biology and function**
- **Blood-tumour barrier (BTB)**
- **CNS bioavailability of drugs in hematologic malignancies**

CNS tumours trigger transformation from BBB to BTB

Incidence of primary and metastatic brain tumours



Blood-Brain Barrier (BBB)

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Brain colonization by a primary or metastatic CNS tumour

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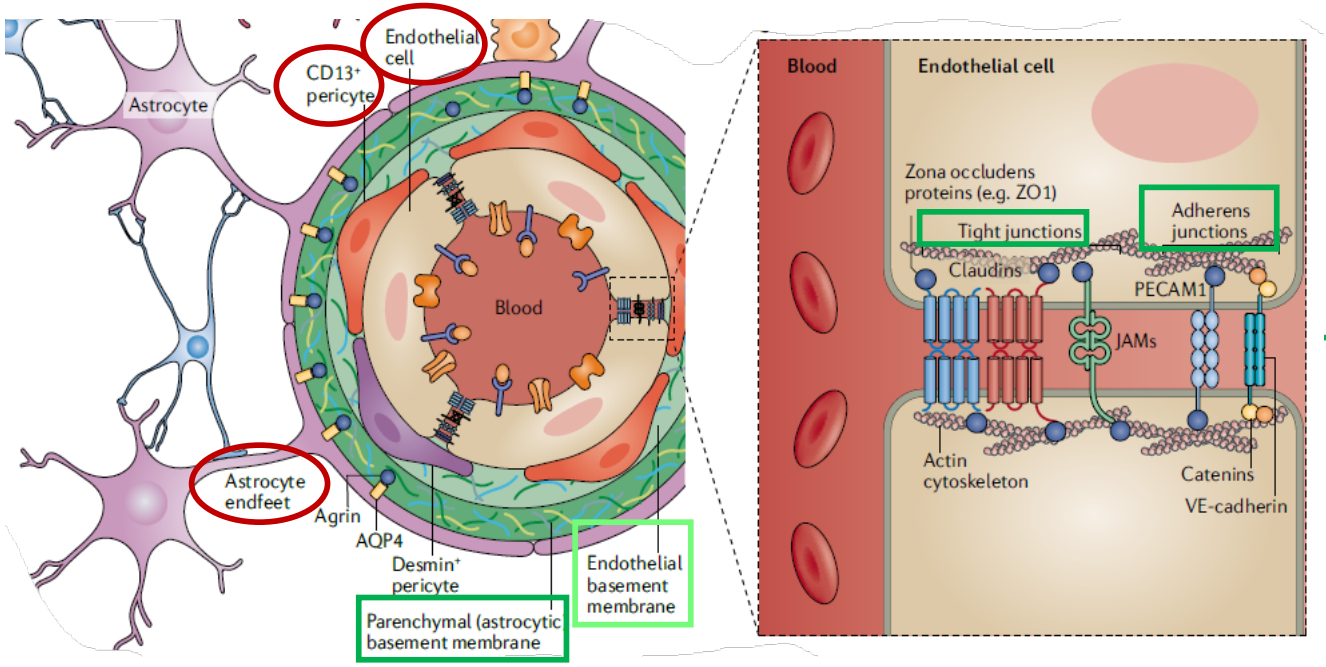
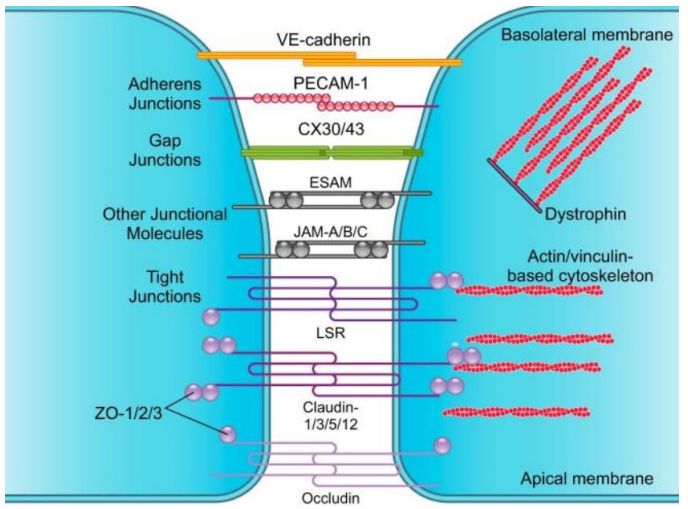
Blood-Tumour Barrier (BTB)

Ostrom *et al., Neuro Oncol*. 2019

BBB forms a tightly regulated neurovascular unit to maintain brain function





Main components of BBB:

- **Endothelial cells**
- **Pericytes**

} **Endothelial basement membrane**

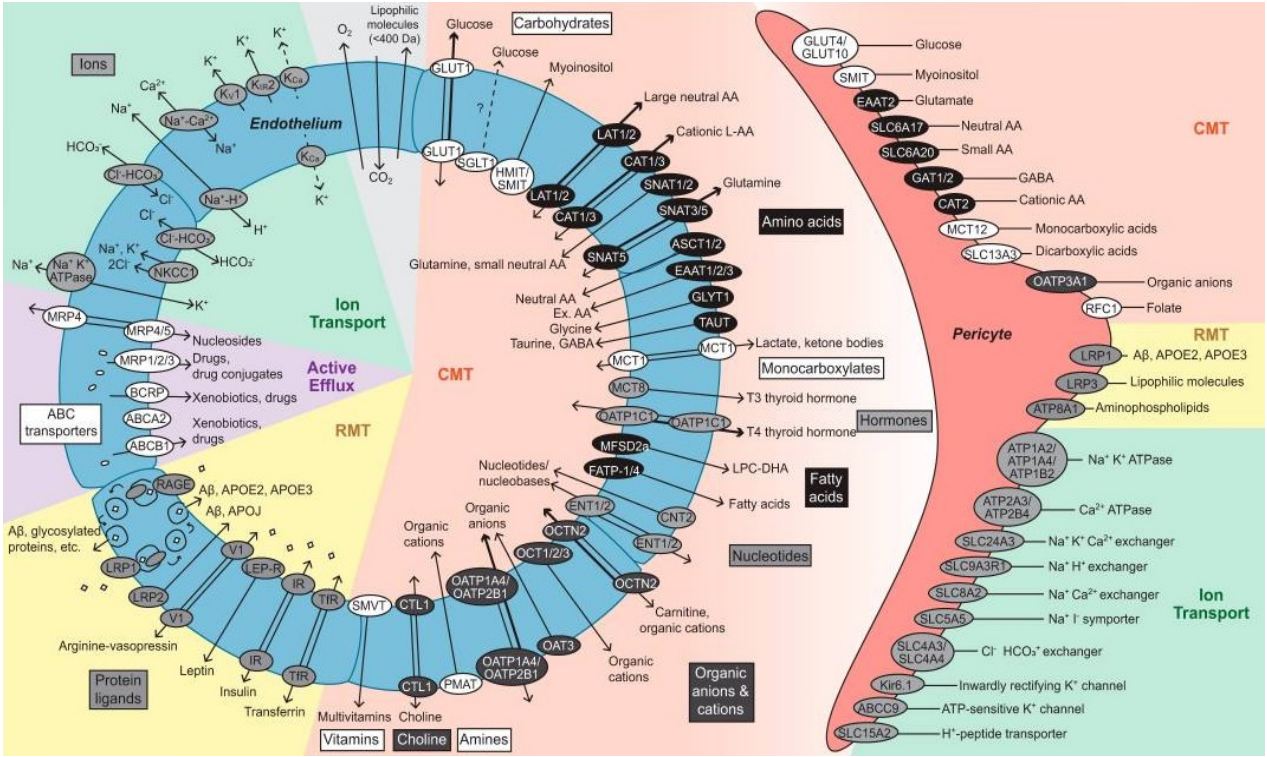
- **Astrocyte endfeet**

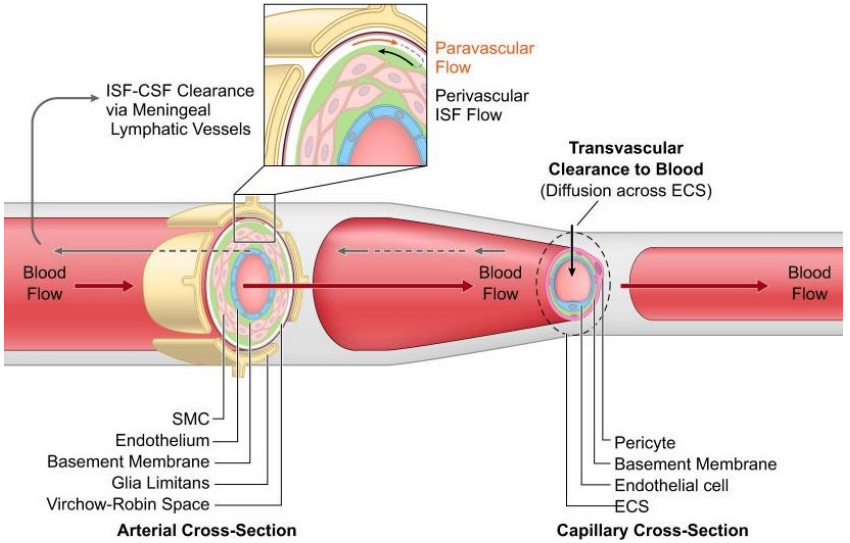
} **Parenchimal basement membrane**

In contrast to leaky capillary endothelium in peripheral organs, in **normal BBB** entry of substances **paracellularly is restricted by adherens junctions and continuous tight junctions**

Sweeney *et al, Physiol Rev*. 2019; Steeg, *Nat Rev Clin Oncol*. 2021

BBB regulation relies on multiple transport systems





Endothelial cells

- Solute carrier-mediated transport
- Receptor mediated transport
- Ion transport
- **Active efflux**

Pericytes

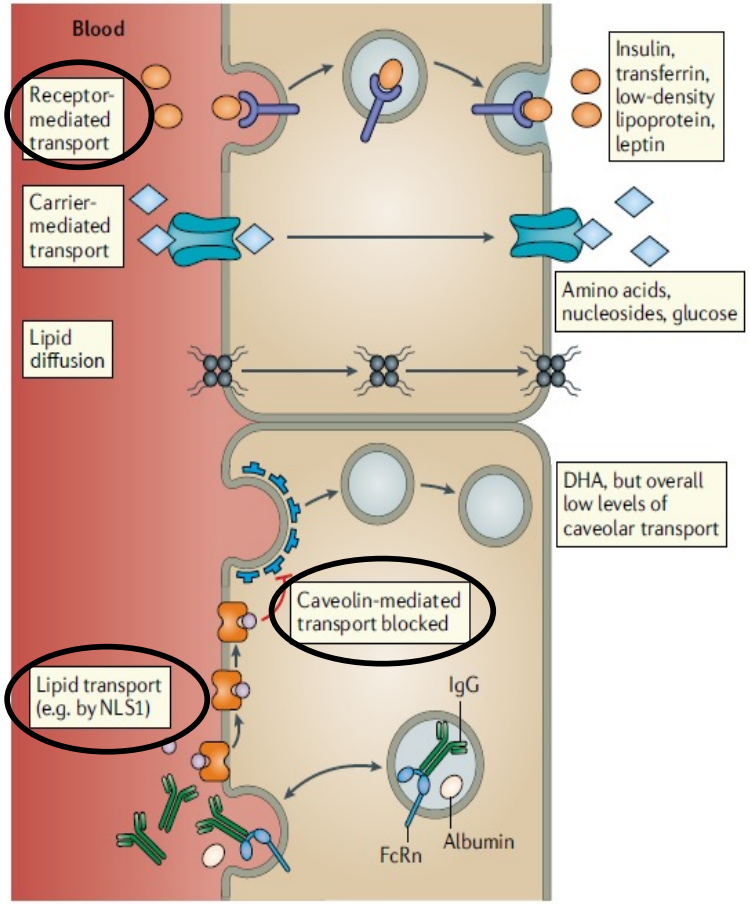
- Solute carrier-mediated transport
- Receptor mediated transport
- Ion transport

Other vascular-mediated transport

Some solutes diffuse across brain endothelial cells and are cleared by the **perivascular interstitial fluid flow**, which travels in the reverse direction of the blood flow and drains into meningeal lymphatic vessels

Sweeney *et al, Physiol Rev*. 2019

BBB endothelial cells express a multitude of uptake and efflux transporters and prevent leukocytes ingress



Apart from receptor-mediated transport, **transcytosis is minimal**

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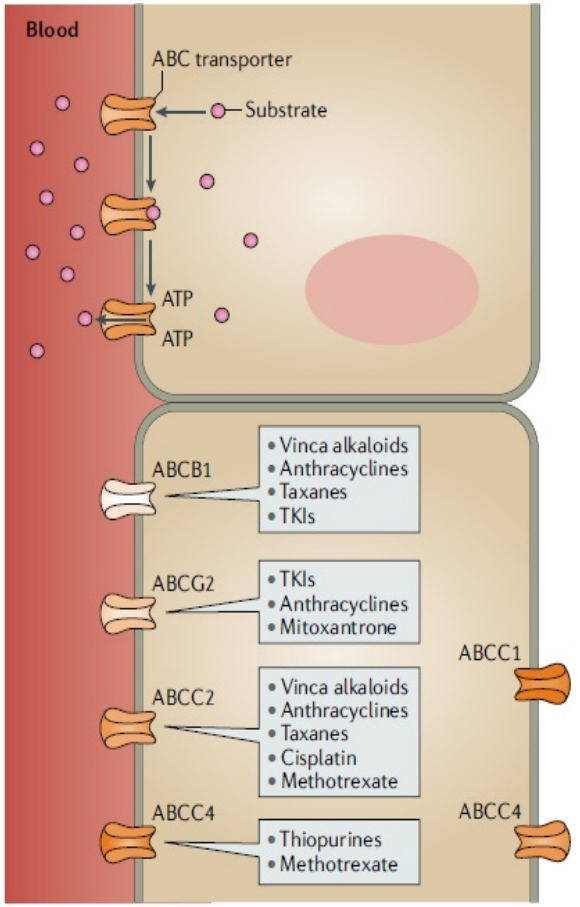
Caveolin-dependent transcytotic pathway is suppressed by **MFSD2A/NLS1**, which **maintains BBB impermeability**

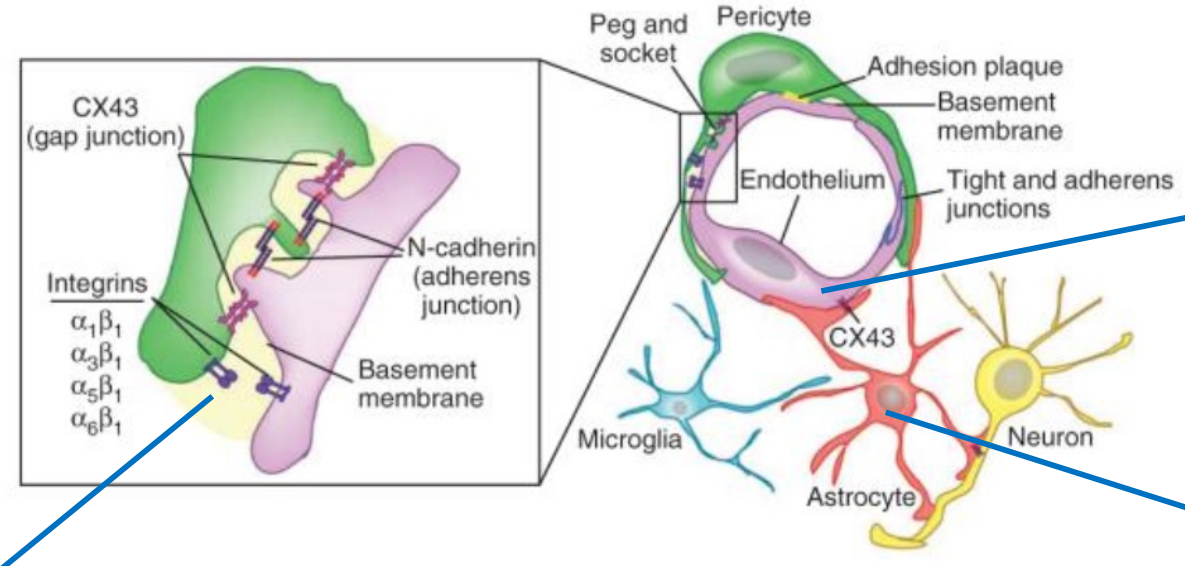
BBB endothelial cells lacks E-selectin and ICAM1 expression



ATP-binding cassette proteins export metabolites and xenobiotics, **reducing anticancer drugs delivery to CNS**

Steeg, *Nat Rev Clin Oncol*. 2021; Villaseñor *et al., Cell Mol Life Sci*. 2019; Ben-Zvi *et al., Nature*. 2014

Pericytes and astrocytes interact with endothelial cells and contribute to BBB stability



Pericytes guide astrocytes endfeet to the BBB

Astrocytes regulate pericyte differentiation

Pericytes interact with endothelial cells **regulating vascular diameter, blood flow and angiogenesis**

Endothelial cells secrete PDGF-B, which is essential for pericytes recruitment and survival

Depletion of **pericytes** or **AQP4 expressed by astrocytes** results in increased BBB permeability in mice

Astrocytes link the **regulation of cerebral flow** to **neuronal activity** (neurovascular coupling)

Astrocytes **promote BBB stability thanks to laminin α2 secretion**

Winkler *et al., Nat Neurosci*. 2011; Yao *et al., Nat Commun*. 2014

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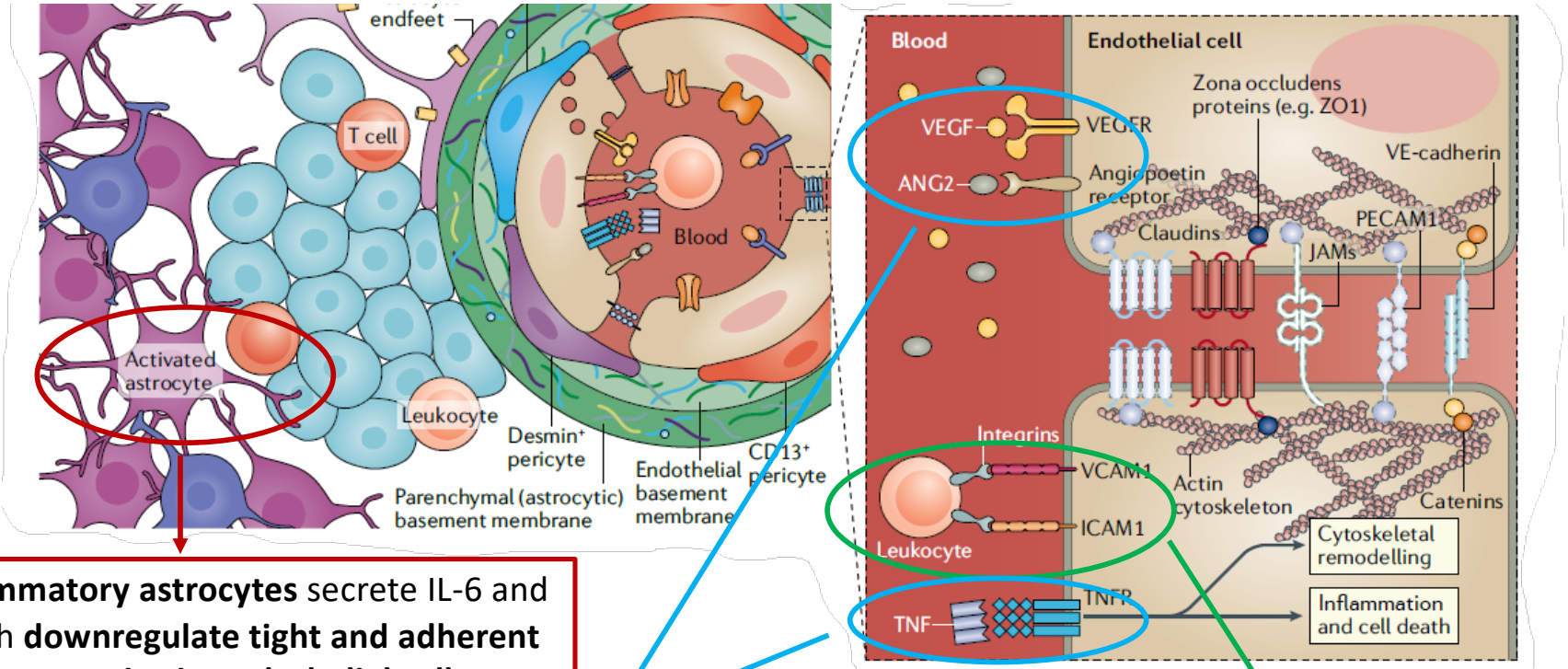
Cancer cell colonization drives transformation of BBB into BTB



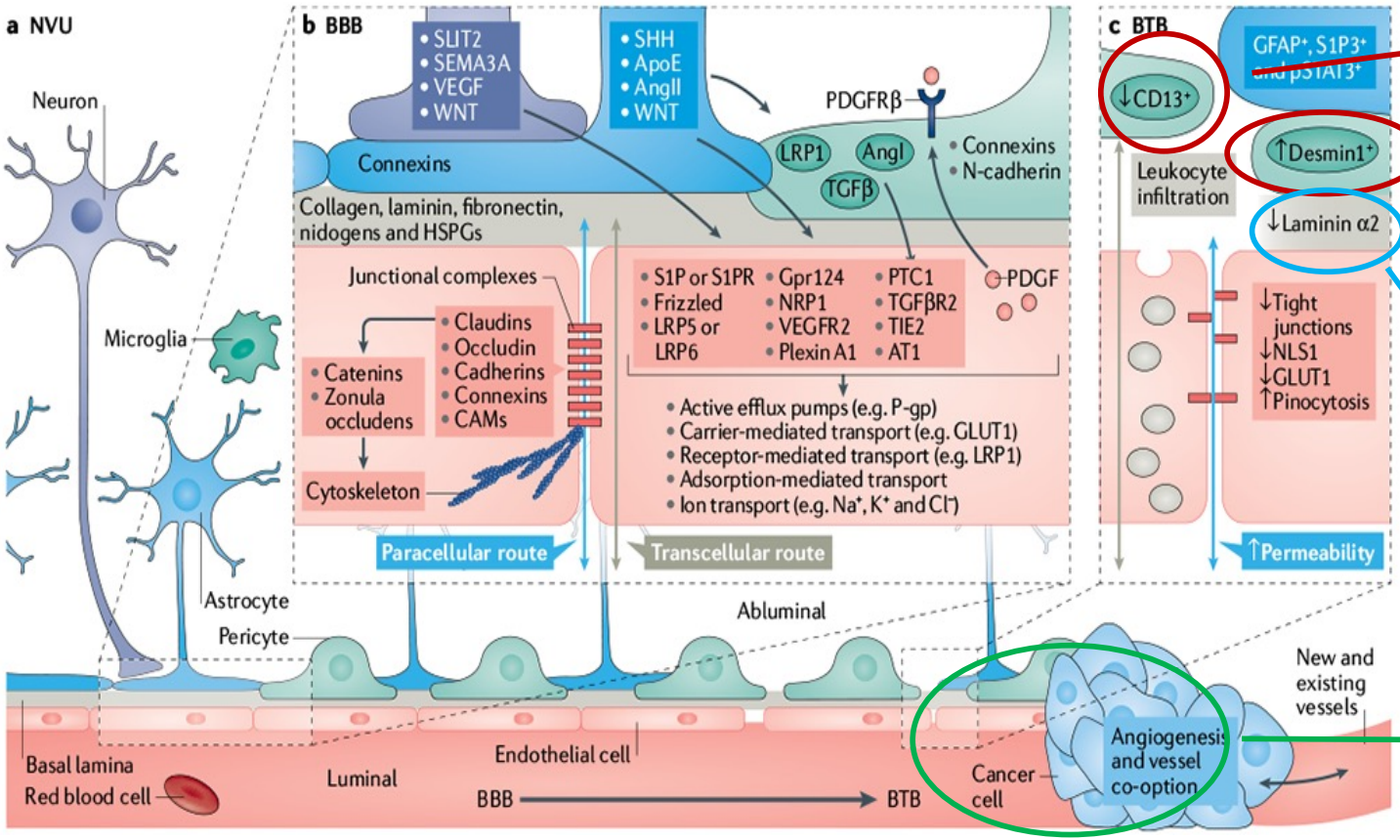
Neuroinflammatory astrocytes secrete IL-6 and CCL2, which **downregulate tight and adherent junction proteins in endothelial cells**

Tumour cell presence leads to VEGF, ANG2 and TNF expression, with **changes in endothelial cells cytoskeleton, junction proteins expression and BTB permeability**

In BTB cell adhesions molecules are upregulated, leading to **immune infiltration**

Sweeney *et al, Physiol Rev*. 2019; Steeg, *Nat Rev Clin Oncol*. 2021

Cancer cell colonization drives transformation of BBB into BTB



The **loss of pericytes expressing CD13** and the **gain of pericytes expressing desmin1** in BTB is associated with **increased paracellular permeability**

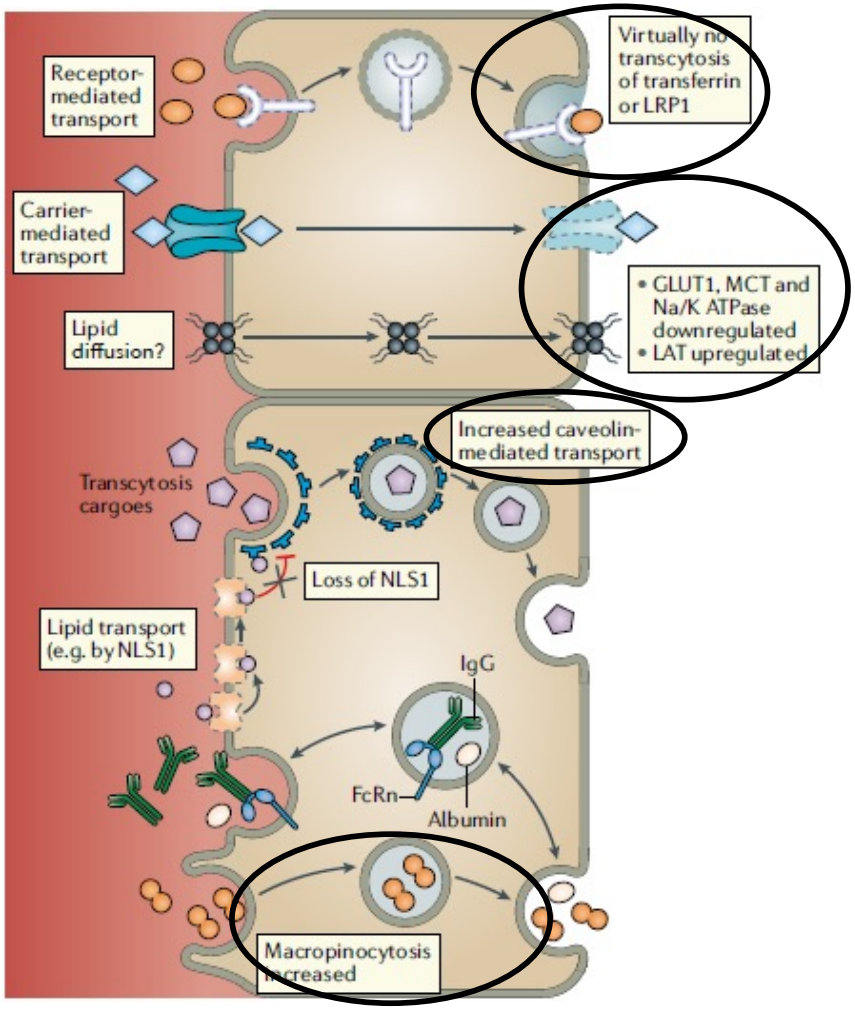
Basement membranes are altered in BTB, with a **loss of laminin α2** and other protein components

Cancer cells cause **astrocytes endfeet displacement**

AQP4 expression is decreased in BTB

Arvanitis *et al., Nat Rev Cancer*. 2020; Steeg, *Nat Rev Clin Oncol*. 2021

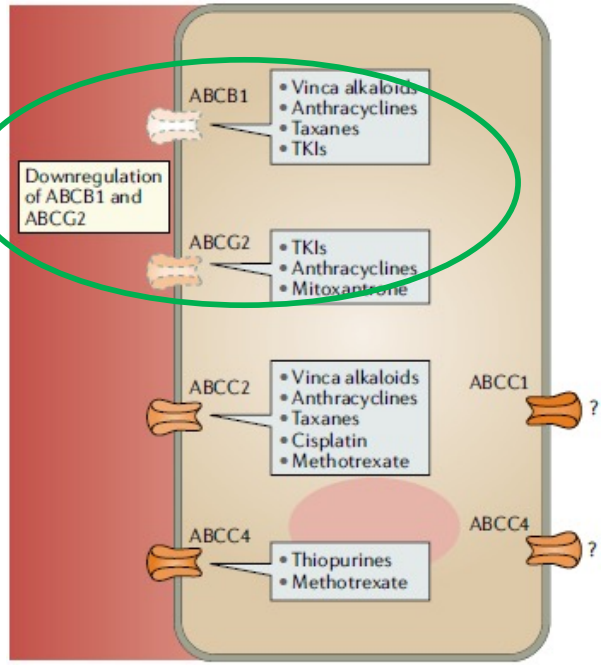
Cancer cell colonization drives transformation of BBB into BTB



Alterations typical of BTB include **downregulation of many receptor-mediated transport pathways**

Loss of NLS1/MFSD2A leads to an increase in caveolin-mediated transcytosis

Micropinocytosis is also increased



Some efflux transporters are downregulated, but many datas are lacking

Steeg, *Nat Rev Clin Oncol*. 2021

BTB: changes in permeability to anticancer drugs

Overall, the BTB is heterogeneously permeable to many drugs, contributing to poor therapeutic efficacy

BTB: changes in permeability to anticancer drugs

Tracers and small-molecule drugs			
TRD	Implanted RG-2 glioma cells; experimental metastases of HER2$^+$ MDA-MB-231BR breast cancer cells	QA and IF	In gliomas, the distribution of TRD was relatively homogeneous, with a 2–5-fold increase in levels over uninvolved brain; in brain metastasis, permeability was heterogeneous[154]
AIB	Spontaneous metastases derived from Walker 256 breast cancer cells subcutaneously implanted into rats	QA	AIB distribution increased with brain metastasis size but remained one-third lower than that of subcutaneous tumours[64]
AIB	Experimental metastasis of PC9 NSCLC cells	QA	Heterogeneous permeability of brain metastases to AIB; cisplatin–etoposide decreased AIB permeability[155]
TRD	Implantation of DIPG cells	IF	Intact BBB; expression of ABCB1, ABCG2 and ABCC1 was unchanged in the BTB relative to the BBB[156]
Paclitaxel, doxorubicin, TRD and AIB	Experimental metastasis of MDA-MB-231 and 4T1 breast cancer cells	Perfusion, QA and IF	Heterogeneous drug and tracer levels; only the ~10% of brain metastases with the highest paclitaxel distribution contained apoptotic cells[52]
Doxorubicin	Implantation of GBM8401 glioblastoma cells	MS	Ratio of drug levels in the tumour versus contralateral brain tissue was ~2 and was further elevated 2.4-fold with FUS[128]
Vinorelbine and TRD	Experimental metastases of MDA-MB-231BR cells	Perfusion, QA and IF	Heterogeneous drug levels; median vinorelbine concentration was fourfold greater in brain metastases than in the uninvolved brain but only 8% of drug levels in extracranial metastases[157]
Temozolomide	Implanted human glioma cells	MS and microdialysis	Heterogeneous drug distribution in tumours; C_{max} in uninvolved brain and tumour was 2.7 and 4.0 μg/ml, respectively, versus 21.9 μg/ml in plasma[53]
Temozolomide	Implanted U87 glioma cells	MS	Heterogeneous distribution in brain tumours, 20–30% of that in plasma; FUS induced a twofold increase in intratumoural drug concentrations[158]
Vincristine and TRD	Implanted SHH subtype and β-catenin-mutant WNT subtype medulloblastoma cells	Perfusion and IF	Dextran distributed to WNT but not SHH subtype tumours — WNT tumours are more sensitive to vincristine[60]
MK-1775 (WEE1 inhibitor)	GBM22 glioblastoma cells implanted into brains or flank	MS	Heterogeneous distribution; MK-1775 levels in brain tumour sections were lower than those in flank tumours[108]
GNE-317 and GDC0980 (PI3K inhibitors) as well as sodium fluorescein	Experimental metastases of RFP-A2058BR3 melanoma cells	Two-photon microscopy through a cranial window	71% of metastases were impermeable to sodium fluorescein and grew more slowly; the BBB-penetrant PI3K inhibitor (GNE-317) slowed the growth of all metastases; the non-BBB-penetrant PI3K inhibitor (GDC0980) only slowed the growth of permeable metastases[85]
GNE-317 and GDC0980	Implantation of GL261 glioblastoma cells	MS	Brain tumours had increased distribution of GNE-317 in brain core and rim, relative to that of GDC0980 (REF.[159])
Osimertinib, gefitinib, rociletinib and afatinib	Experimental metastasis of PC9 cells and H1975 NSCLC cell implantation	QA and MS	Non-malignant brain tissue to plasma ratio was 3.4, 0.2, <0.1 and <0.1 for osimertinib, gefitinib, rociletinib and afatinib, respectively; in the H1975 xenograft model, osimertinib had a C_{max} of 1.9, 1.0 and 0.7 for plasma, brain and tumour tissues, respectively, and inhibited experimental brain metastases[138]
Lapatinib and TRD	Experimental metastasis of MDA-MB-231 cells transfected with HER2	Perfusion, QA and MS	Heterogeneous levels of lapatinib in brain metastases, with a 7–9-fold greater distribution than in uninvolved brain but only 10–20% of the levels in extracranial metastases[81]

Experimental brain-directed therapeutics			
HA-conjugated paclitaxel and unconjugated paclitaxel	Experimental metastasis of triple-negative breast cancer	Perfusion and IF	HA-conjugated paclitaxel infiltrated brain metastases; OS of mice was longer with HA–paclitaxel than with free paclitaxel; in vitro data suggests an endocytic mechanism of uptake for HA–paclitaxel[89]
ANG1005 (paclitaxel conjugated to angiopep 2), angiopep 2 and paclitaxel	Experimental metastasis of MDA-MB-231 cells	QA with vascular correction	Greater penetration of ANG1005 versus angiopep 2 or paclitaxel into non-malignant brain tissue; ANG1005 distributed to both brain metastases and the uninvolved brain[86]
Paclitaxel ± angiopep 2, angiopep 2 and angiopep 2–TAT (a cell-penetrating peptide)	Implanted U87 glioblastoma cells	ELISA of brain homogenate	Free paclitaxel, angiopep 2 and angiopep 2–TAT were more abundant in the brain parenchyma than in tumours, although the levels of angiopep 2 and angiopep 2–TAT were higher than those of free paclitaxel[87]
Irinotecan, liposomes and liposomal irinotecan	Experimental metastasis of MDA-MB-231BR cells	Perfusion, IF and MS	Tumour to plasma ratio of active metabolite was 0.05–0.90 for liposomal irinotecan but was unmeasurable for free irinotecan; labelled liposomes were detected in and prevented metastases[90]
Antibody-based agents			
^{125}I-trastuzumab and TRD	Experimental metastasis of MDA-MB-231BR cells transfected with HER2	IF and QA	3% of serum trastuzumab reached the non-malignant brain, whereas ~5% reached brain metastases; drug uptake in the most permeable lesions was eightfold over that in uninvolved brain; heterogeneous permeability within and between metastases correlated with TRD distribution but not with tumour size[80]
^{89}Zr-trastuzumab	Implanted tumours from HER2$^+$ GEM	PET 1–5 days post-injection	Percent injected dose per gram of tissue was 24% and 9.2% in brain tumours and contralateral brain tissue, respectively; trastuzumab extended mouse survival[160]
Trastuzumab and T-DM1	Implanted HER2$^+$ BT-474 breast cancer cells	Western blotting of tumour tissue	Heterogeneous levels of trastuzumab and T-DM1; permeability to each drug was similar; vessel density and vascular volume not significantly different between antibody-treated and control groups[76]
Doxorubicin, T-DM1 and trypan blue	Implanted HER2$^+$ BT-474 cells	FUS and intravital microscopy	FUS increased doxorubicin uptake into tumour over a 10-minute time course; uptake was highest in tissue nearest the vasculature; FUS with microbubbles accelerated T-DM1 entry into tumours[129]
Trastuzumab and HRP	Experimental metastasis from syngeneic 4T1 cells	Histology and single-photon emission tomography	<20% of metastases showed any uptake of HRP; trastuzumab was also excluded from brain metastases; TNF and lymphotoxin transiently increased permeability[62]
Biparatopic anti-HER2 ADC and TRD	Experimental metastasis of HER2$^+$ JIMT-1 and SUM190 breast cancer cells	IF using anti-human IgG	ADC permeated a median of 4–6% of the area of JIMT-1 lesions, with a maximum of ~18%, and a median of 7–17% of the area of SUM190 lesions, with a maximum of >80%; ADC uptake was observed without TRD staining and was associated with epithelial endocytosis in vitro[67]
Angiopep 2 conjugate to anti-HER2 antibody (ANG4043)	Implantation model of HER2$^+$ tumour	IF	Increased uptake of ANG4043 in brain tumour and uninvolved brain compared with the anti-HER2 antibody alone; ANG4043 conjugate increased survival[88]
^{125}I-trastuzumab–melanotransferrin conjugate (BT2111), trastuzumab and Texas red sulfur-rhodamine	Experimental metastasis of HER2$^+$ MDA-MB-231BR cells	QA and IF	Heterogeneous and overall higher uptake of BT2111 in brain metastases, predominantly in small lesions, as compared with trastuzumab. Mean K_in at 8 hours post-injection 11×10^{-7} for trastuzumab, $10–12 \times 10^{-6}$ for conjugate; conjugate substantially reduced the formation of brain metastases[62]

Many studies have investigated BTB permeability to anticancer drugs in *in vivo* models

BTB: changes in permeability to anticancer drugs

- **BTB is partially and heterogeneously permeable**: different levels of drugs and tracers between *different metastases within the same brain* and often between *areas within a single lesion*

- Brain metastases are always less permeable than other metastases of the same tumour: **drug and tracer distribution through the BTB remains limited in most cases**

- The development of new approaches for drug distribution through the BTB, exploiting mainly **paracellular permeability**, is a high priority to achieve additional clinical progress in the treatment of primary or secondary brain tumours

Arvanitis *et al., Nat Rev Cancer*. 2020; Steeg*, Nat Rev Clin Oncol*. 2021

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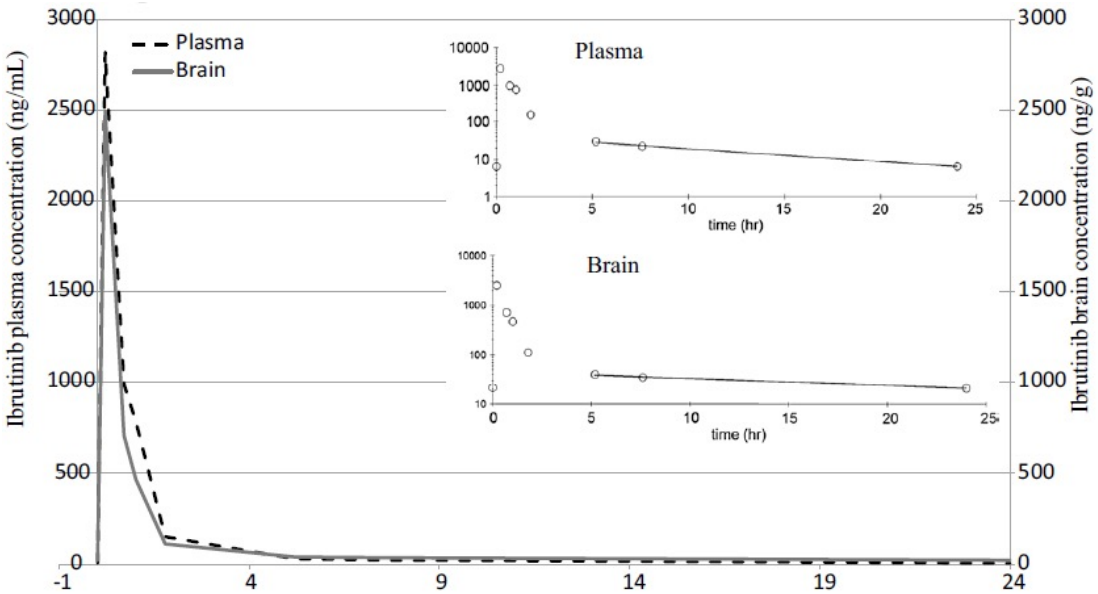
- Blood-tumour barrier (BTB)
- CNS bioavailability of drugs in hematologic malignancies

Ibrutinib vs BBB crossing chemotherapy in mantle cell lymphoma CNS relapse

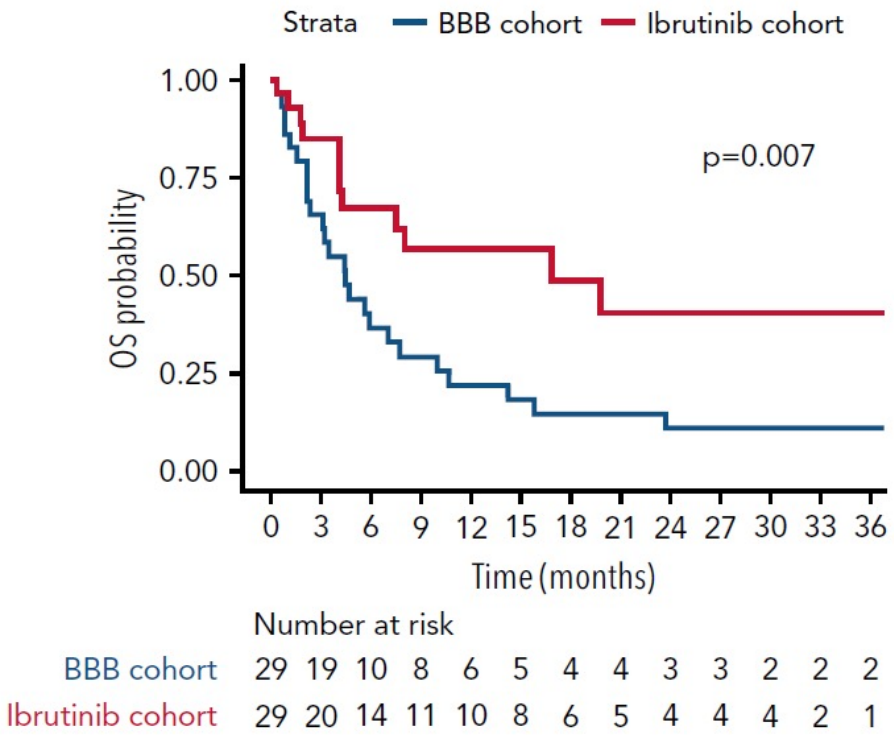


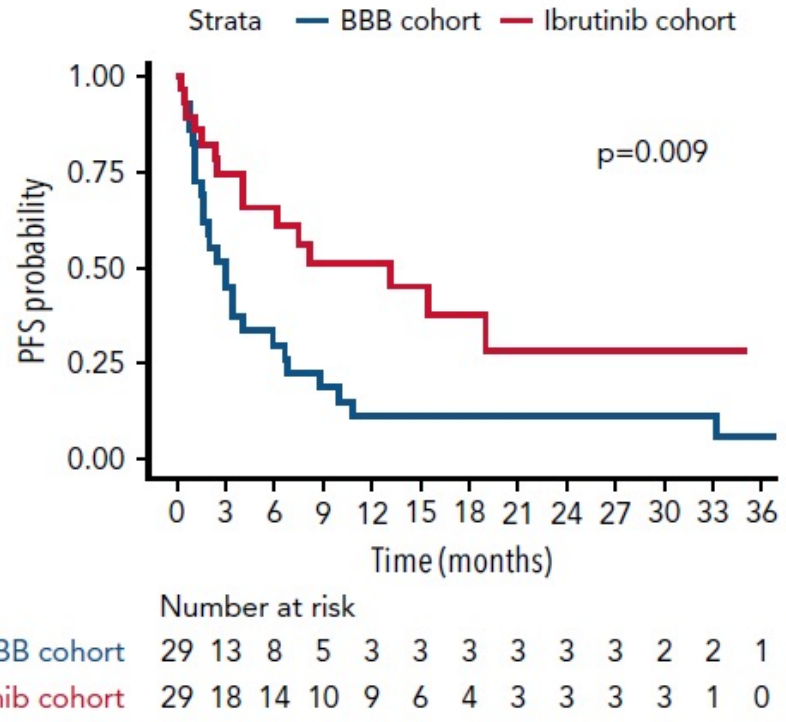
Preclinical studies have shown that **ibrutinib penetrates BBB and highly distributes in brain tissue**, supporting its use in a range of B-cell malignancies

Multicenter retrospective observational study to **compare ibrutinib vs BBB crossing chemotherapy** (HD-MTX, HD-Ara-C) in **mantle cell lymphoma CNS relapse**, a rare phenomenon with a dismal prognosis

Goldwirt *et al., Cancer Chemoter Pharmacol*. 2018; Rusconi *et al., Blood*. 2022

Ibrutinib vs BBB crossing chemotherapy in mantle cell lymphoma CNS relapse

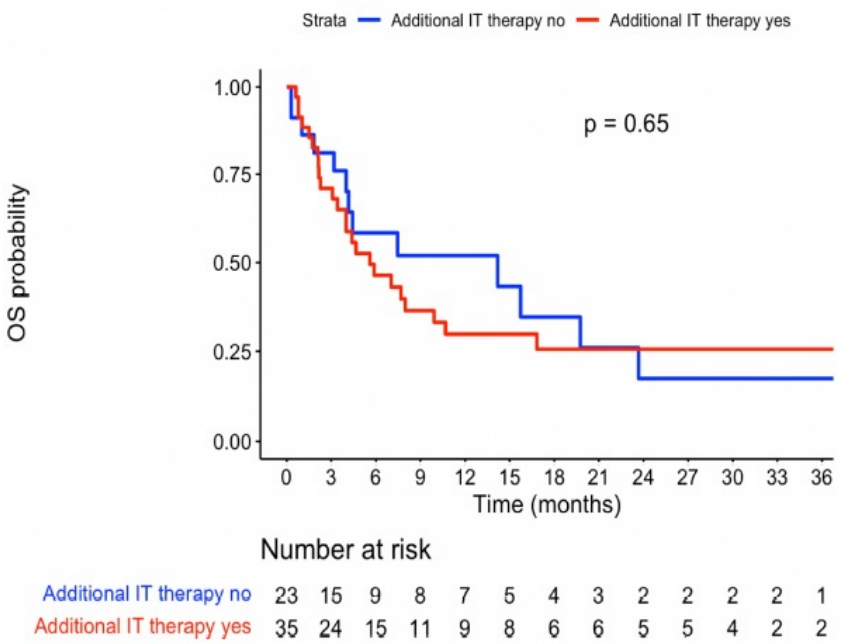


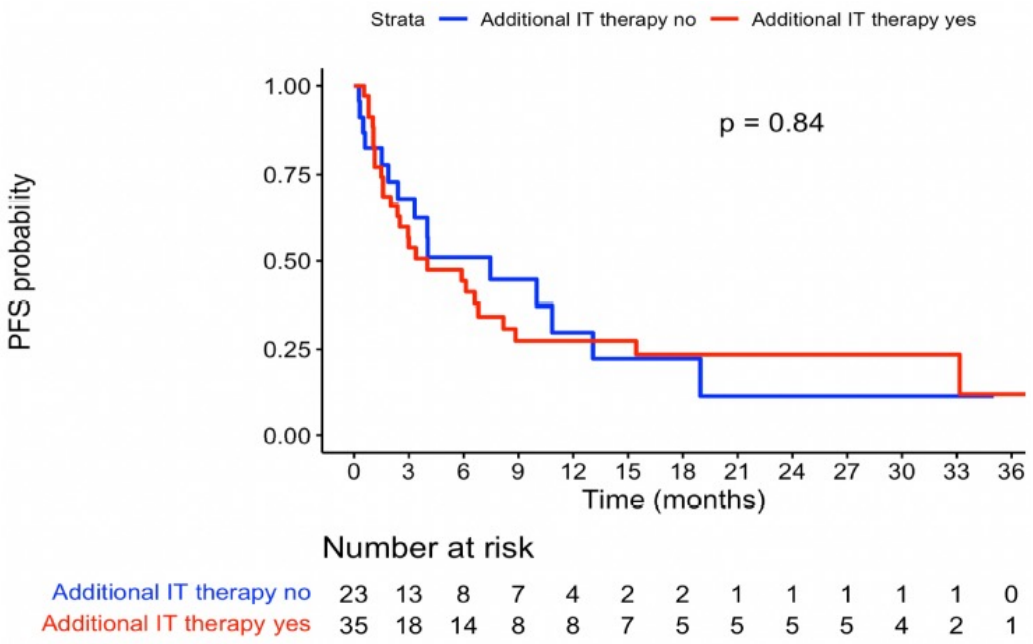


Ibrutinib therapeutic choice was the strongest independent favorable predictive factor for both OS and PFS
ORR was higher in the ibrutinib cohort (p=0.031)

Rusconi *et al., Blood*. 2022

Ibrutinib vs BBB crossing chemotherapy in mantle cell lymphoma CNS relapse

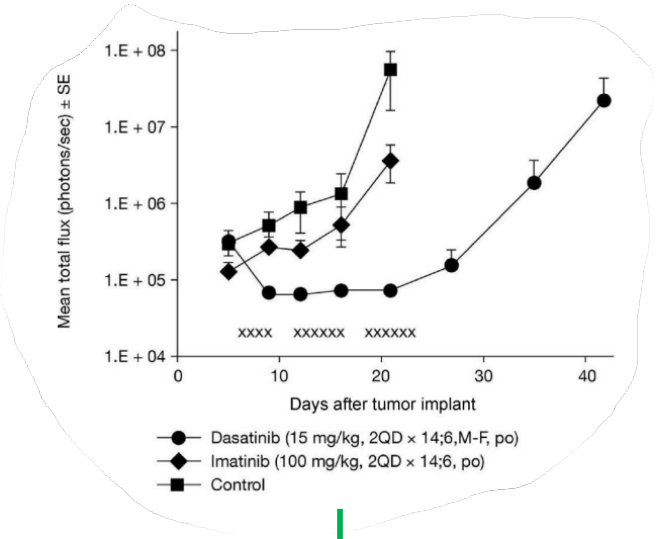




There was no PFS and OS benefit from the addition of intrathecal chemotherapy, known to be associated with morbidity and logistic burden, irrespectively of CNS-directed systemic therapy

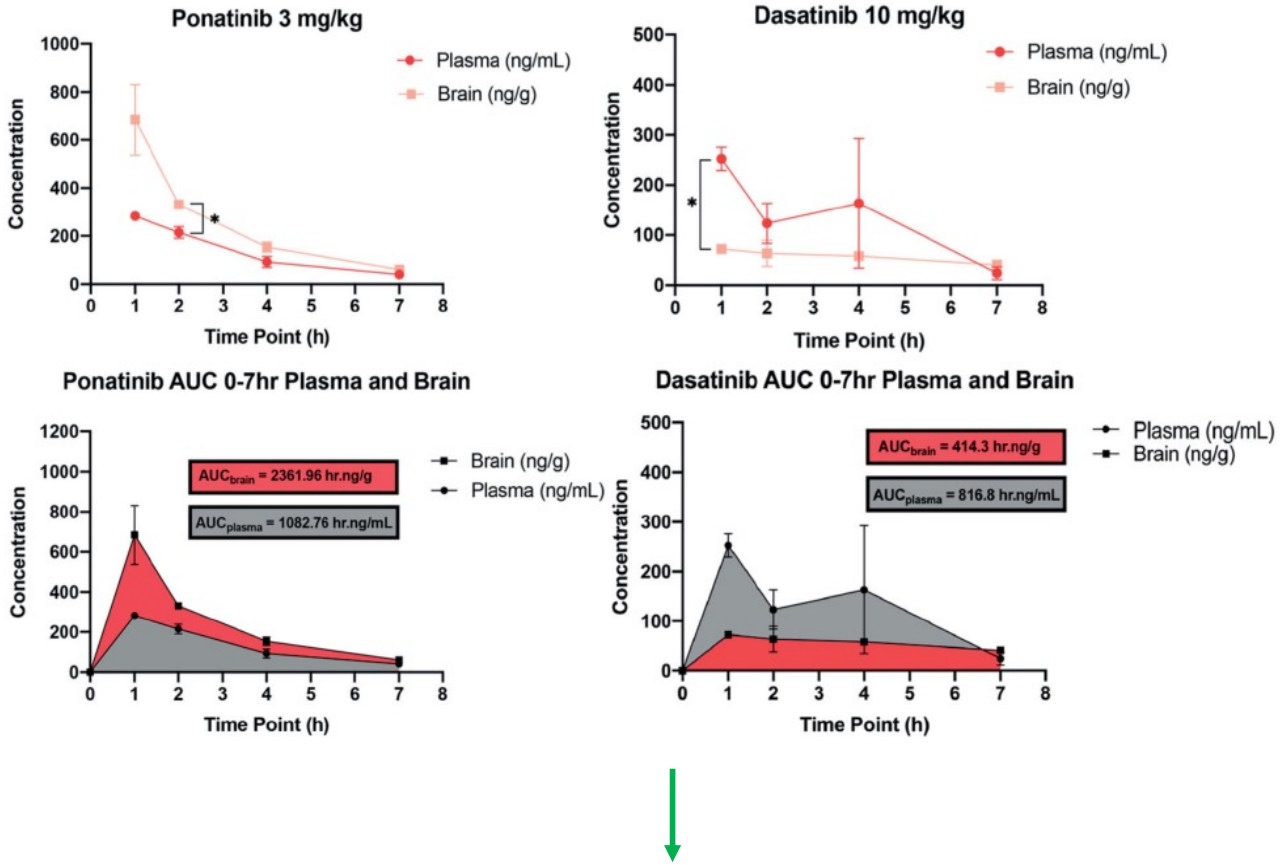
Rusconi *et al., Blood*. 2022

TKIs CNS penetration and activity in Philadelphia-positive leukemia



Up to **20% of imatinib-treated patients** with either lymphoid or myeloid BC-CML or Ph ALL develop **CNS relapses** due to **poor imatinib penetration into CSF**

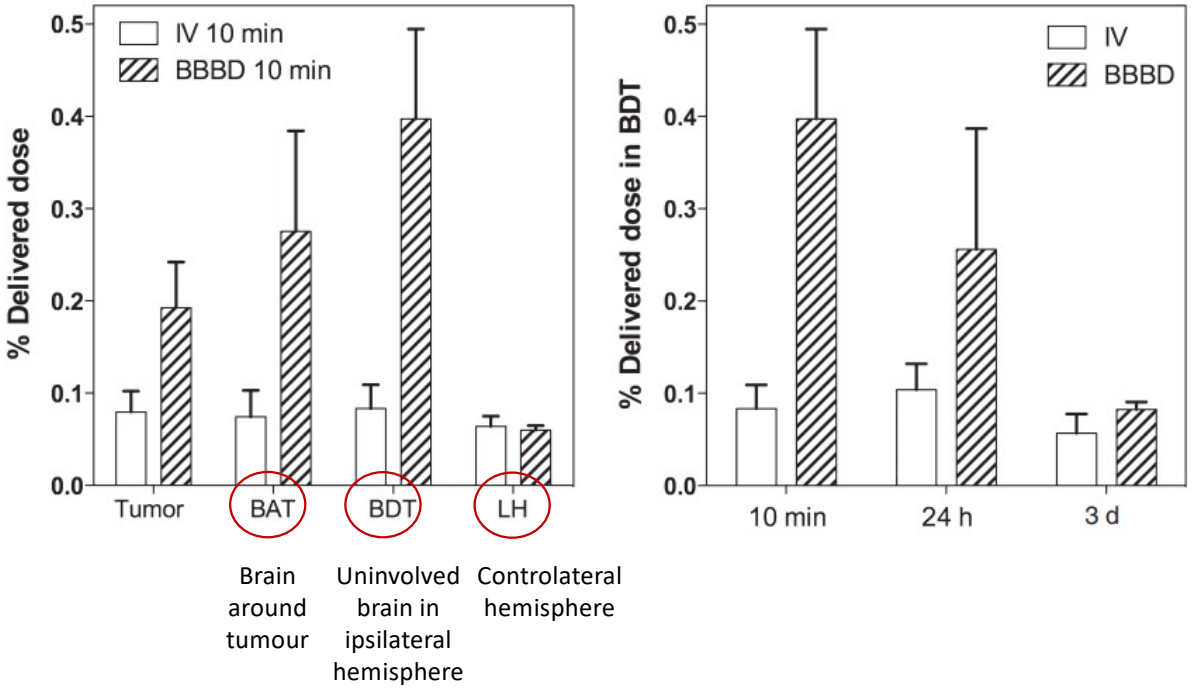
In preclinical studies, **dasatinib** **increased survival and reduced tumour growth** compared to imatinib

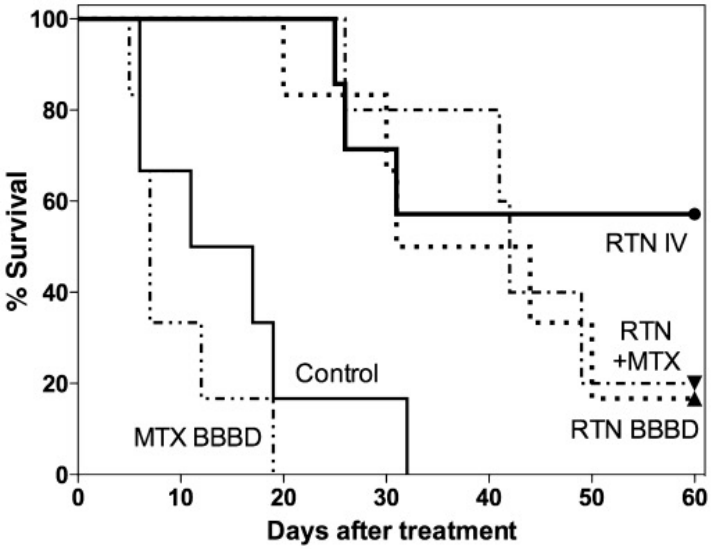


Ponatinib, when compared to **dasatinib**, achieves **greater therapeutic concentrations in the CNS** in animal models

Porkka *et al., Blood*. 2008; Ravi *et al., Leuk Lymphoma*. 2021

Rituximab immunotherapy in animal models of PCNSL



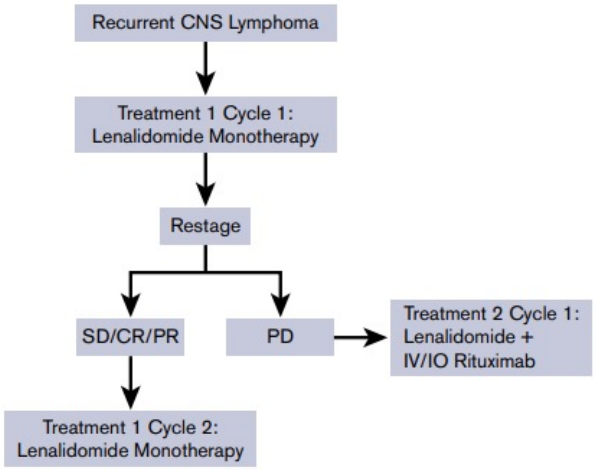


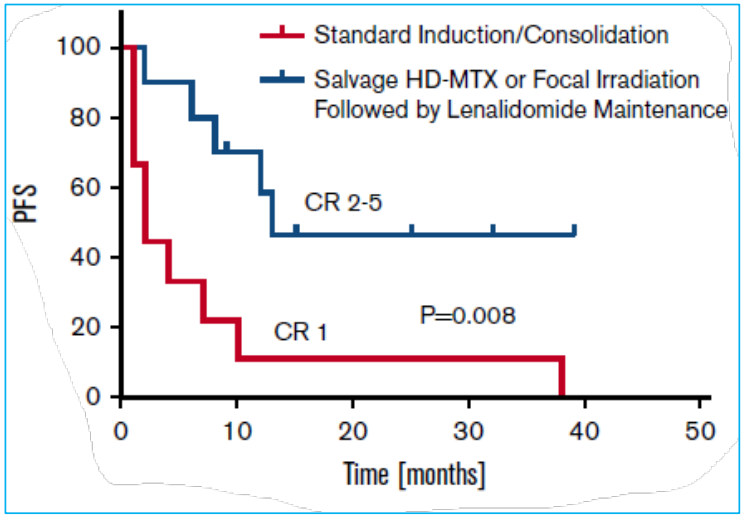
BBB disruption increases rituximab delivery in rat models of PCNSL

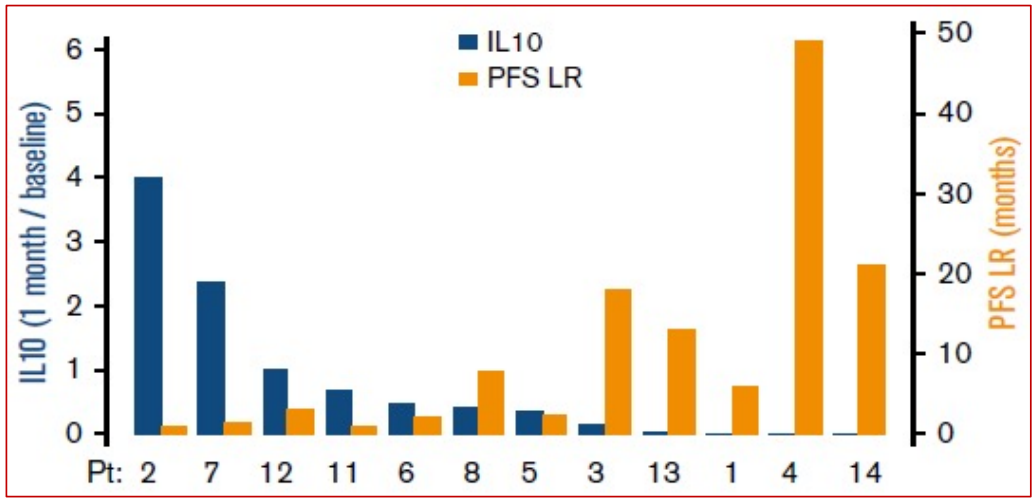
Rituximab increased survival in rat models of PCNSL, irrespective of adding MTX or BBB disruption

Muldoon *et al., Clin Cancer Res*. 2011

Lenalidomide +/- rituximab in relapsed PCNSL (phase 1 trial)



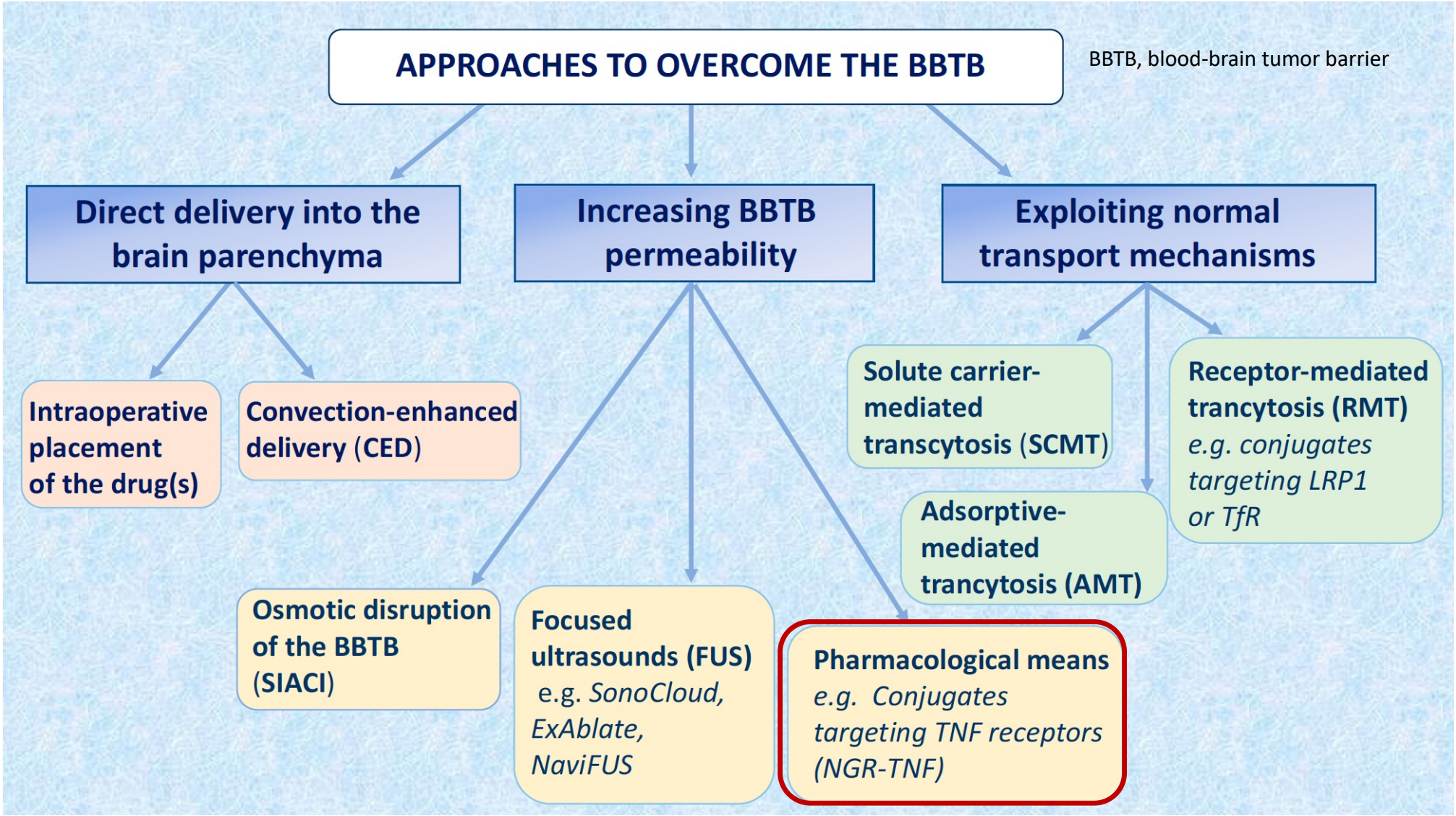




Lenalidomide had a good CSF penetration and is active as monotherapy (inverse correlation between IL10 CSF levels and response)

Maintenance lenalidomide is feasible and may potentiate response duration after salvage in relapsed PCNSL and delay WBRT.

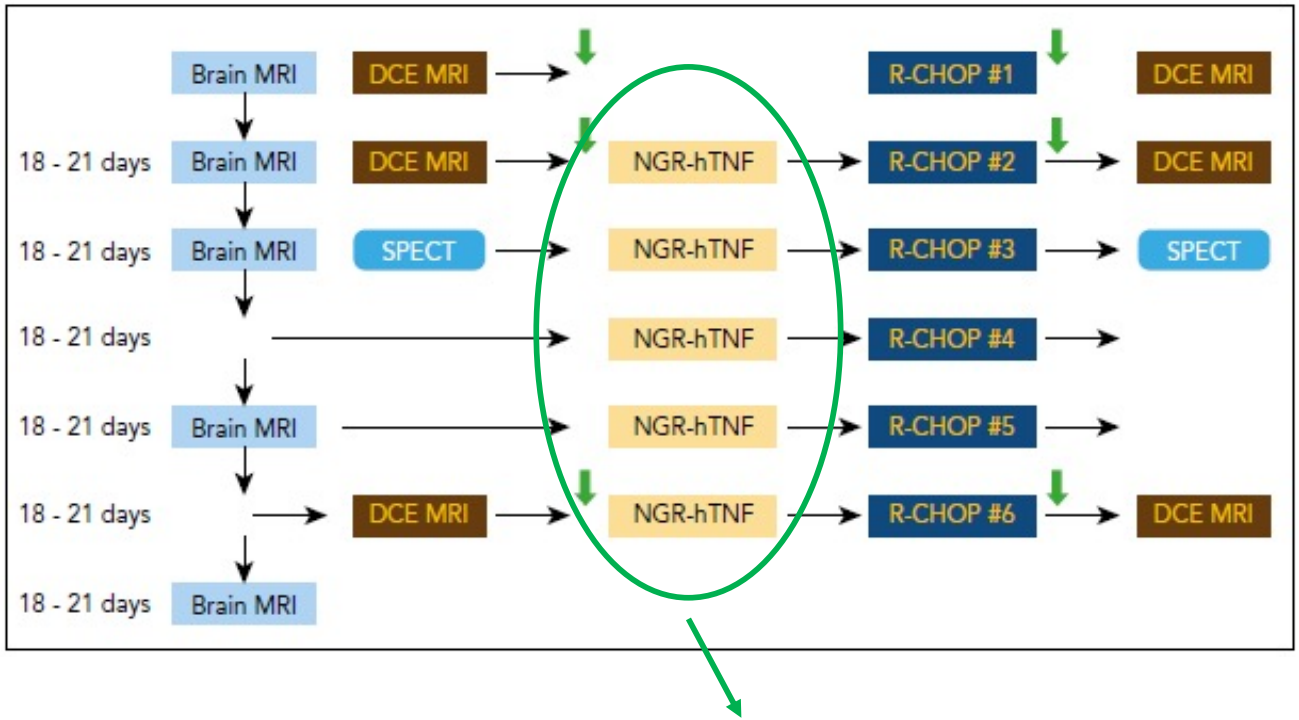
Rubenstein *et al., Blood Adv*. 2011



Marcucci et al., Cancers 2021

R-CHOP preceded by NGR-hTNF-α in R/R PCNSL

R-CHOP regimen may improve survival and management of PCNSL patients, but **BBB penetration of related drugs is poor**

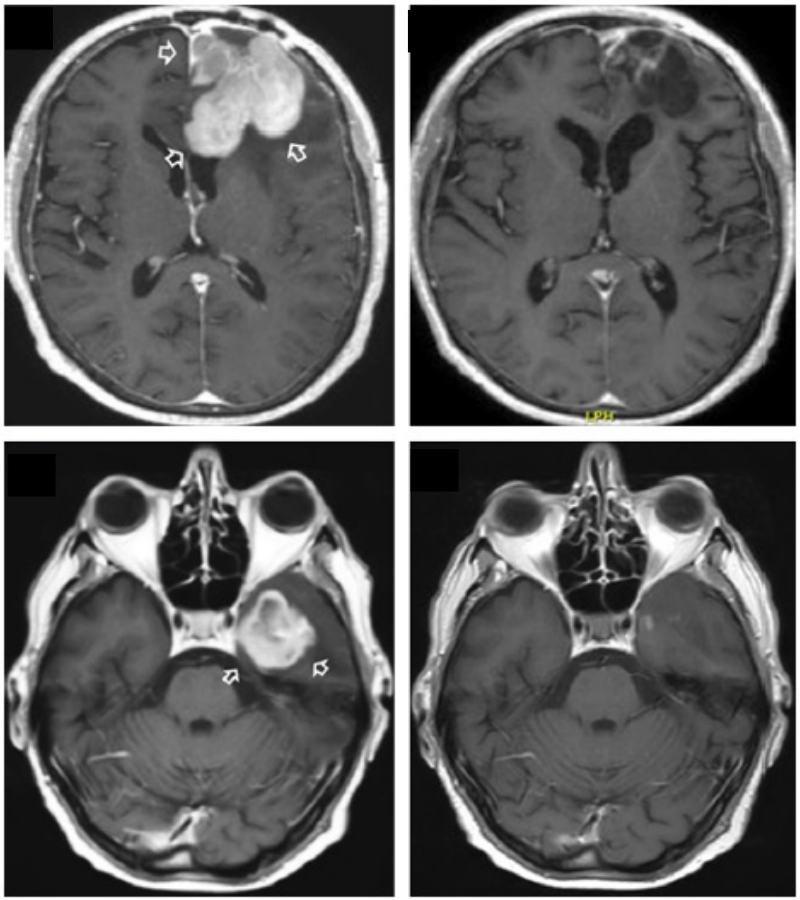


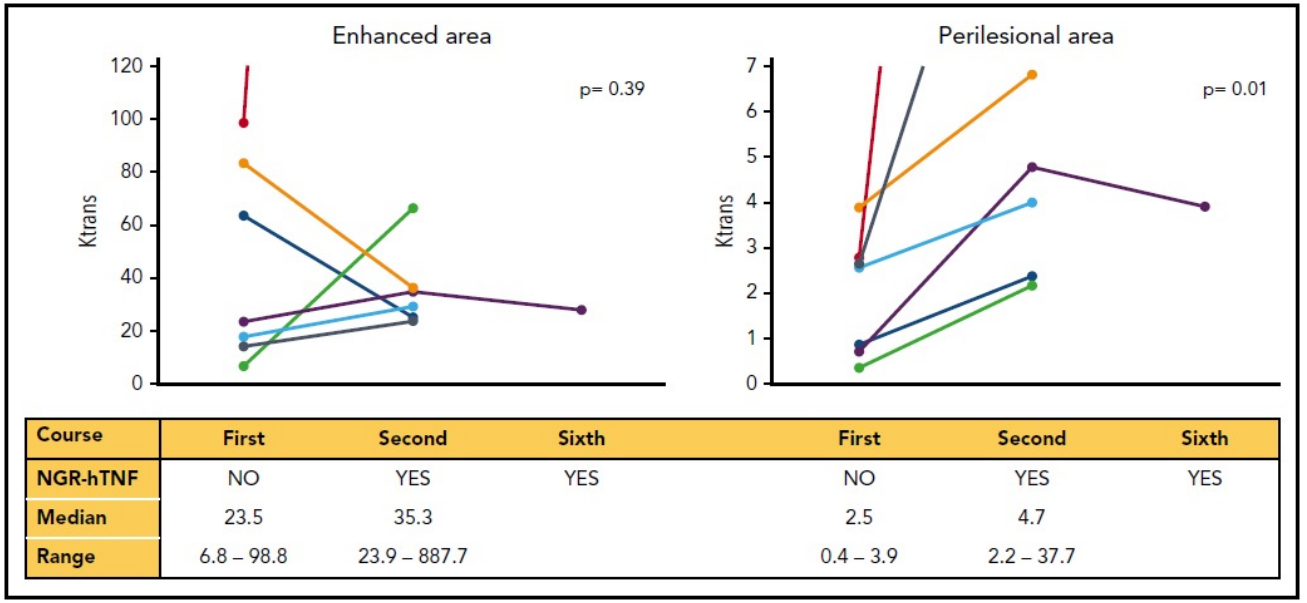
Exploratory phase: 10 patients received 1 cycle of R-CHOP alone and 5 cycles of R-CHOP preceded by **engineered tumor necrosis factor-α**

NGR-hTNF-α targets CD13+ tumor vessels and increases vascular permeability selectively in tumor/peritumoral areas of PCNSL.

Ferreri *et al., Blood*. 2019

R-CHOP preceded by NGR-hTNF-α in R/R PCNSL





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	

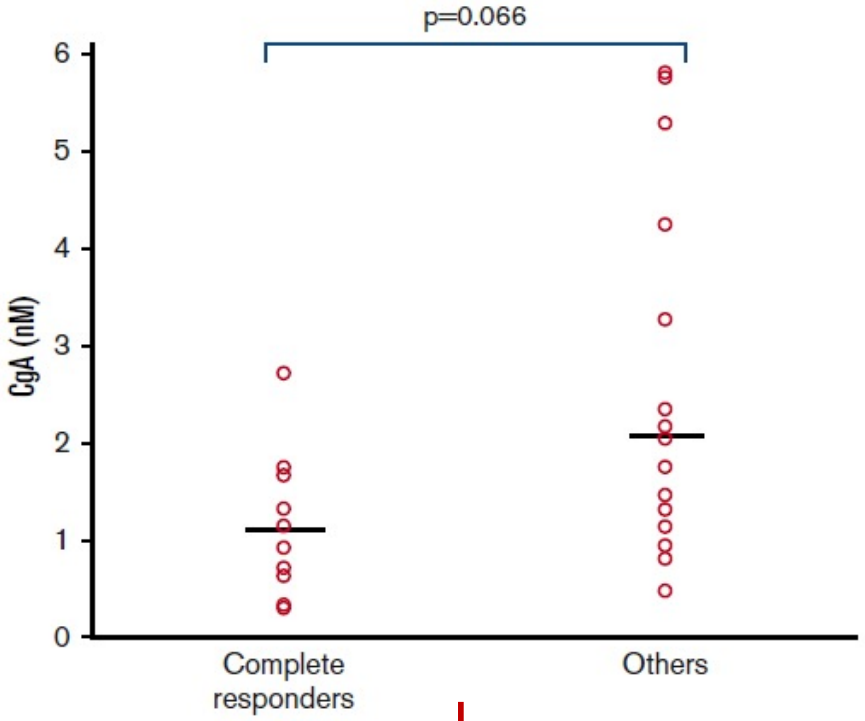
In responders DCE-MRI analysis showed that **vascular permeability was increased after the first NGR-hTNF infusion**

Ferreri *et al., Blood*. 2019

R-CHOP preceded by NGR-hTNF-α in R/R PCNSL

	Grade 1-2, n (%)	Grade 3, n (%)	Grade 4, n (%)	Grade 5
Neutropenia	9 (7)	17 (13)	57 (43)	—
Thrombocytopenia	34 (26)	25 (19)	26 (20)	—
Anemia	86 (65)	12 (9)	2 (2)	—
Febrile neutropenia	—	5 (4)	1 (1)	—
Hepatotoxicity	27 (20)	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)	—	—	—
Constipation	2 (2)	1 (1)	—	—
Nausea and vomiting	4 (3)	—	—	—
TNF infusion reaction*	9 (7)	—	—	—

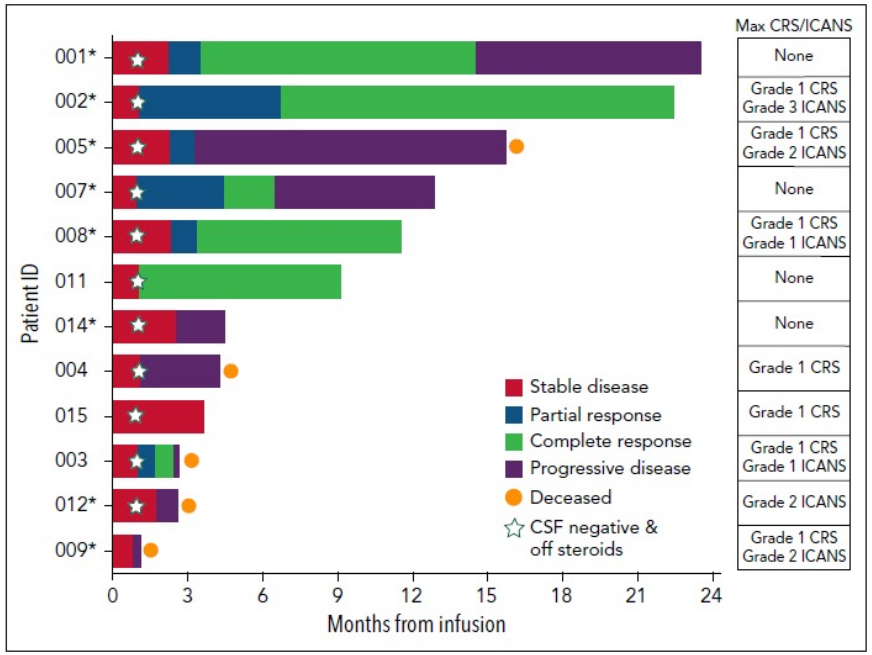
No unexpected toxicities, no interruptions or dose reductions due to toxicity
36/132 missed courses due to PD
Only 6 courses delayed



CgA levels correlated with lower CR rates
Treatment with PPI was associated with high CgA levels

Ferreri *et al., Blood Adv*. 2020

Tisagenlecleucel in primary CNS lymphoma (phase 1/2 trial)



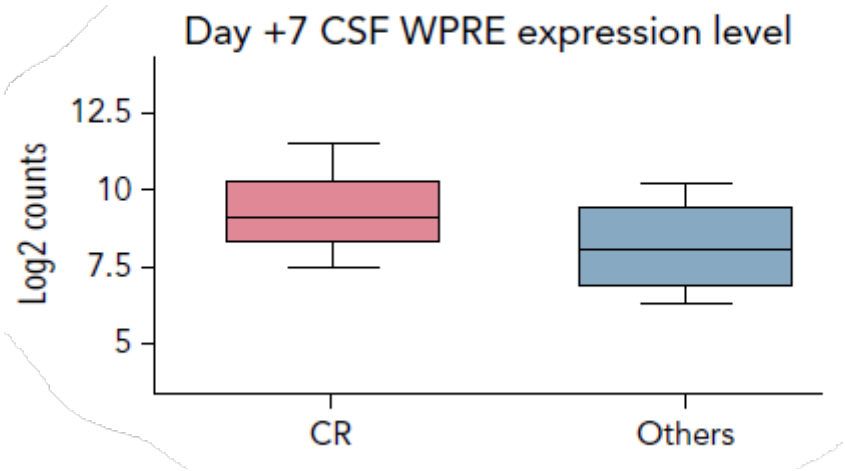
7/12 patients (58.3%) demonstrated response, with CR in 6 patients (50%)
After a median follow-up of 12.2 months, 7 patients alive and 3 patients with no PD

Characteristics	Patients (n = 12)
CRS*	
Any CRS	7/12
Grade 1	7/12
Grade 2	—
Grade 3	—
Grade 4	—
Required tocilizumab	—
Median onset of CRS (day postinfusion)	4
Median duration of CRS (day postinfusion)	2
ICANS*	
Any ICANS	6/12
Grade 1	3/12
Grade 2	2/12
Grade 3	1/12
Grade 4	—
Required corticosteroids	
At time of infusion for disease control†	4/12
Additional provided for ICANS following infusion	6/12
Median onset (day postinfusion)	5
Median duration (day postinfusion)	3

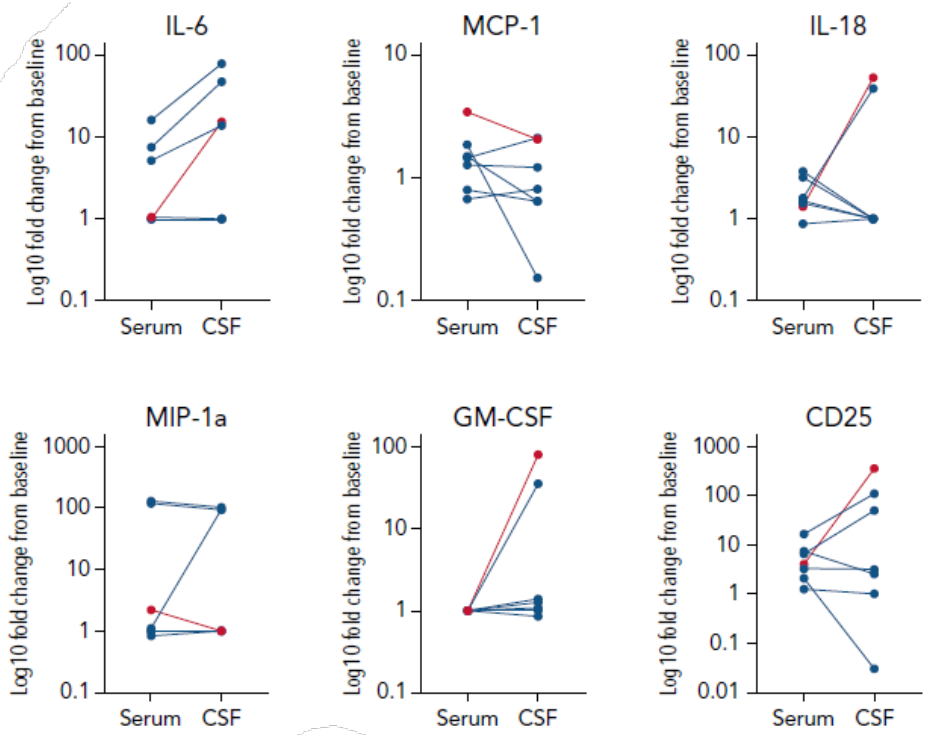
Although any patients experienced toxicity, all of them but 1 experienced **low grade CRS and/or ICANS → acceptable safety profile**

Frigault *et al., Blood*. 2022

Tisagenlecleucel in primary CNS lymphoma (phase 1/2 trial)



Patients who achieved a CR as their best response demonstrated higher levels of CAR transgene RNA in the CSF



Along with CNS trafficking of CAR-T cells, there was a trend toward **increased inflammatory cytokines compared with preinfusion baseline within the serum and CNS 1 week after tisagenlecleucel infusion**

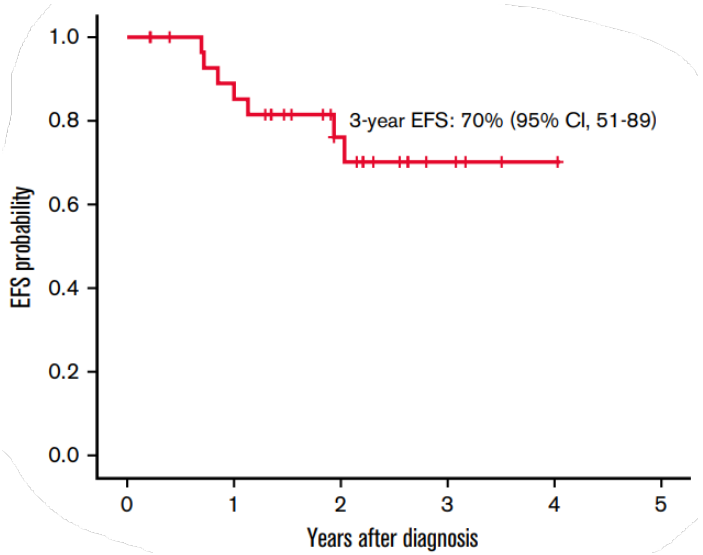
Frigault *et al., Blood*. 2022

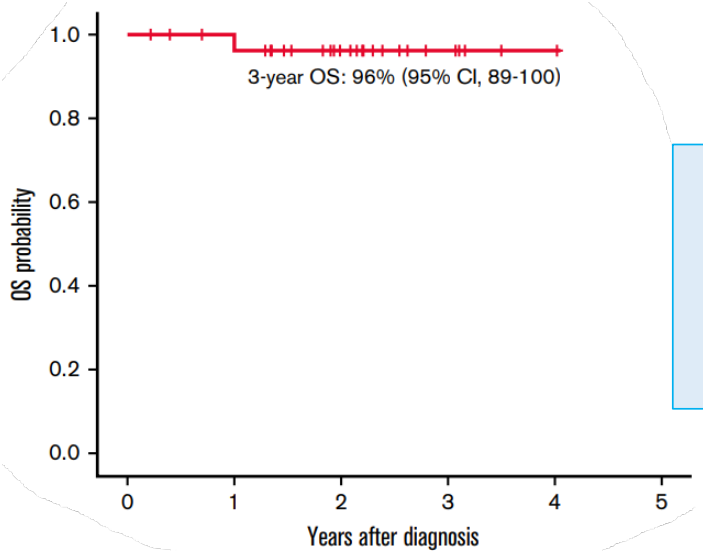
Conclusions

- The BBB (blood brain barrier) prevents neurotoxic plasma components, blood cells and pathogens from entering the brain
- The BBB regulates transport of molecules into and out of the CNS, which maintains tightly controlled chemical composition of the neuronal milieu that is requested for neuronal functioning
- Endothelial cells, pericytes and astrocytes are the main cellular players of the BBB
- Cancer cells disrupt the normal physiology of the BBB and induce a BTB (blood-tumor barrier)
- The BBB penetration by novel pathway inhibitors and mAbs requires detailed studies
- Novel strategies for facilitating drug delivery across the BBB are under investigation

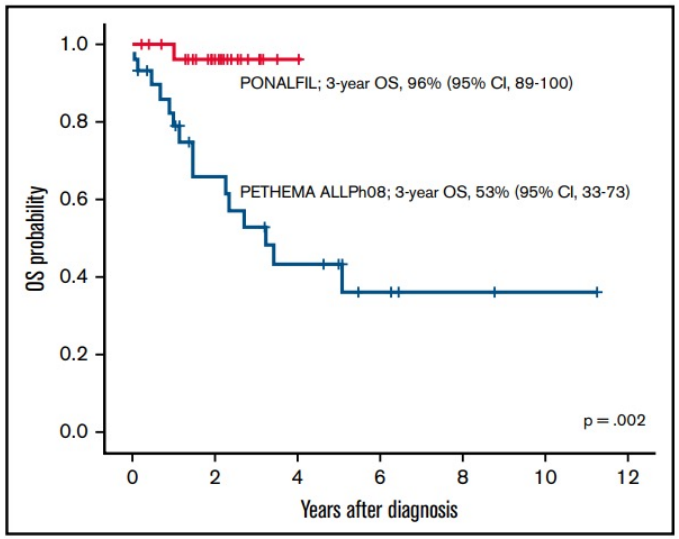
Back-up slides

Ponatinib, chemotherapy, and transplant in adults with Philadelphia-positive ALL (PONALFIL)





High efficacy (**CMR 71%** at the end of consolidation)
High rate of alloHSCT performance (26/30 patients)
Promising EFS and OS



PONALFIL showed a significant improvement in OS when compared to ALLph08 (Chemotherapy and Imatinib in Young Adults With Acute Lymphoblastic Leukemia Ph positive)

Ribera *et al., Blood Adv*. 2022