

# What the neurologist needs to know in choosing the right assay for autoantibody testing

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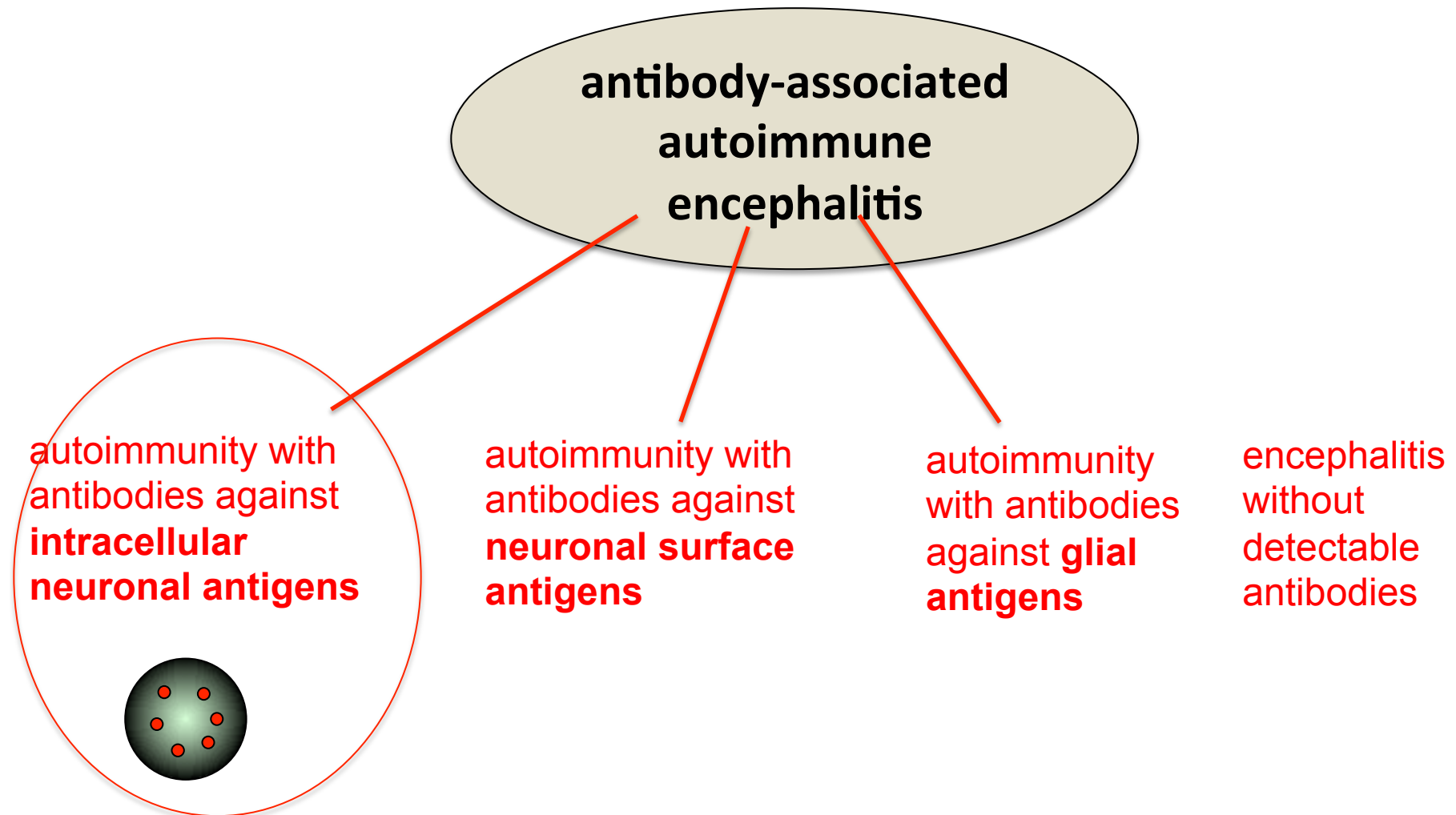
5th EAN Congress Oslo, June 29 – July 2, 2019  
Teaching Course 9: Antibodies: From autoimmune encephalitis to  
paraneoplastic myelopathies (Level 2)

## **Disclosures**

Received travel support from Novartis

Speaker's honoraria from Euroimmun, Novartis, Biogen and Sanofi-Aventis GmbH

# Classification autoimmune encephalitis

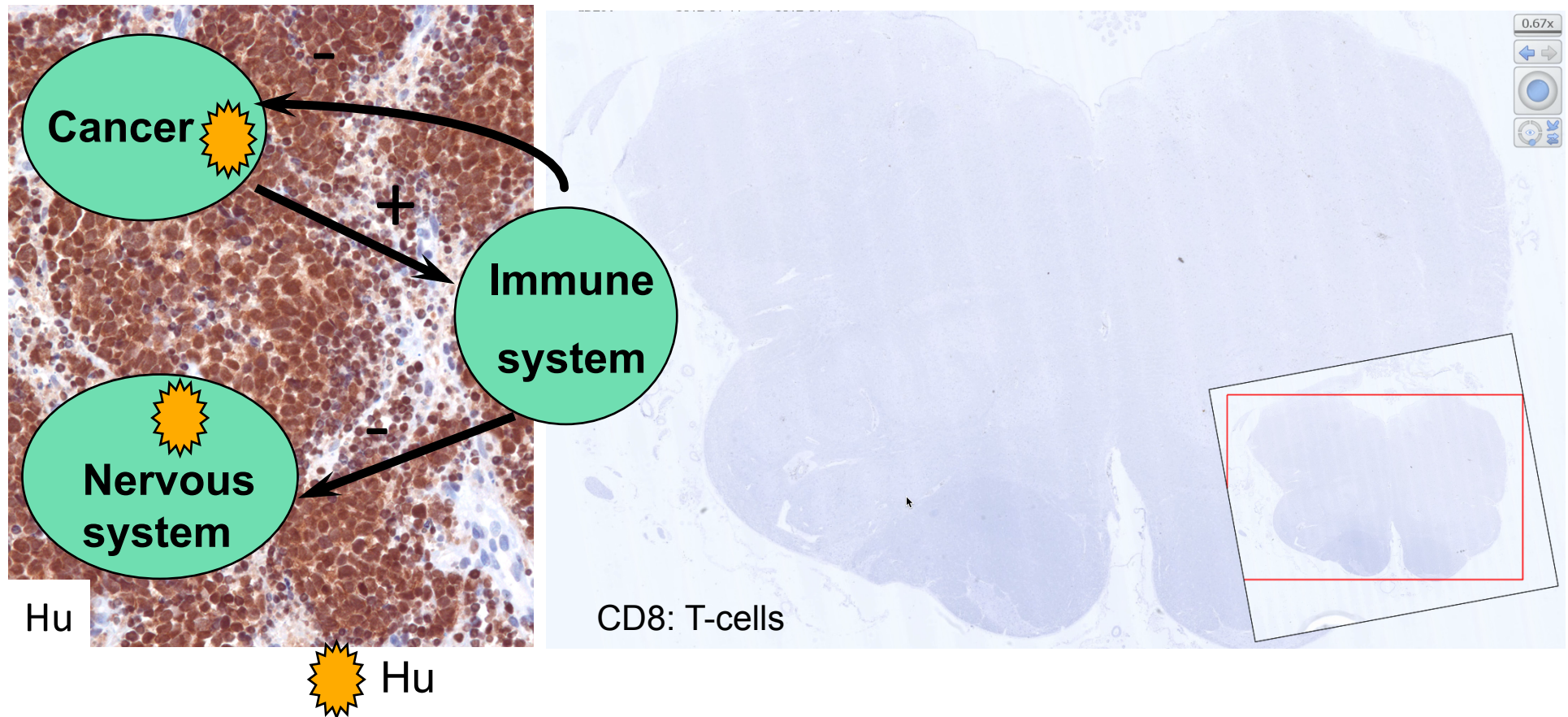


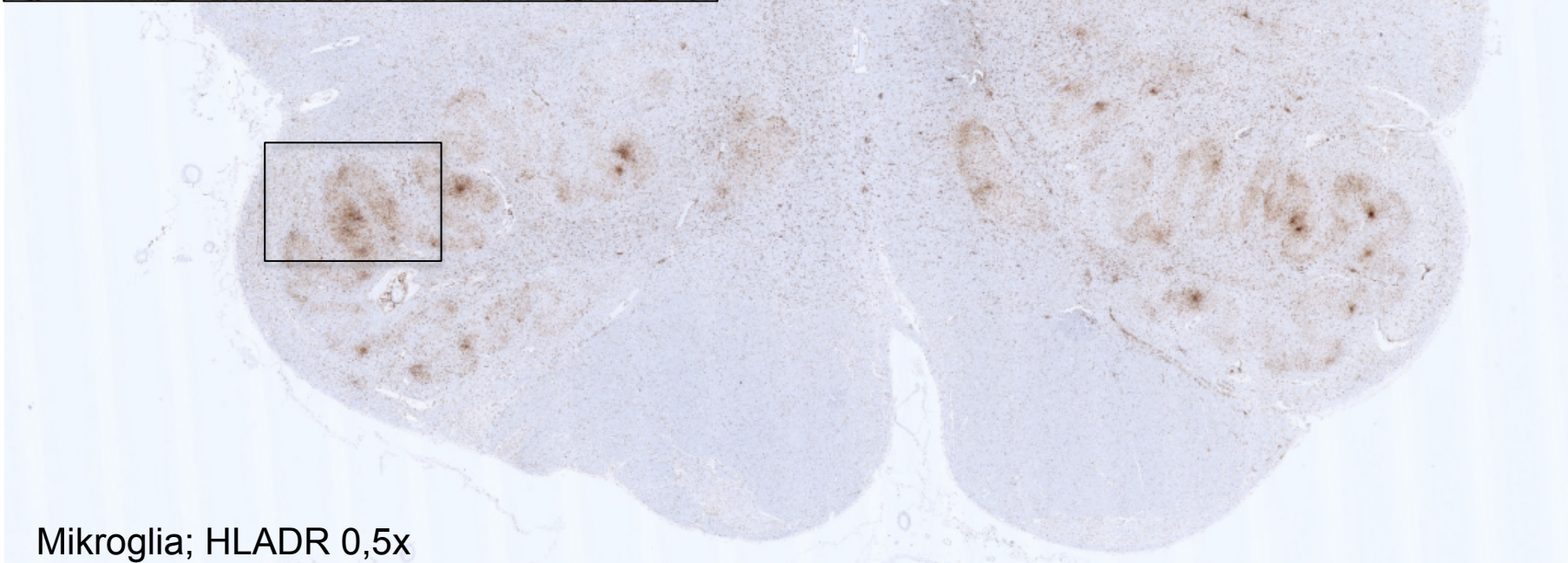
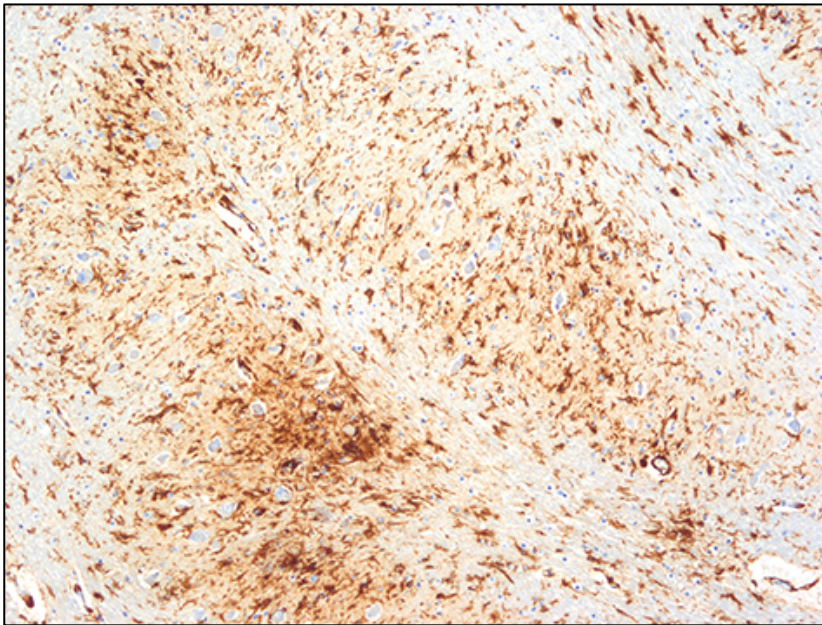
T cell mediated  
antibodies epiphenomenon  
paraneoplastic syndromes

# Pathogenetic mechanisms

SCLC

Medulla oblongata





Mikroglia; HLADR 0,5x

# Well-characterised onconeurological antibodies

68-year old female; breast cancer 5 yrs ago treated with surgery and chemotherapy; ataxia progressing over 6 mo, now wheelchair bound; no response to immunotherapy

Antibody	Associated tumor	Syndrome
Hu (ANNA1)	SCLC, others	encephalomyelitis, brainstem-encephalitis, LE, PCD, sensory neuropathy
Yo (PCA1)	gynecological tu, mammary	cerebellar degeneration
Ri (ANNA2)	mammary, SCLC	cerebellar ataxia, opsoclonus
Tr (DNER)	Hodgkin's lymphoma	cerebelläre Degeneration
CV2/ CRMP5	SCLC, thymoma, others	encephalomyelitis, uveitis, neuropathy
Ma-1/2	testicular germ cell tumors, others	limbic, diencephalon, brainstem-encephalitis
Amphiphysin	mammary, SCLC	stiff-person syndrome, encephalomyelitis

# Well-characterised onconeuroal antibodies

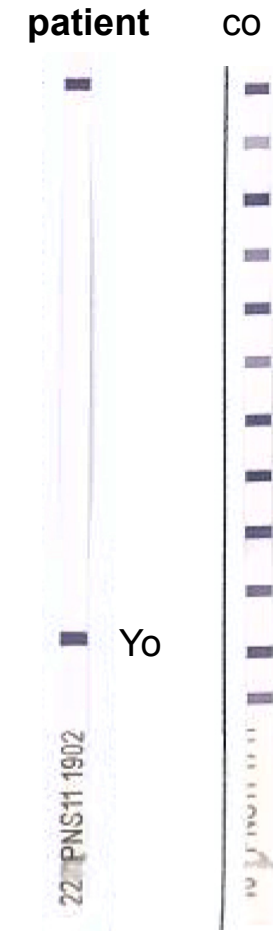
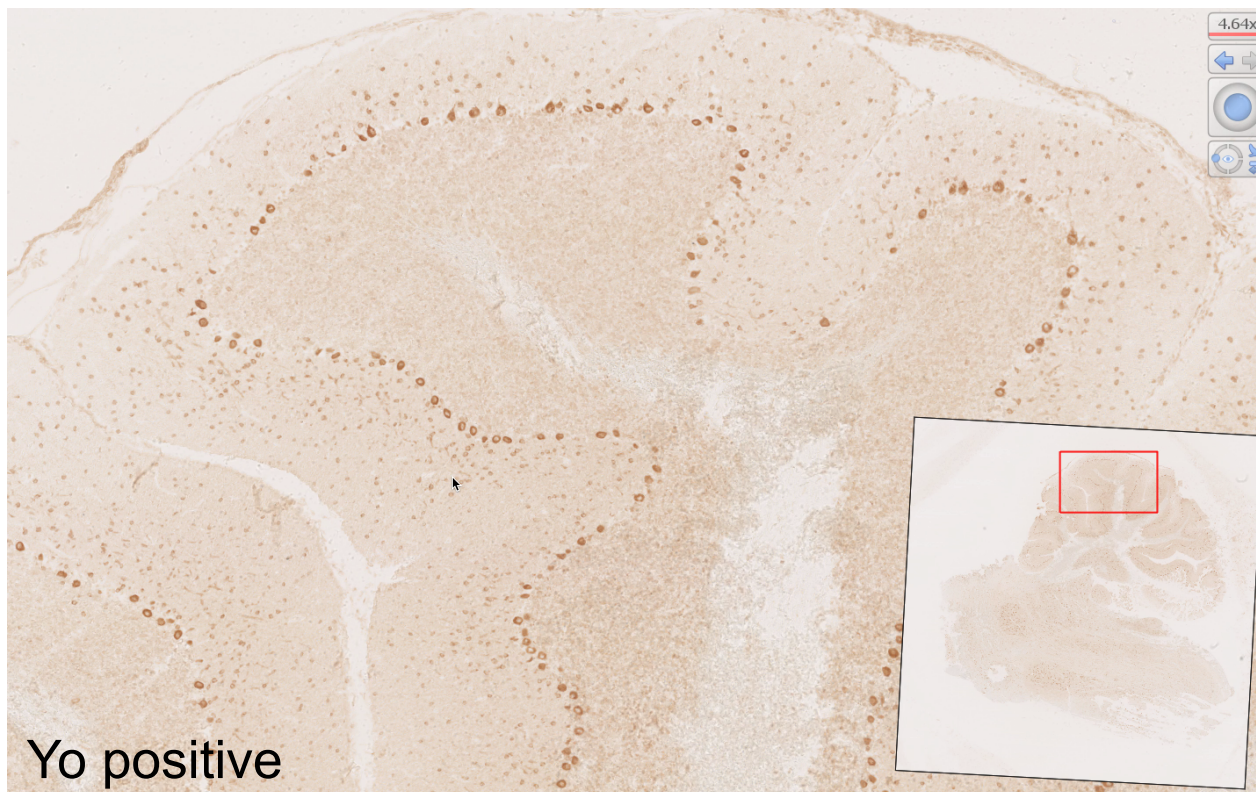
80-year old female; breast cancer in patient's history; now rigidity, stiff person syndrome

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# Diagnostic assay for intracellular/onconeuronal antibodies

Confirmation of  
specificity in Line Assay

Detection of a recognisable pattern in tissue based assay  
on fixed rat cerebellum



Yo-positive



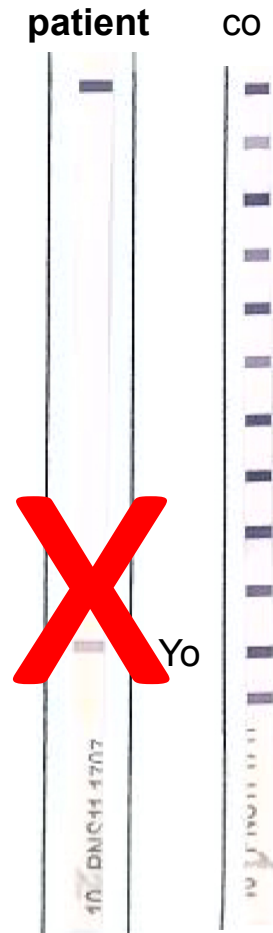
# Diagnostic assay for intracellular/onconeuronal antibodies

25-year old man with seizures



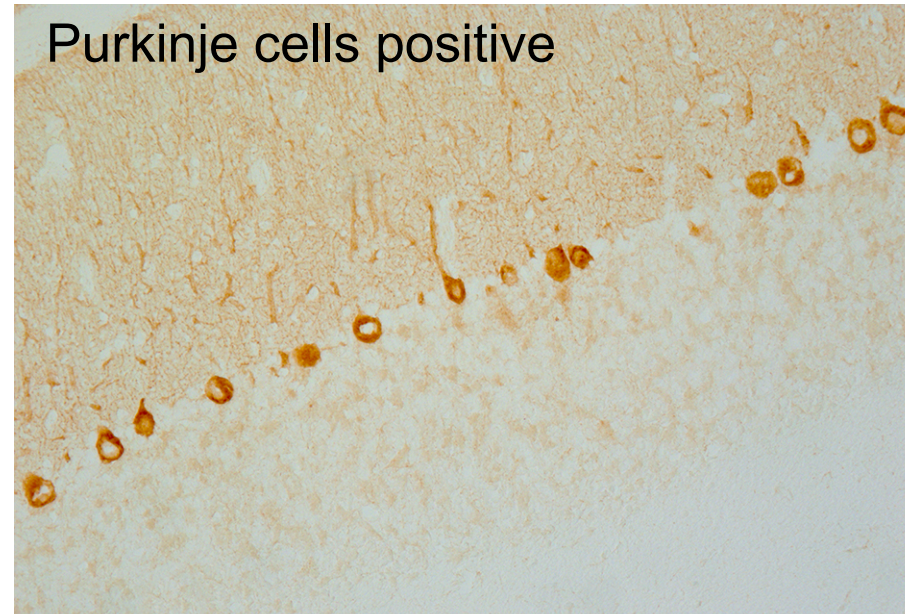
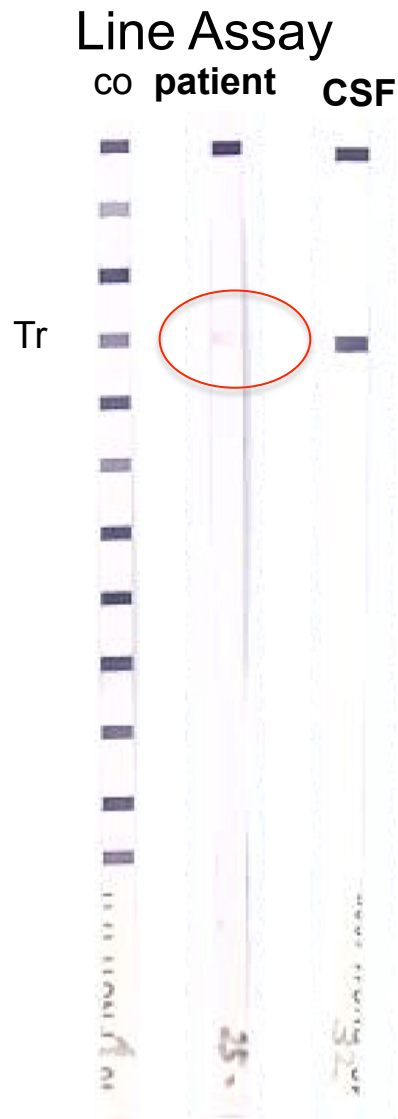
Negative

Line Assay

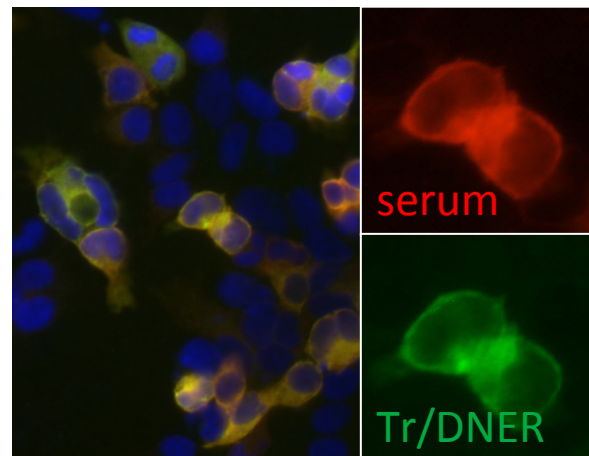


unspecific ?

50-year-old male patient with vertigo; several months later progressive ataxia, dysarthria, nystagmus



delta/notch-like epidermal growth factor-related receptor

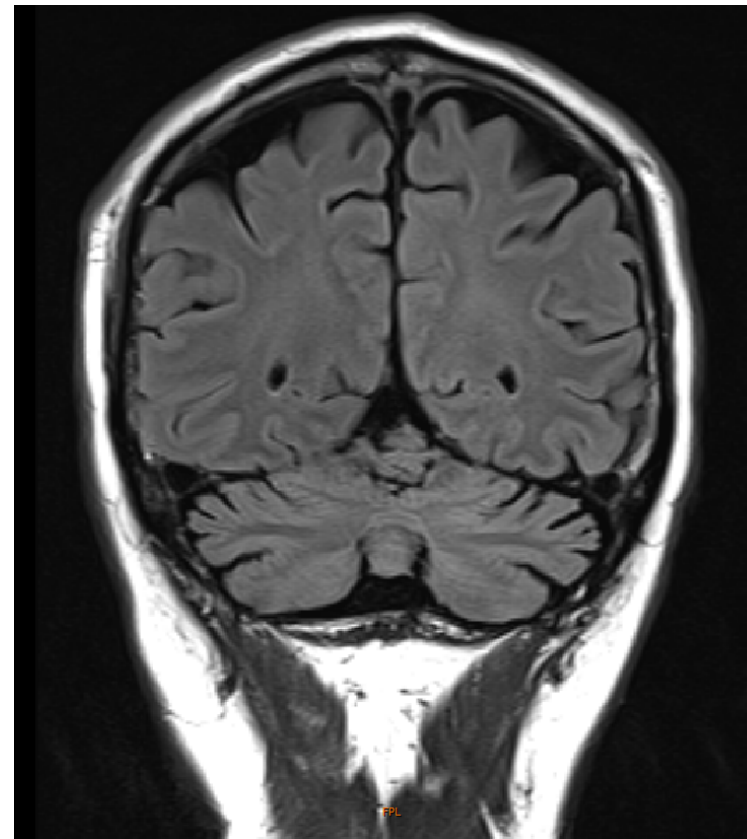
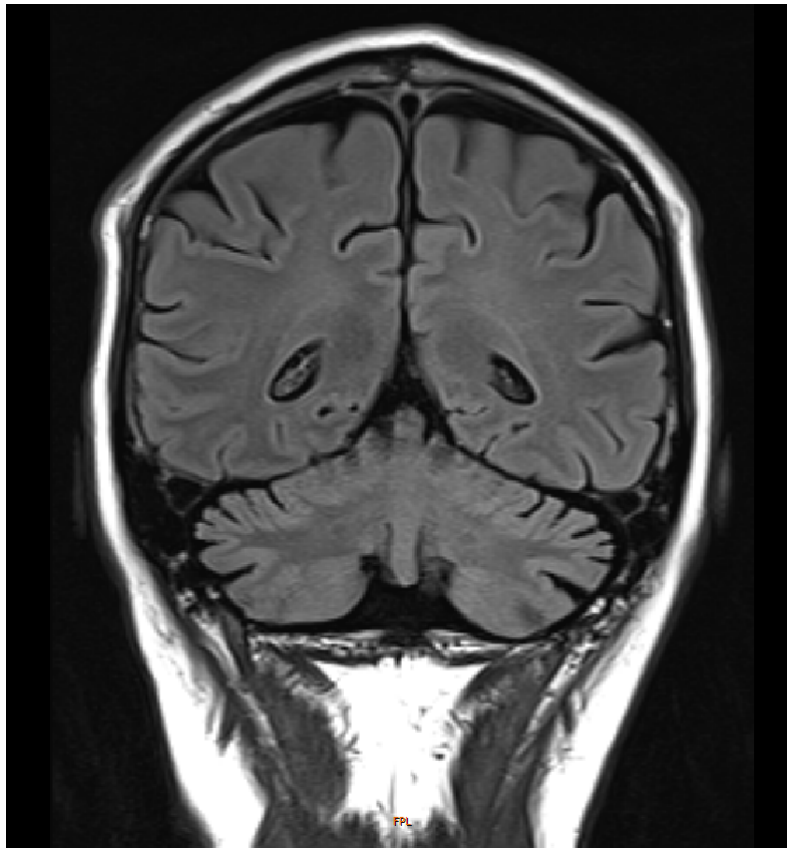


De Graaff et al, Ann Neurol 2012



FDG-PET  
Hodgkin lymphoma

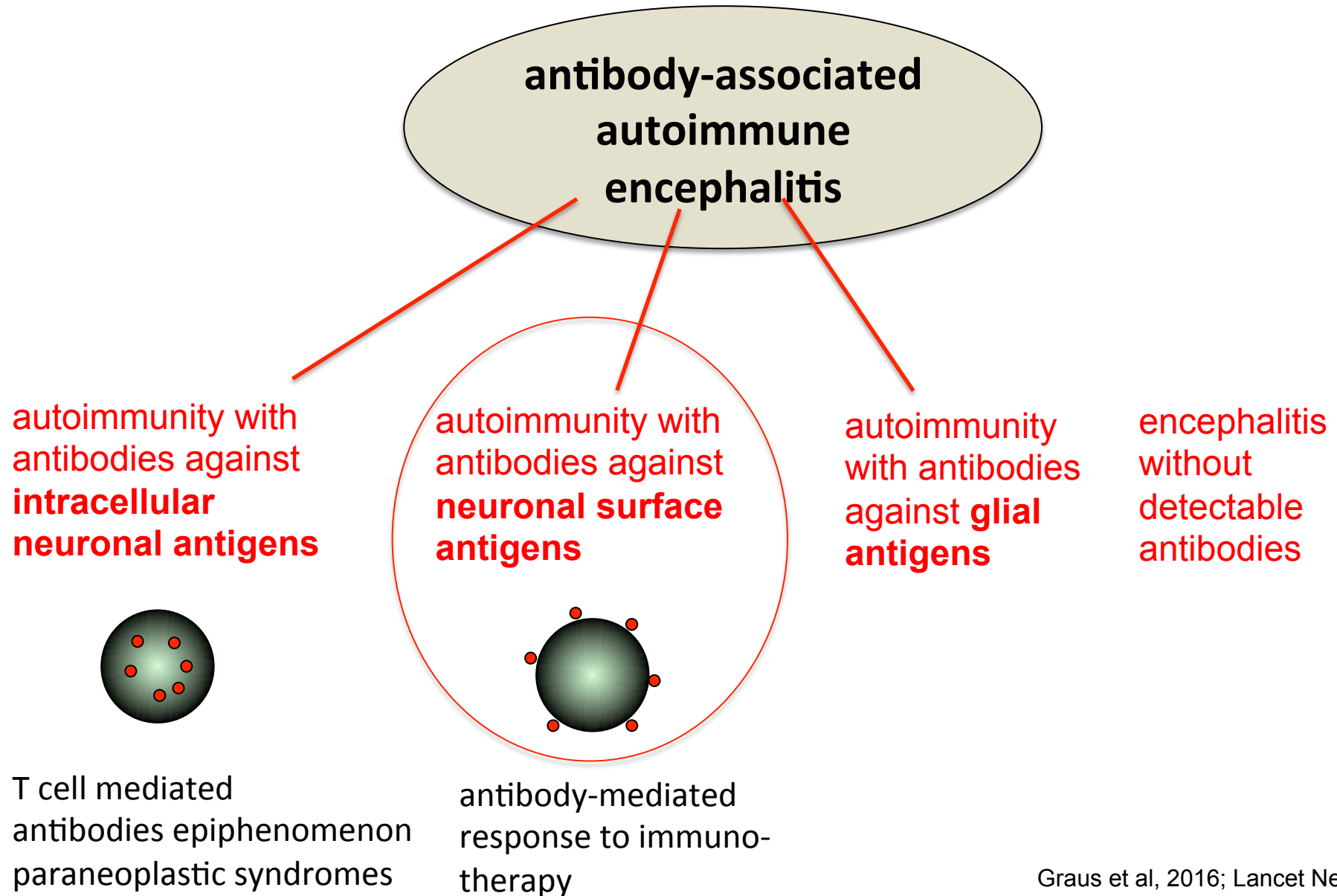
Despite treatment of Hodgkin lymphoma and immunotherapy, patient did not respond and has severe dysathria and ataxia; needs walker to move around



cerebellar atrophy in MRI

with courtesy of Dr. Hainberger and Dr. Lanzinger

# Classification autoimmune encephalitis

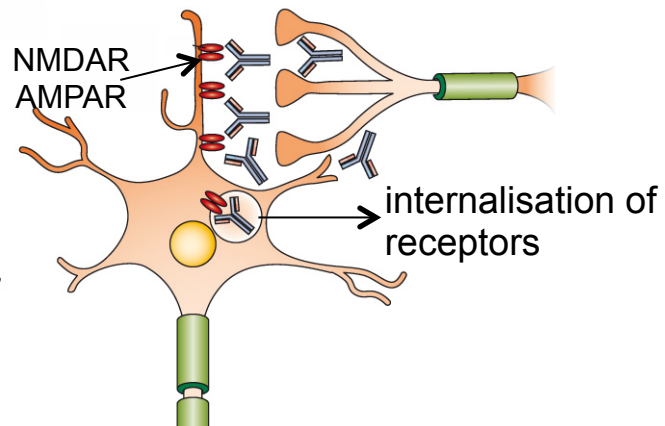


## Synaptic/surface protein autoimmunity

Antigen	Syndrome and main clinical characteristics
NMDAR	Anti-NMDAR encephalitis
AMPA	Limbic encephalitis, psychosis
DPPX (Kv4.2)	Hallucinations, myoclonus, tremor, seizures, diarrhea
GABA(A)R	Status epilepticus, refractory seizures
GlyR	Stiff person syndrome, PERM, OMS, optic neuritis
GABA (B) R	Limbic encephalitis with prominent seizures, status
mGluR1	Cerebellitis (+/- Hodgkin's Disease)
mGluR5	Limbic dysfunction, movement disorders; 55% paraneoplastic
Dopamin D2 Neuroexin 3 alpha	Basal ganglia encephalitis, Sydenham's chorea Seizures, orofacial dyskinesias
LGI1	Limbic encephalitis, faciobrachial dystonic seizure, hyponatremia
CASPR2	Encephalitis and/or neuromyotonia
IgLON5	"REM sleep behavior disorder", brainstem-limbic dysfunction
Neurofascin155	Atypical CIDP, tremor, ataxia
Contactin 1, CASPR1	Atypical CIDP, tremor

# Pathogenetic mechanisms

## NMDAR antibodies



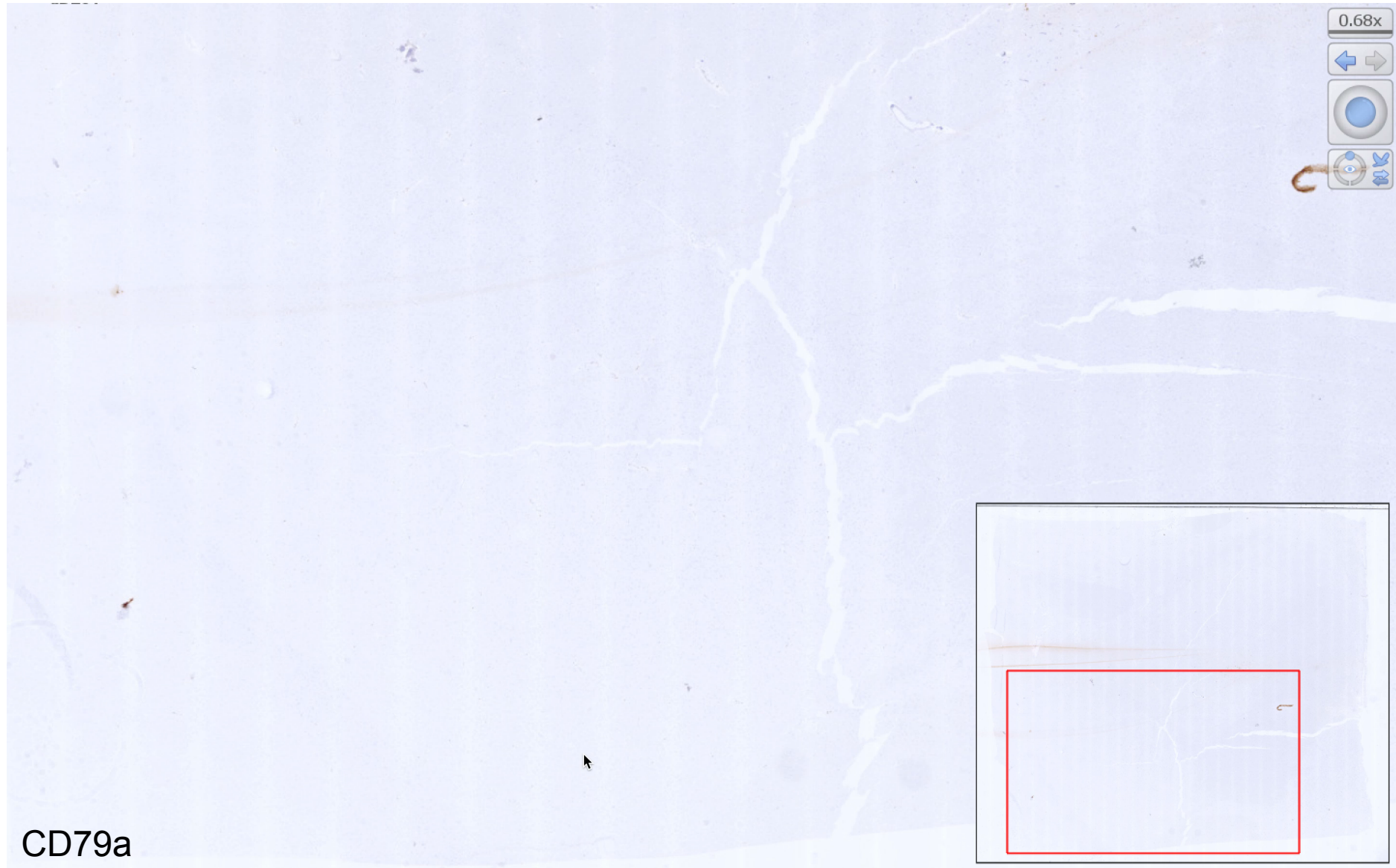
Antibodies of IgG1 subclass target an extracellular and **conformation-dependent** epitope

**Dysfunction of neurons**



**Response to immunotherapy**

# Neuropathology of anti-NMDAR encephalitis



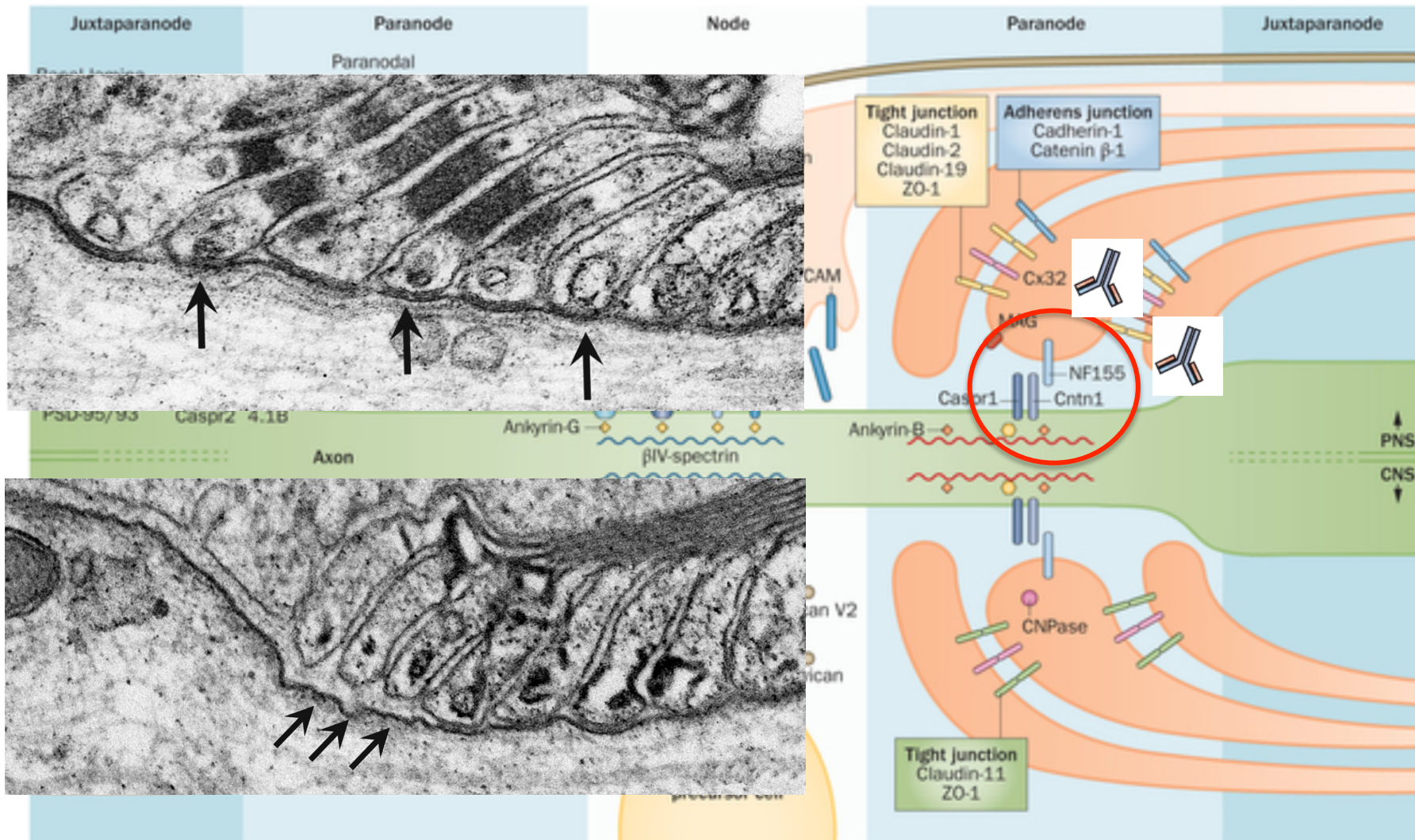
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## Synaptic/surface protein autoimmunity

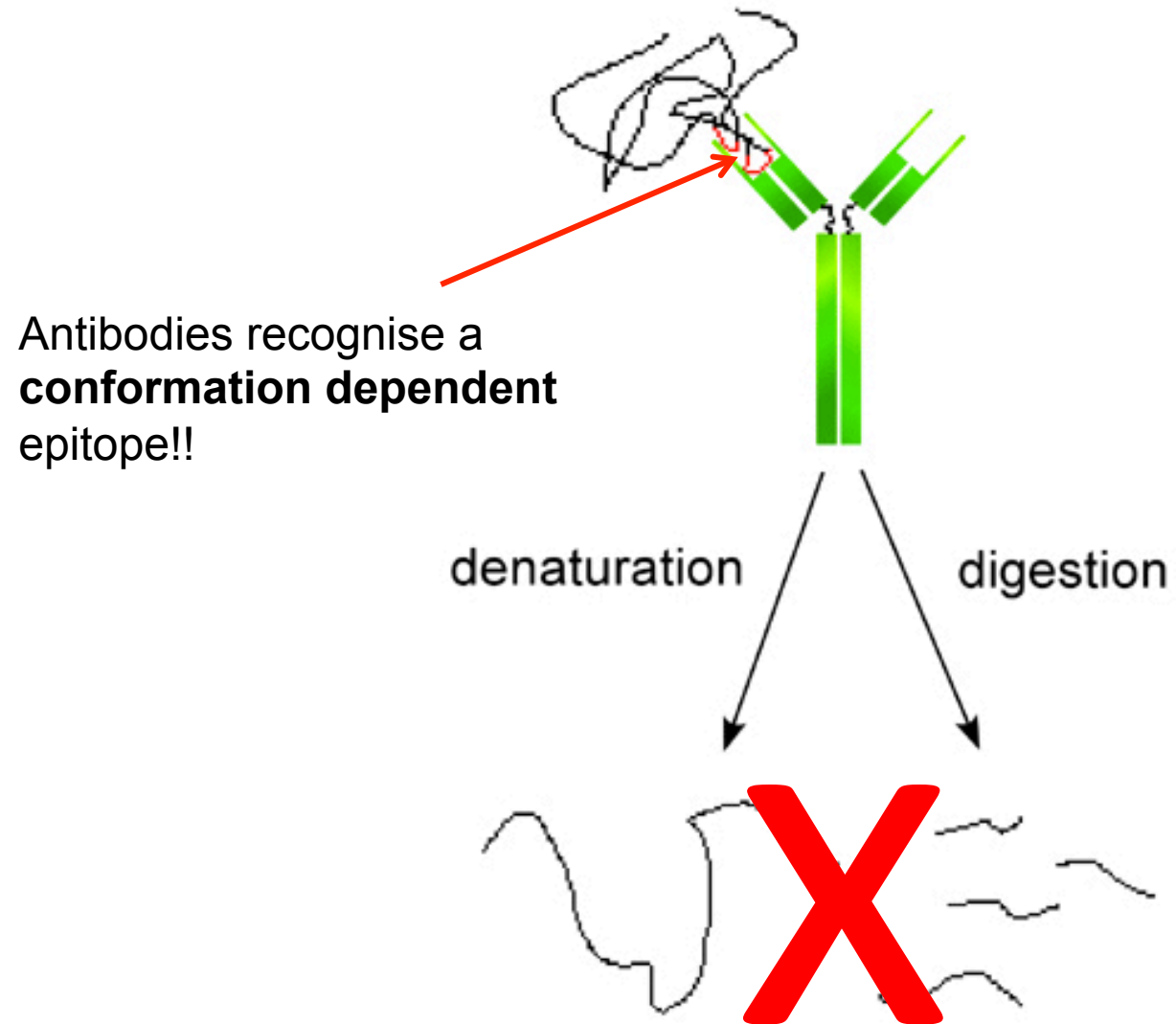
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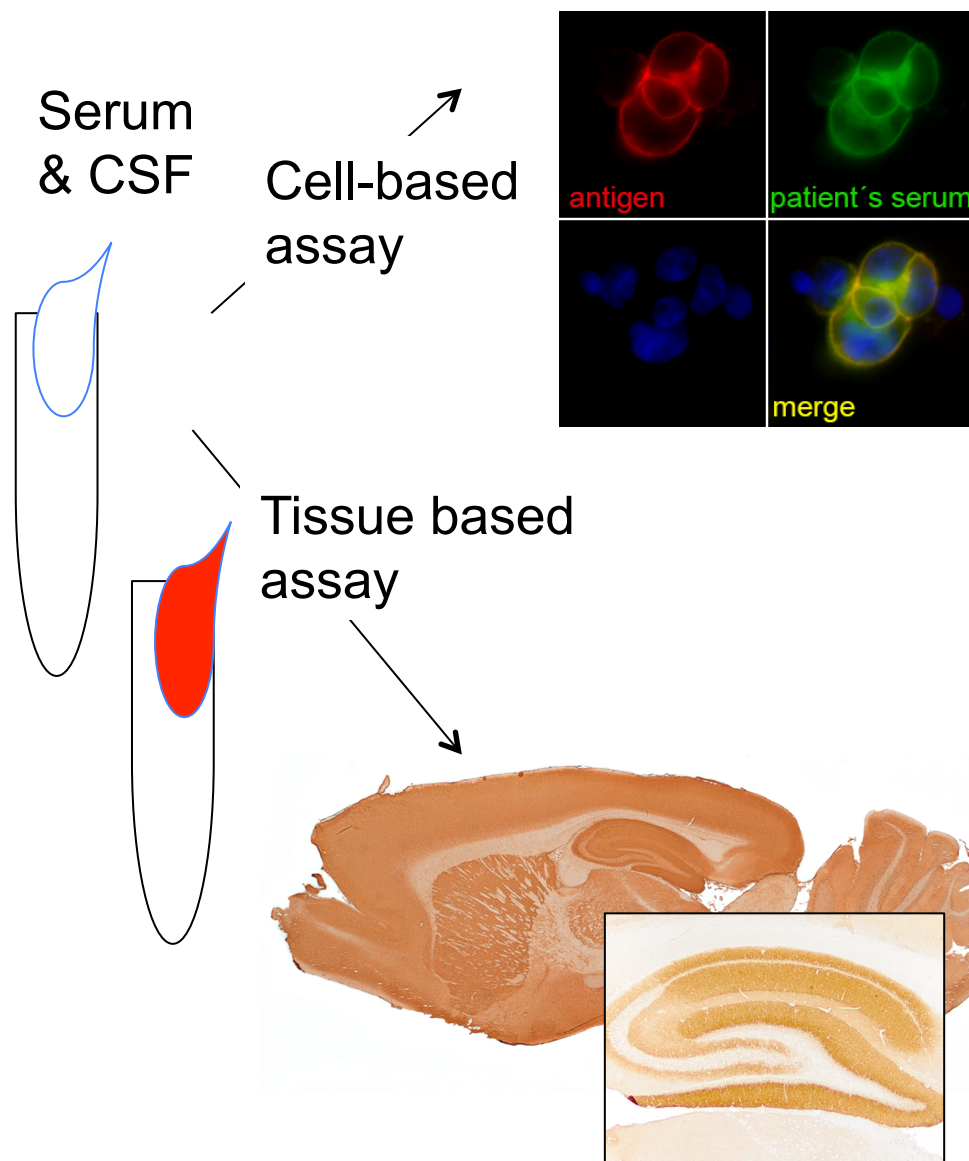
## Detachment of terminal myelin loops

Koike et al, JNNP 2016;

Stathopoulos et al et al, Nat. Rev. Neurol 2015



# Screening for neuronal surface antibodies



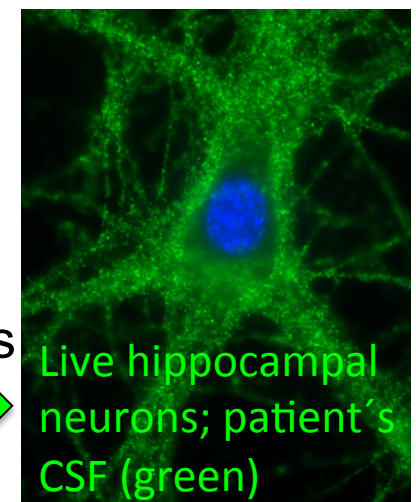
**Commercially available:**  
 NMDAR  
 AMPAR  
 GABA(B)R  
 LGI1, CASPR2  
 DPPX, IgLON5,  
 GlyR

**In-house:**  
 GABA(A)R,  
 mGluR5,  
 mGluR1,  
 neurexin3alpha

**disadvantage:** only antibodies that you ask for are tested

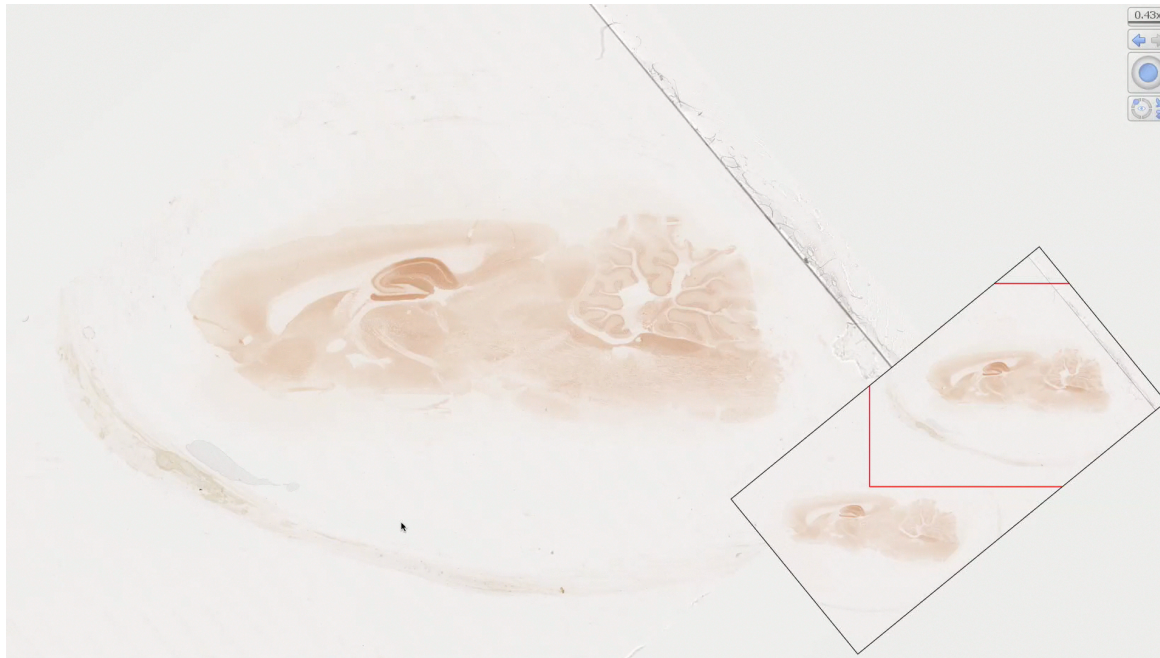
detection of most currently known surface antibodies

also new antibodies can be detected →

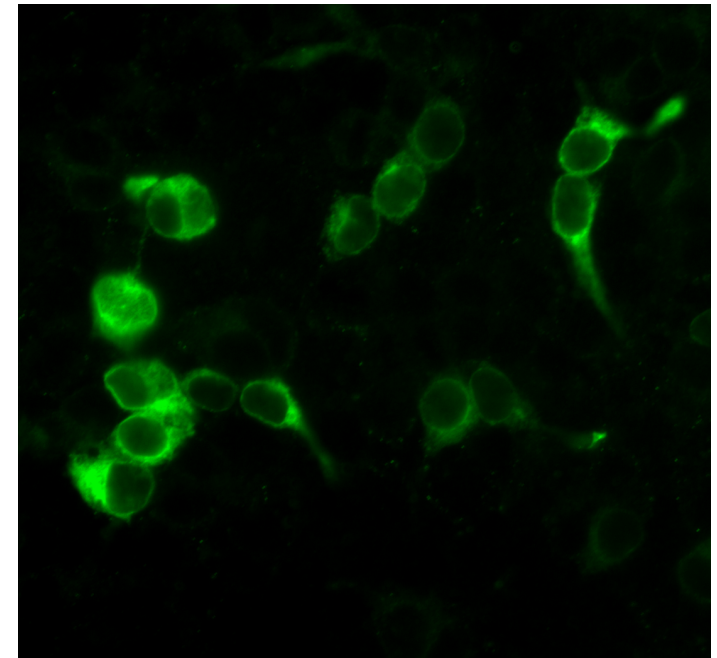


# Diagnostic assay for surface antibodies

Neuropil staining pattern in post-fixed tissue based assay on rat brain

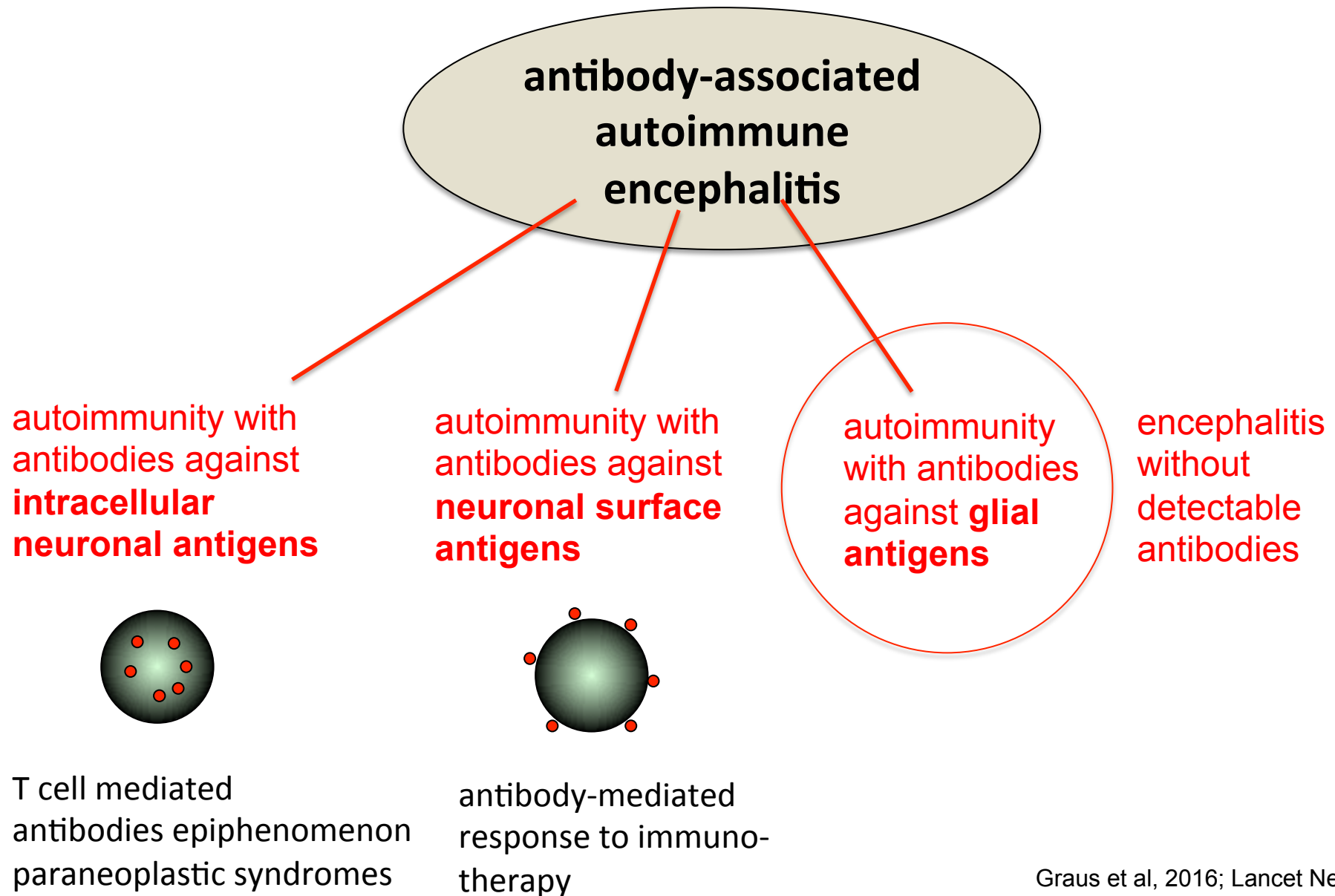


Confirmation of specificity in CBA



NMDAR positive

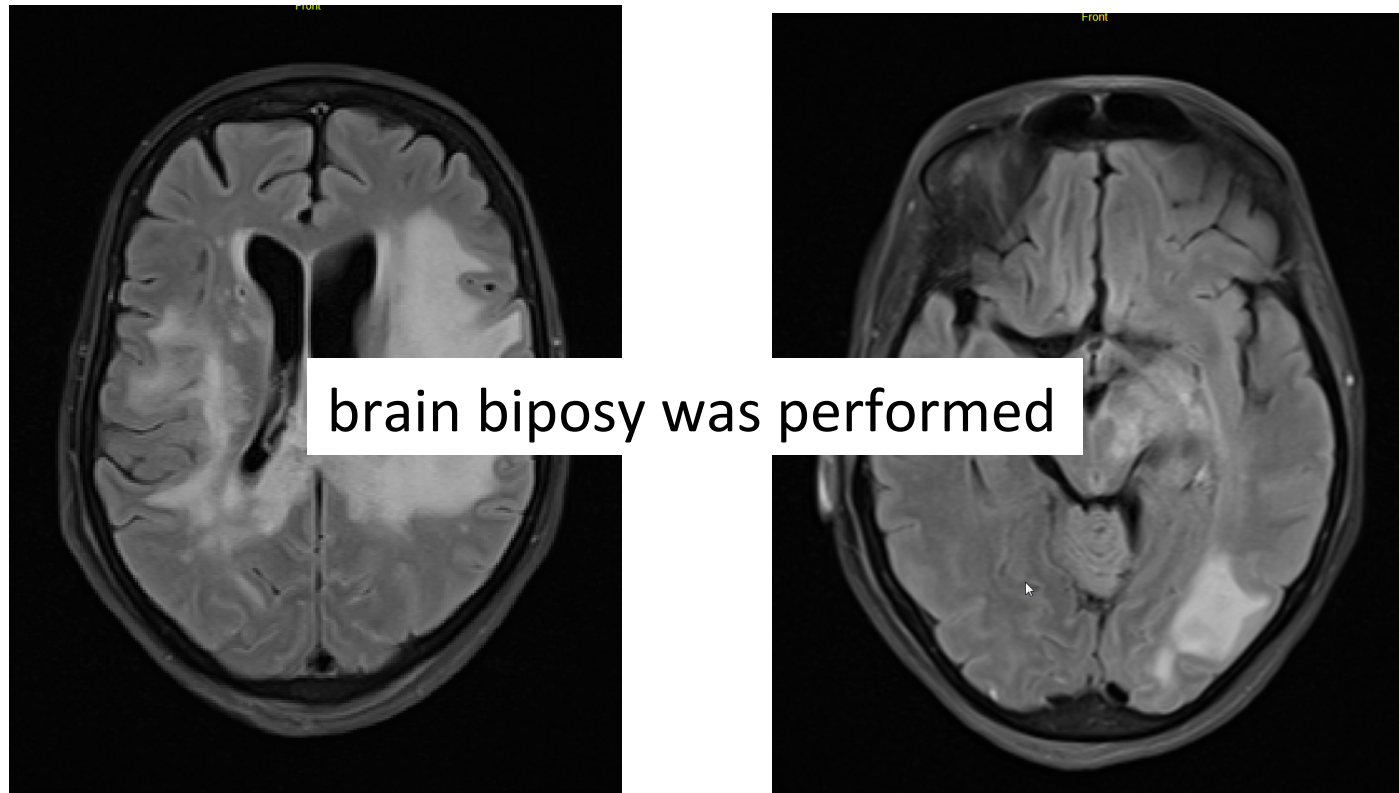
# Classification autoimmune encephalitis



# Case: 67-year old patient

Previous history: unremarkable

After pneumonia: headache, dysarthria, hemiparesis; on day of admission to hospital status epilepticus only stoppable with barbiturate



highdose steroid therapy, nevertheless rapid progression of symptoms

Suspicion on lymphoma

Antibody testing for MOG:  
**MOG-antibody positive**  
titer: serum 1:320

## **Diagnosis:**

**ADEM associated with MOG-antibodies**

patient did not respond to immunotherapy (steroids, PLEX, rituximab) and died due to multiorgan failure



# Autoimmunity associated with MOG-antibodies

Neuromyelitis optica spectrum disorder

clinically isolated syndrome / MS

MS rarely associated with MOG

atypical demyelination  
monophasic      recurrent

ADEM

multiphasic  
ADEM (MDEM)

optic neuritis

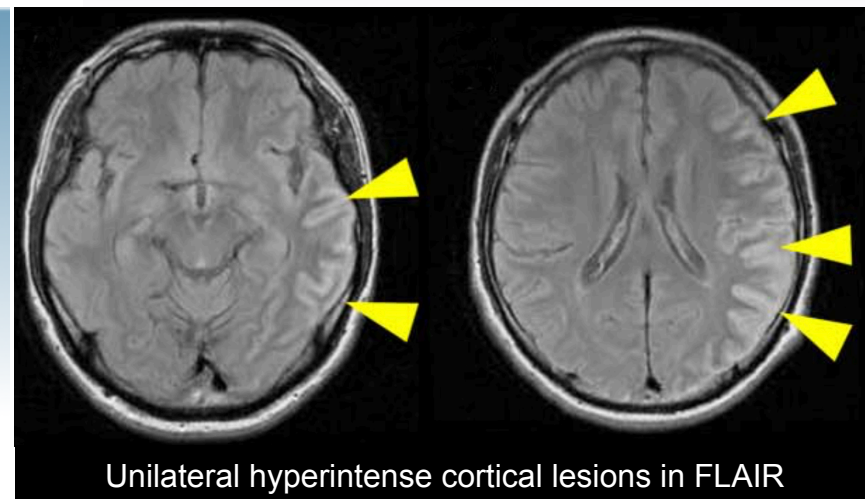
ADEM-ON

Transverse  
myelitis (TM)

recur. ON

recur. TM

Encephalitis with steroid-responsive seizures



Unilateral hyperintense cortical lesions in FLAIR

Ogawa et al, 2017

Rostasy et al, 2012; Kitley et al; 2013, Sato et al 2011; Höftberger et al, 2014; Baumann et al 2014; Reindl et al

## prominent cortical demyelination in MOG-spectrum disorder

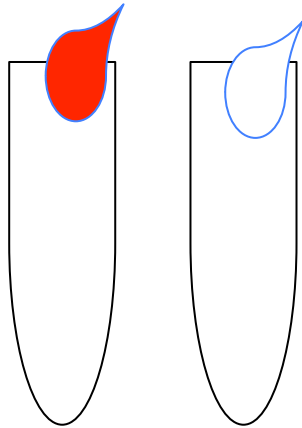
MOG; orange: cortical demyelination, green: white matter demyelination

### **CAVEAT:**

MOG-antibodies recognise a HUMAN SPECIFIC epitope

-> tissue based assay on rat brain is negative

# Antibody Screening



**CSF:** NMDAR, GABA(B)R, AMPAR

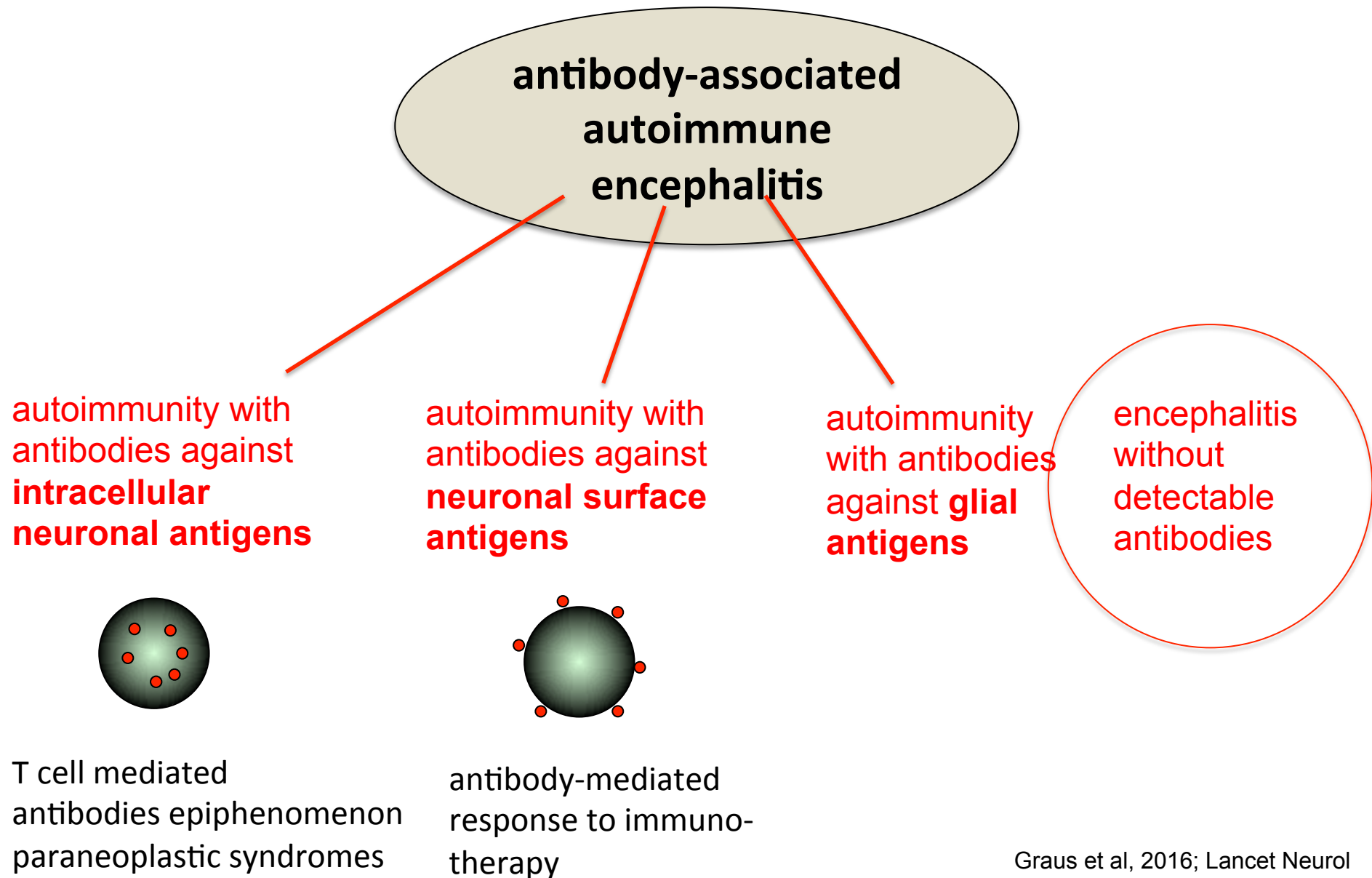
- 1 out of 7 patients would be missed
- higher risk for false positive results

**CSF and serum:** CASPR2, LGI1, GABA(A)R, GlyR

**Serum (CSF):** MOG, AQP4

To avoid false positive / negative results or delay in the diagnosis it is recommended to always test both, **serum AND CSF**

# Classification autoimmune encephalitis



## Syndroms that are often antibody negative

### Diseases of cerebellum and brainstem

- Acute cerebellitis → Few cases with mGluR1, GlyR;
- Opsoclonus myoclonus Syndrome → 11% intracellular (Ri), 11% surface ab  
(GlyR; HNK-1 IgM-antibodies  
(Human natural killer1); Armangue 2016

### Diseases of basal ganglia

- Tourette Syndrome → Few cases with D2R; Dale et al 2012
- PANDAS

Do our current test methods fail to detect  
the antibody?

### Other rare syndromes

- Narcolepsia → Other immunemechanisms than antibodies?  
Liblau et al Lancet Neurol 2015; Varadkar et al Lancet  
Neurology 2015
- Rasmussen Encephalitis

## Summary

1. Onconeuronal and surface antibodies are tested with different assays
2. In case of an unexpected positive/negative result discuss with laboratory and consider retesting or sending for second opinion
3. Interpretation of test results should be done in context with clinical presentation
4. Tissue based assay for surface antibodies can detect most of the currently known surface antibodies; some limitation for MOG (DD ADEM!) and glycin-receptor antibodies (PERM and related syndromes)
5. Indication of main clinical symptoms in the referral sheet will help the laboratory physicians to perform the right assay
6. Send serum AND CSF for testing!!

# Thank you for your attention

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