

Diagnostica di laboratorio delle encefaliti immunomediate

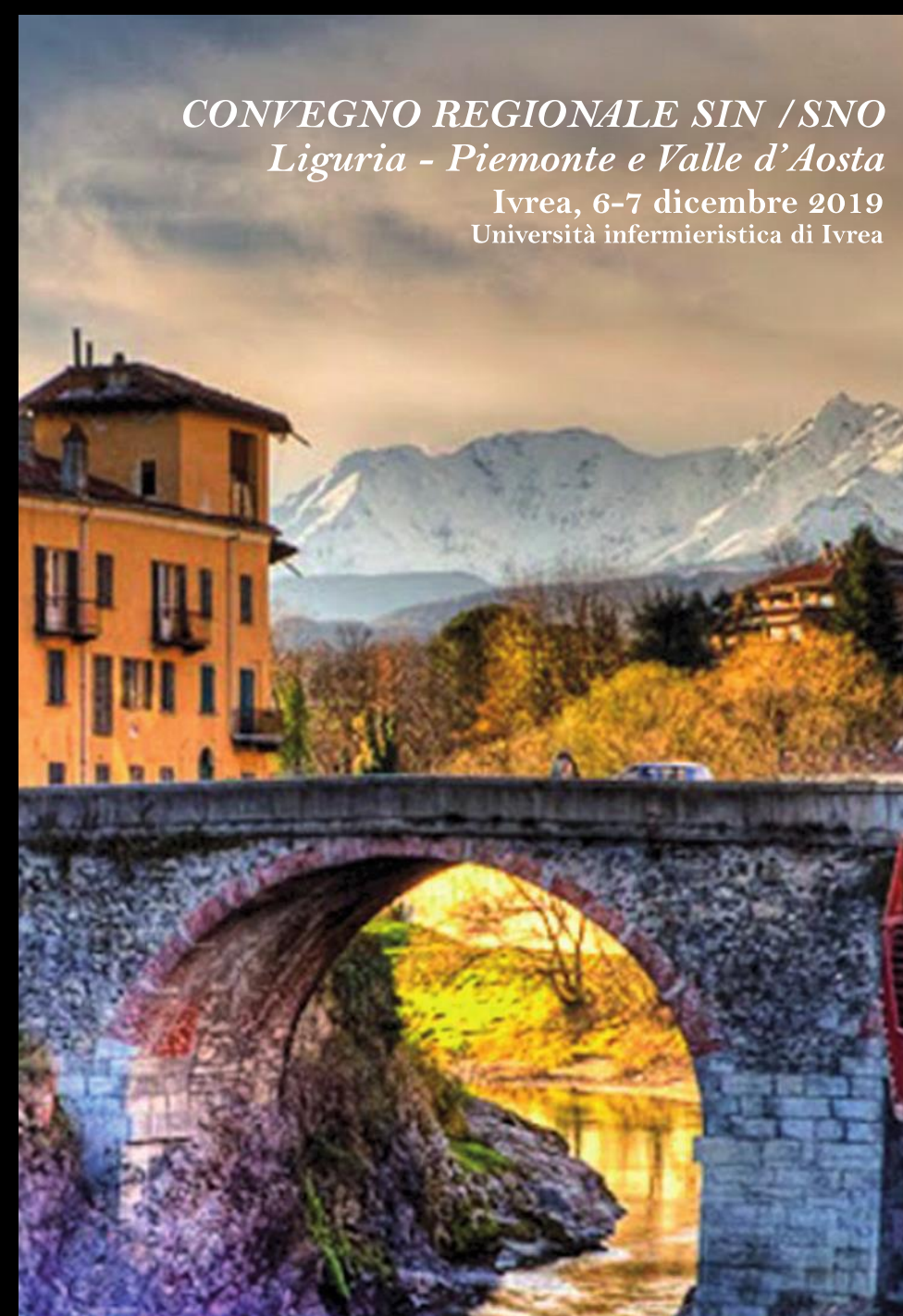
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CONVEGNO REGIONALE SIN / SNO

Liguria - Piemonte e Valle d'Aosta

Ivrea, 6-7 dicembre 2019

Università infermieristica di Ivrea



Dennyse 25 anni



Dicembre 2017: stato confusionale ad esordio acuto, cefalea, impaccio nell'eloquio e nei movimenti nella mano sx...



Esame citochimico del liquor (1°): glucosio 44 mg/dl, proteine 41 mg/dl, cellule 100/mmc



Acyclovir + ceftriaxone



Film-array NEGATIVO

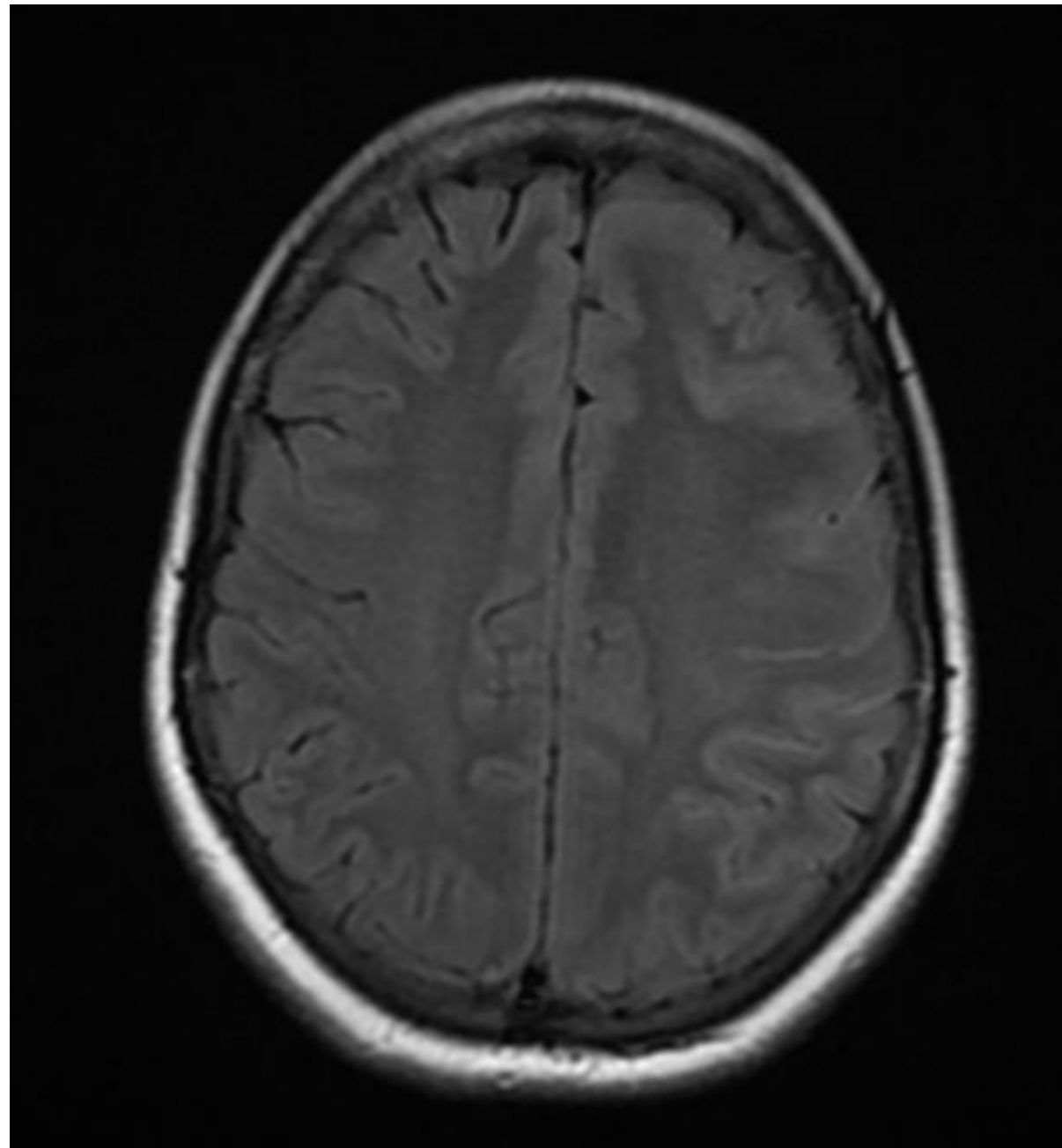


PCR per virus neurotropi NEGATIVA

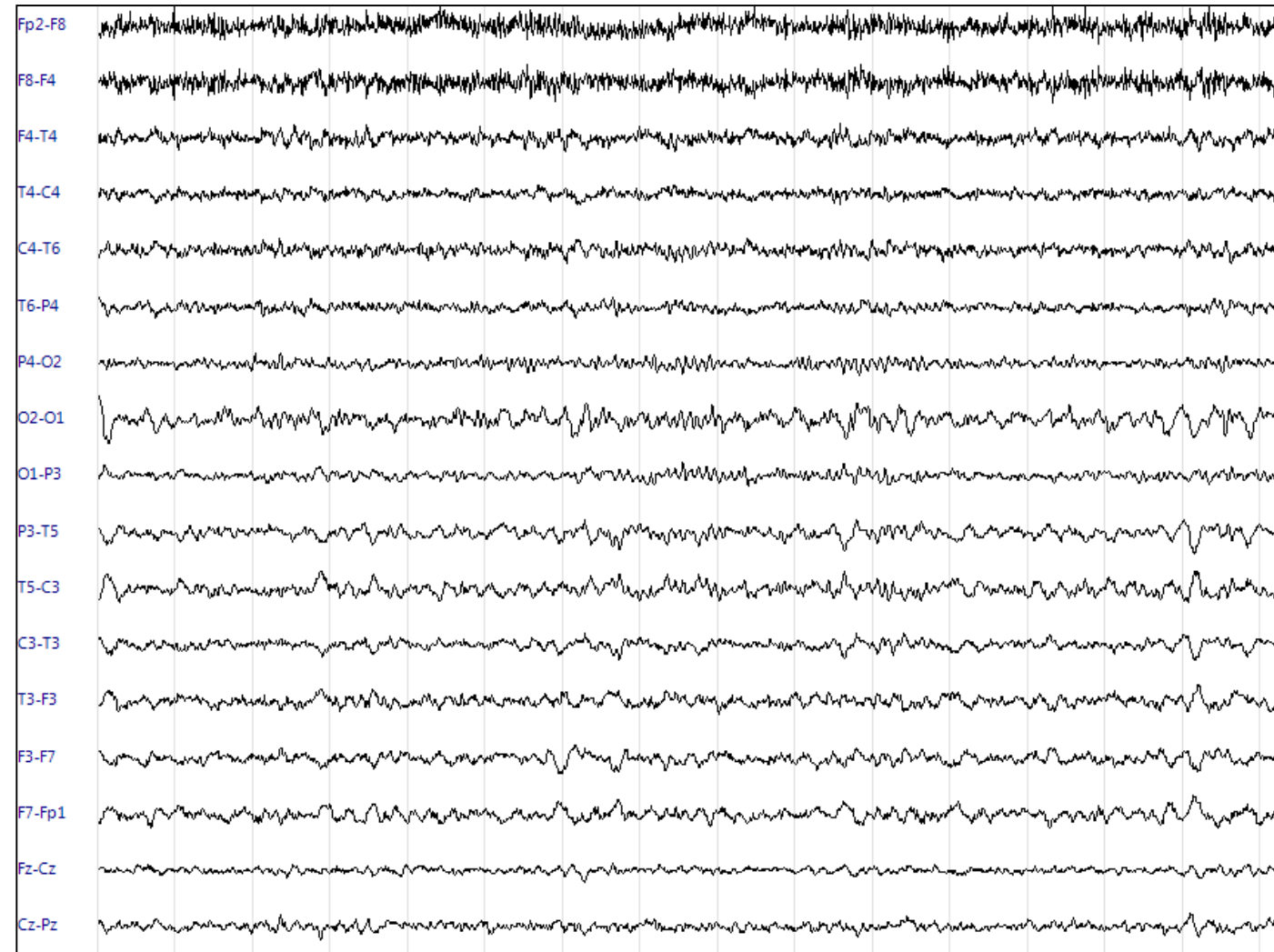


Esame citochimico del liquor (2°): cellule 36/mmc

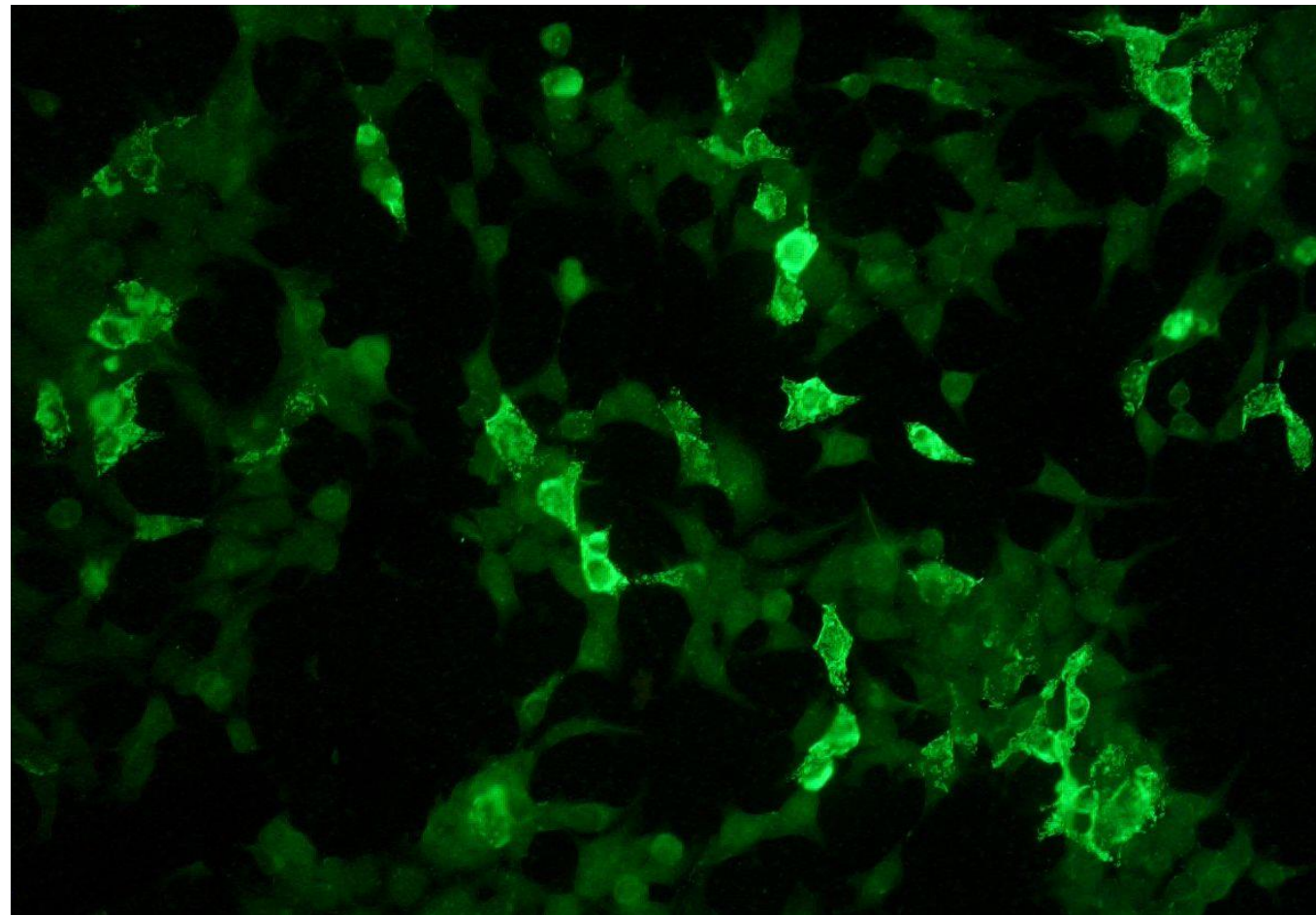
Dennyse
25 anni



Dennyse 25 anni

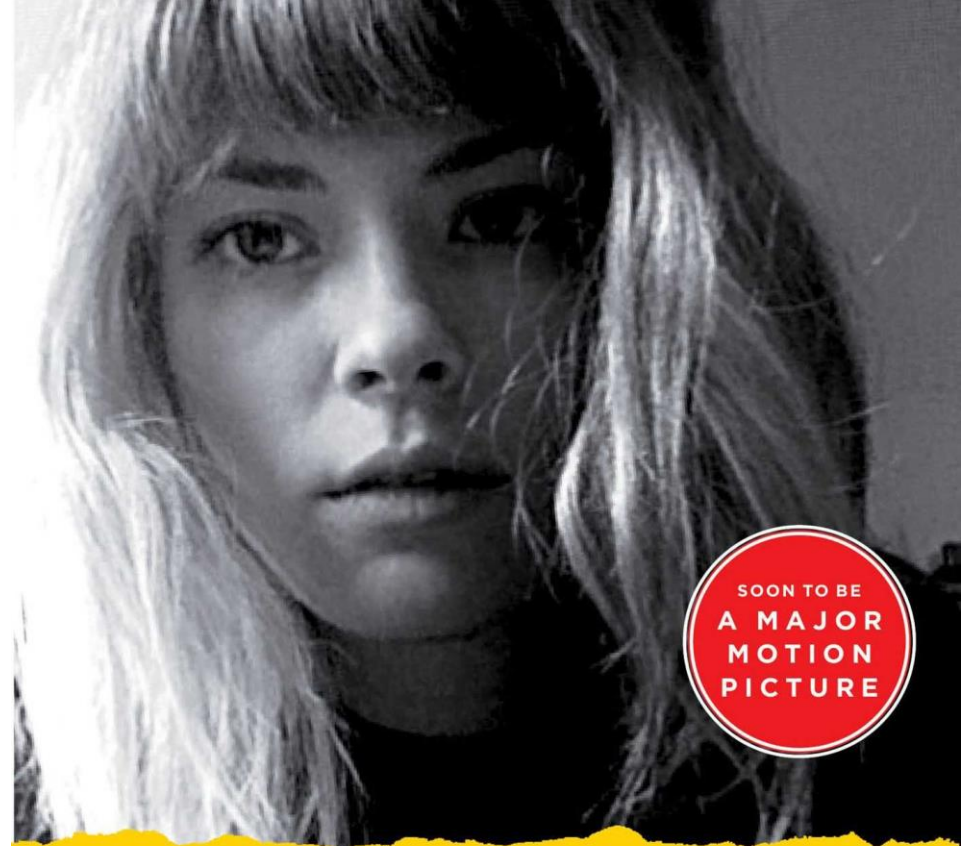


Dennyse
25 anni



Dennyse
25 anni

Encefalite
da anticorpi
anti
NMDA-R



#1 NEW YORK TIMES BESTSELLER

“Stunningly brave . . . an unexpected gift of a book from one of America’s most courageous young journalists.” —NPR

BRAIN ON FIRE

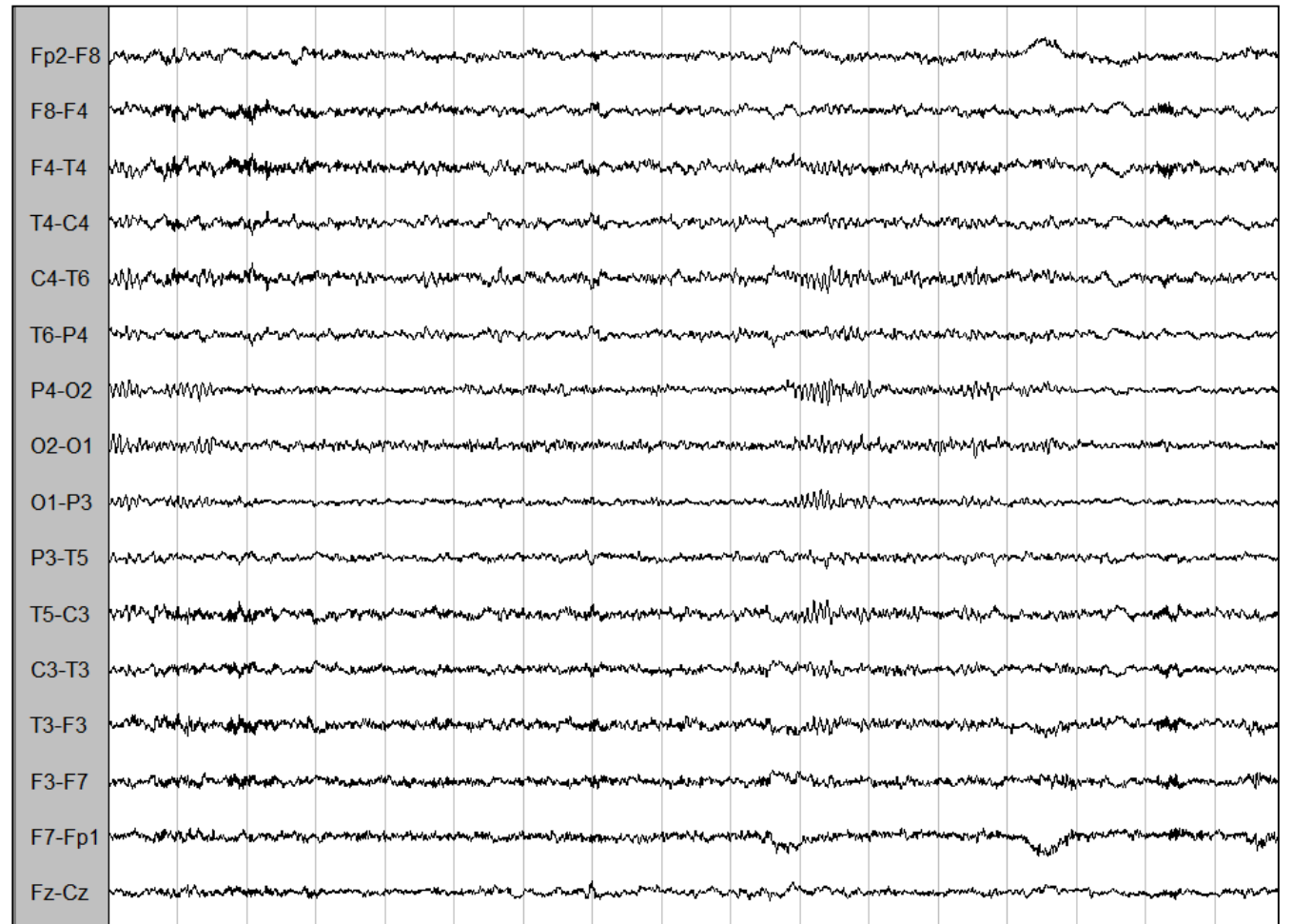
— *My Month of Madness* —

SUSANNAH CAHALAN

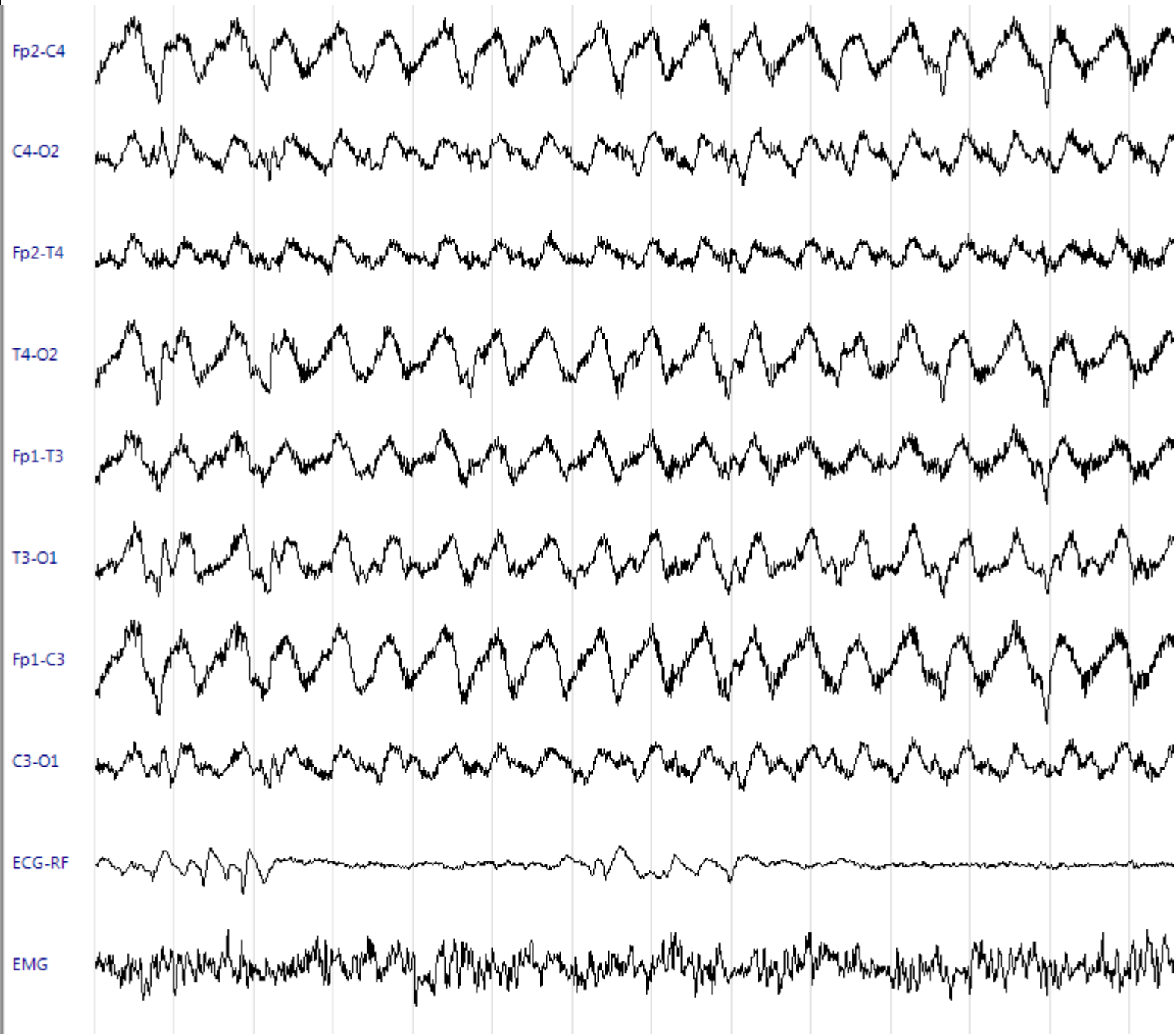
WITH A NEW AFTERWORD

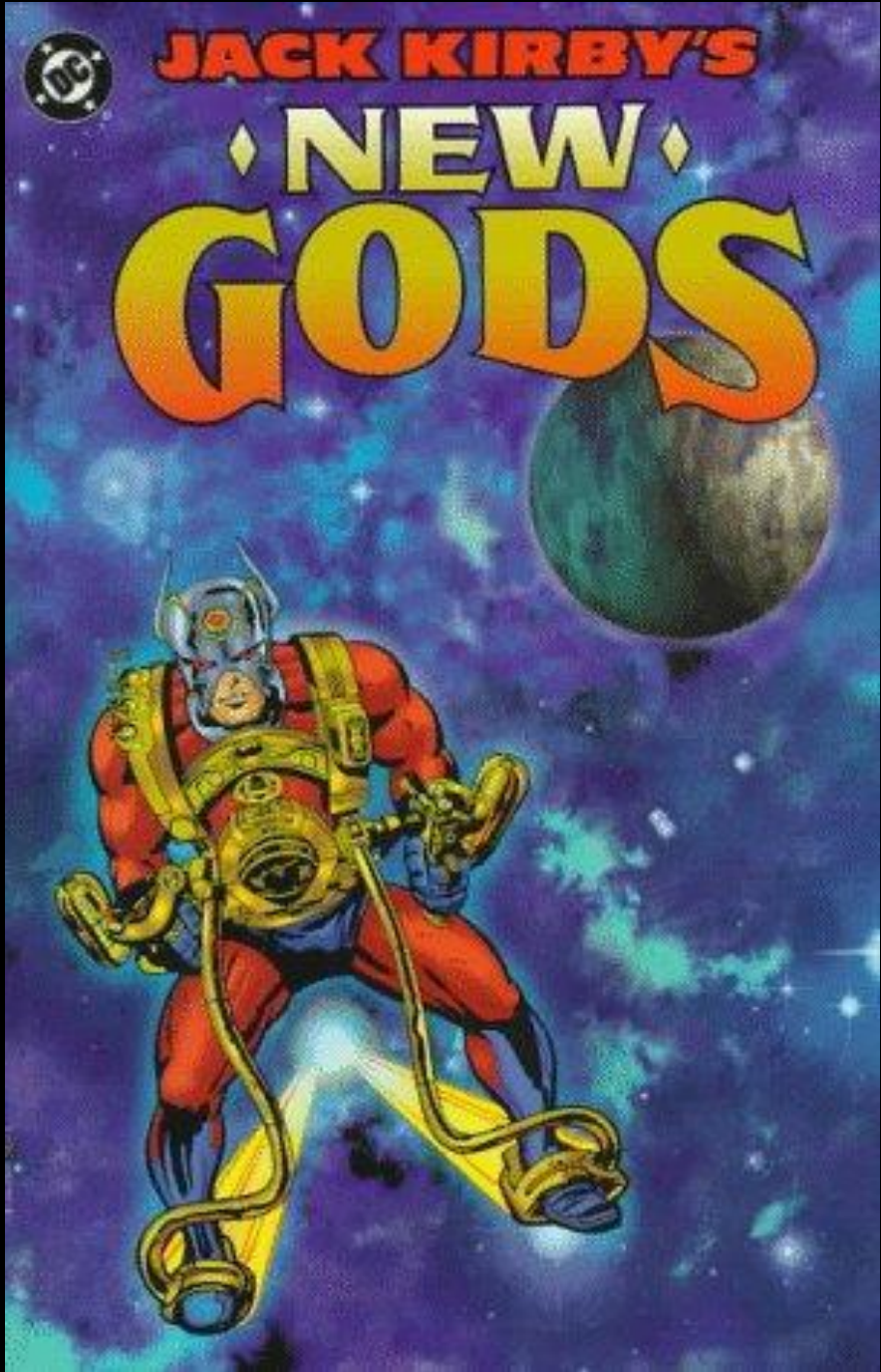


Dennyse 25 anni



Ana
33 anni





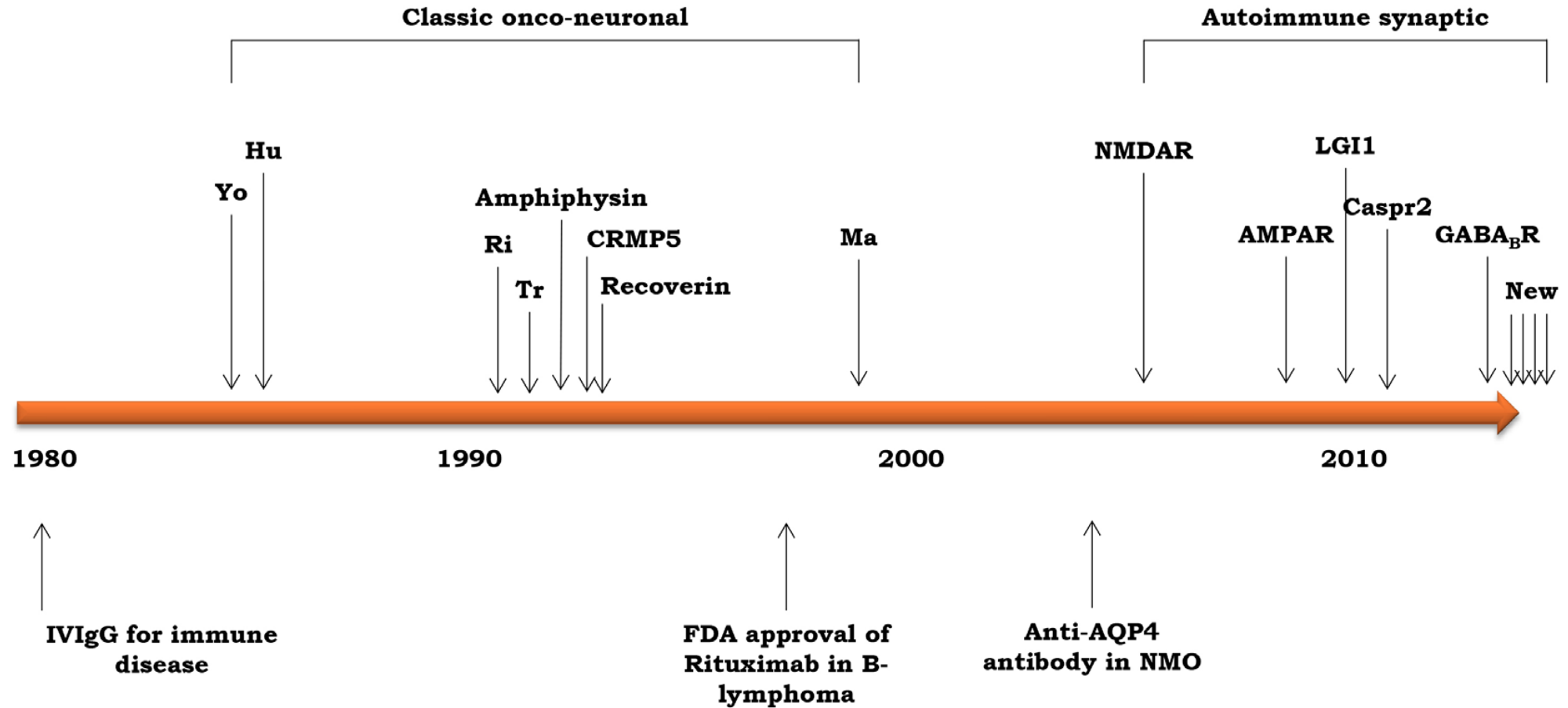
Encefaliti immuno-mediate

- Antineural antibodies
 - e.g. anti-NMDAR, anti-LGI1, anti-Hu
- Hashimoto's encephalopathy (also known as SREAT)
- Systemic disease
 - e.g. SLE, Sjogren's syndrome, sarcoidosis
- Demyelinating disease
 - e.g. ADEM, MOG encephalitis, NMO
- Iatrogenic
 - e.g. CAR-T cell or checkpoint inhibitor-associated encephalitis
- Neurodegenerative
 - e.g. CAA-related inflammation

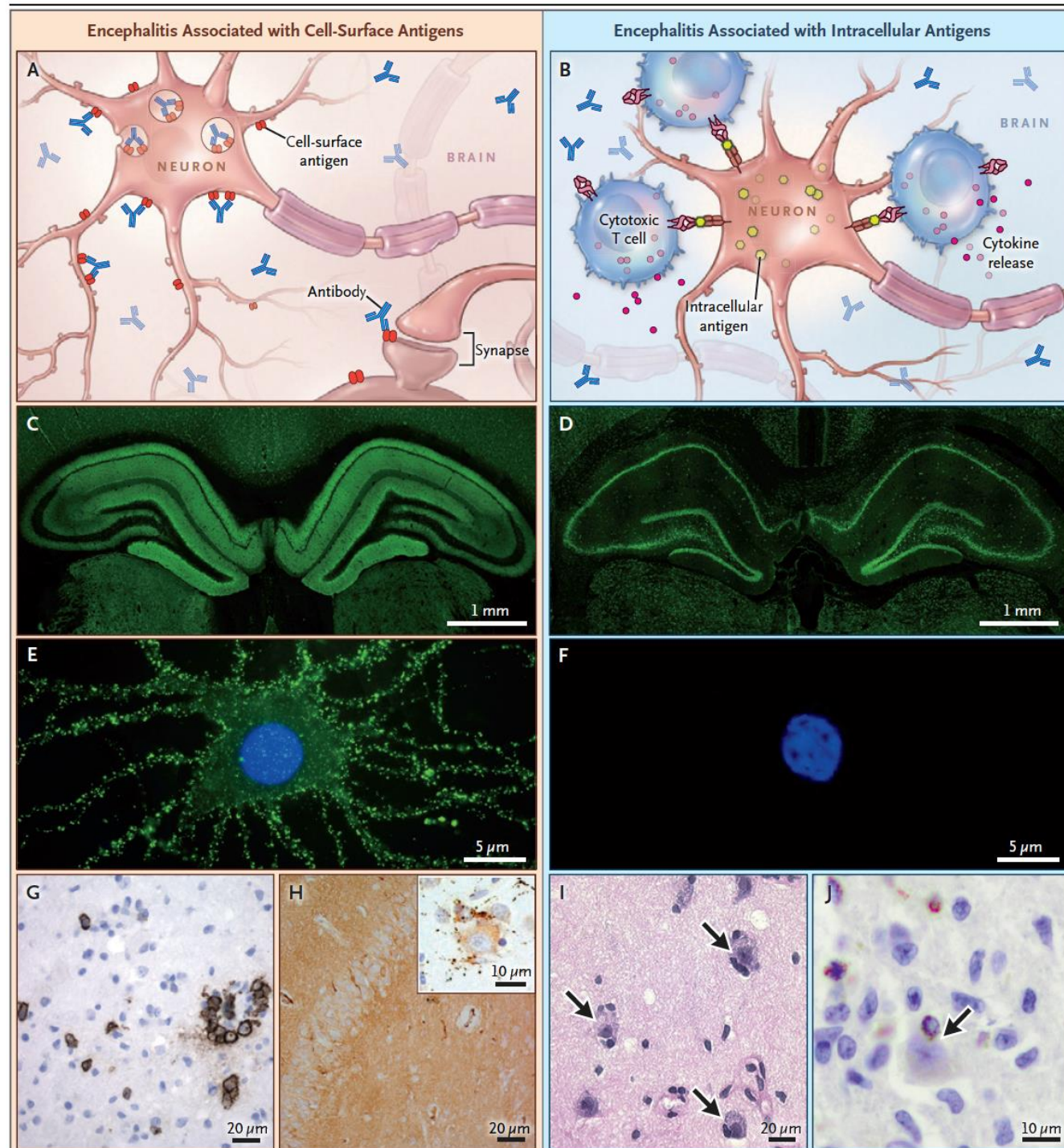
Encefaliti immuno-mediate

- Anni '60-'70
 - Encefalite limbica
 - Termine «paraneoplastica»
 - Encefalopatia associata a tiroidite di Hashimoto
- Anni '80-'90
 - Identificazioni di autoanticorpi diretti contro antigeni intraneuronali (anti-Hu, anti-Yo, anti-Ma) associati a particolari sindromi neurologiche paraneoplastiche (encefalite limbica, degenerazione cerebellare, neuronopatia sensitiva)
- Anni 2000
 - Identificazioni di autoanticorpi diretti contro antigeni di superficie (anti-VGKC) associati a sindromi neurologiche anche non paraneoplastiche

Encefaliti immuno-mediate



- Encefaliti immunomediate associate ad anticorpi diretti contro antigeni superficiali dei neuroni
- Encefaliti immunomediate associate ad anticorpi diretti contro antigeni intracellulari dei neuroni
- Encefaliti immunomediate senza dimostrazione di una specificità anticorpale associare



Antigeni intracellulari («classici»)

| Antibody | Oncological association | Frequency of tumour | Response to immunotherapy | Neurological manifestations |
|------------------|--|---------------------|---------------------------|--|
| ANNA-1 (anti-Hu) | Small-cell carcinoma. | >90% | Poor | Limbic, cortical encephalitis. Autonomic neuropathies, sensory neuronopathy and other peripheral neuropathies. |
| ANNA2 (anti-Ri) | Small-cell carcinoma, breast adenocarcinoma and bladder cancer. | >60% | Poor | Brainstem encephalitis (opsoclonus–myoclonus, laryngospasm, trismus and cranial neuropathy) and cerebellar degeneration. |
| ANNA3 | Small-cell carcinoma. | >60% | Poor | Limbic and brainstem encephalitis, sensory and sensorimotor neuropathies and myelopathy. |
| PCA2 | Small-cell carcinoma. | >90% | Poor | Brainstem or limbic encephalitis and cerebellar degeneration. |
| Ma1, Ma2 | Testicular (Ma2); breast, colon and testicular (Ma1). | >90% | Moderate | Ma2 Limbic encephalitis, diencephalitis, brainstem encephalitis; Ma1 and Ma2 brainstem encephalitis and cerebellar degeneration. |
| CRMP-5 | Small-cell carcinoma and thymoma. | >75% | Poor | Encephalitis. Optic neuritis and retinitis, myelopathy, neuropathy and Lambert–Eaton myasthenic syndrome. |
| Amphiphysin | Small-cell carcinoma and breast adenocarcinoma. | >90% | Poor | Limbic encephalitis. Myelopathy, stiff-man syndrome and cerebellar degeneration. |
| GAD65 | Thymoma; neuroendocrine tumours, breast or colon adenocarcinoma. | <10% | Moderate | Stiff-person syndrome, stiff-person phenomena, brainstem encephalitis and cerebellar degeneration. |
| GFAP | None described to date. | | Good | Meningoencephalomyelitis, headache, papillitis and cerebellar ataxia. |

ANNA, antineuronal nuclear antibody; CRMP-5, collapsin response mediator protein-5; GAD65, glutamic acid decarboxylase 65; GFAP, glial fibrillar acidic protein; PCA, Purkinje cell cytoplasmic antibody.

Antigeni di superficie o sinaptici

| Antibody | Oncological association | Frequency of tumour | Response to immunotherapy | Neurological manifestations |
|----------------------|---|-------------------------------------|---------------------------|--|
| VGKC complex LGI1 | Thymoma, small-cell lung cancer. | <10% | Good | Limbic encephalitis, hyponatremia and faciobrachial dystonic seizures. |
| CASPR2 | Thymoma | 40% | Good | Isaacs syndrome, Morvan's syndrome and limbic encephalitis. |
| NMDAR | Ovarian teratomas, testicular germinoma and neuroblastoma. | Varies with age, sex, and ethnicity | Good | Psychiatric disturbances, dyskinesias, catatonia, central hypoventilation and autonomic instability, and opsoclonus–myoclonus. |
| AMPAR | Thymic tumours, lung carcinoma and breast adenocarcinoma. | 70% | Good | Limbic encephalitis and nystagmus. |
| GABA-A receptor | Thymoma, small-cell lung cancer and rectal cancer. | 40% | Good | Status epilepticus, epilepsy partialis continua, psychosis, behavioural disturbances, orolingual dyskinesias and chorea. |
| GABA-B receptor | Small-cell lung carcinoma and other neuroendocrine neoplasia. | 70% | Good | Limbic encephalitis and orolingual dyskinesias. |
| mGluR5 receptor | Hodgkin's lymphoma. | >90% | Good | Cerebellar ataxia and limbic encephalitis (Ophelia syndrome). |
| GlyR | Thymoma, breast cancer and Hodgkin's lymphoma. | <10% of published cases | Moderate | Progressive encephalomyelitis with rigidity and myoclonus, oculomotor disturbances, dysautonomia, hyperekplexia and respiratory failure. |
| DPPX | None described to date. | | Moderate | Encephalitis, sleep disturbances, myoclonus, hyperekplexia, dysautonomia and gastrointestinal dysmotility. |
| IgLON5 | None described to date. | | Poor | Non-REM parasomnias, REM sleep behaviour disorder, apnoea, stridor and cognitive decline. |

AMPAR, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; Caspr2, contactin-associated protein-like 2; DPPX, dipeptidyl-peptidase-like protein-6; GABA-A, γ -aminobutyric acid--A; GABA-B, γ -aminobutyric acid-B; GlyR, glycine receptor; LGI1, leucine rich glioma inactivated protein 1; mGluR5, metabotropic glutamate receptor 5. NMDAR, N-methyl-D-aspartate receptor; REM, rapid eye movement; VGKC, voltage gated potassium channel;

| | Intracellular Antigens | intracellular synaptic antigens | Cell-surface/Synaptic Antigens |
|-----------------------------|--|---|---|
| Antibodies detected | Hu, Yo, Ri, Ma2, CRMP5/CV2, Zic4, etc. | GAD, amphiphisin | LGI1, CASPR2, NMDAR, AMPAR, GABAA/BR, GlyR, D2R, DPPX, etc. |
| Pathogenic mechanism | considered markers of paraneoplastic syndromes with poor prognosis and treatment response, in which autoimmunity is mainly effected by cytotoxic T-cells; the antigens are deemed to be inaccessible in vivo | controversial role in the pathogenesis, their antigen could be transiently accessible during synaptic vesicle fusion and uptake | considered pathogenic as they target proteins that are accessible in vivo and, typically, play an important role in synaptic transmission, plasticity or excitability |

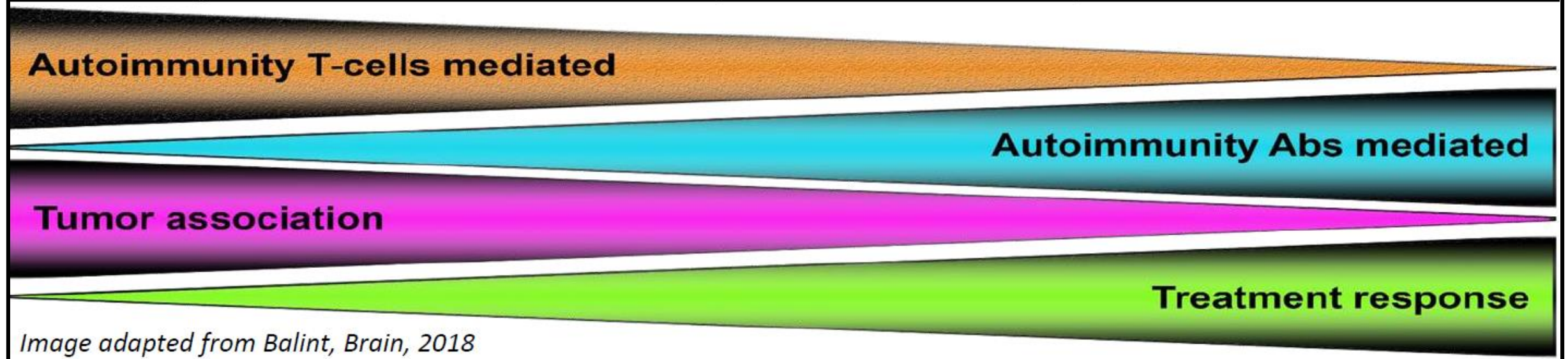
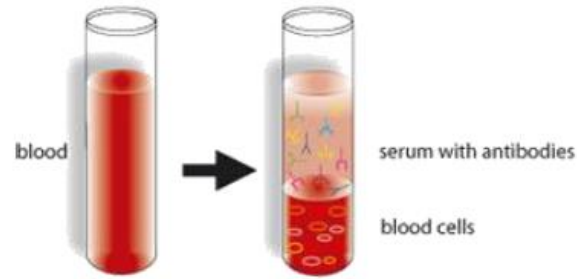


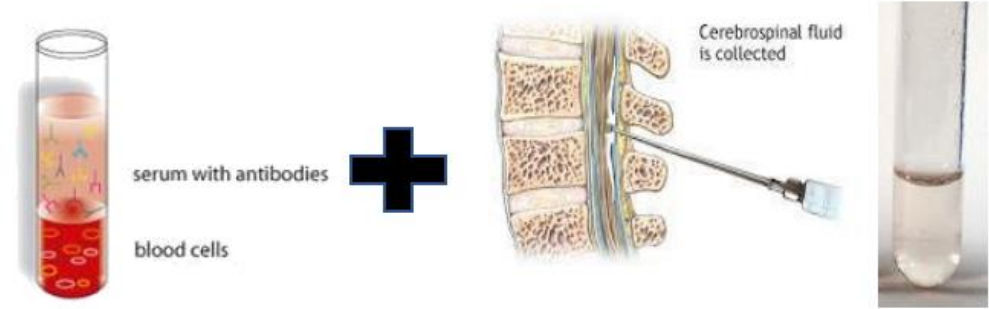
Image adapted from Balint, Brain, 2018

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SERUM

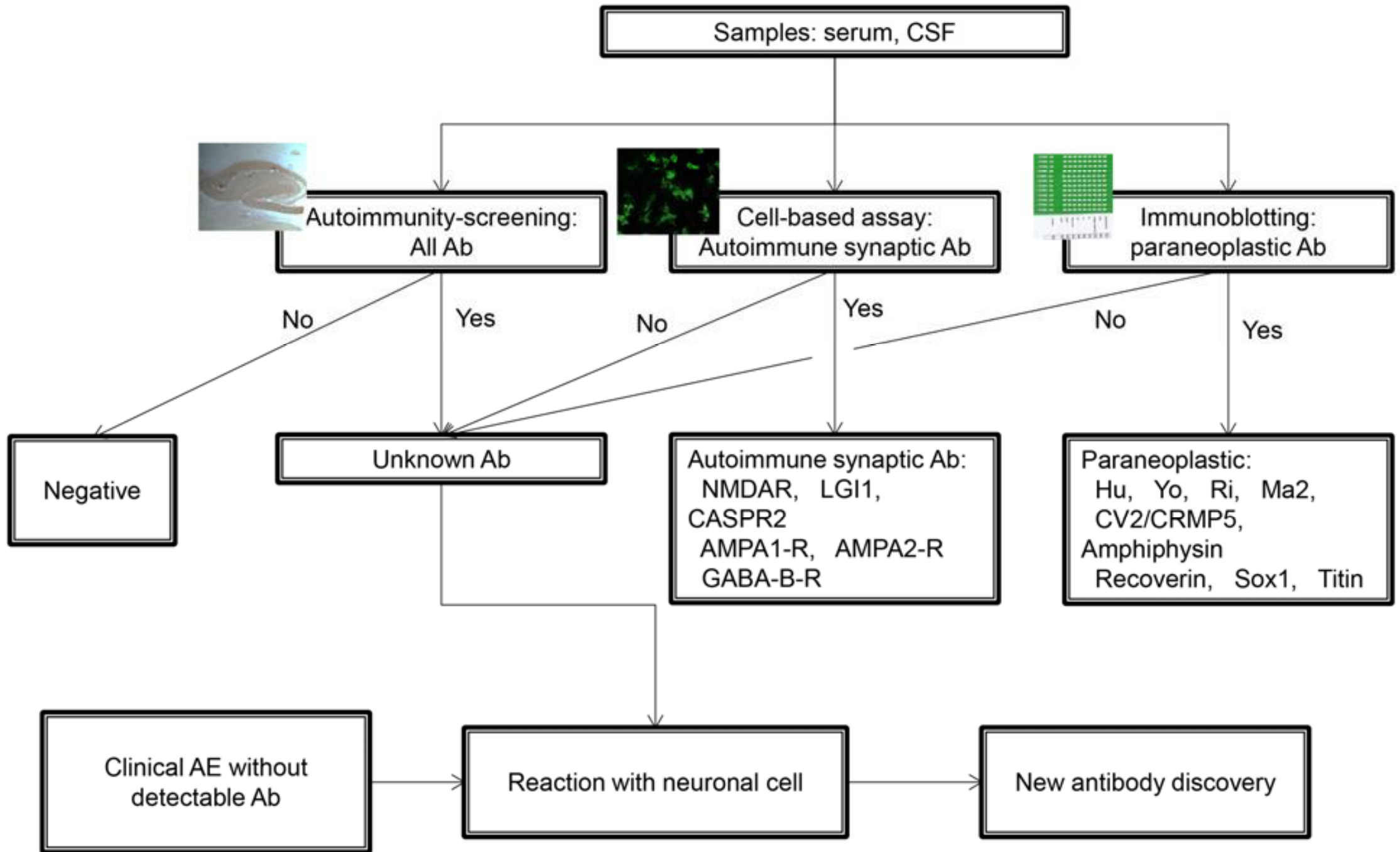


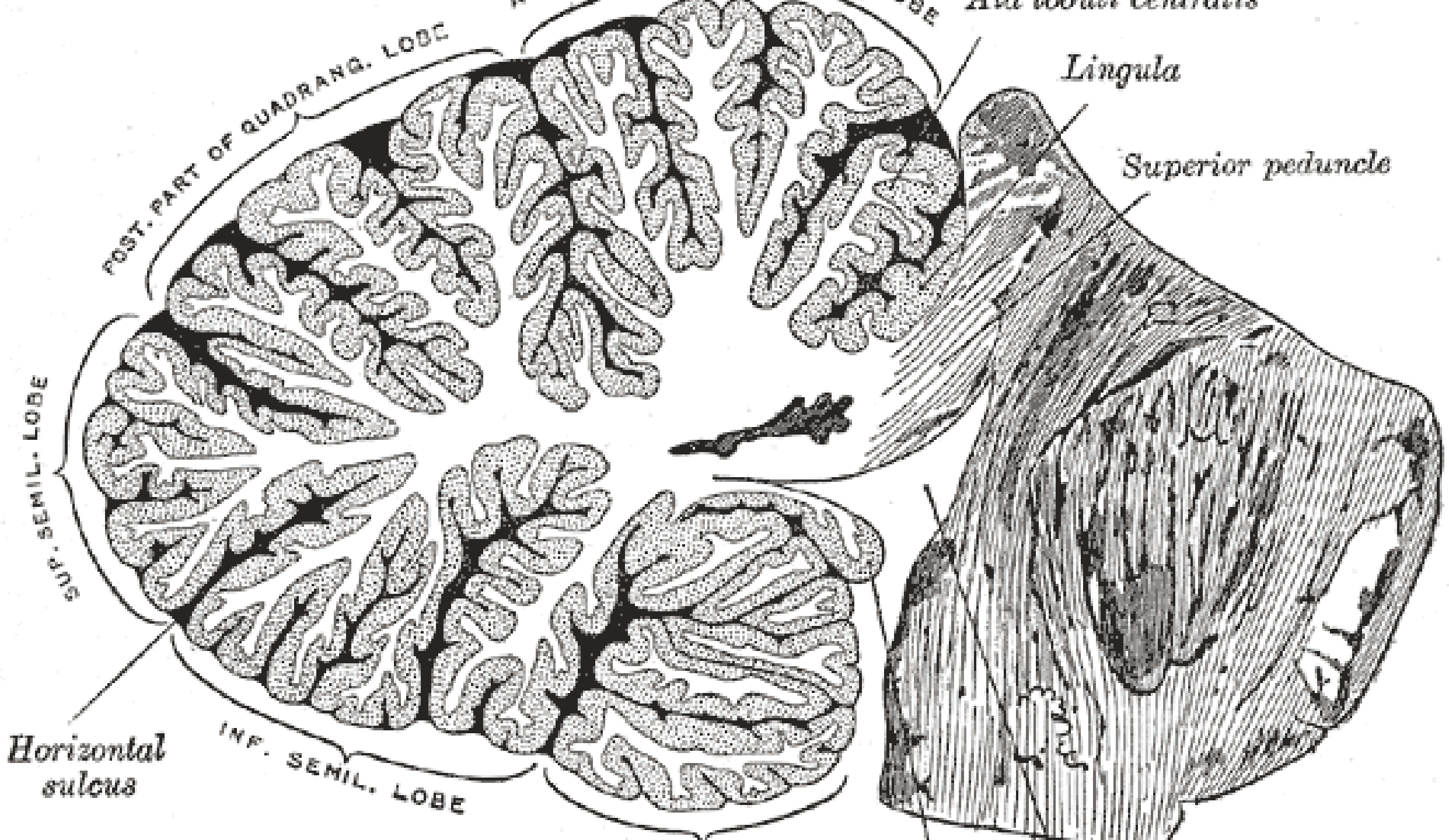
SERUM & CSF

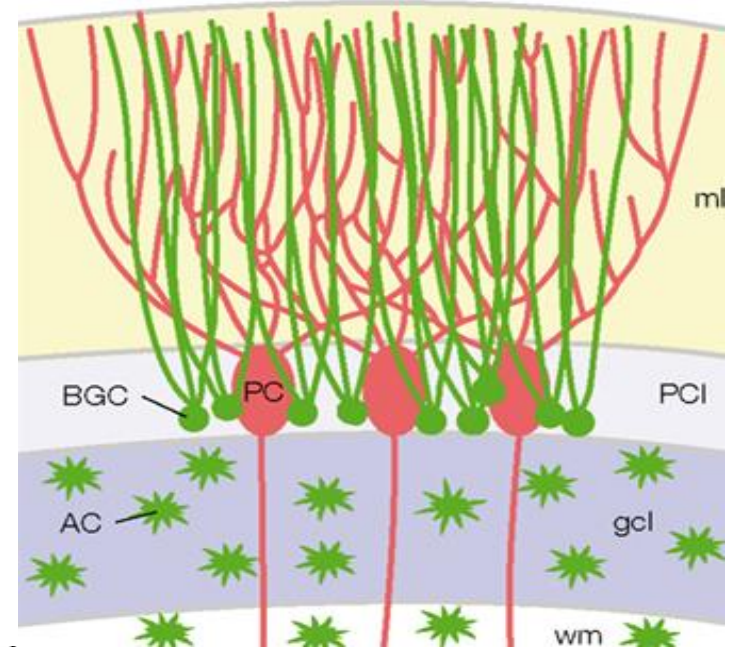
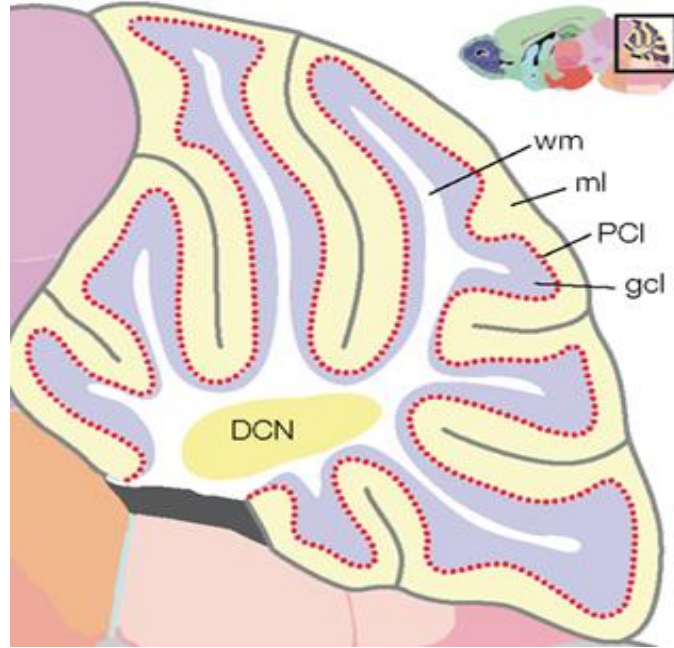
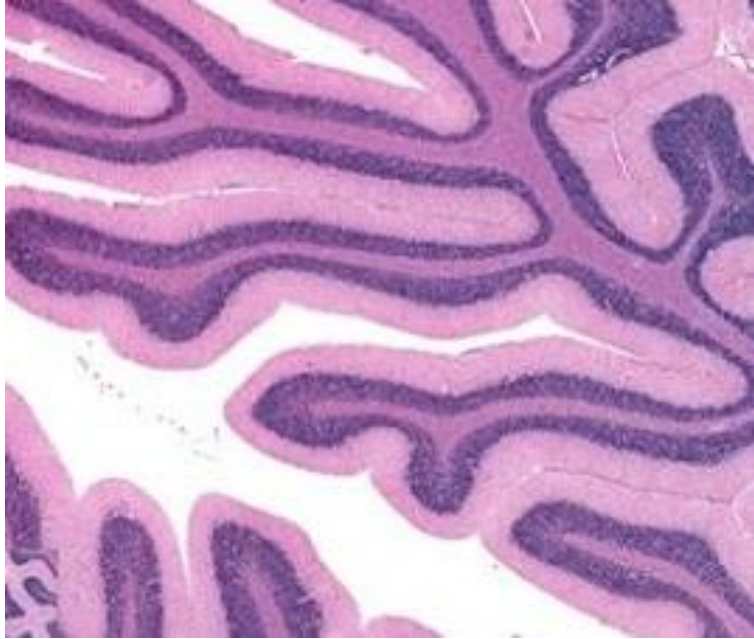


| | SERUM | SERUM & CSF |
|------------------------|---|---|
| INTRACELLULAR ANTIGENS | Hu, Yo, Ri, amphiphysin, CV2, recoverin, titin, SOX1, Zic4, PCA-2, ANNA-3 | GAD65 |
| SURFACE ANTIGENS | Tr/DNER, AQP-4, MOG | NMDAR, AMPAR, LGI1, CASPR2, GABA _B R, DPPX, IgLON5 |

Leyboldt & Dalmau, (2015); Graus *et al.*, (2016); Waters *et al.*, (2016)

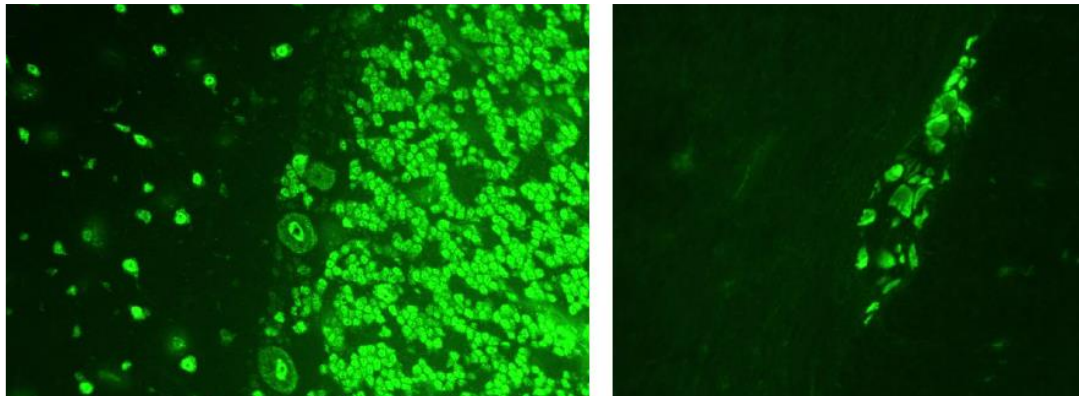






Pattern fluoroscopici su cervelletto di primate

Anti-Hu

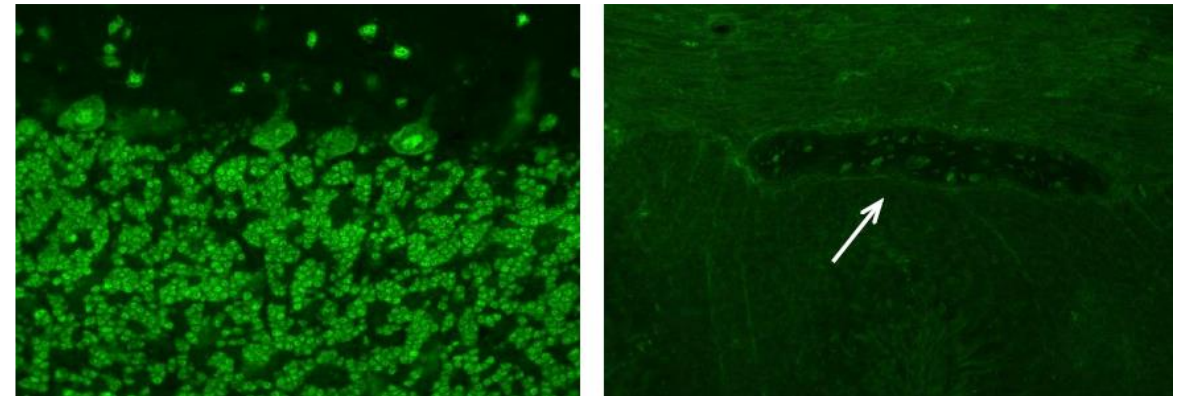


Most commonly occurring paraneoplastic neurological antibody

Neurological syndrome: Cerebellar ataxia, encephalomyelitis, sensory neuropathy

Associated tumour: Small cell lung carcinoma and neuroblastoma

Anti-Ri

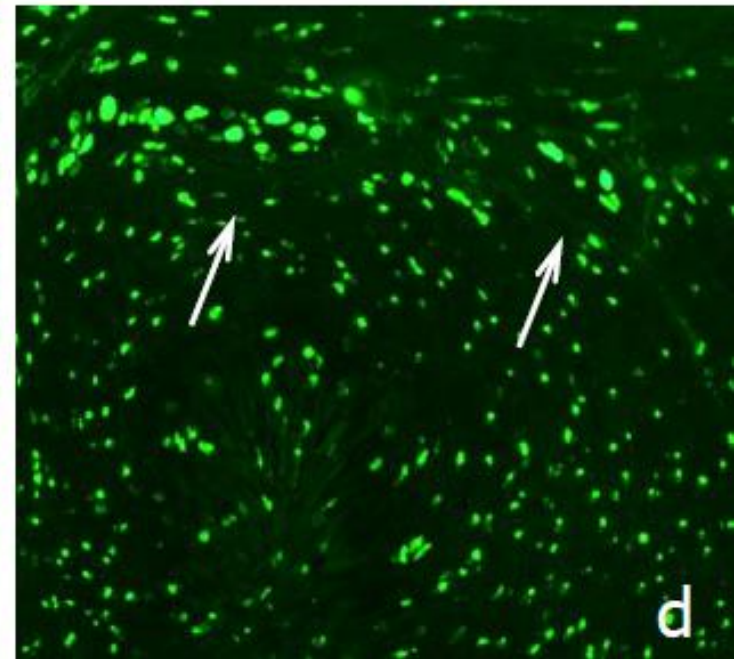
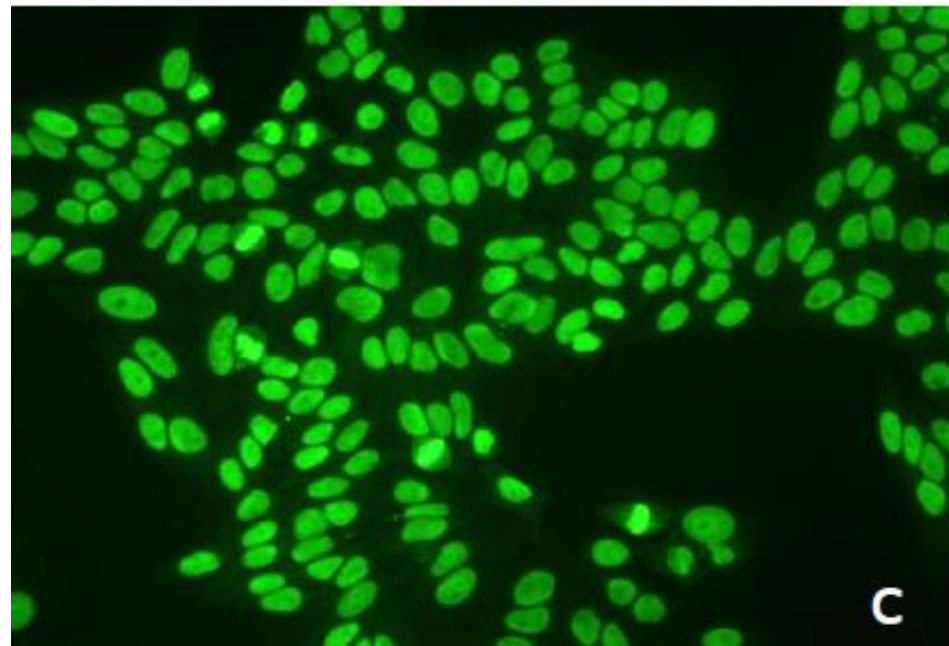
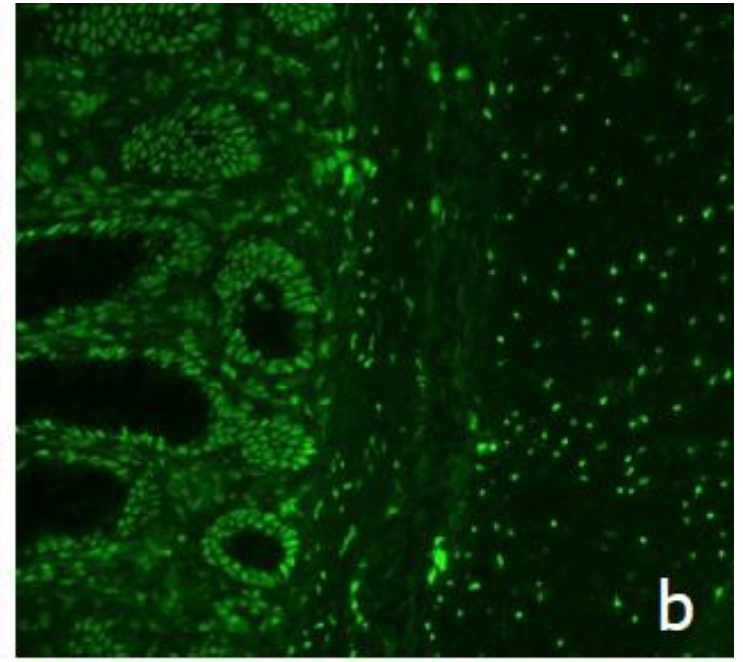
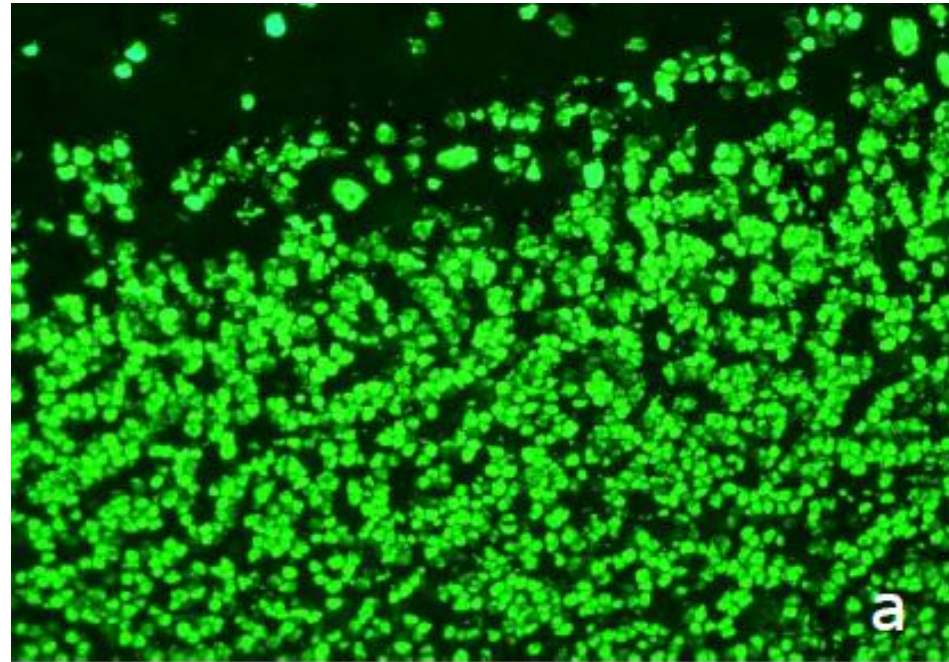


Rare antibodies (IgG)
Similar staining pattern as anti-Hu antibody

Neurological syndrome: Cerebellar degeneration, opsoclonus/myoclonus

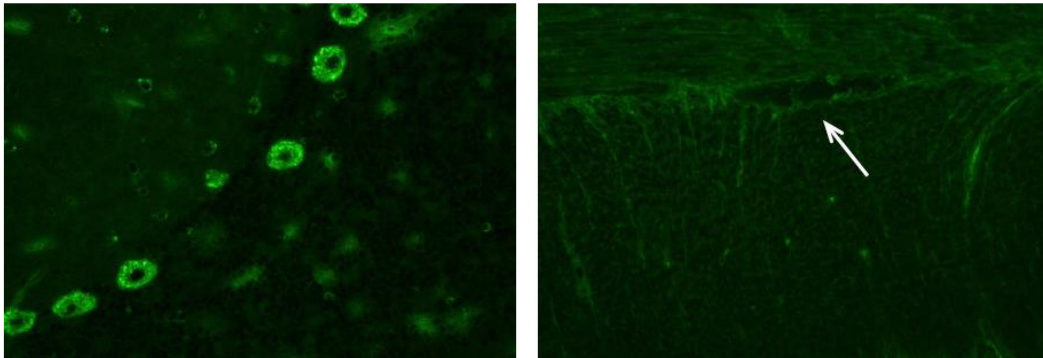
Associated tumours: Breast, gynaecological and small cell lung carcinoma

ANA



Pattern fluoroscopici su cervelletto di primate

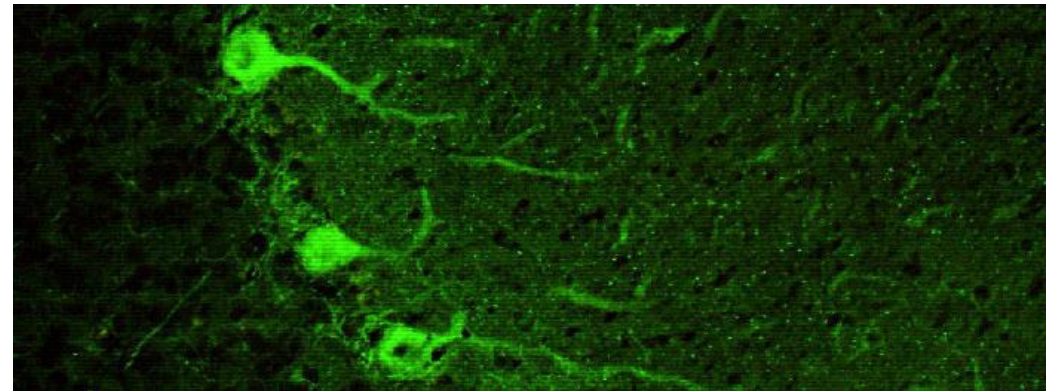
Anti-Yo



Clinical indication: Cerebellar degeneration

Associated tumours: Breast and ovarian carcinomas. With only few exceptions PCA are found exclusively in female patients

Anti-Tr



Cytoplasm of Purkinje cells together with the dendrites.

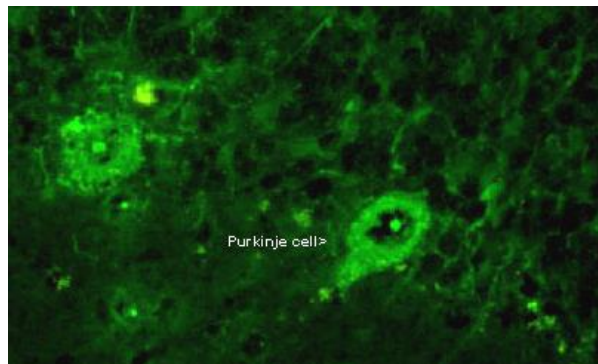
Neurological syndrome: Cerebellar degeneration

Associated tumour: Hodgkin's disease

[L]
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Pattern fluoroscopici su cervelletto di primate

Anti-Yo

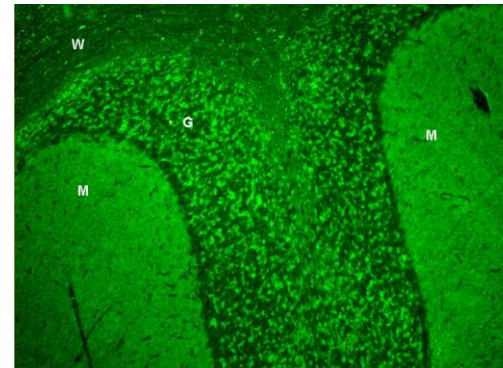


Ma is located in nucleoli of the molecular and Purkinje neurones

Tumour: Ma1: Various tumours. Ma2 usually found in younger males with testicular tumour.

Syndrome: Ma1: Brainstem/cerebellar syndrome. Ma2; Brainstem, cerebellar, limbic signs.

Anti-Amphyphysin



The cell processes in the molecular layer are stained more intensely than the nerve terminals in the granular layer

Clinical conditions: Stiff person's syndrome (5%), paraneoplastic encephalomyelitis.

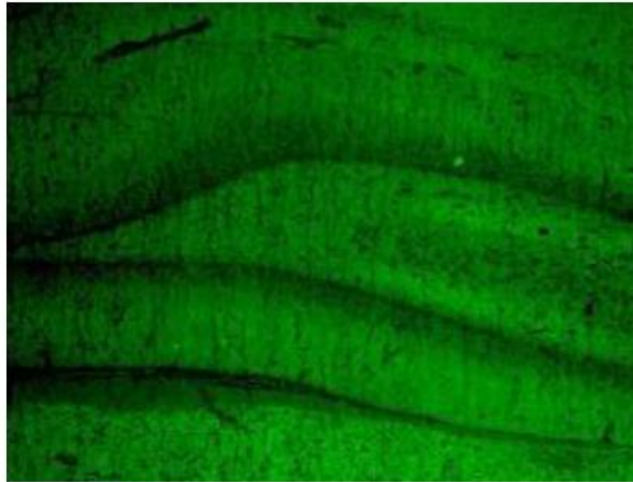
Associated tumours: small cell lung carcinoma and breast cancer

PHASE 1- Screening with TBA on tissue

PHASE 2- Confirmation/further investigation

Surface antigens

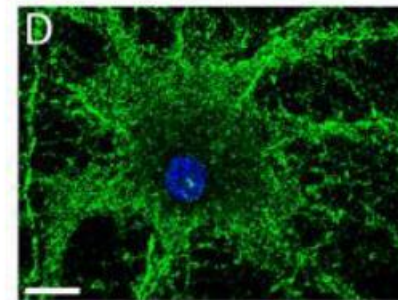
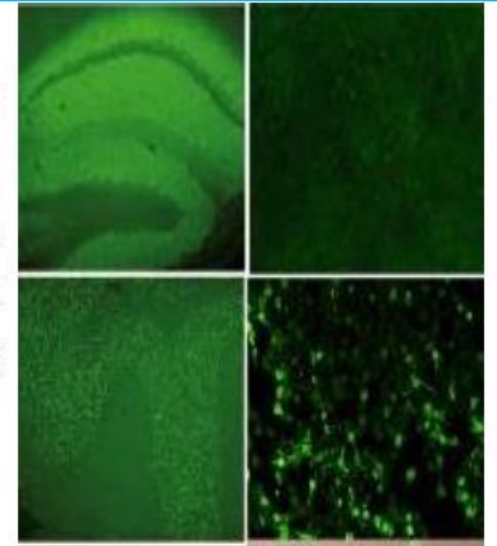
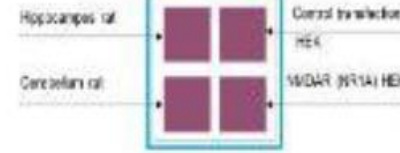
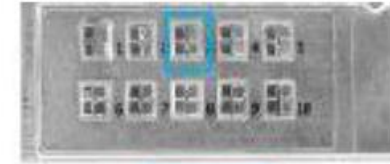
Postfixed brain



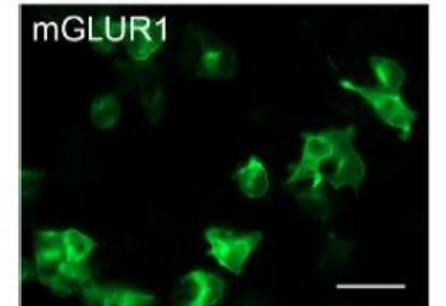
NEG

POS

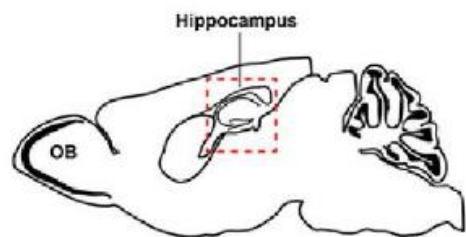
Dubious or clinical data highly suggestive



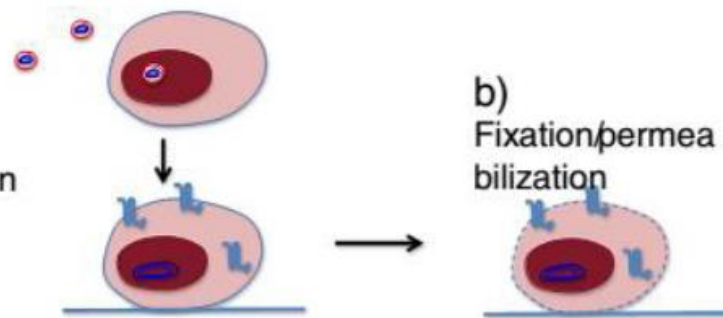
Live murine neurons



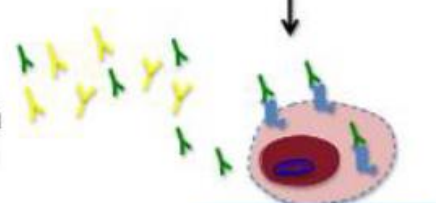
CBA (home made)



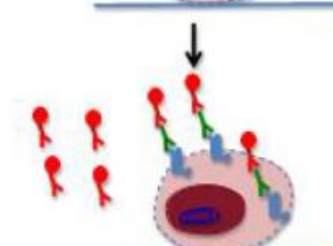
- 1 Transfection
- 2 Surface antigen expression



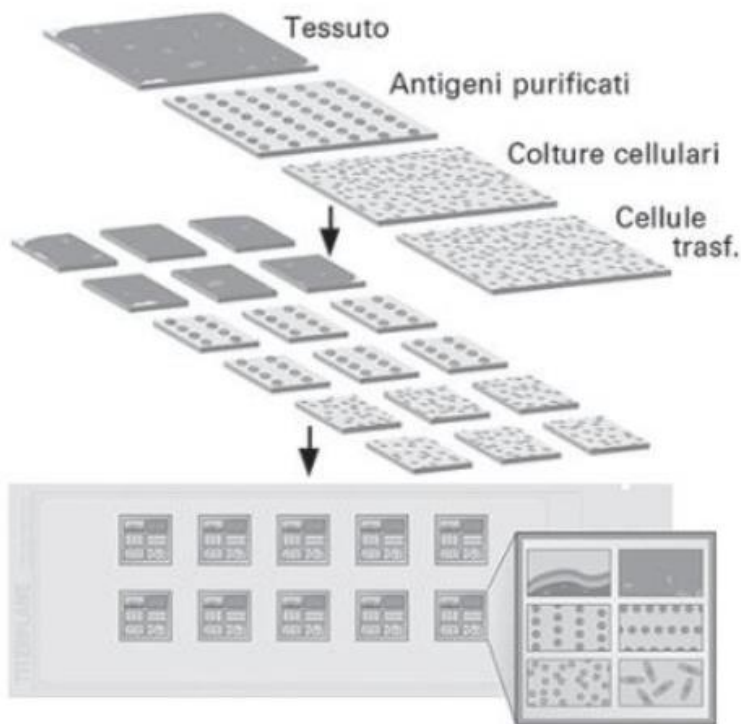
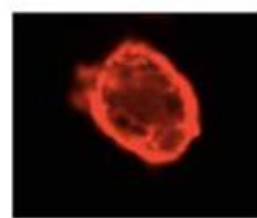
- 3 Incubation with patient serum



- 4 Incubation with fluorescent anti-human IgG

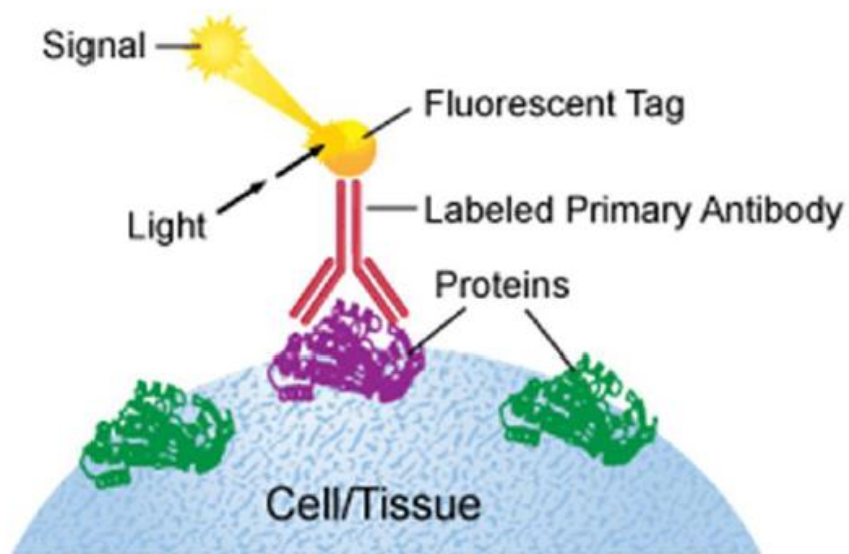


- 5 Microscopic evaluation



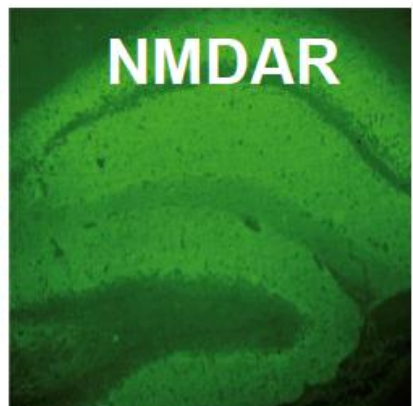
Tecnologia e Mosaici BIOCHIP

Immunofluorescence

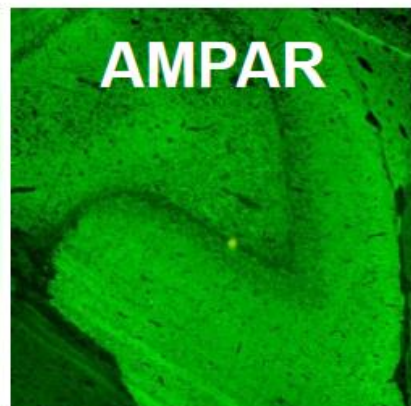


HIPPOCAMPUS

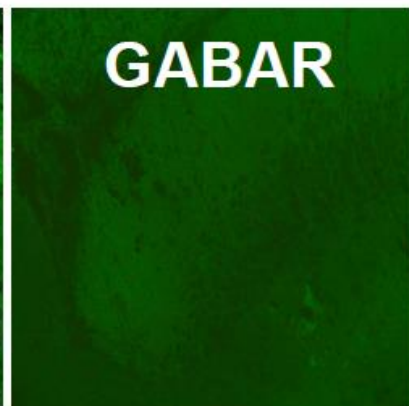
NMDAR



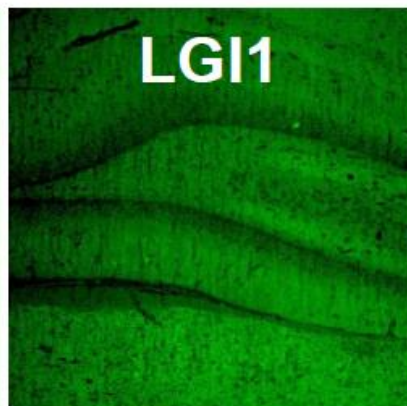
AMPA



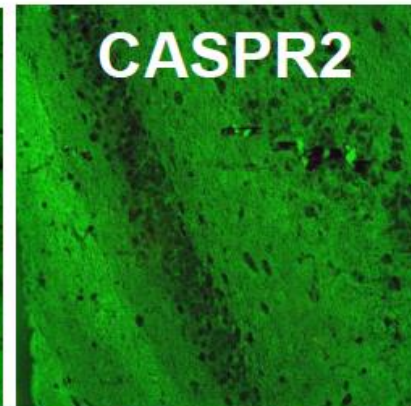
GABAR



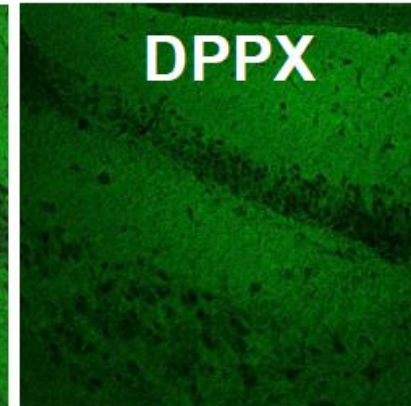
LGI1



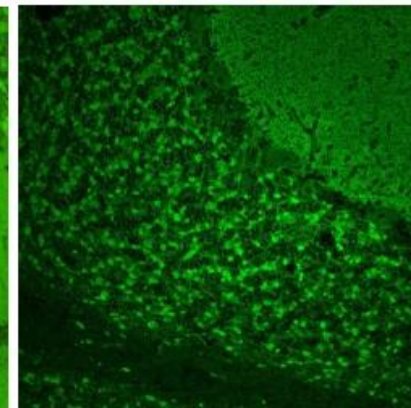
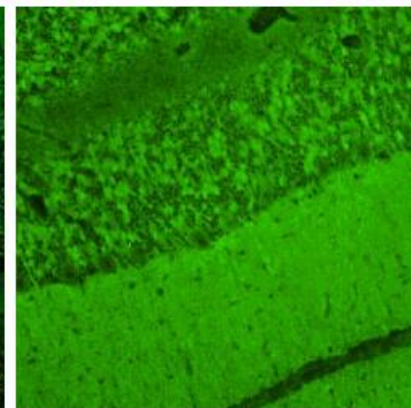
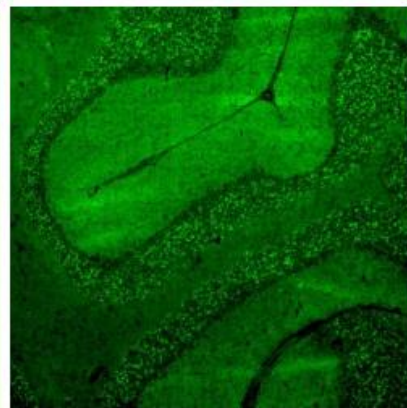
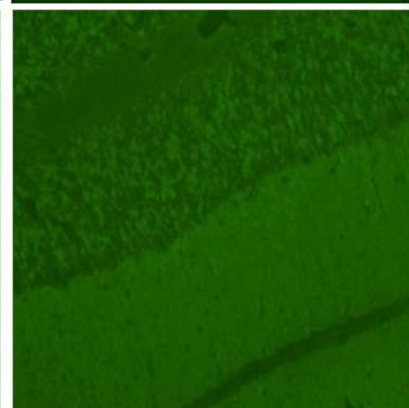
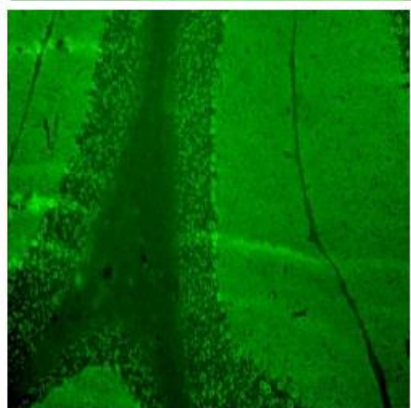
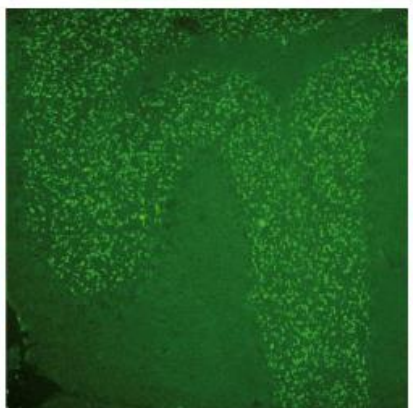
CASPR2



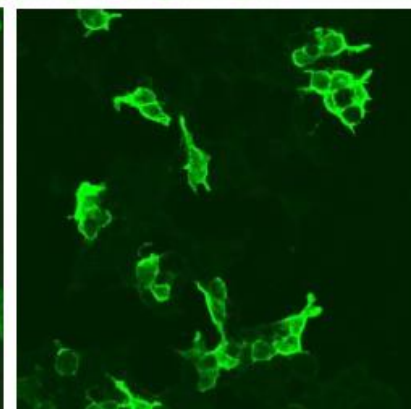
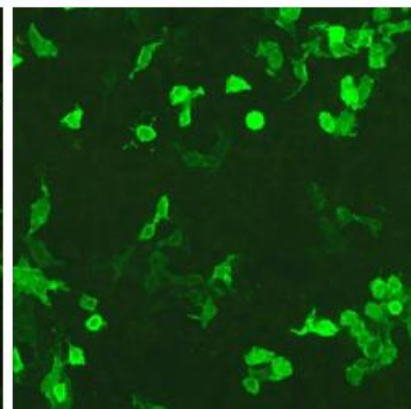
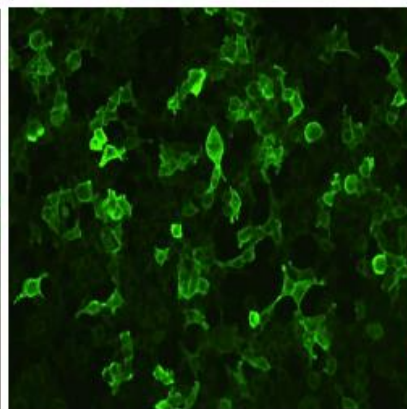
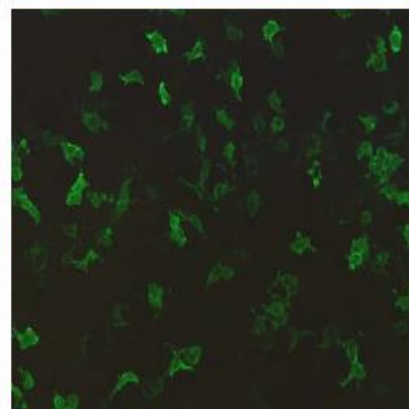
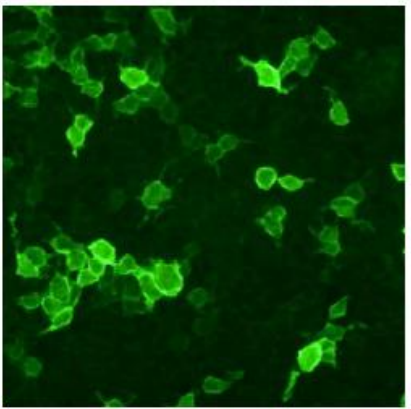
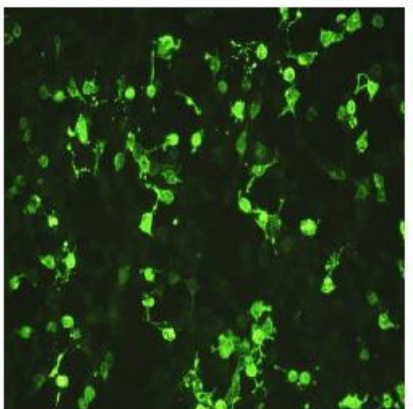
DPPX



CEREBELLUM



HEK CELLS



Approcci diagnostici: vantaggi e svantaggi

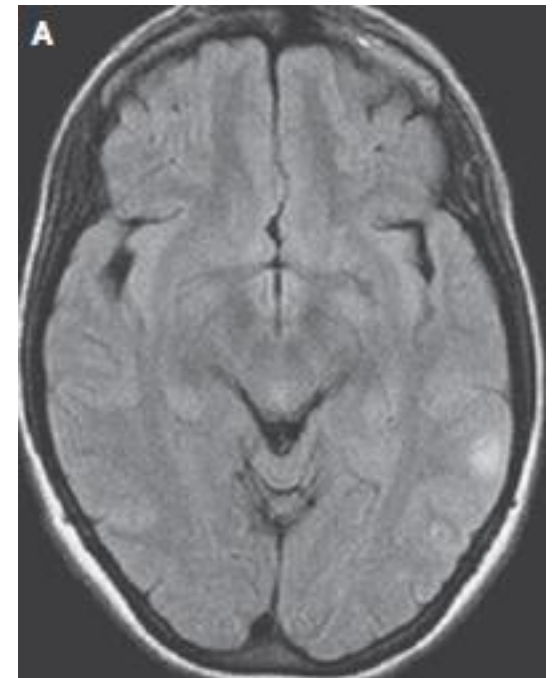
| Clues | Advantages | Disadvantages |
|---|---|---|
| Syndrome-based criteria | <ul style="list-style-type: none">• Enable early immunotherapy• Useful for inclusion criteria of clinical studies | <ul style="list-style-type: none">• Incorrect• Not helpful for differential diagnosis• Can lead to over-immunotherapy |
| Response to immunotherapy | <ul style="list-style-type: none">• Useful in retrospective case analysis | <ul style="list-style-type: none">• Many patients with AE do not respond to 1st-line immunotherapy• Not useful for initial decision |
| Clinicians' "gut feeling" based on clinical course, MRI, and CSF test | <ul style="list-style-type: none">• Case-specific approaches | <ul style="list-style-type: none">• Requires experience• Incorrect |
| Antibody test | <ul style="list-style-type: none">• Confirmatory• Determines comorbidities, tumors, long-term treatment, and prognosis | <ul style="list-style-type: none">• Half of AE cases are antibody negative• Time and availability for antibody tests• False positive or asymptomatic antibodies |

Sindromi cliniche

- Aspetti RMN e CSF simili alle encefaliti virali
- Evoluzione acuta/subacuta (giorni-settimane)
 - Decorso subdolo in pazienti con anti CASPR2, LGI1, DPPX
- 60% prodromi aspecifici (febbre, malessere, cefalea)
- Alcuni elementi clinici presentano una certa specificità
 - Crisi distoniche facio-brachiali e episodi vertiginosi acuti in pazienti con anti-LGI1
 - Diarrea severa e calo ponderale iniziali nei pazienti con anti-DPPX

Encefalite da anti-NMDA-R

- Bambini e giovani adulti
- F:M=4:1
- Teratoma ovarico nel 60% dei casi
- Sintomi psichiatrici, allucinazioni, idee deliranti, fenomeni catatonici -> disturbi cognitivi
- Instabilità autonoma, ipoventilazione
- Ricerca anti-NMDAR su CSF (siero meno attendibile, FN)
- RMN alterata fino al 30% dei casi (polimorfa)



Encefalite limbica

- Diverse specificità antigeniche
- Confusione, disturbi comportamentali, crisi comiziali, deficit cognitivi
- Generalmente alterazioni RMN bilaterali
- Se unilaterali...
 - Crisi subentranti
 - Encefalite da HSV-1
 - Neoplasie gliali
- Anti LGI1
 - Iponatremia nel 65% dei casi
- Anti GABA-B-R, Anti AMPA-R
 - Neoplasie nel 50-60% dei casi
- Anti Hu, anti Ma2, anti GAD65

Situazioni particolari...

- Stato di male refrattario
 - Anti GABA-A-R
- Encefalopatia, insonnia disautonomia, ipereccitabilità periferica, dolore neuropatico (sindrome di Morvan)
 - Anti CASPR2
- Mioclono, tremori, ipereklesia
 - Anti DPPX

Red flags

- Generalmente vi è una lieve pleiocitosi liquorale (<100 cell/mmc)
 - ... ma una conta cellulare normale non esclude la diagnosi
- Le encefaliti da anti LGI1 e anti DPPX sono quelle che più frequentemente non si associano ad alterazioni RMN e liquorali
- Solo in circa metà dei pazienti con encefaliti anticorpo-mediate, si riescono a dimostrare autoanticorpi «specifici»
- Nelle encefaliti da anti LGI1 gli autoanticorpi possono essere presenti nel siero ma non nel liquor
- Trattare se vi è il sospetto... non aspettare il dato di laboratorio

The FOREVER PEOPLE!

THEY'RE FROM A PLACE THAT MEN
HAVE SOUGHT BUT NEVER FOUND--
WE'VE SEEN THEM LIKE BEFORE--
IN DIFFERENT AGES-- IN DIFFERENT
GUISES-- BUT NEVER LIKE THIS--
YET, ALWAYS LIKE THIS-- WHEN
MAN'S CIVILIZATION FACES
DESTRUCTION...

IN SEARCH
OF A
DREAM!

EDITED, DRAWN
AND WRITTEN BY
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(OUR MAN IN THE
BOOM TUBE)
INKED BY
VINCE COLLETTA

GUEST-
STARRING
THE IMMORTAL
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