FERRO E DEMENZA: MODELLIZZAZIONE DEL MECCANISMO FISIOPATOLOGICO E DEL POSSIBILE APPROCCIO TERAPEUTICO

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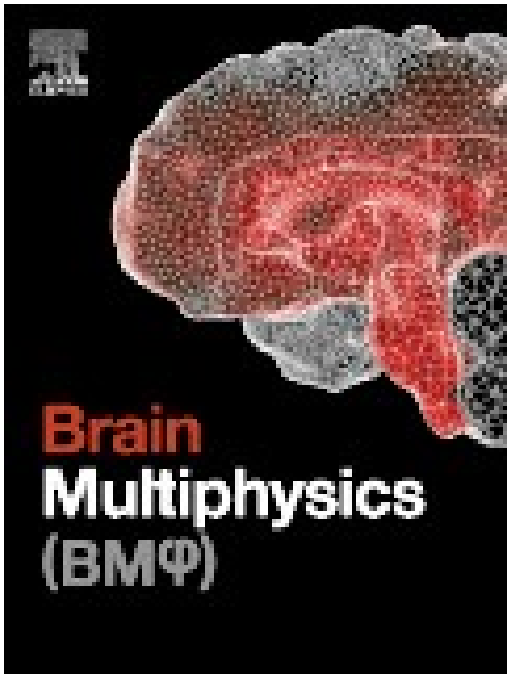
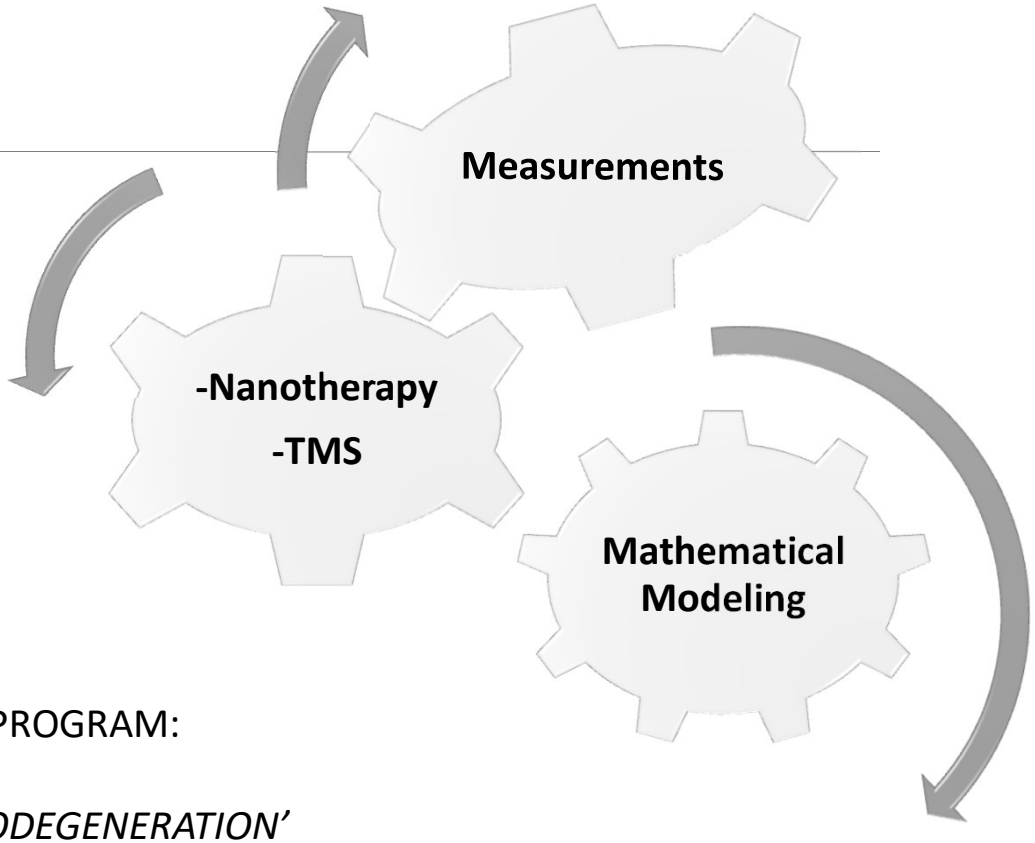
DBios

Physics?



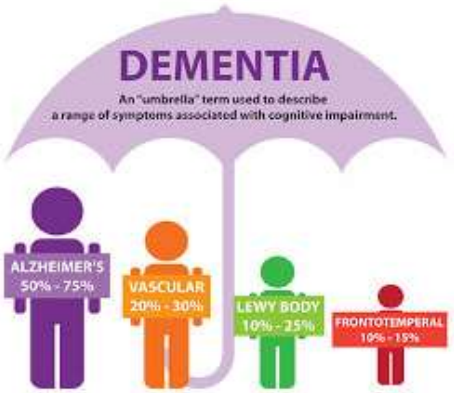


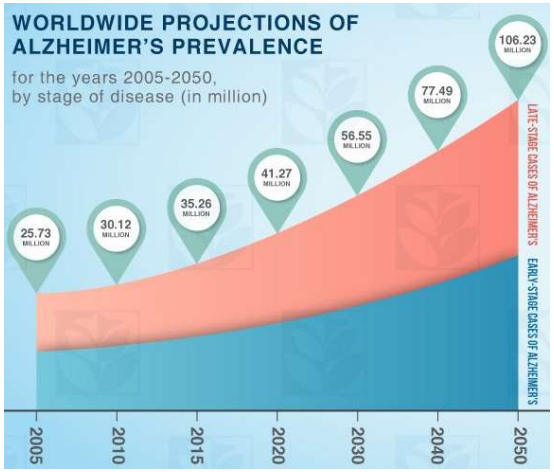
CALL FOR COST ACTION PROGRAM:

'BASIC SCIENCES 4 NEURODEGENERATION'

New Elsevier publication

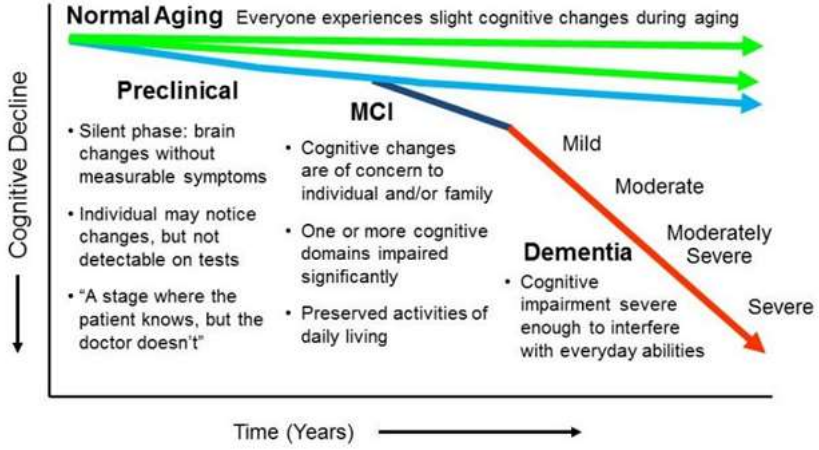
Dementia and Alzheimer's Disease: a social emergency





Alzheimer's disease is the most common and severe type of dementia

- Related to progressive population aging
- No effective therapy counteracting the pathology
- Very costly for SSN/insurance agencies



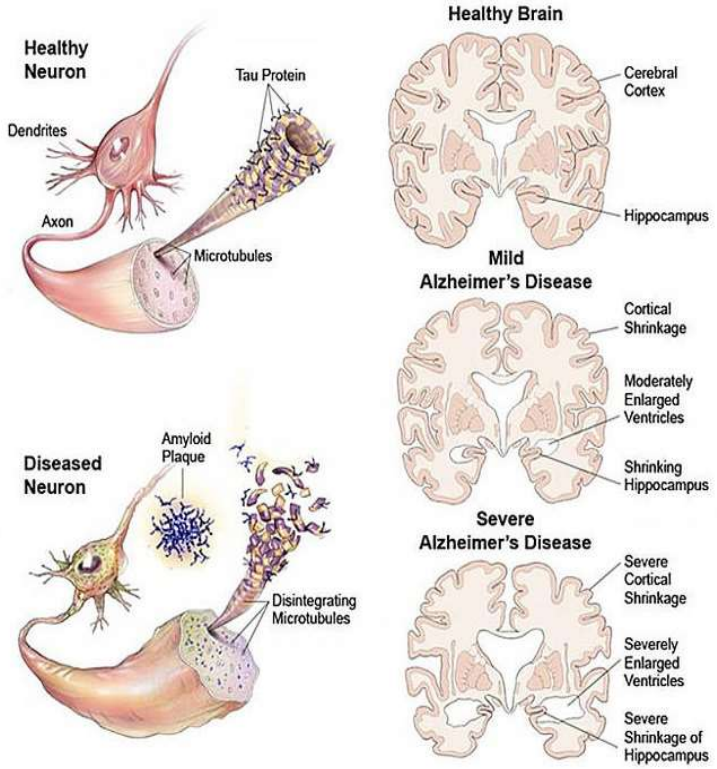
Clinical target:

Predict AD at pre-clinical stages to counteract progression!

MCI = Mild Cognitive Impairment

Alzheimer's Disease: Etiology (old hypotheses)

Ex-vivo

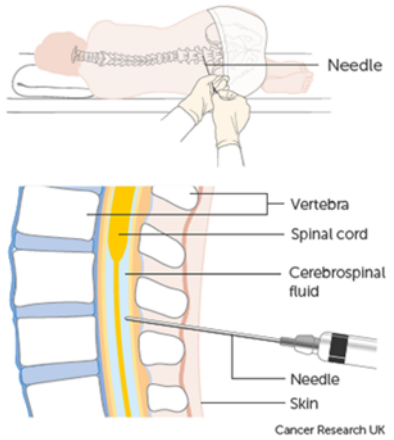


(https://www.brightfocus.org/alzheimers-disease/infographic/progression-alzheimers-disease)

In-vivo (Cerebro Spinal Fluid)

Routine Biomarkers in CSF

Aβ and τ protein

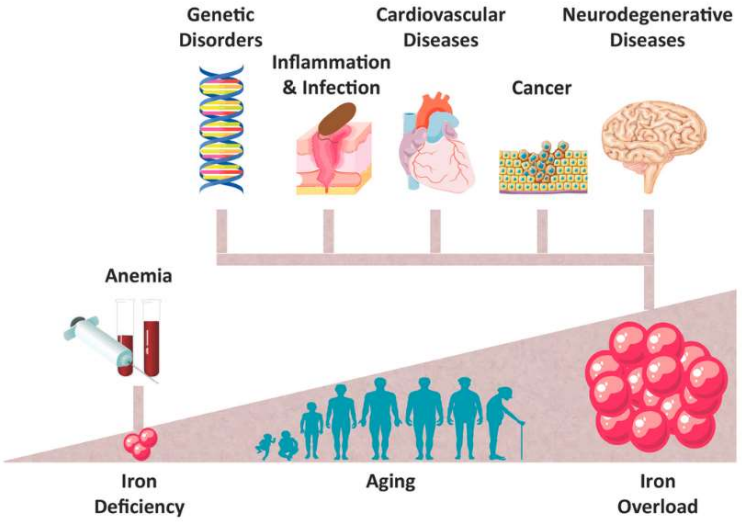


Reference values in CSF

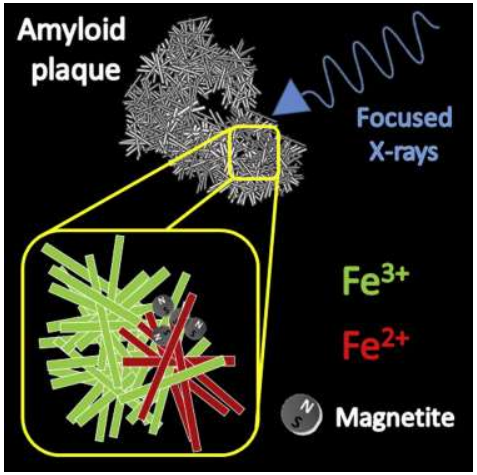
	Years	Normal
t-tau protein (pg/mL)	1-50	< 300
	51-70	< 450
	71-93	< 500
p-tau (pg/mL)		< 61
Amyloid Beta(1-42) (pg/mL)		> 500

Iron in neurodegenerative diseases: ferroptosis hypothesis

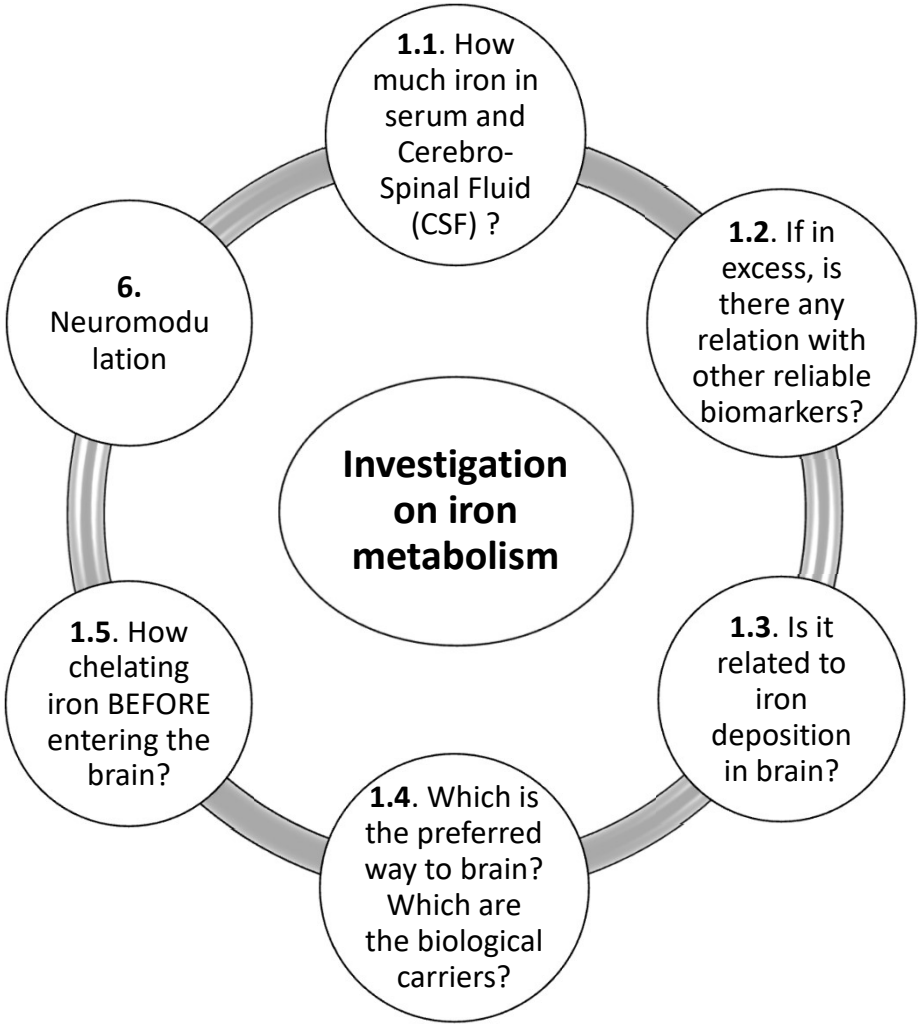
- Iron deposition within the brain parenchyma (mostly **hippocampus** and **cortex**) observed via **MRI** due to possible dysfunction of iron homeostasis at brain barrier level (Ward et al. 2014 Lancet Neurol.)



(Gozzelino and Arosio 2016, Int. J. Mol. Sc.)



(Telling et al. 2017 Cell Chemical Biology)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6. Neuromodulation
Investigation on iron metabolism

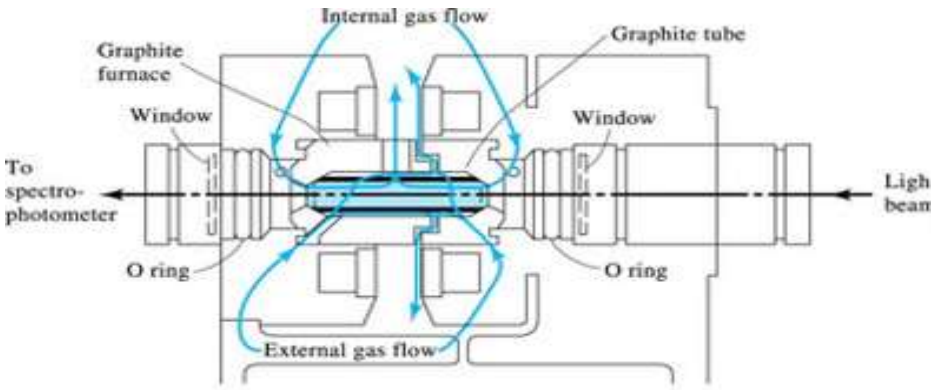
Well-established protocol for total iron detection in CSF

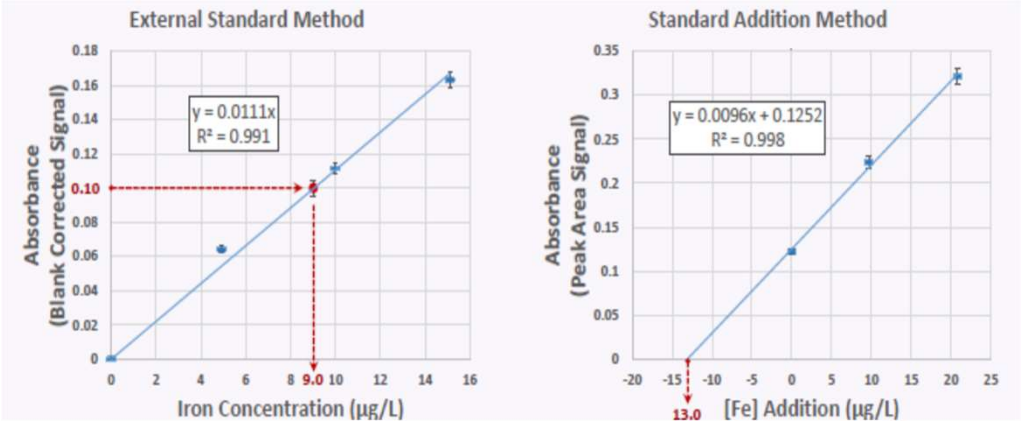
Graphite Furnace Atomic Absorption Spectrometry (GF-AAS)

- Low limit of detection (< 0.5 µg/L for iron)
- Small volumes of samples with minimum pre-treatment (dilution 1:3)
- Good linearity and reproducibility
- Matrix modifier (magnesium nitrate) to enhance signal
- Standard Addition Method to minimize matrix effect

4-step temperature protocol

Step	Temperature	
Drying	130°	Removal of acqueous component
Pyrolisis	1400°	Removal of organic matter
Atomization	2100°	Atomization of analyte
Cleaning	2450°	Clean-out of the furnace





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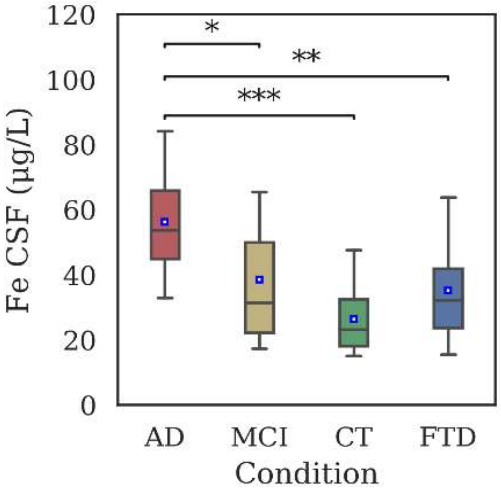
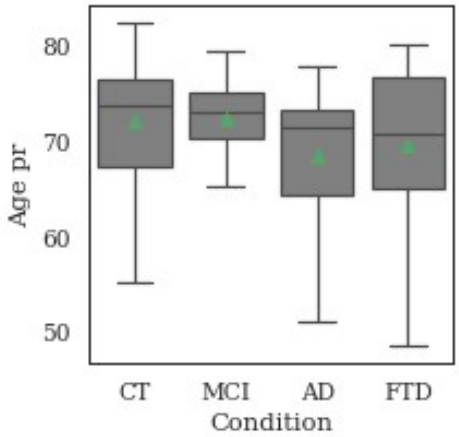


Iron content in CSF

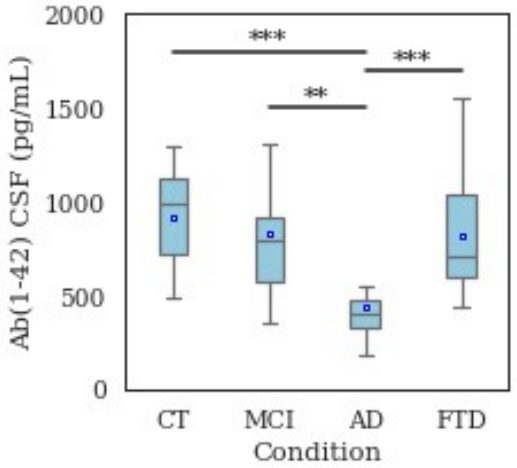
Kruskall-Wallis+Dunn's Post-Hoc Test
(***P<0.001,**P<0.01, *P<0.05)

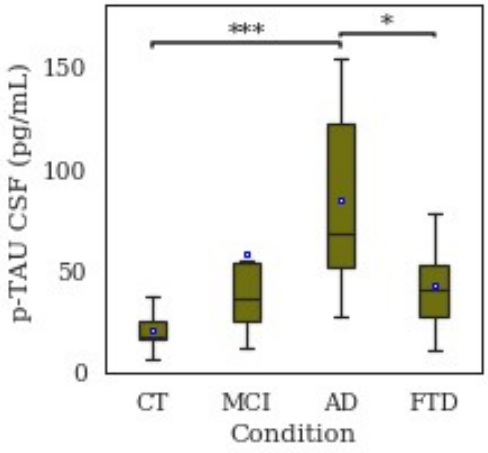
Total of **69** patients

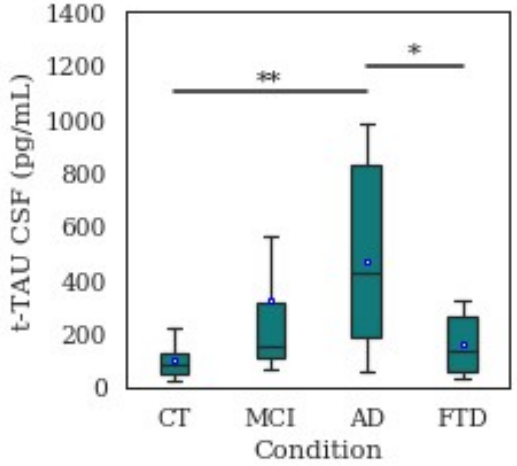
Increase of total iron concentration in CSF of patients affected by Alzheimer's disease with respect to control groups.



Biomarkers (Aβ, total-Tau, phosphorilated-Tau) Kruskall-Wallis+Dunn's Post-Hoc Test (***P<0.001,**P<0.01, *P<0.05)



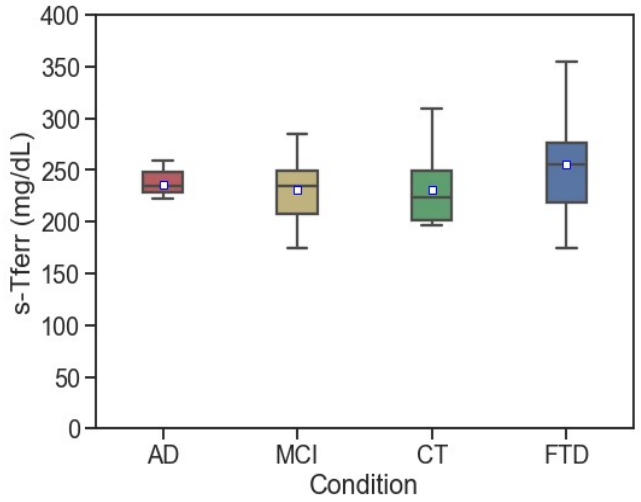




1. How much iron in serum and Cerebro-Spinal Fluid (CSF) ?

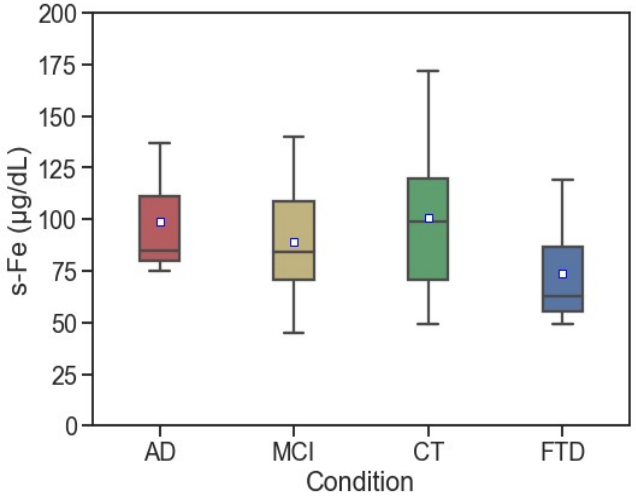
Iron in serum : clinical dosage

Transferrin: plasmatic protein involved in the blood iron transport.
It bounds only trivalent iron and is normally saturated with iron for 50%.
Reference Values: 200 - 400 mg/dL.



N=40 pts

Iron not bound to haemoglobin → **Serum iron** (sideremy)
Men: 80-170 µg/dL
Women: 60-140 µg/dL
Elders: 40-80 µg/dL



N=44 pts

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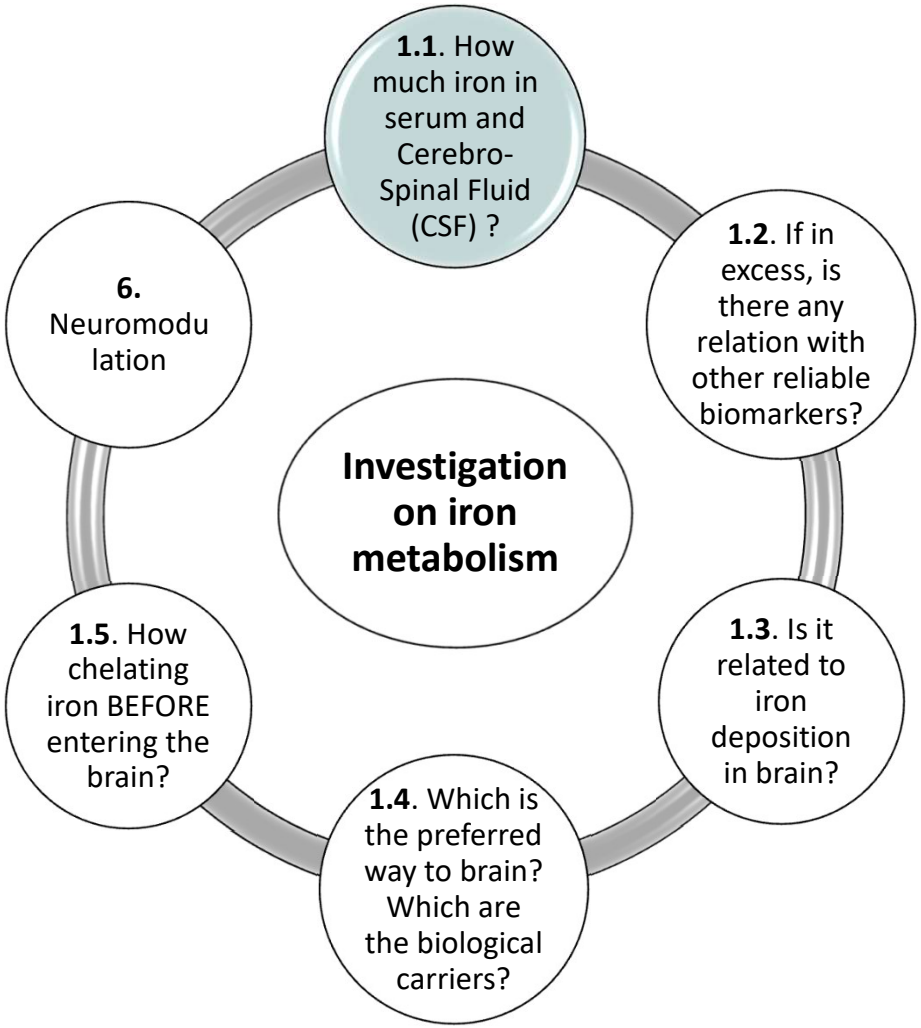
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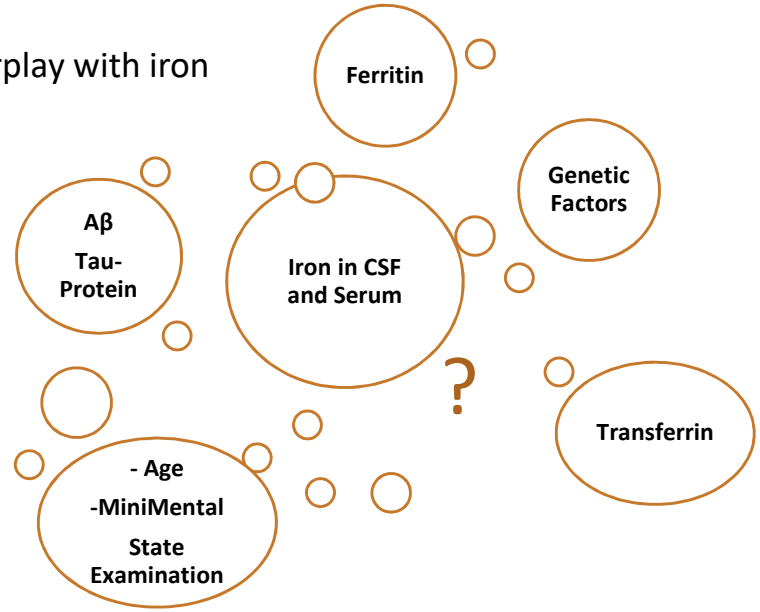
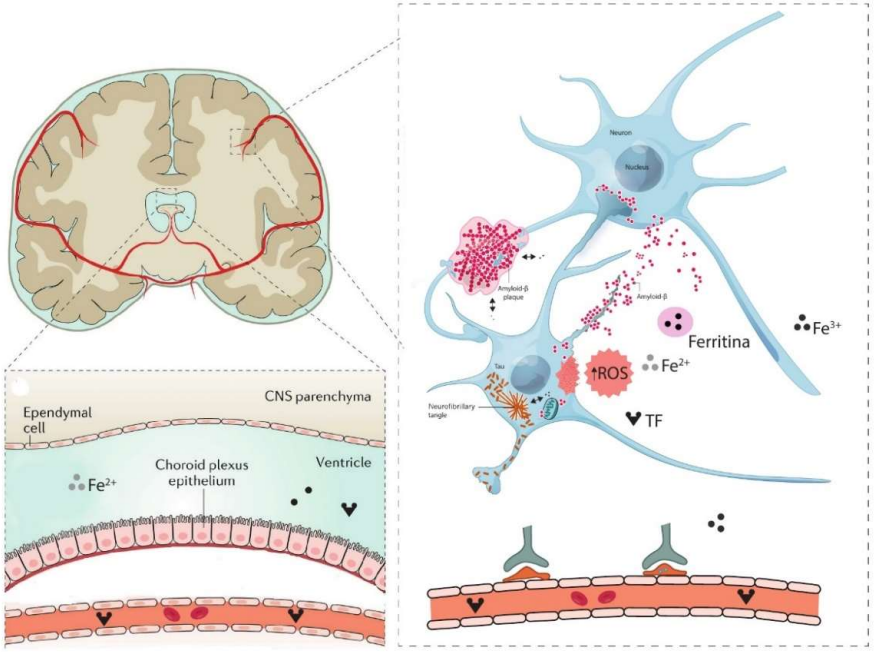
Iron *vs* ‘ CONSOLIDATED’ biomarkers

- Quantify the interplay with iron



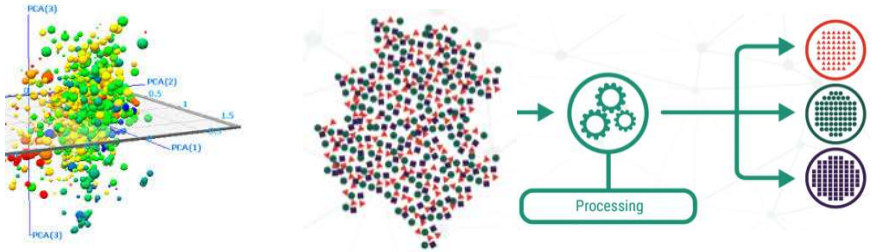


Modified from (https://www.nature.com/articles/d41586-018-05718-5)

- Different machine learning algorithms (unsupervised and supervised) applied to identify potential **correlations between parameters**

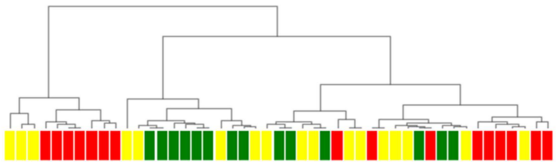
Discover structure in data → Identify sub-groups (Clustering)

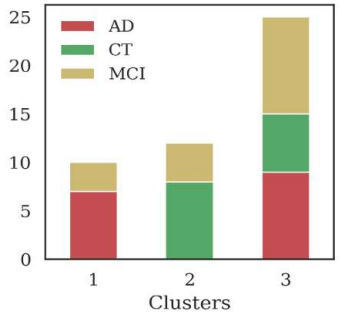
Classification Algorithms with different sets of features

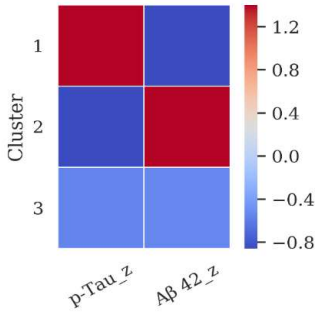


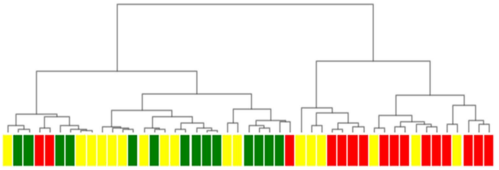
2. If in excess, is there any relation with other reliable biomarkers?

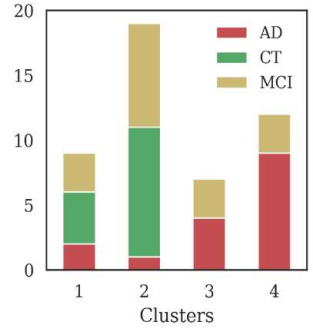
Hierarchical Clustering

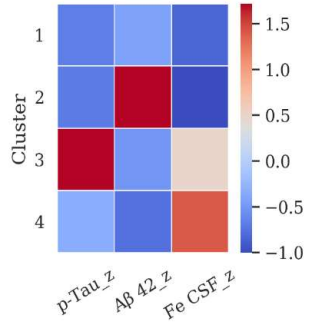


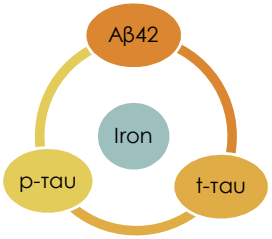


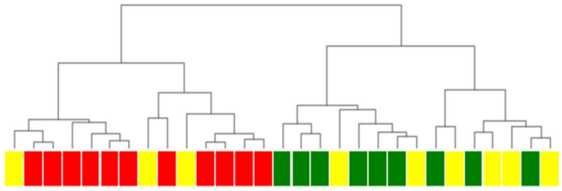


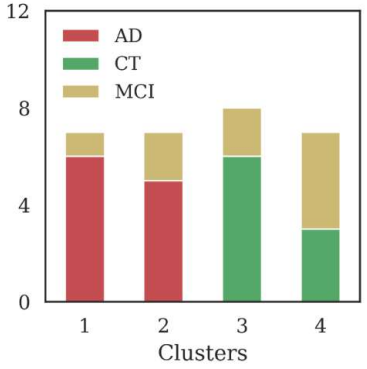


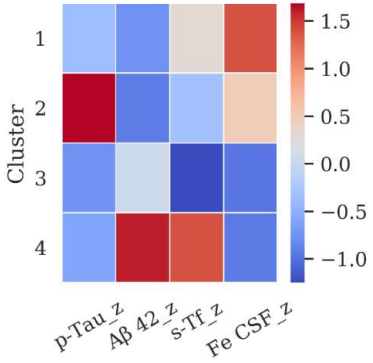












Information on Transferrin in Serum improved correct discrimination of patients

Interaction Fe CSF / p-Tau in intermediate state of dementia?

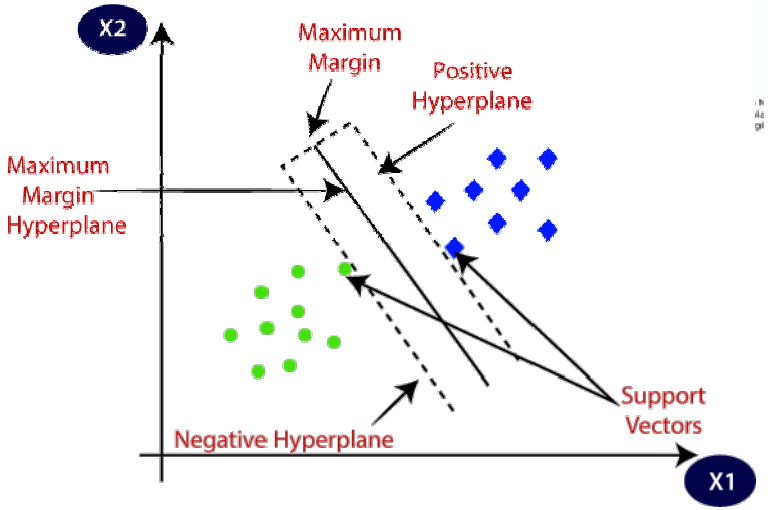
2. If in excess, is there any relation with other reliable biomarkers?

Receveir operating characteristic (ROC) curves showed the improvement of multi-classification performances of [Fe CSF + biomarkers] respect to biomarkers features set.

CT MCI AD

Linear Support Vector Model

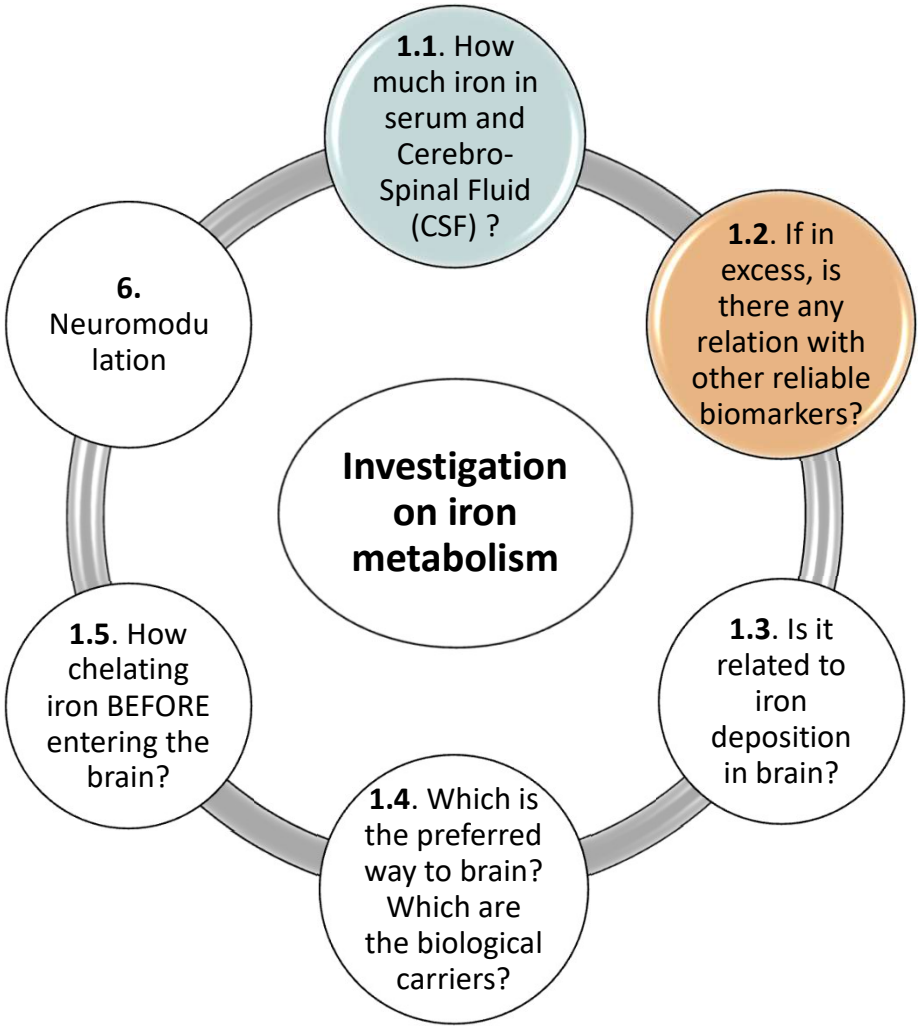
	Model	AUROC	Weight of features
Biomarkers (Abeta, pTau)	Linear SVM (C=10)	0.74 ± 0.11	1. P-Tau= (0.080±0.0939) 2. Aβ =(0.072±0.1261)
Biomarkers + Iron CSF	Linear SVM (C=1)	0.73 ± 0.12	1. Fe CSF=(0.0715±0.0877) 2. P-Tau= (0.0625±0.0965) 3. Aβ =(0.0282±0.1259)



https://www.javatpoint.com/machine-learning-support-vector-machine-algorithm

Weights of feature based on repeated permutations method: **importance of iron**

2. If in excess, is there any relation with other reliable biomarkers?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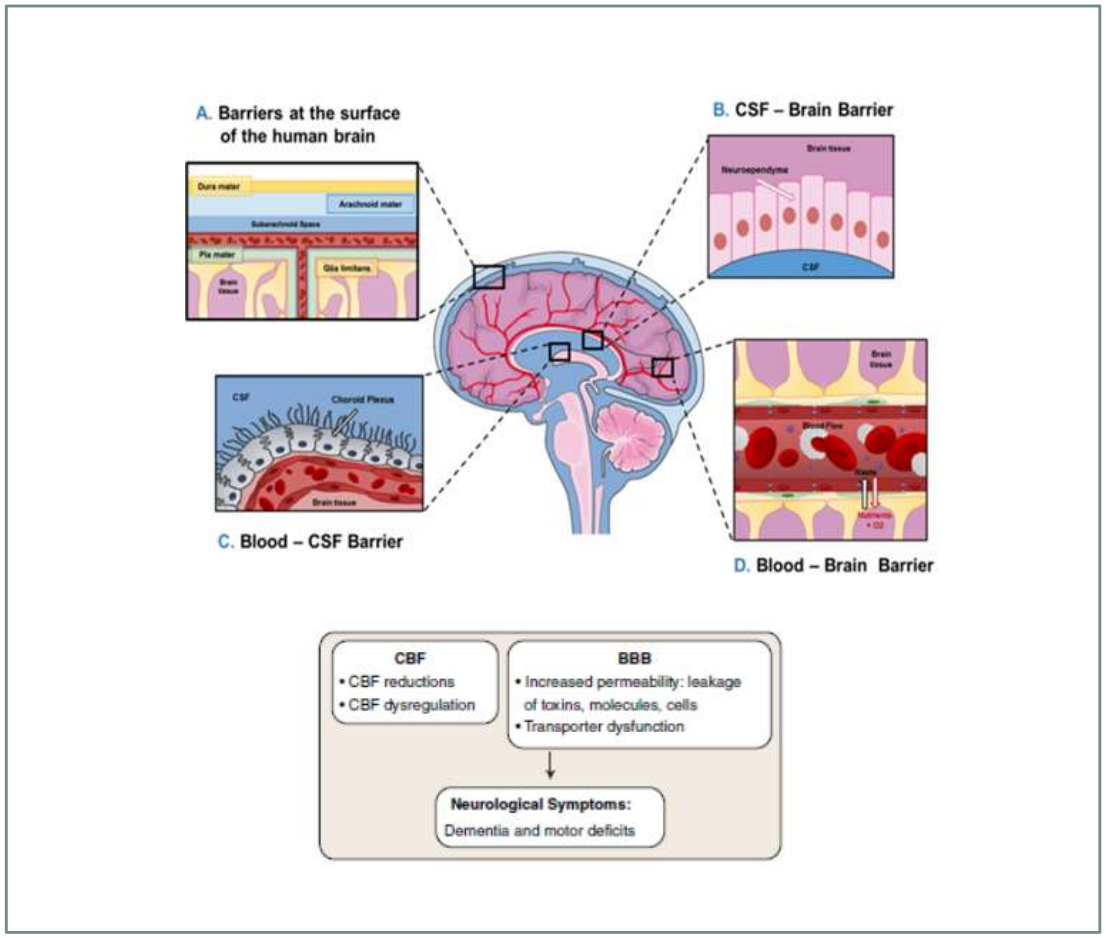
Investigation on iron metabolism

Further investigation of total iron concentration in biological fluids as new biomarker

Iron uptake

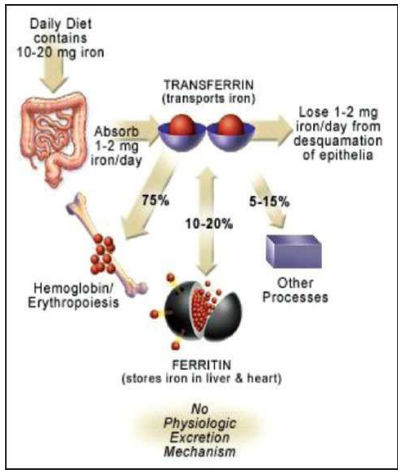


Iron brain deposition

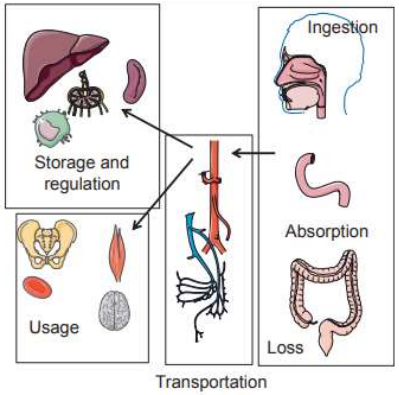
Black box

3. Is it related to iron deposition in brain?

Iron metabolism in blood
(intake E and excretion K)



(Abbaspour et al. 2014, J Res Med Sci)

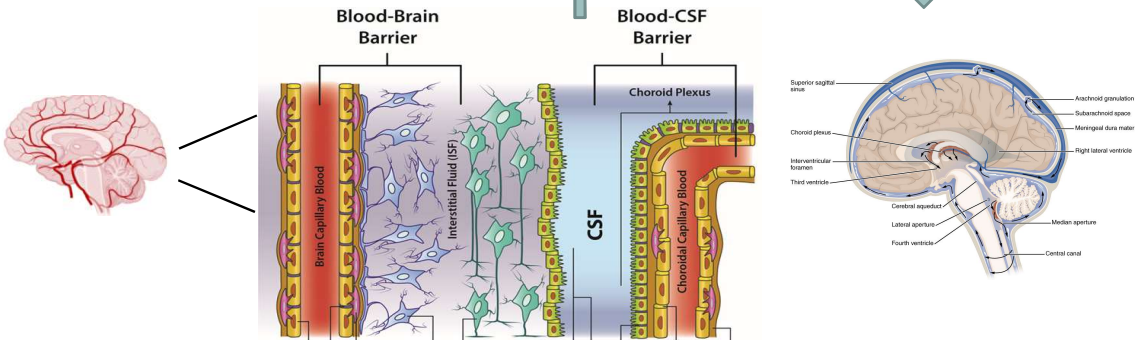


(Waldvogel-Abramowskia et al. 2014, Transfus Med Hemother)

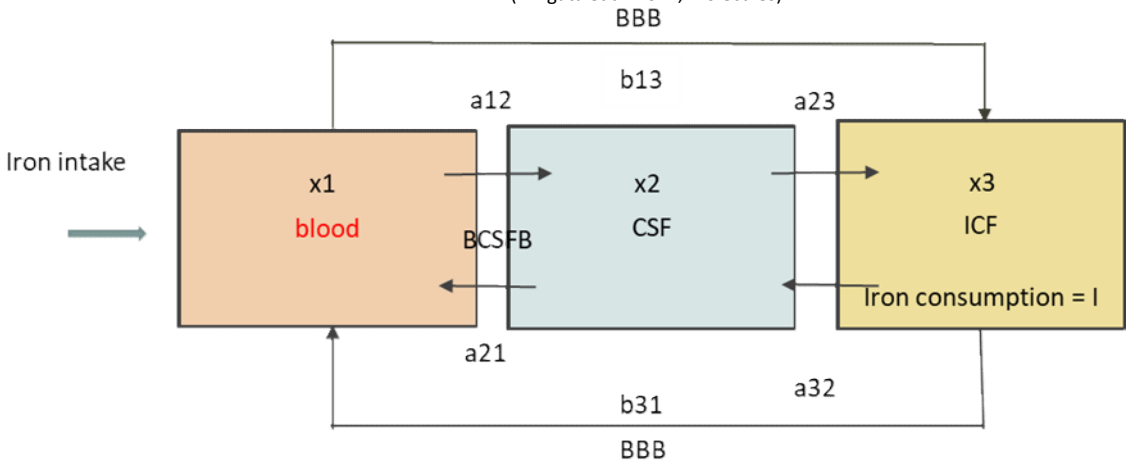
Iron uptake : from blood

Directly to brain tissues

to Cerebro-Spinal Fluid and to brain



(D'Agata et al. 2017, Molecules)



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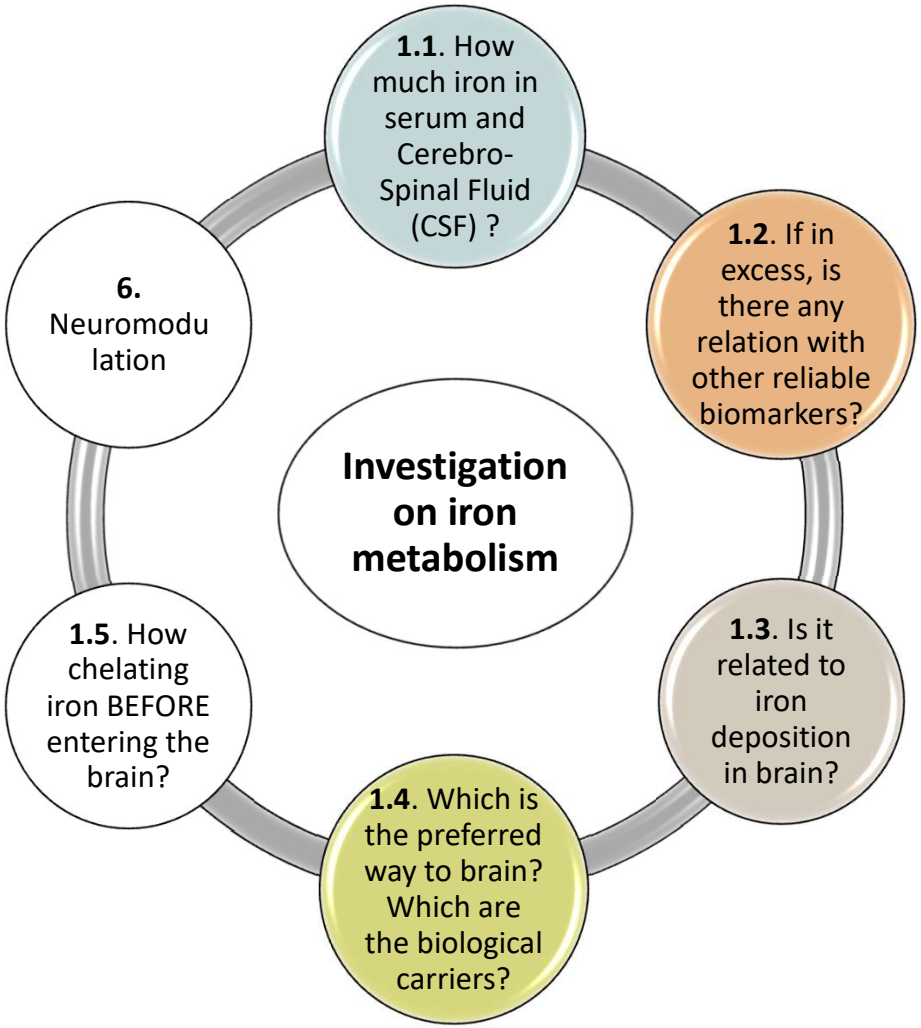
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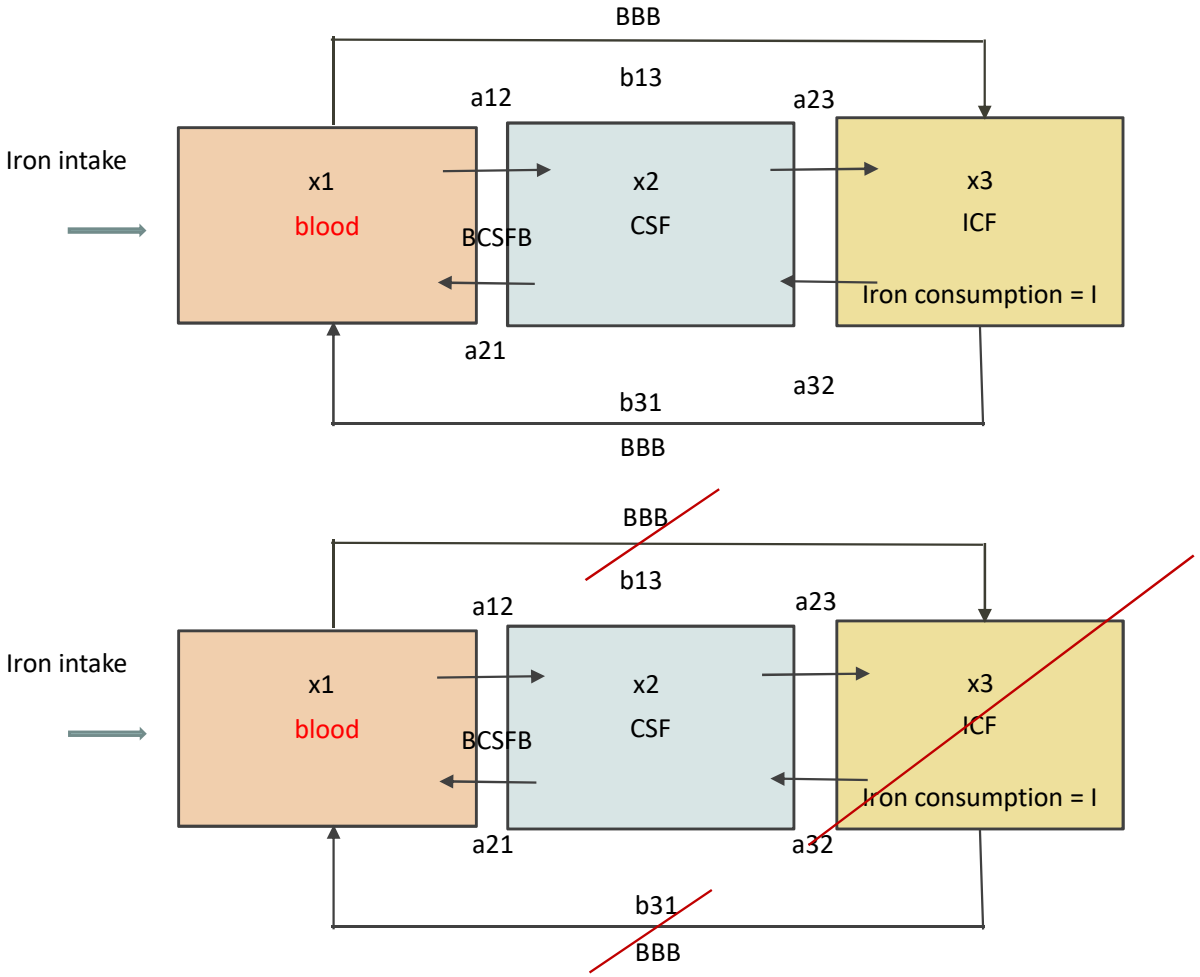
Mathematical modeling



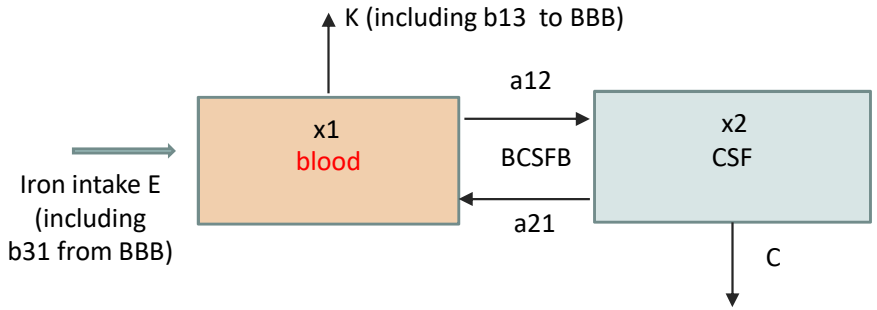
Presently we have no data on iron concentration in the brain…..

We therefore disregard the third compartment and the passage of iron from CSF to ICF.

Step-by-step approach

4. Which is the preferred way to brain ? Which are the biological carriers?

Simplified 2 compartments model



a12 >> b13 ???
b31 = 0 ???

2-compartimental model: non-homogeneous system of ODEs describing iron concentration rate from blood (X1) to brain (X2).

$$\begin{cases} \frac{dx_1}{dt} = -(a_{12} + k)x_1 + a_{21}\,x_2 + \mathrm{E} \\ \frac{dx_2}{dt} = a_{12}\,x_1 - (a_{21} + c)x_2 \end{cases}$$

X1= sideremy, x2=total iron estimated by GF-AAS

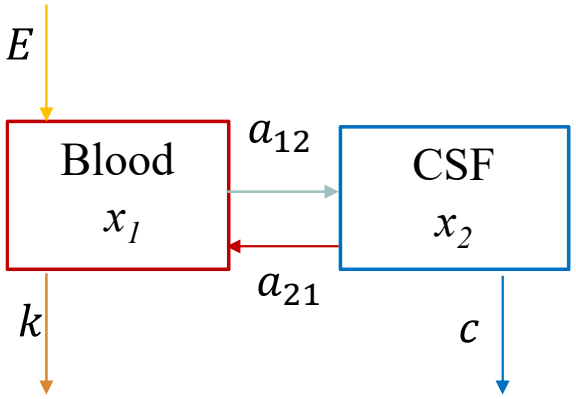
- E = Iron intake (hypothesis: fixed quantity [mg/day])
- K= Iron consumption from blood (tipically from metabolism and bleeding; considering also iron that enter direct in the brain from BBB)
- a12= kinetic constant rate for iron entering from blood to CSF
- a21= kinetic constant rate for iron returning from CSF to blood
- c= consumption of iron in the CSF (iron metabolism in the brain)

Our questions

1. Differences between physiological and pathological conditions in the regulation of iron passage between blood and CSF?
2. Which parameter(s) mostly affect iron concentration?

4. Which is the preferred way to brain ? Which are the biological carriers?

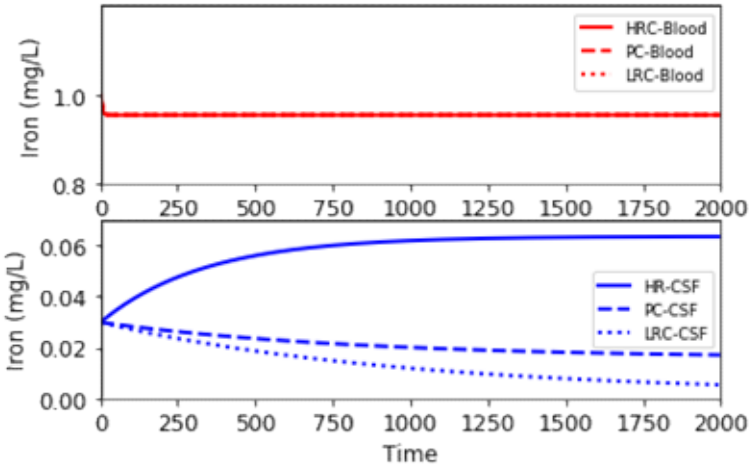
First Step A two-compartmental model for **blood-cerebrospinal fluid barrier (BCSFB)**



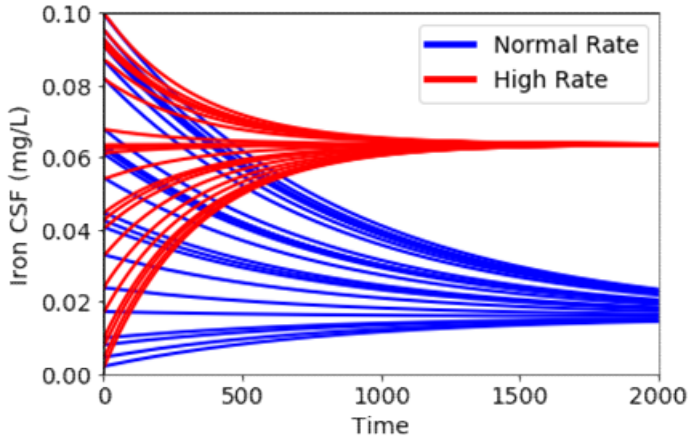
$$\begin{cases} \dfrac{dx_1}{dt} = -(a_{12} + k)x_1 + a_{21}\,x_2 + \mathrm{E} \\ \dfrac{dx_2}{dt} = a_{12}\,x_1 - (a_{21} + c)x_2 \end{cases}$$

Parameter	Physiological Condition	Low Rate Condition	High Rate Condition
E (mg/L)	0.22	0.22	0.22
k	0.23	0.23	0.23
a_{12}	$2 \cdot 10^{-5}$	$2 \cdot 10^{-6}$	$2 \cdot 10^{-4}$
a_{21}	$2 \cdot 10^{-4}$	$2 \cdot 10^{-5}$	$2 \cdot 10^{-3}$
c	$1 \cdot 10^{-3}$	$1 \cdot 10^{-3}$	$1 \cdot 10^{-3}$

τ = t/T (T=1 day time scale for normalization)



(Ficiarà E. et al., 42nd Ann. Int. Conf. IEEE Eng. Med. Biol.)

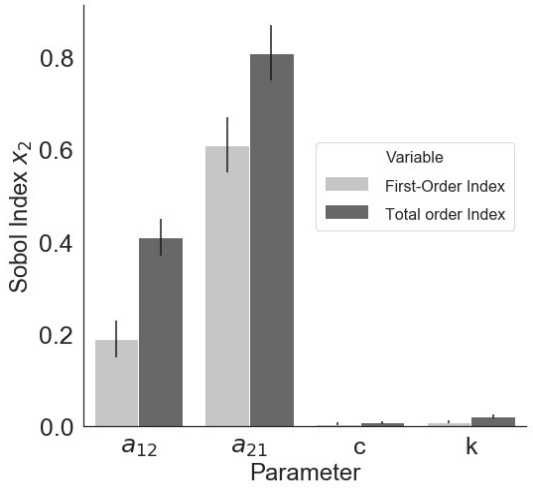


4. Which is the preferred way to brain ? Which are the biological carriers?

Global Sensitivity Analysis

- Overall effects of the model inputs on the model output by perturbing model input parametrs within large ranges
- Variance-based methods

- Variance-based Sobol method (first- , second-order and total effect sensitivity indices)



(Ficiarà E. et al., 42nd Ann. Int. Conf. IEEE Eng. Med. Biol.)

Iron in CSF

Strong contribution of the parameter a_{21} and a minor one from a_{12} for iron concentration in CSF

The interaction of the two parameters a_{12} and a_{21}, showed the strongest impact on the variability of iron concentration in CSF, reporting a significant value of the second-order index ($S_2 = 0.20 \pm 0.08$)

Parameter	CT	AD	MCI	FTD
a_{12}	$5 \cdot 10^{-5}$	$5 \cdot 10^{-4}$	$2 \cdot 10^{-4}$	$1 \cdot 10^{-4}$
a_{21}	$1 \cdot 10^{-3}$	$7 \cdot 10^{-3}$	$4 \cdot 10^{-3}$	$2 \cdot 10^{-3}$

MODEL ESTIMATED PARAMETER VALUES FOR CONDITION OF DEMENTIA AND NEUROLOGICAL CONTROL

Different rates for iron exchange in the various forms of dementia (AD, MCI and FTD) and CT

Alteration of the biological condition for the iron transport in the CSF in AD patients?

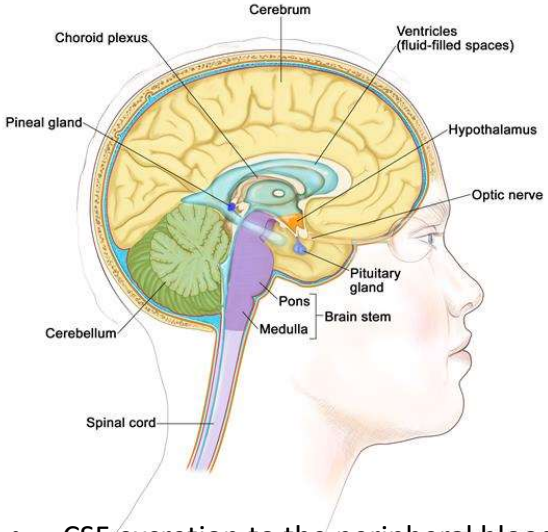
4. Which is the preferred way to brain ? Which are the biological carriers?

Understanding the BCSFB (Blood- CSF- barrier) !!!!!!!

a. Where ?

b. How? Biological models for Iron exchange

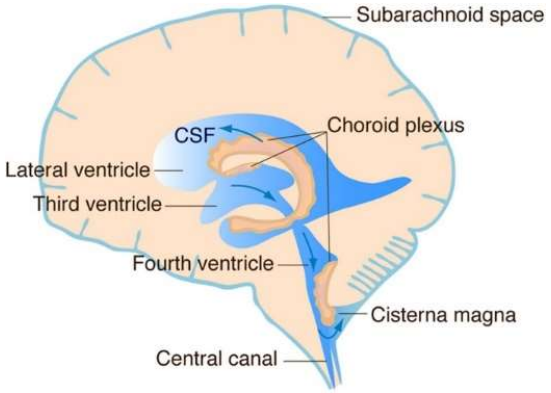
c. Physical models for Iron exchange



- The Choroid Plexus: CP

CP protrudes into all 4 ventricles in the brain, **closely apposing the hippocampus**, produces the filling CSF.

It produces daily 150 ml of Cerebrospinal Fluid which bathes the interstitium of the CNS

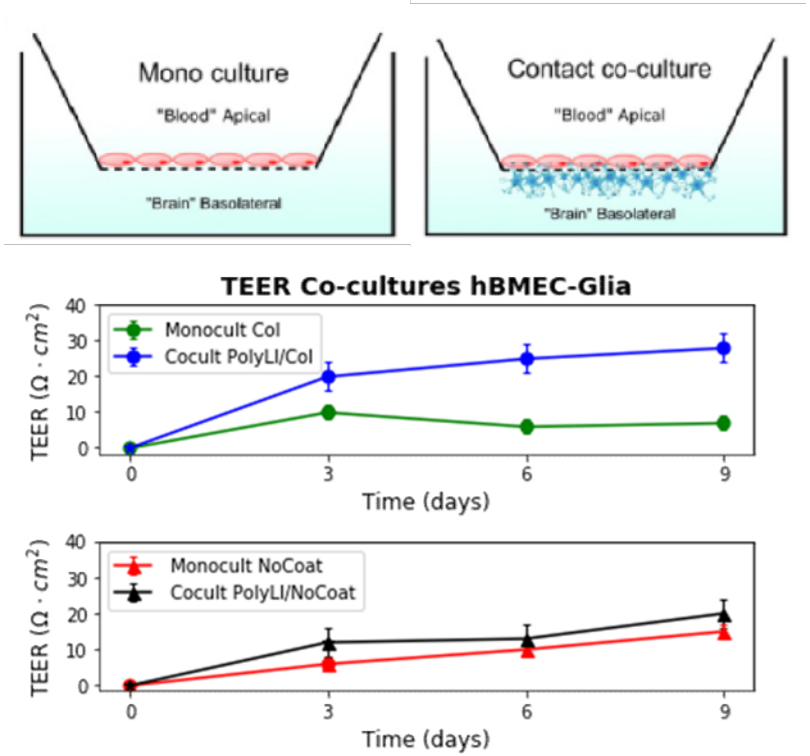


- CSF excretion to the peripheral blood and limphatic circulation (g-limphatic system) is still under study
- **Clearance** functions for toxins and metabolites (and Aβ peptited) activating the so-called ABC transporters
- It becomes atrophic and thicker with age when brain structures shrink

4. Which is the preferred way to brain ? Which are the biological carriers?

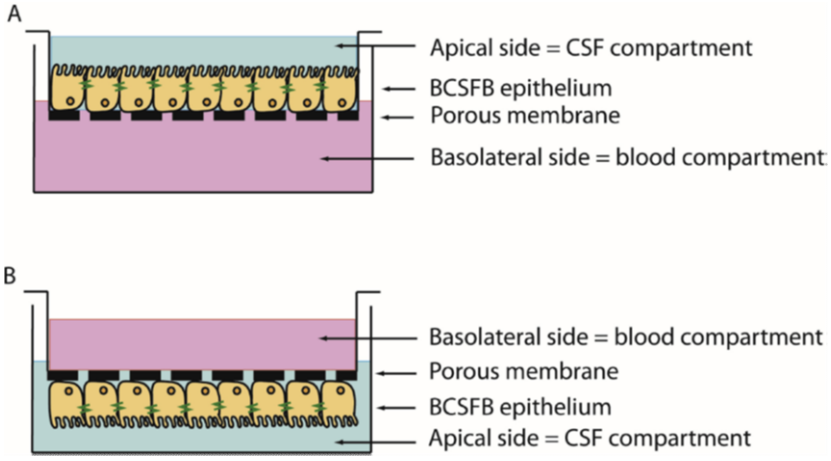
BBB

Primary Human Brain Microvascular Endothelial Cells (hBMECs)+ Primary Glia from mouse

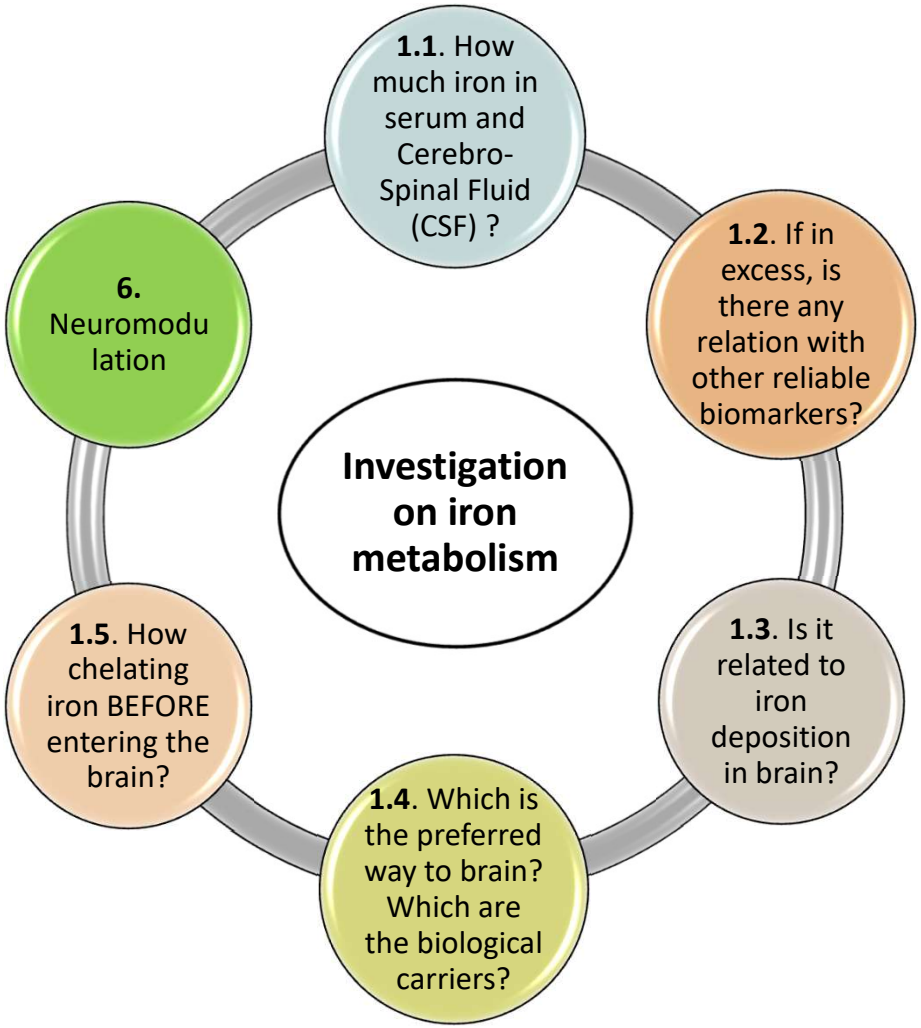


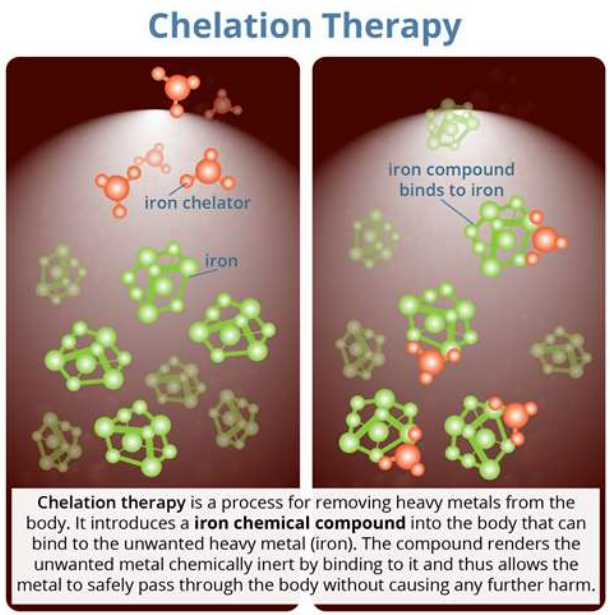
BCSFB

Primary cells	Immortal and immortalised cell lines
Primary murine and rat BCSFB epithelial cells can be prepared from dissected CP of naïve, mutated or tumour bearing animals.	Simian virus 40 large T-antigen (SV40-TA) immortalised rat CP epithelial cell line Z310 and TR-CSFB3
Human primary CP epithelial cells (HCPEpiC) can be obtained from aborted embryos, directly after surgical removal or post mortem(Redzic, 2013). HCPEpiC can be bought commercially.	Immortalised CP tumour cells (CPC-2 cell line). In CPC-2 cells, tight junction proteins, occluding and Cldn-1, were discontinuously expressed or mislocalised in the nucleus, respectively Cldn-2 and ZO-1 were not detected on protein level by immunohistochemistry. (Szmydynger-Chodobska et al., 2007)



(Erb et al. Journal of Neuroscience Methods 329,2020)


1.1. How much iron in serum and Cerebro-Spinal Fluid (CSF) ?
1.2. If in excess, is there any relation with other reliable biomarkers?
1.3. Is it related to iron deposition in brain?
1.4. Which is the preferred way to brain? Which are the biological carriers?
1.5. How chelating iron BEFORE entering the brain?
6. Neuromodulation
Investigation on iron metabolism



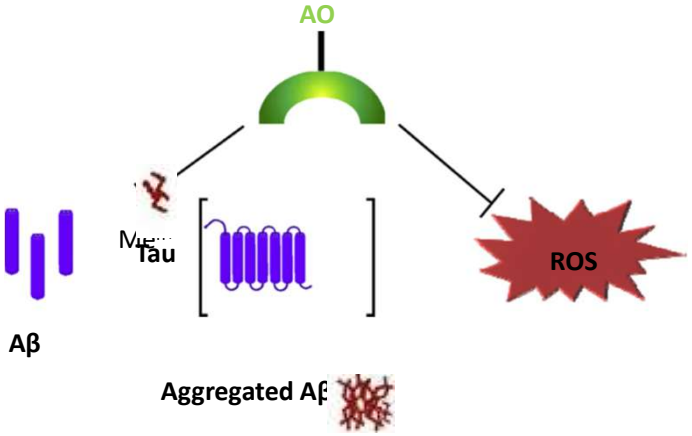
However:

1) **Therapy is NOT personalized, i.e. not related to the specific patient iron concentration**

2) **There are important side effects**

3) **Oral or systemic delivery**

Chelation therapy makes sense provided we can prevent iron from entering the brain!



Redox Metals (Me^{n+}) and the Role of Multifunctional Drugs Possessing Metal-Chelating (Green Semicircle) and Antioxidant (AO) Properties.

These drugs are designed to target the metal ions (chelation effect, inhibition of Ab and Tau aggregation) and reactive oxygen species (ROS; radical-scavenging effect).

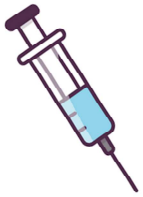
If iron is involved → the chelation approach

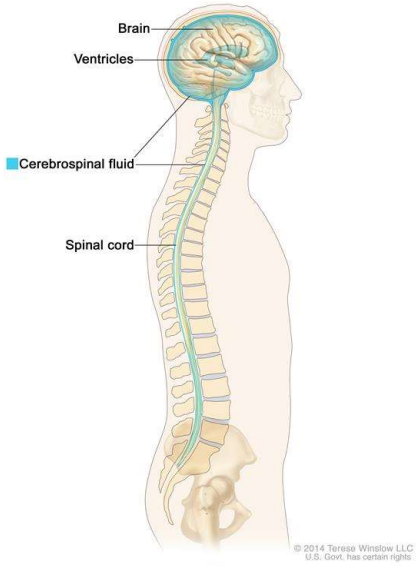
Therapeutic hypothesis:

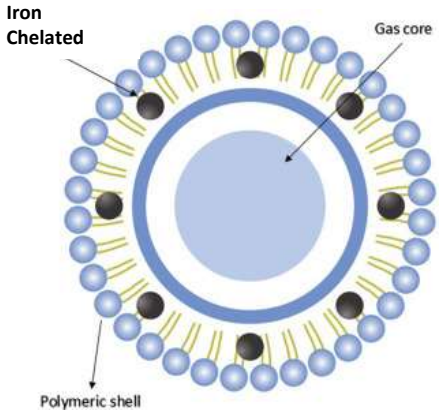
1) Prevent iron imbalance in the brain by accessing the brain via CSF

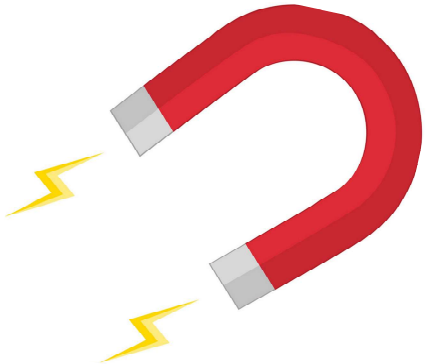
2) nanochelating biocompatible and biodegradable agents delivered via direct spinal injection

3) Provided iron-chelated nanovectors acquire magnetic properties (or providing magnetic properties by linking SPIONS on the surface) , they maybe forced accross BCSFB (to brain) using proper and safe static magnetic fields.

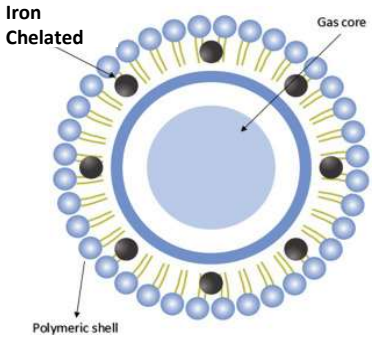








- **A novel system of chelation therapy through the use of nanocarriers which have ability to chelate iron.**



Chitosan is a biodegradable and less toxic polymer which has ability to Absorb both Fe^{+2}, Fe^{+3} .
Formulated **chitosan nanobubbles and Glycol- chitosan EDTA nanobubbles**.
(Dept of Pharmaceutical Science and Technology, University of Turin)

- **UV method for identification:** Optimized a UV Ferrozine and Deferoxamine method for the identification of Fe^{+2} and Fe^{+3} respectively.

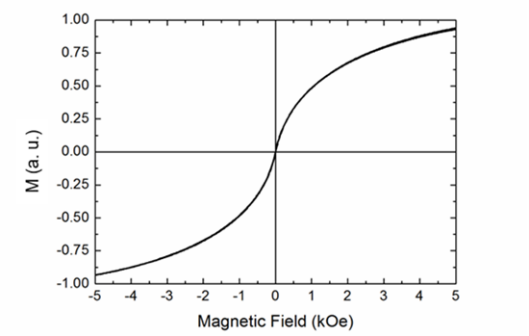
Nanobubbles	**% of chelation of Fe^{+2}**	**% of chelation of Fe^{+3}**
Chitosan nanobubbles	88.35	41.78
Glycol-Chitosan+EDTA Nanobubbles	97.22	52.25

SIMPLIFIED MODEL….MOLNB

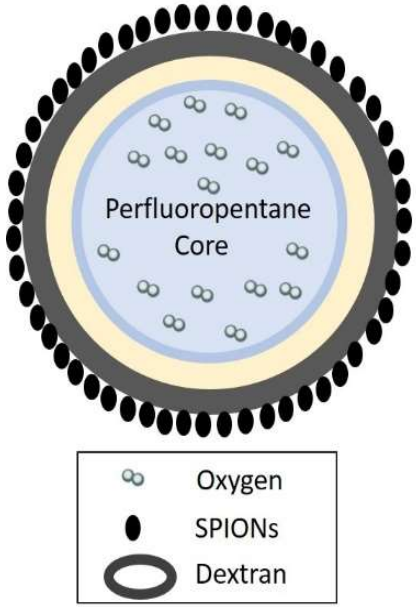
Novel Multipurpose Theranostic Carriers in the Central Nervous System

Physically Drivable Magnetic Nanobubbles

Magnetic Oxigen Loaded Nanobubbles (MOLNBs): Dextran NB covered with Superparamagnetic iron Oxide nanoparticles (SPIONs)



(Zullino et al., 2019, *Frontiers in Pharmacology*)



US Sonography

- Enhancing of O_2 release by sonication
- Monitoring and Imaging by US

Magnetic Driving

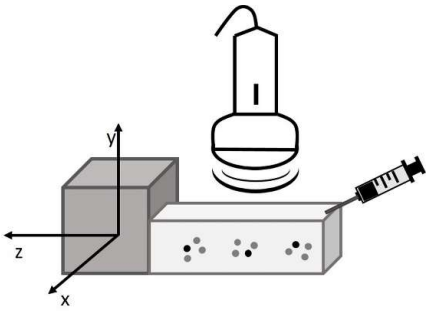
- Targeting of brain tumors by *ad hoc* tailored magnetic fields
- Helping BBB crossing by magnetic force
- Monitoring and Imaging by MRI

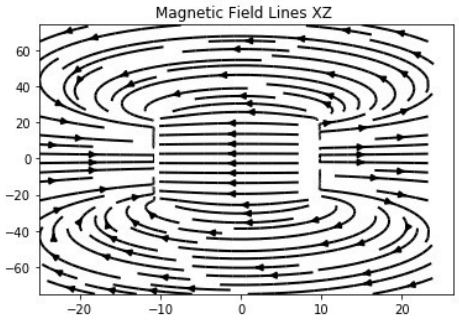
Oxygen and/or Drug Loading

- Loading and delivery of O_2 by diffusion for radiotherapy enhancement in CNS
- Loading and delivery of chemotherapy drugs to target brain tumors

(Ficiarà E. et al., 2020 *Molecules, 25,2104*)

Magnetic driving of MOLNs (and iron-chelated NBs ????) investigated by B-mode US imaging





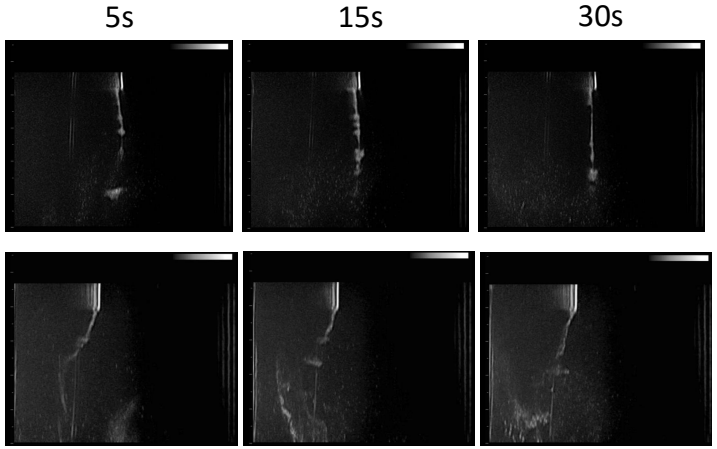
B=1.26-1.29 T

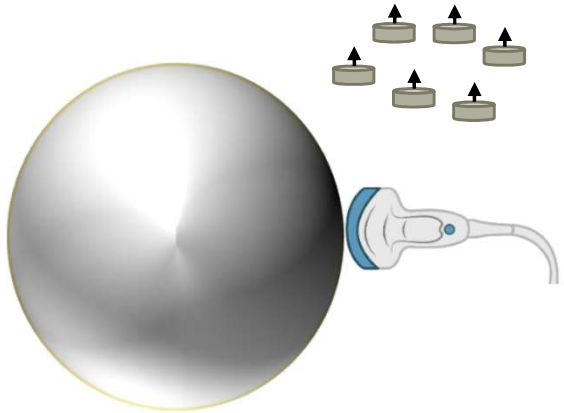
SETUP: a permanent magnet of neodymium coated with Ni-Cu-Ni of cuboid form (50 x 50 x 20 mm) positioned in axial direction proximally to the plastic (7.5 x 3 x 2.5 cm) tank where the NBs will be sonicated.

- The field lines investigated using iron filings showed intense magnetic induction almost parallel along the central axis.

- Sonication by a diagnostic US device (MyLab™25Gold Esaote, Genova, Italy), equipped with a linear array transducer (LA523, 7.5 MHz central frequency) operating in B-mode (small parts imaging preset) and acquiring B-mode cineloop.

Snapshots from US imaging

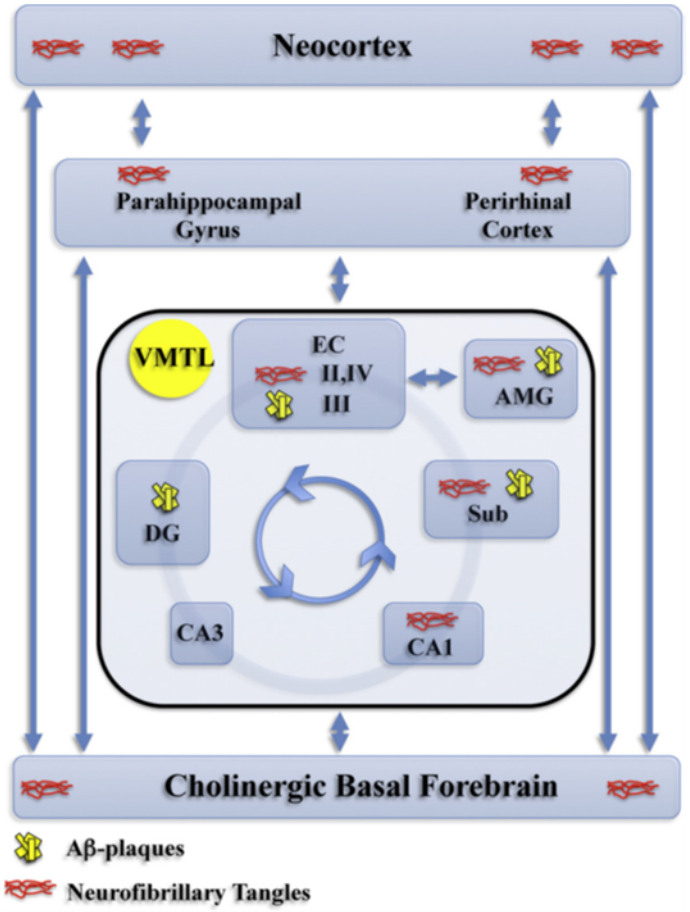




Setup simulating brain (see Ficiarà Presentation)

6. Neuro (re)modulation (‘electro-ceutics’)

Abeta plaques and
Tau tangles
are responsible for
‘circuit oxydation’
and altered electric
conduction



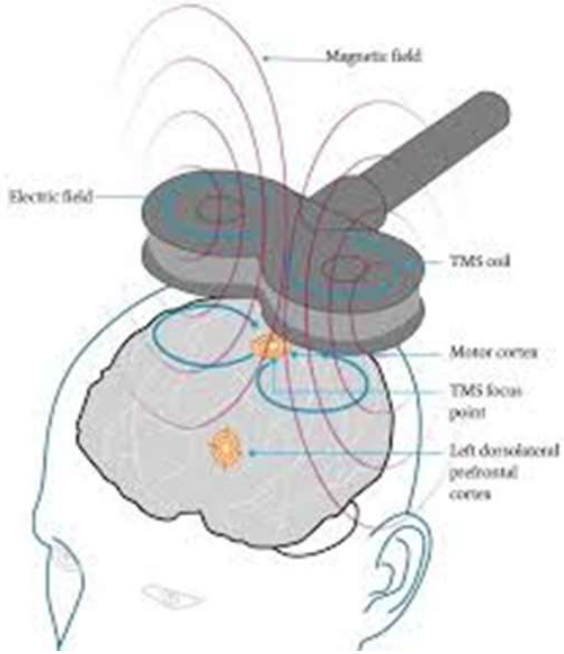
The Amyloid Precursor Protein C-Terminal Domain Alters CA1 Neuron Firing, Modifying Hippocampus Oscillations and Impairing Spatial Memory Encoding
By: Pousinha, Paula A.; Mouska, Xavier; Bianchi, Daniela; et al.
CELL REPORTS Volume: 29 Issue: 2 Pages: 317-+ Published: OCT 8 2019

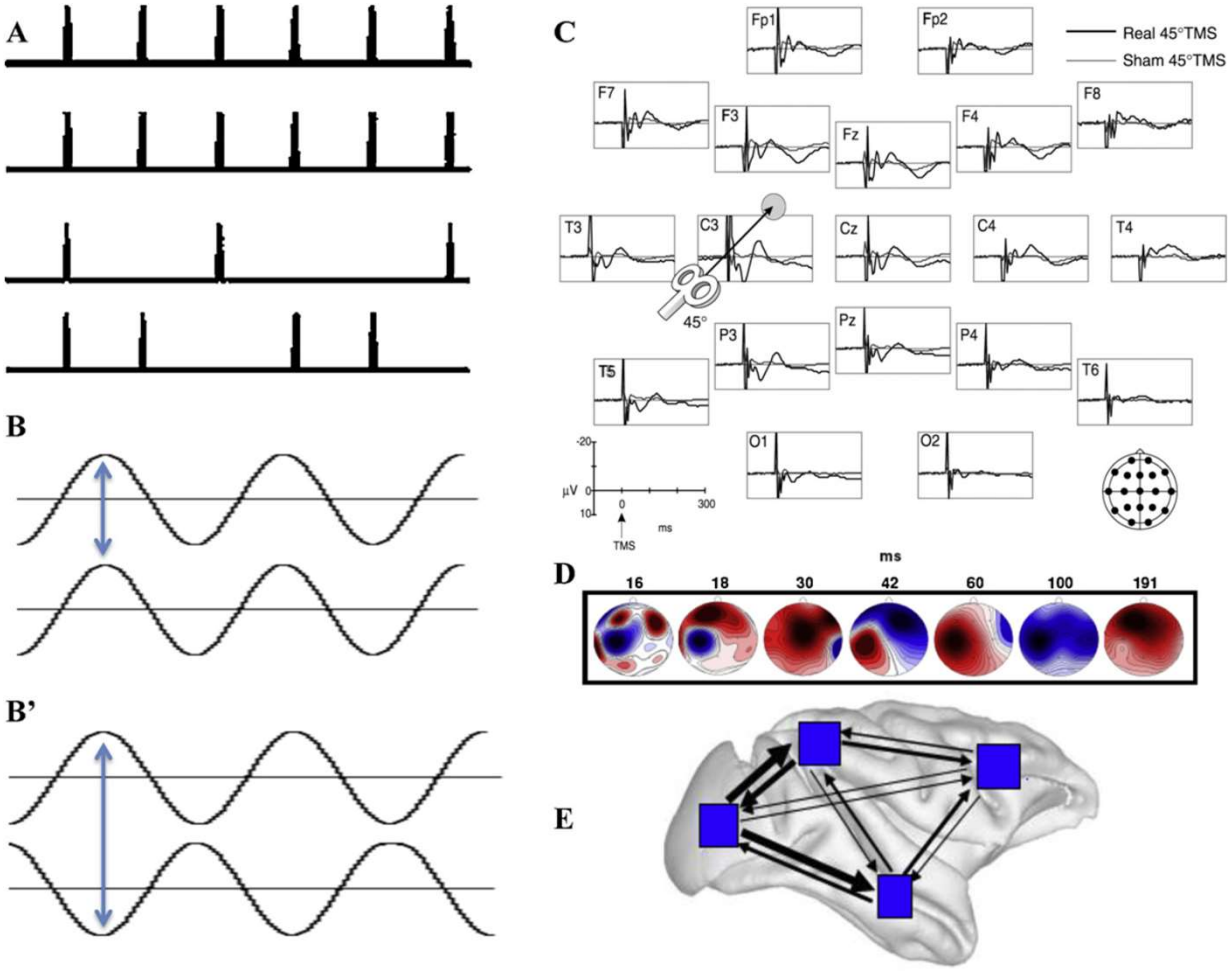
Early Alterations of Hippocampal Neuronal Firing Induced by Abeta42
By: Gavello, Daniela; Calorio, Chiara; Franchino, Claudio; et al.
CEREBRAL CORTEX Volume: 28 Issue: 2 Pages: 433-446 Published: FEB 2018

Brain Arrhythmias Induced by Amyloid Beta and Inflammation: Involvement in Alzheimer's Disease and Other Inflammation-related Pathologies
By: Pena-Ortega, Fernando
CURRENT ALZHEIMER RESEARCH Volume: 16 Issue: 12 Pages: 1108-1131 Published: 2019

TMS

Transcranial Magnetic Stimulation





M. D'Amelio, P.M. Rossini / Progress in Neurobiology 99 (2012) 42–60

BBB opening?

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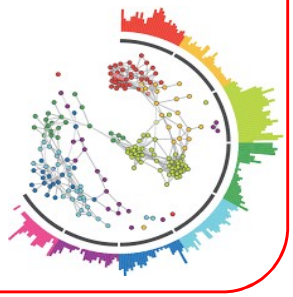

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Città della Salute e della Scienza di Torino

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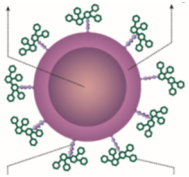

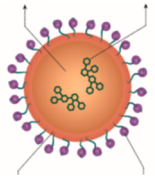

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DBios