Pharmacology of immunosuppressants used in cats and dogs

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Virginia-Maryland College of Veterinary Medicine

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Outline

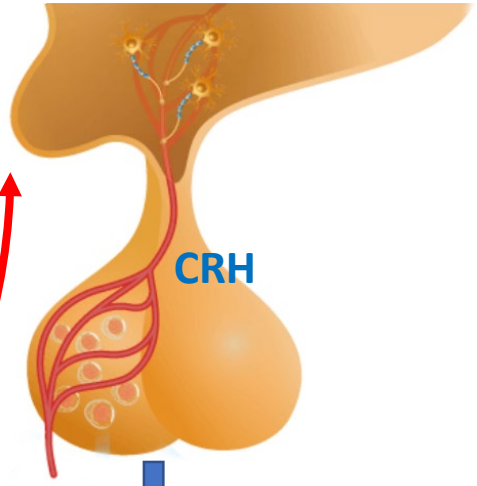
- Immunosuppressants:
  - Glucocorticoids
  - Cyclosporine
  - Azathioprine
  - Chlorambucil
  - Mycophenolate mofetil
  - Oclacitinib
  - Bruton's tyrosine kinase inhibitor
- **Mechanism of action**
- Autoimmune dermatological uses in cats and dogs
- Adverse effects

HPA axis and cortisol

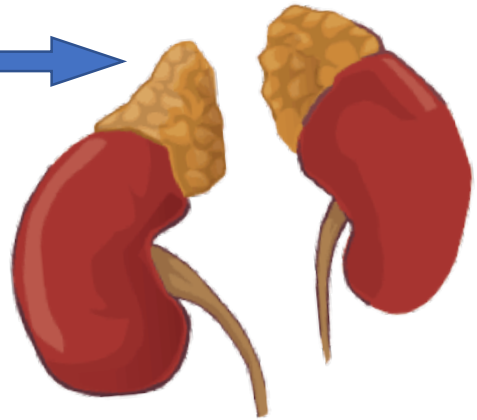
Circadian cycle

Inflammation

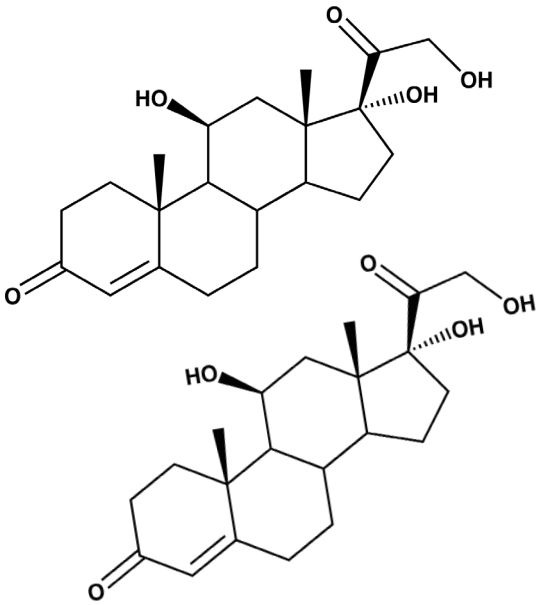
STRESS!!



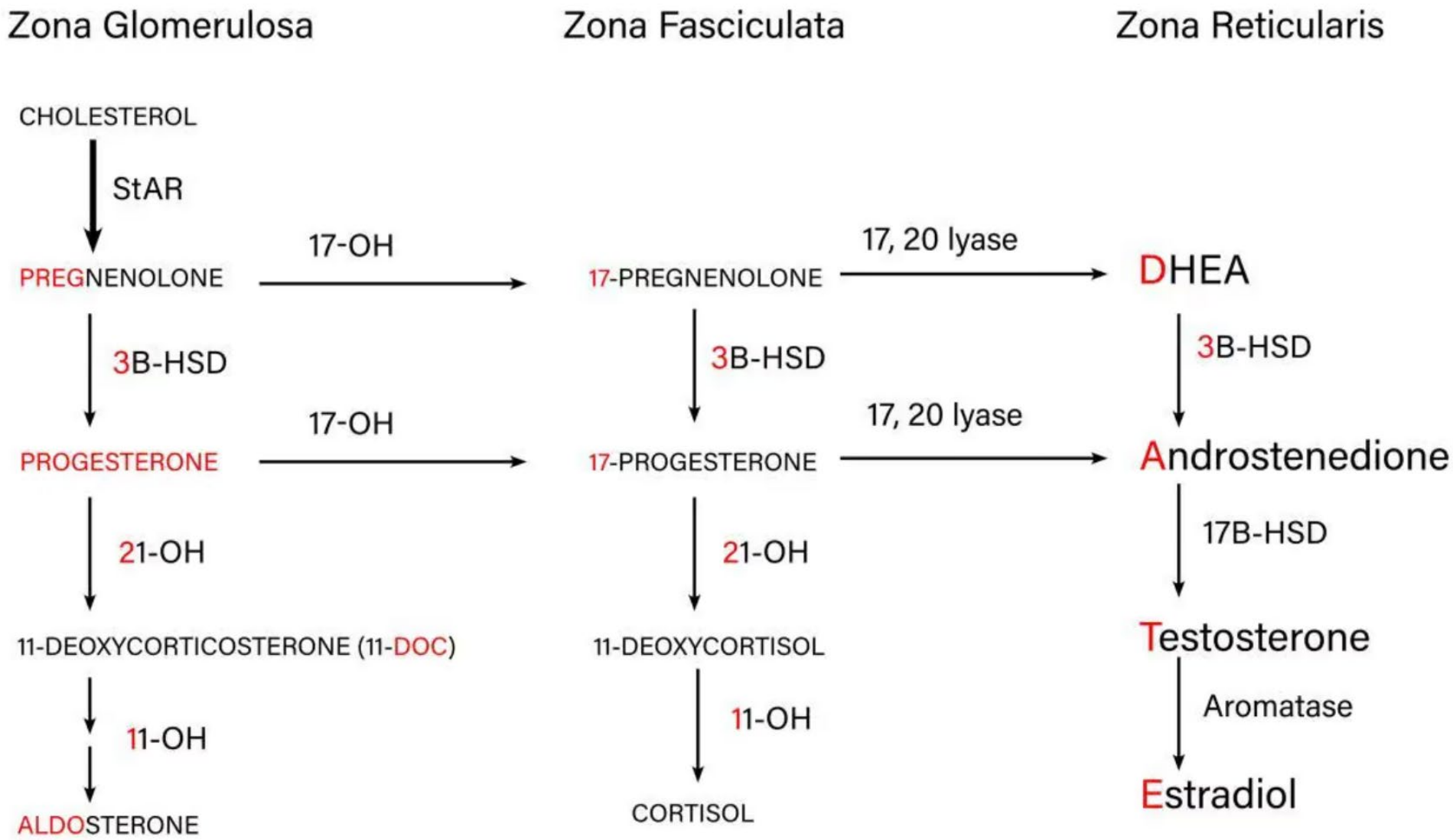
ACTH



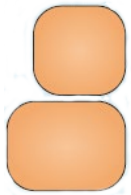
Cortisol



Steroid biosynthesis pathway

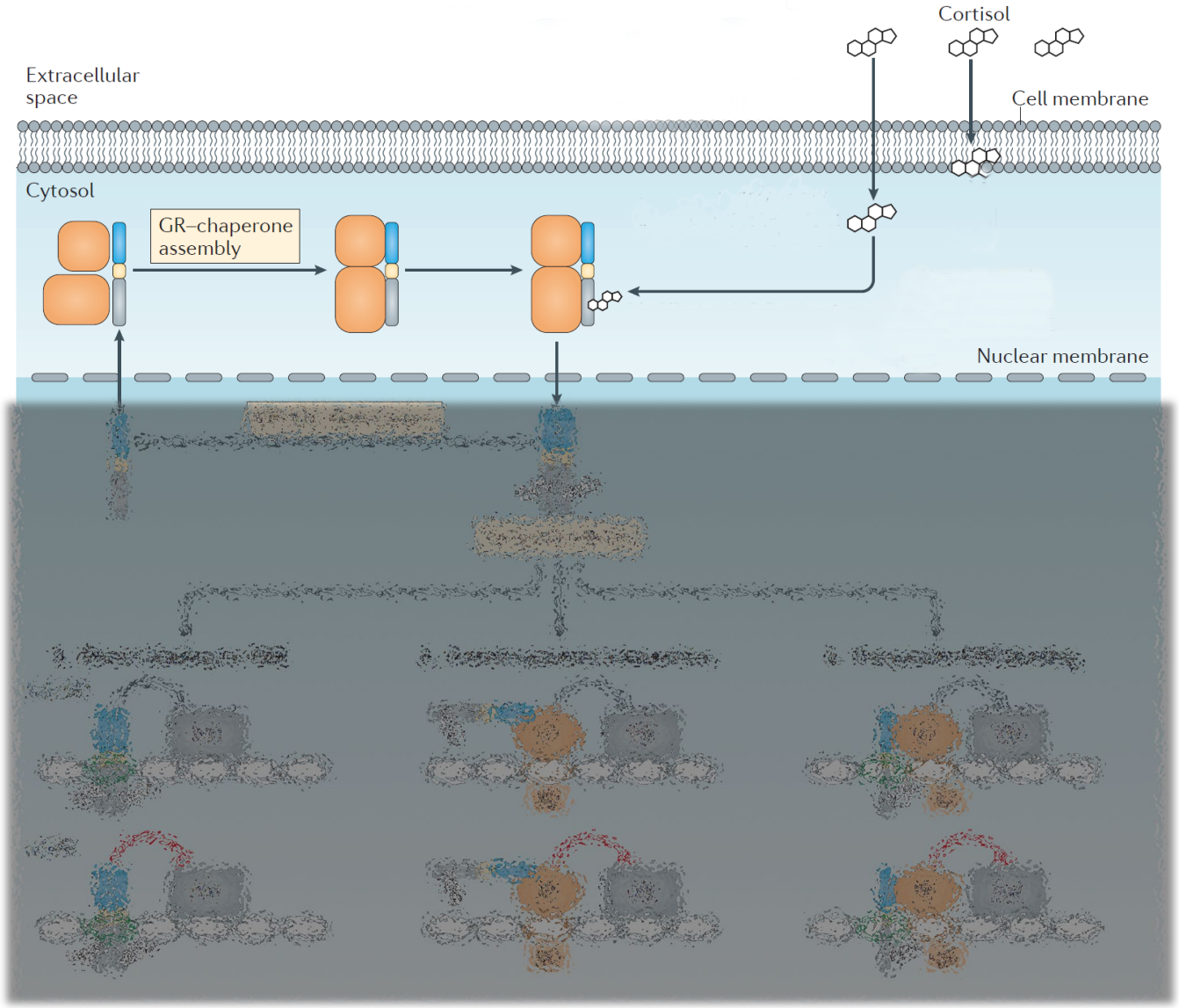


Glucocorticoids: Mechanism of action



Chaperone

GC receptor (GR)



(Modified from Cain, Nature Rev Immunol 2017)

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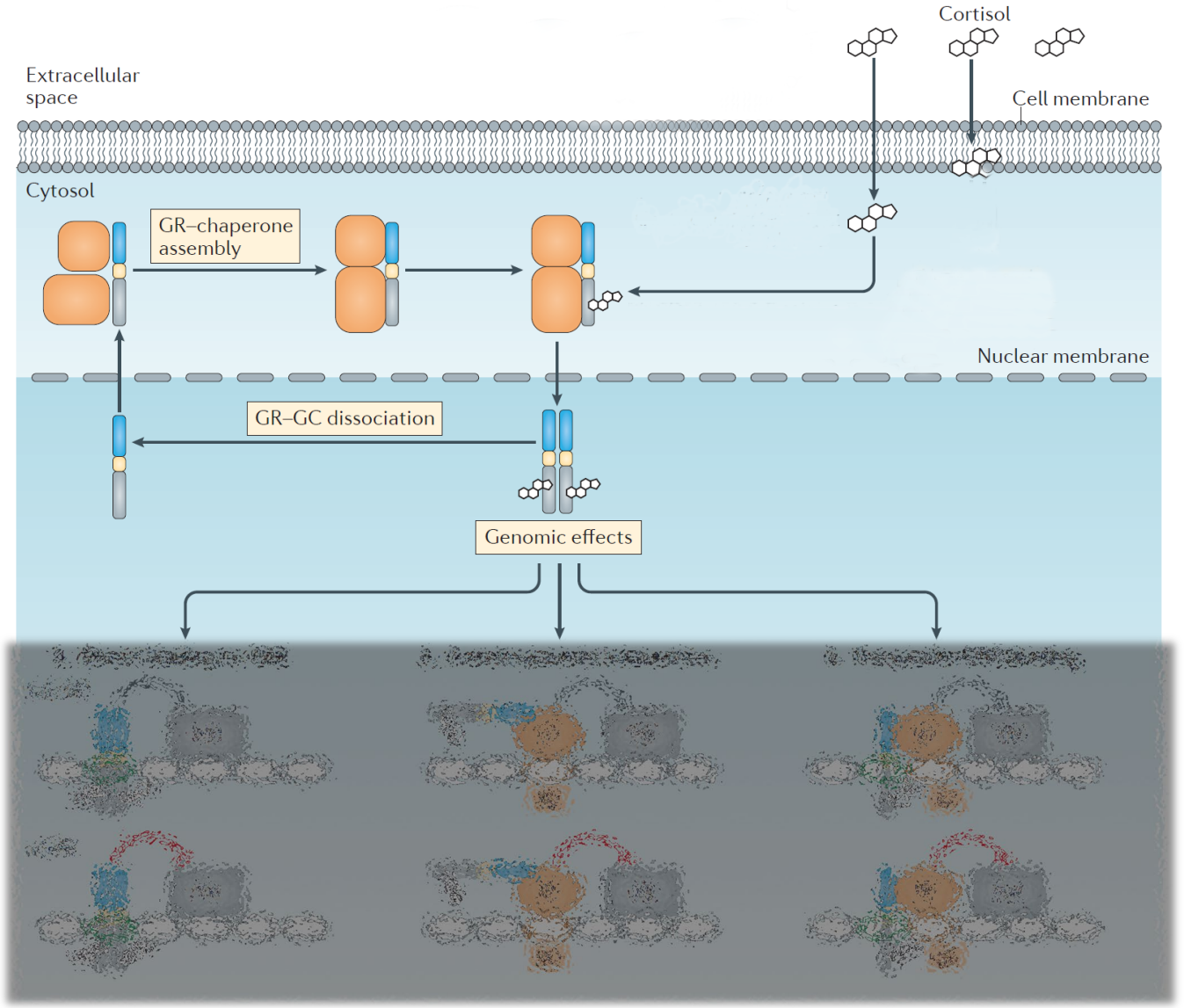

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Glucocorticoids: Mechanism of action

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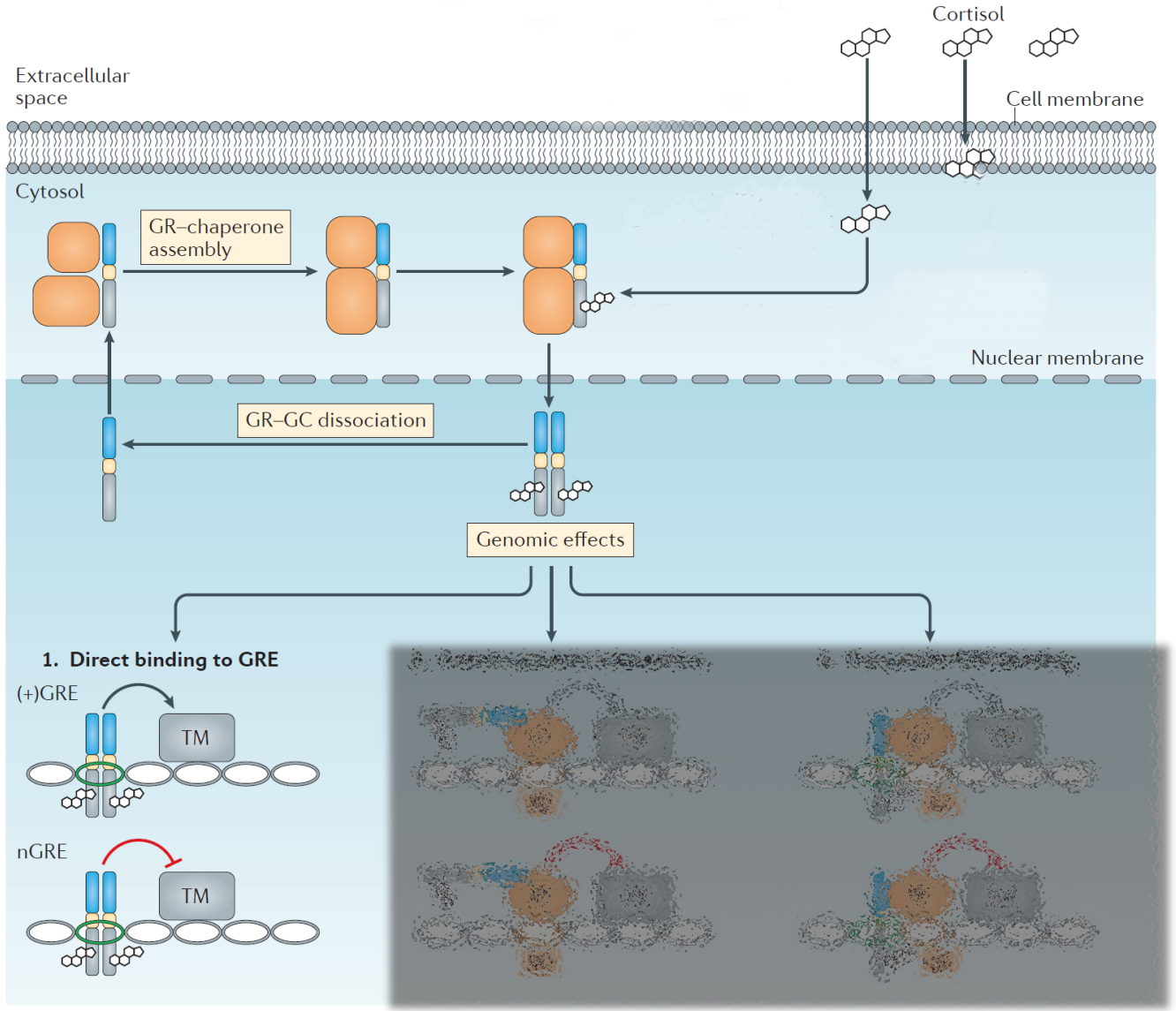

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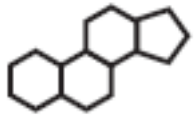
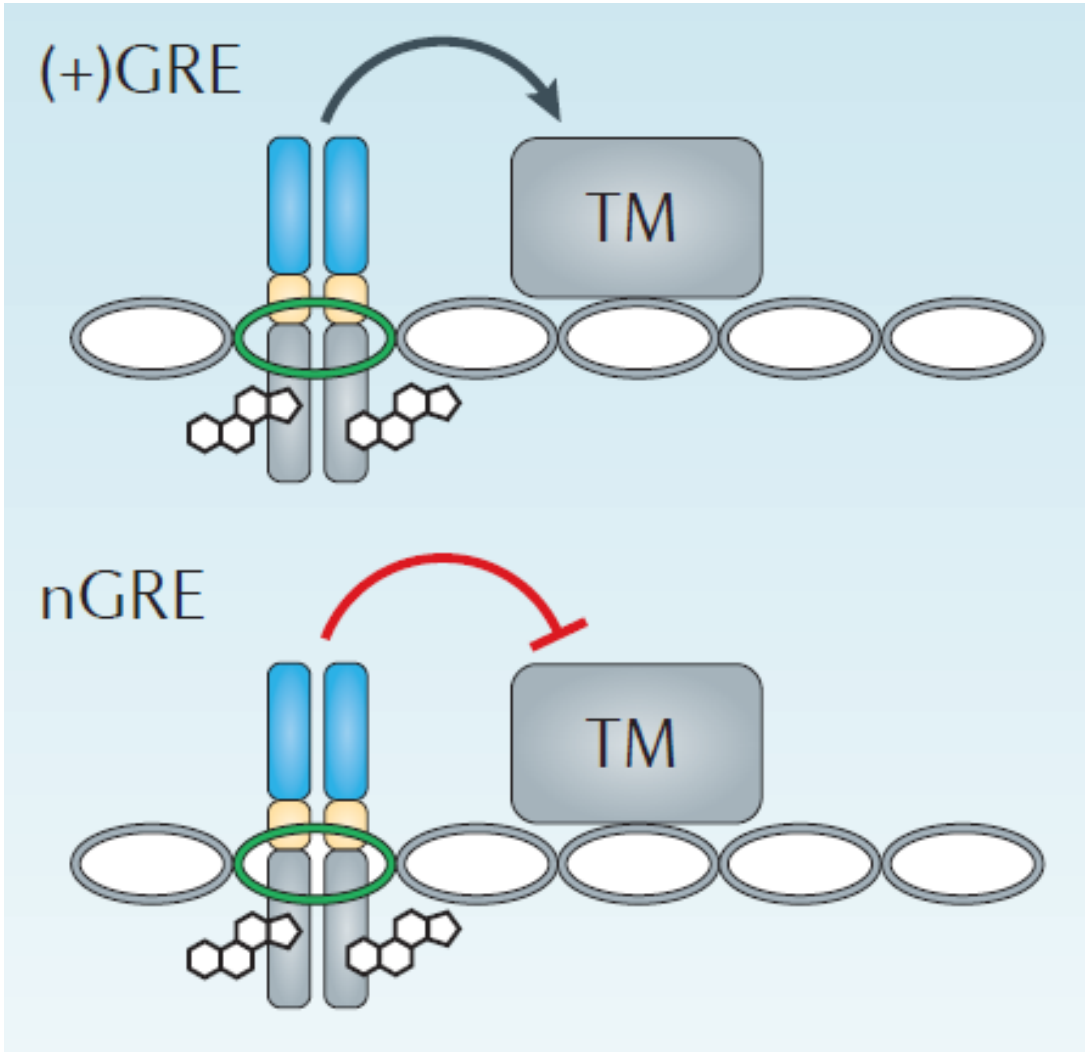
Direct GRE binding



Glucocorticoid

GC receptor (GR)

Transcriptional machinery



Direct binding to GC Receptor Element (GRE)

+ GRE

Induces transcription of genes that have anti-inflammatory properties:

- Annexin-1
- GC-induced leucine zipper (GLIZ) ⊣ **NFκB,** MAPK
- Mitogen-activated protein kinase phosphatase 1 (MPK1)

nGRE

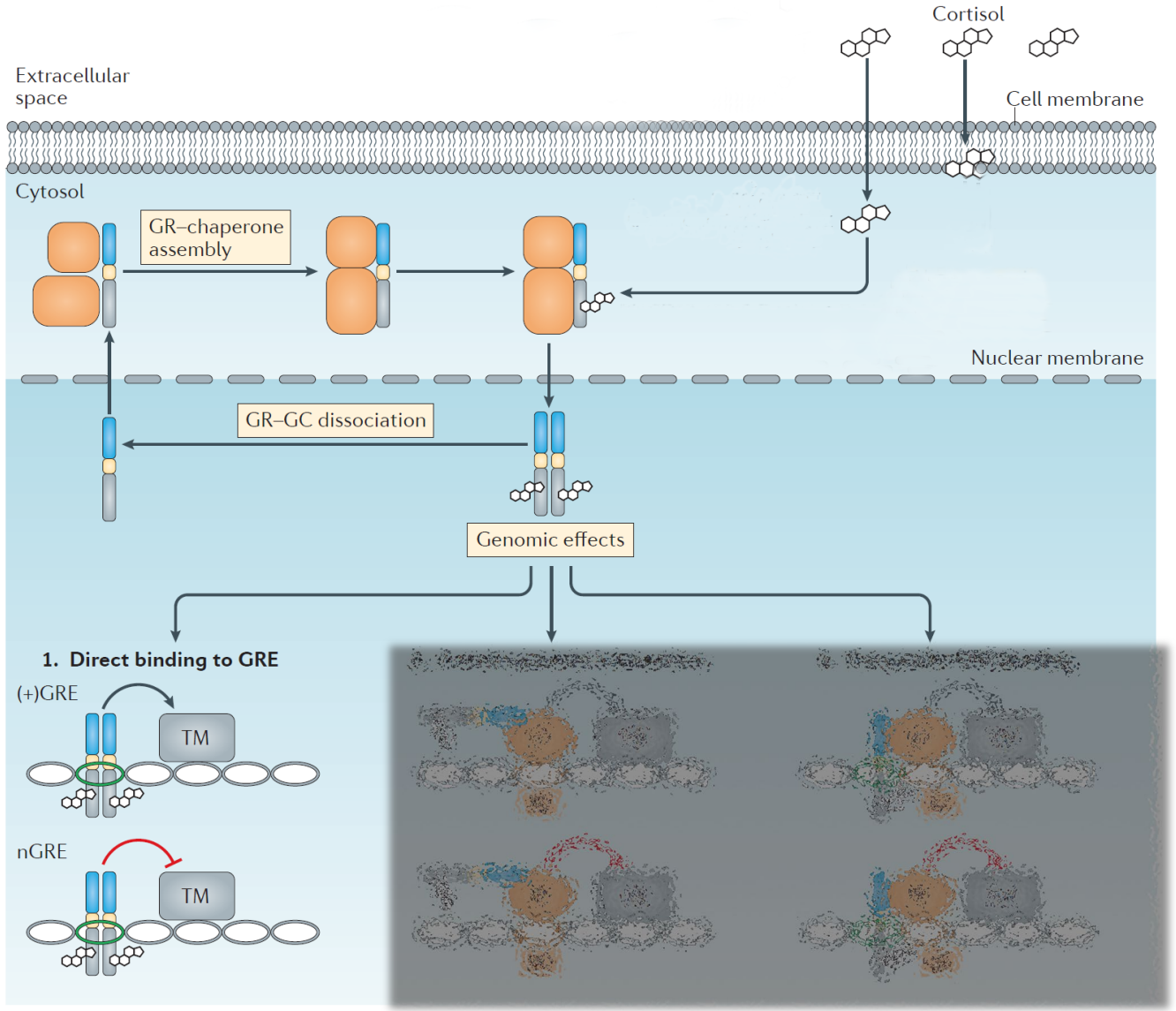
Inhibits transcription of genes:

- Corticotrophin-releasing hormones (CRH)
- β-endorphins
- Melanocyte-stimulating hormones (MSH)

Glucocorticoids: Mechanism of action

Chaperone

GC receptor (GR)



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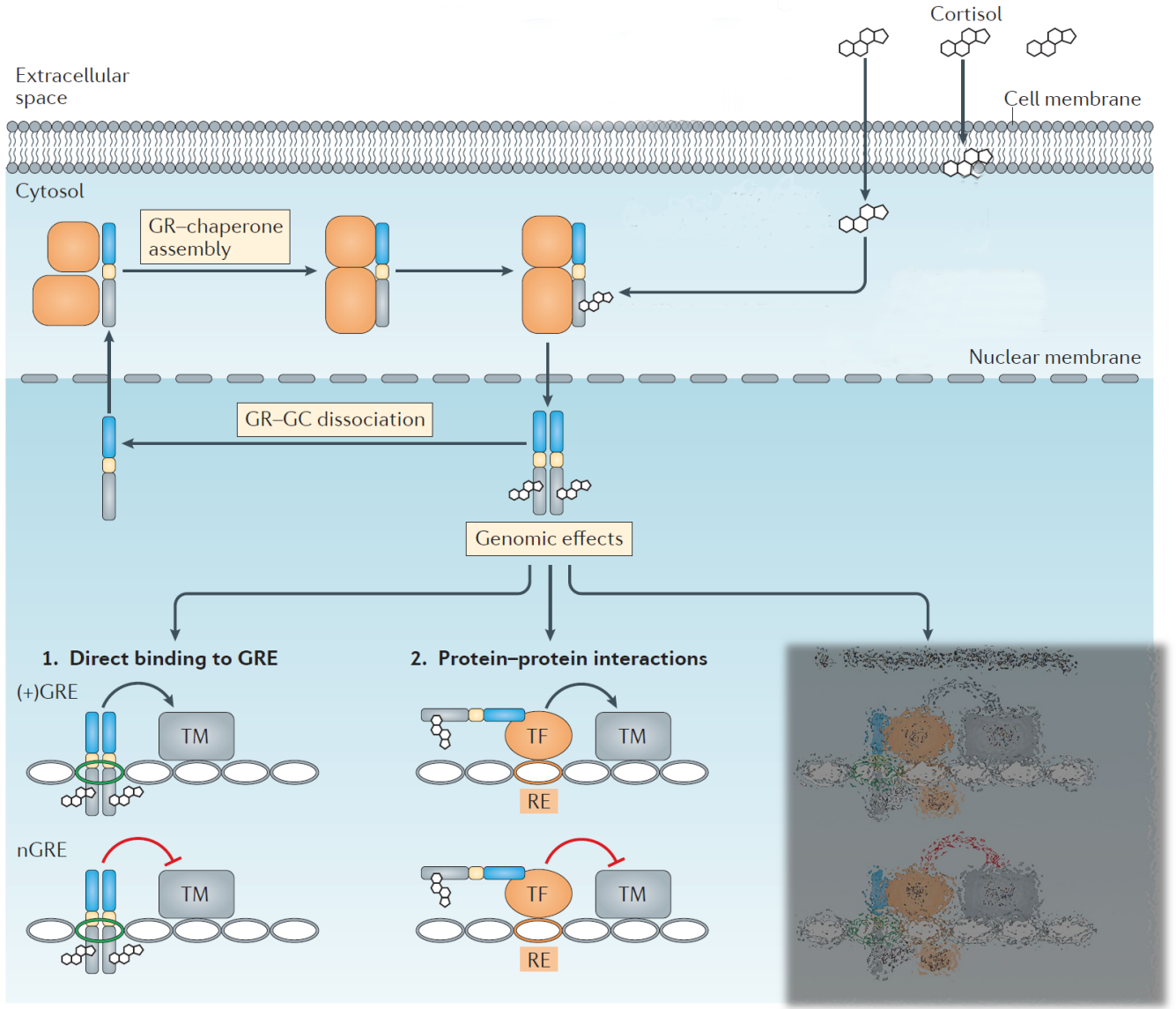
Glucocorticoids: Mechanism of action

Chaperone

GC receptor (GR)



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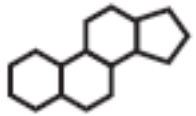
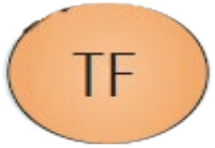
Tethering



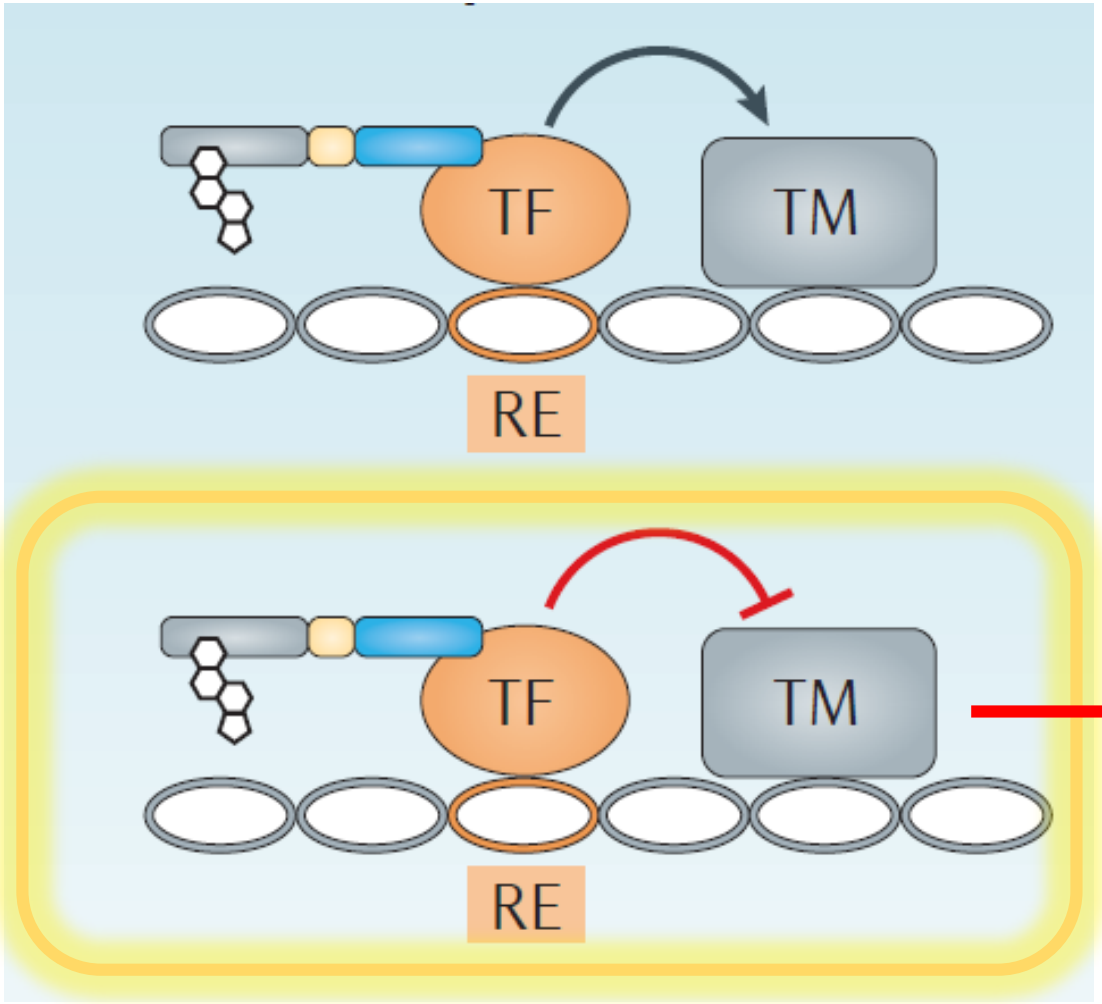
Glucocorticoid

GC receptor (GR)



Transcription factor

Transcriptional machinery



NFκB

AP-1

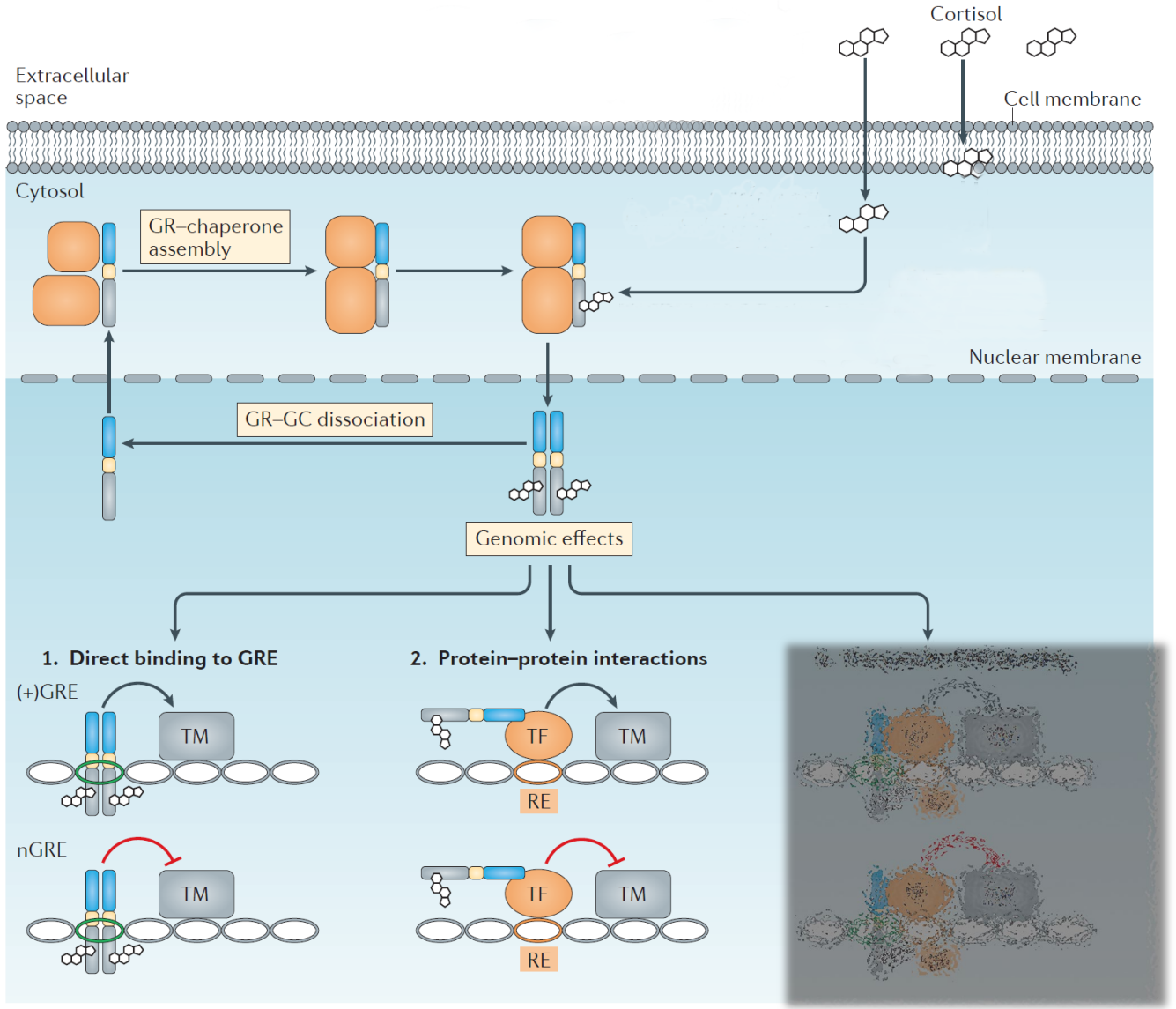
STAT

NFAT

Glucocorticoids: Mechanism of action

Chaperone

GC receptor (GR)



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Glucocorticoids: Mechanism of action

Chaperone

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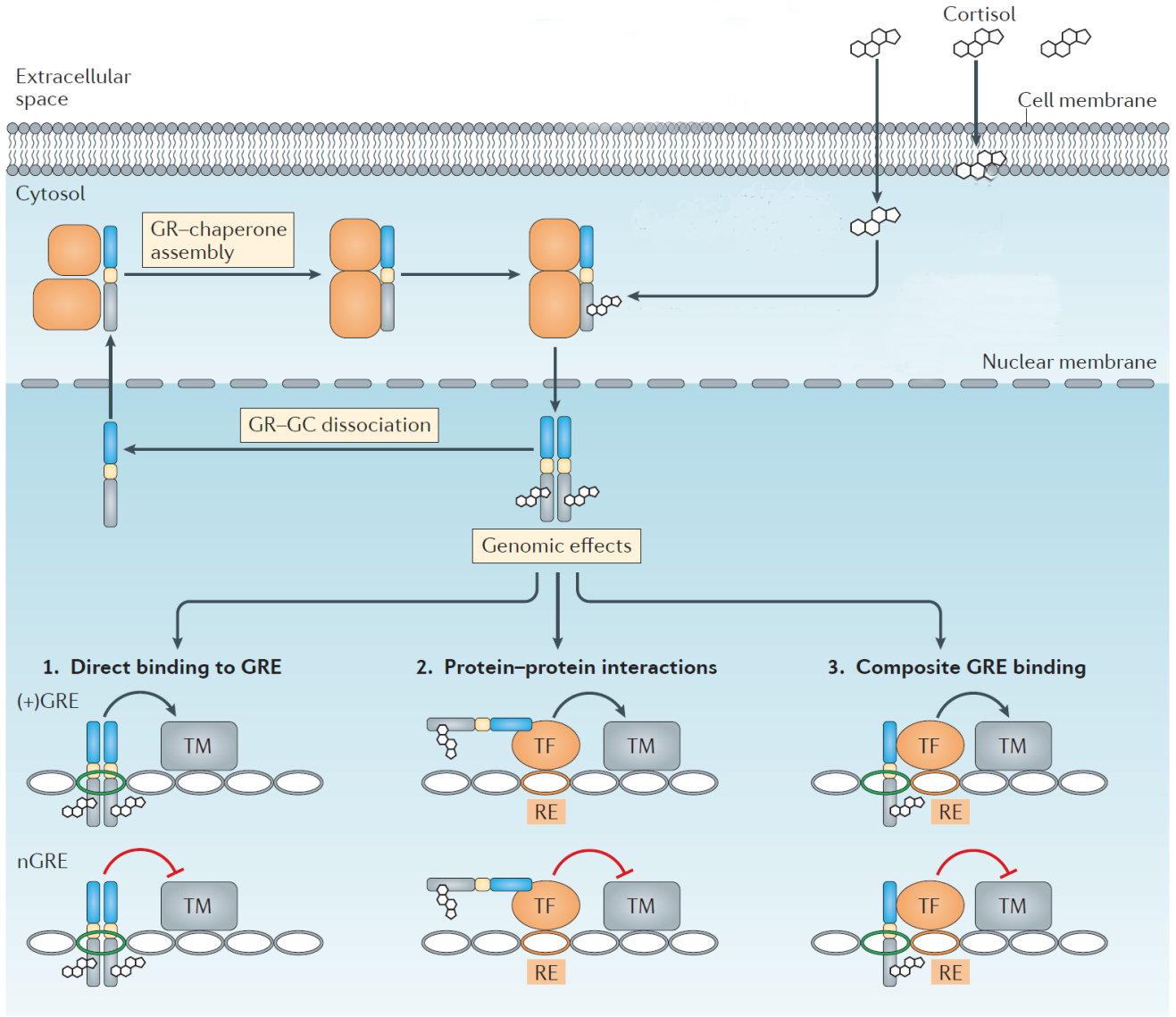

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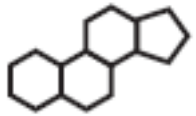
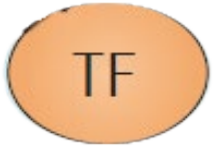
Composite binding



Glucocorticoid

GC receptor (GR)



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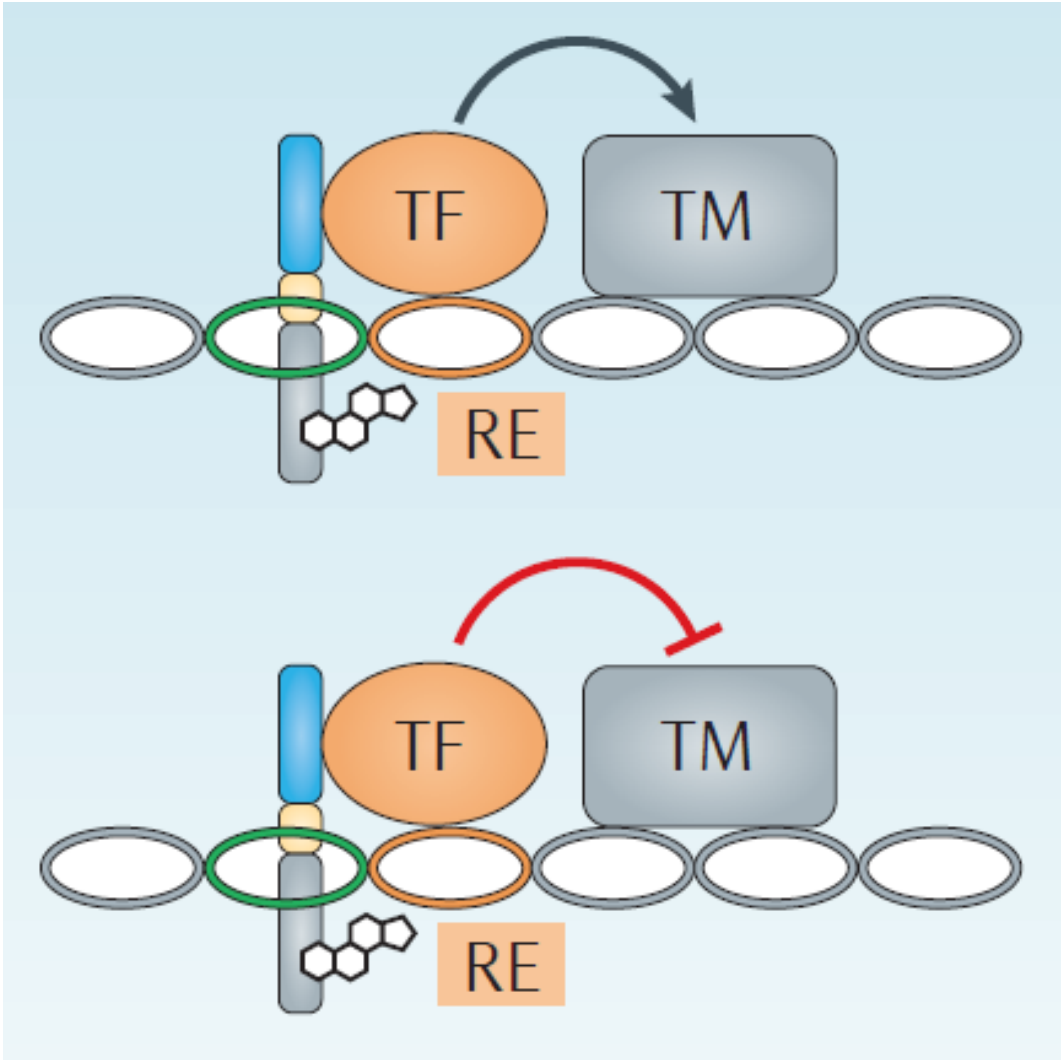
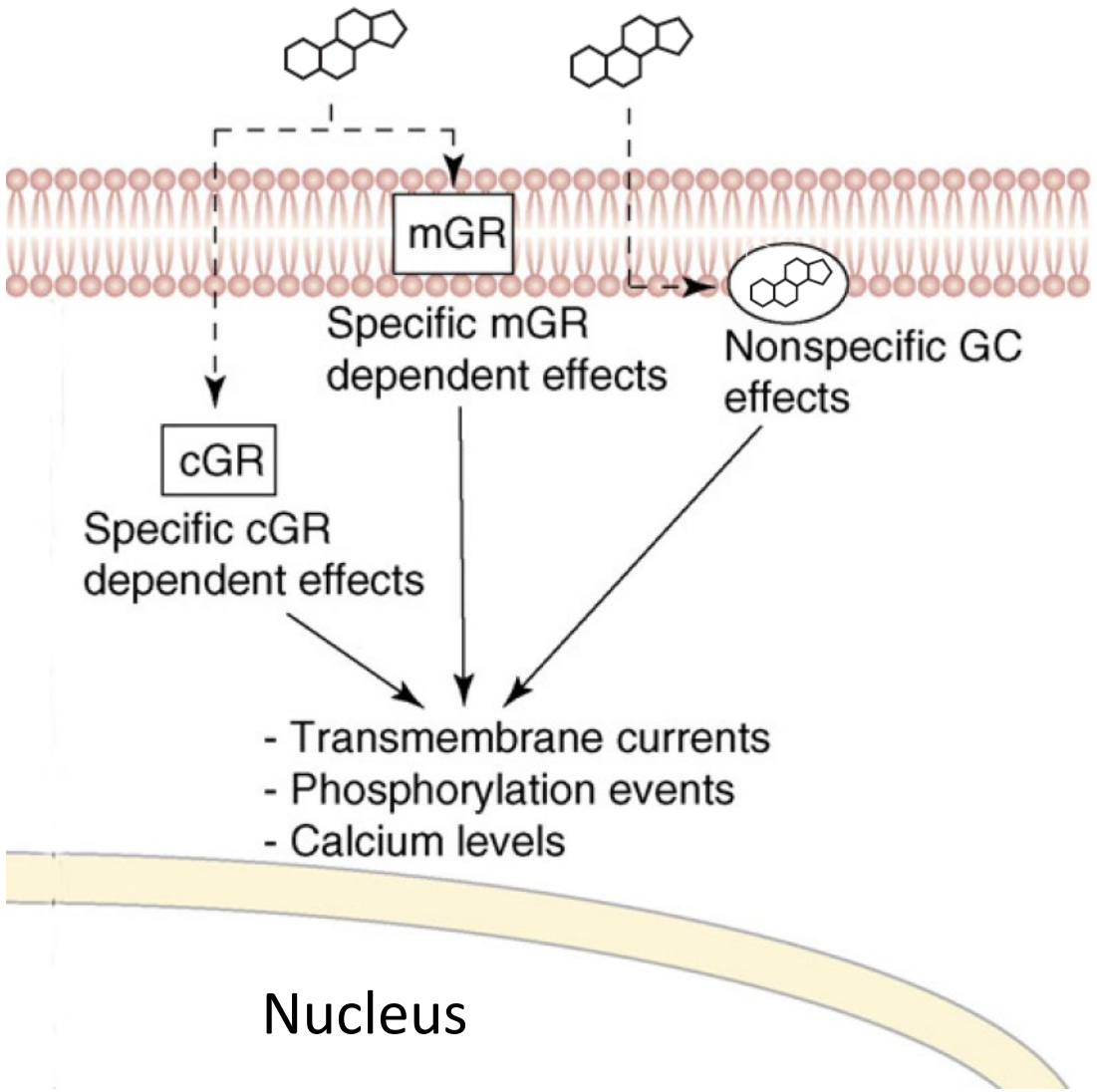
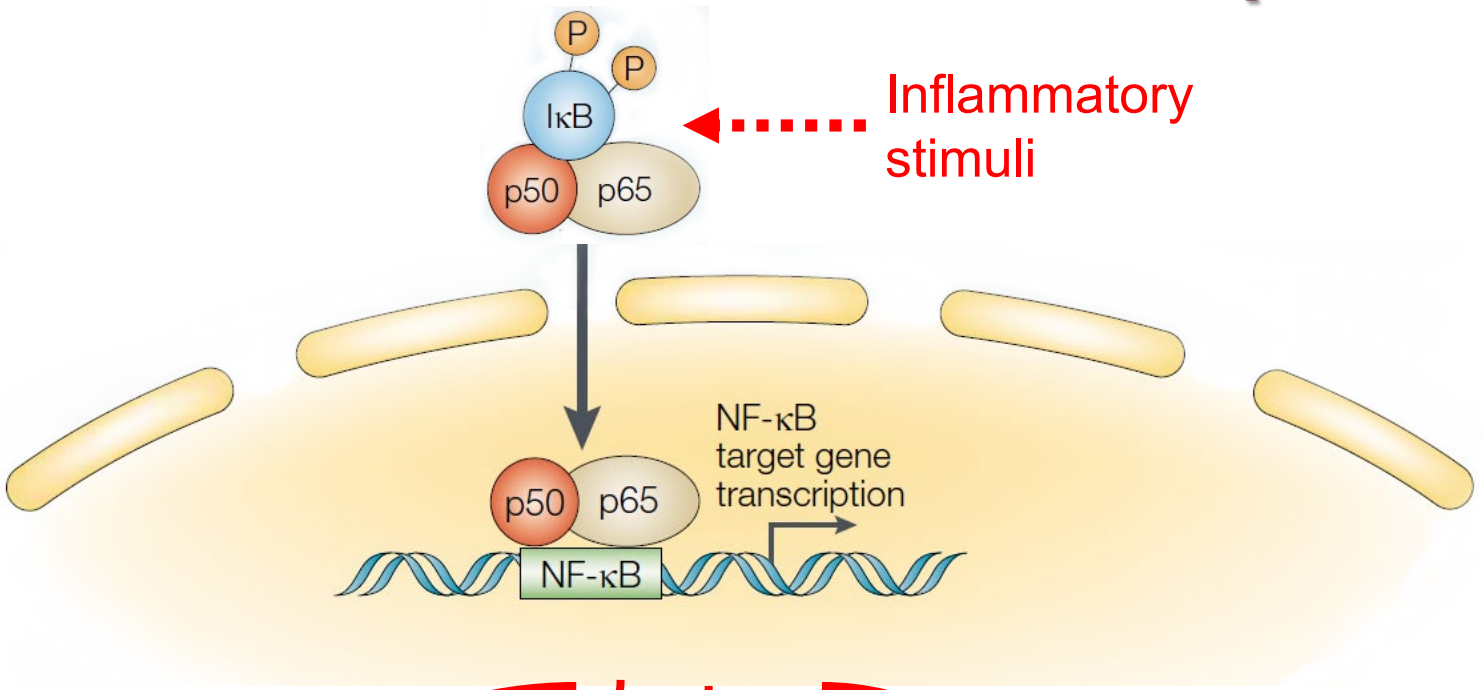


Glucocorticoid: Nongenomic mechanism



(Modified from Lowenberg, Trends Mol Med 2007)

Nuclear factor- κB (NFκB)



5 NFκB family members:

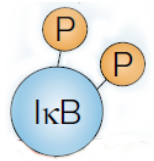
- p50
- p65
- p52
- REL
- RELB

Pro-inflammatory cytokines and chemokines (TNF, IL1, IL6, CXCL8)

Anti-apoptosis proteins (BCL-2)

Lymphocyte survival & activation

Adhesion molecules (ICAM1, VCAM1)



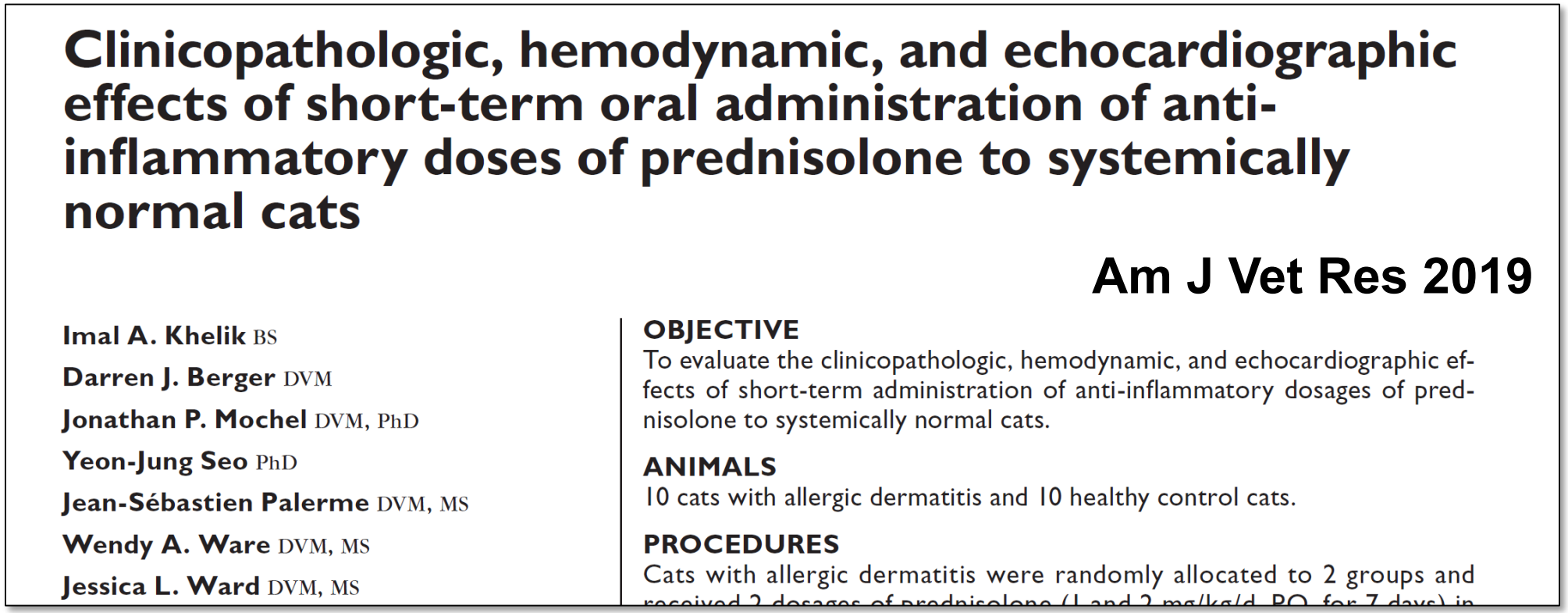
NFκB inhibitor

Glucocorticoid (GC)
Dermatological uses

- Pemphigus foliaceus (PF), autoimmune subepidermal blistering dermatoses (AISBD), cutaneous lupus erythematosus, pemphigus vulgaris (PV), uveodermatological syndrome (UDS)
- Oral GC pulse therapy in canine PF (Bizikova, *Vet Dermatol* 2015):
  - Higher proportions of CR in the first 3 months
  - Lower average maximal GC use
- Oral GC monotherapy: most common drug at the time of disease control in feline PF (Bizikova, *BMC Vet Res* 2019)
- Higher doses of oral GC +/- immunosuppressants needed for PV and canine UDS (Tham, *BMC Vet Res* 2020)

Glucocorticoid
Adverse effects

- Wide distribution of GR in all nucleated cells
- iatrogenic hyperadrenocorticism
- gastrointestinal ulceration
- cutaneous atrophy
- diabetes mellitus due to insulin resistance
- opportunistic infections
- delayed wound healing



Clinicopathologic, hemodynamic, and echocardiographic effects of short-term oral administration of anti-inflammatory doses of prednisolone to systemically normal cats

Am J Vet Res 2019

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Jonathan P. Mochel DVM, PhD
Yeon-Jung Seo PhD
Jean-Sébastien Palerme DVM, MS
Wendy A. Ware DVM, MS
Jessica L. Ward DVM, MS

OBJECTIVE
To evaluate the clinicopathologic, hemodynamic, and echocardiographic effects of short-term administration of anti-inflammatory dosages of prednisolone to systemically normal cats.

ANIMALS
10 cats with allergic dermatitis and 10 healthy control cats.

PROCEDURES
Cats with allergic dermatitis were randomly allocated to 2 groups and

- the anti-inflammatory dose of oral prednisolone (1-2mg/kg/day) given to healthy cats with allergic dermatitis for 14 days did not result in significant hemodynamic and echocardiographic changes
- But is that true for immunosuppressive doses??

Cyclosporine (CsA)

- Derived from the soil fungus *Beauveria nivea*
- Ultramicronized (microemulsified) preparation, in which absorption is more consistent and predictable.

Generic modified cyclosporine achieved _____ blood concentrations at 1 hour post-administration than Atopica after a single oral administration in normal healthy dogs.

A lower

B higher

C similar

D I don't know

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Veterinary Dermatology

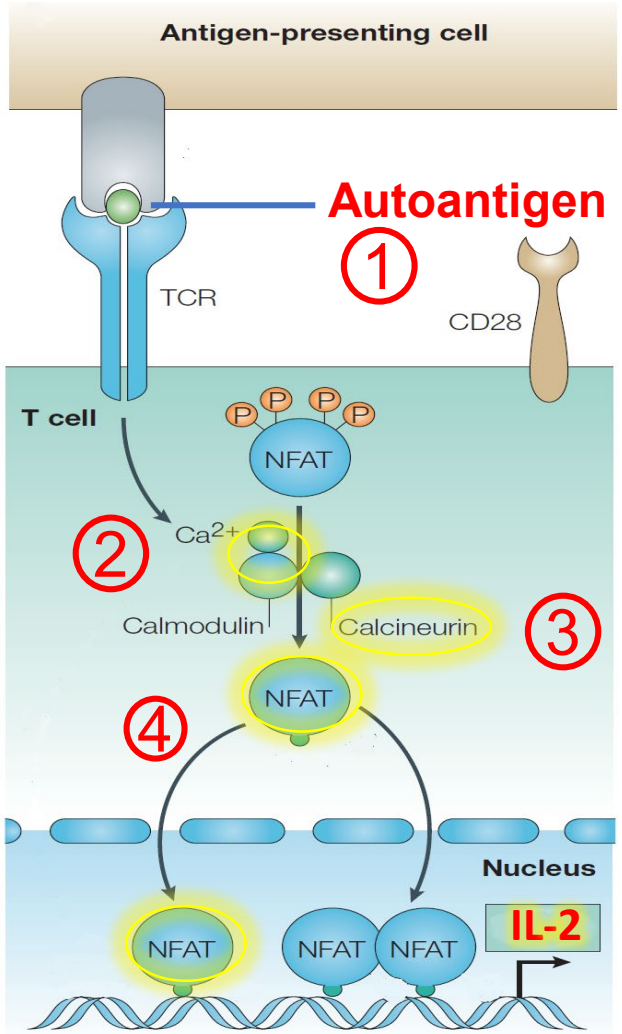
ORIGINAL ARTICLE

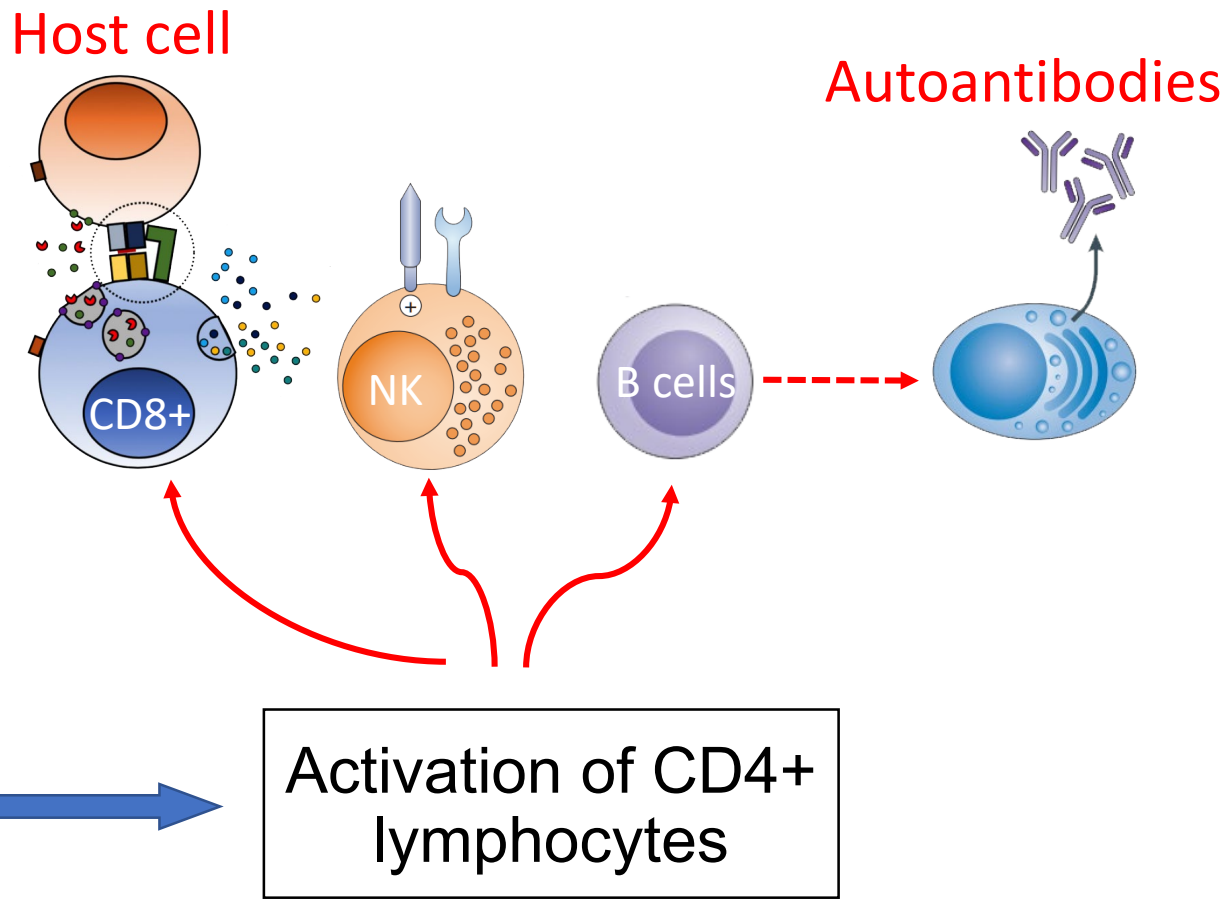
Comparison of whole blood concentrations of oral human generic modified ciclosporin capsules with microemulsified ciclosporin capsules approved for canine atopic dermatitis following a single oral administration to healthy dogs

Cheryl Vargo | Michaela Austel | Frane Banovic

- 1 hour post-administration CsA blood concentration: generic > Atopica
- 1.5 hour post administration: no significant difference
- Need Cmax and AUC to determine bioequivalence (PK studies)

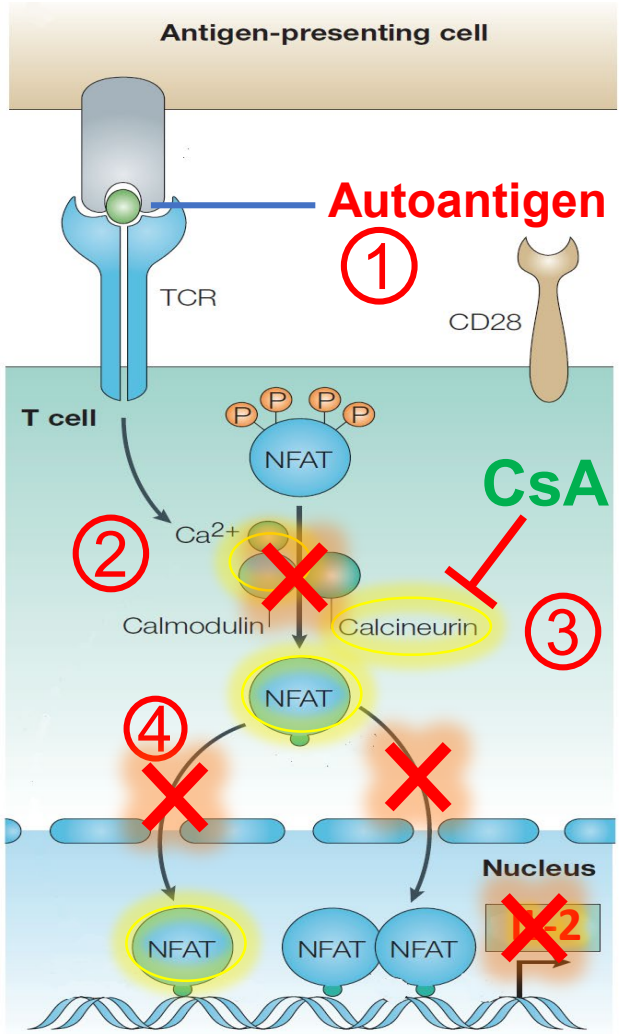
Nuclear factor of activated T cell (NFAT)

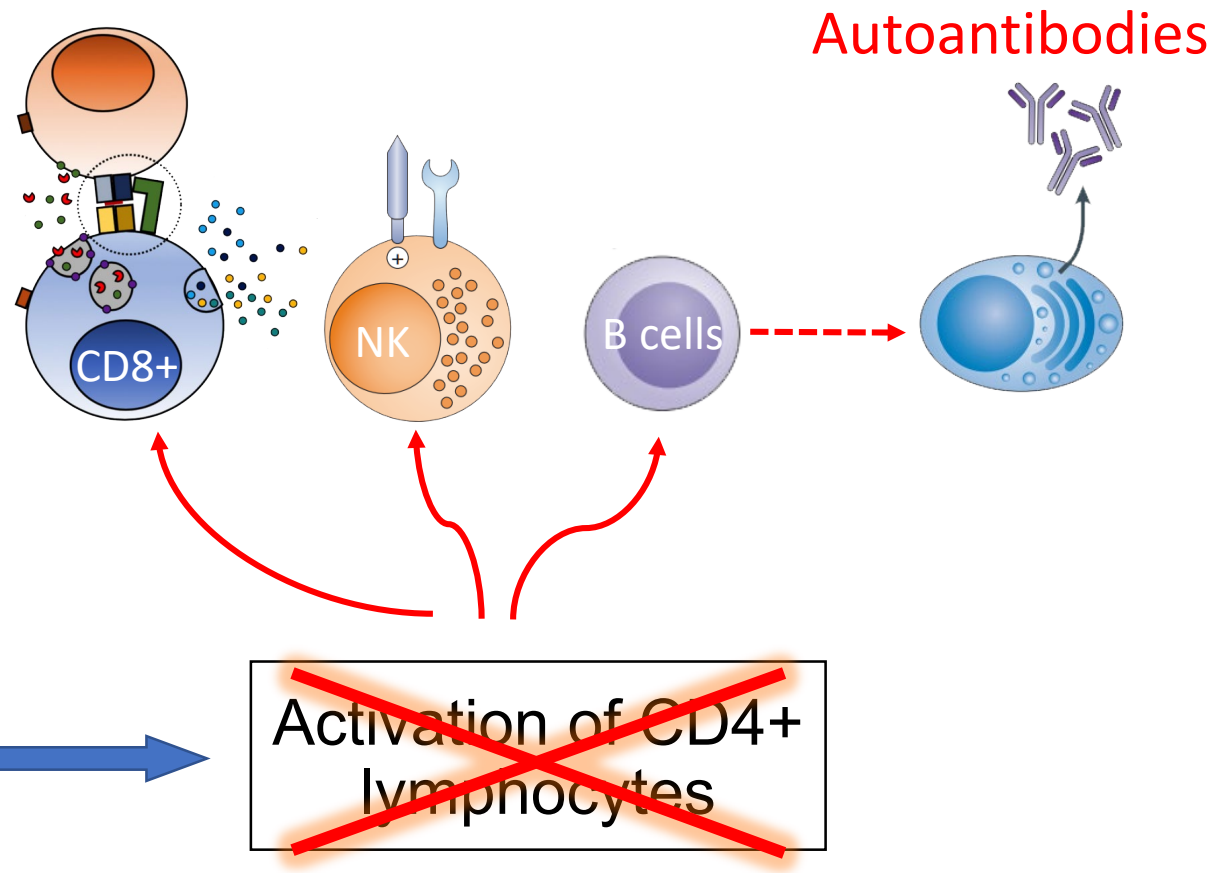




(Modified from Macian, Nature Rev Immunol 2005)

Cyclosporine (CsA): Mechanism of action





(Modified from Macian, Nature Rev Immunol 2005)

Cyclosporine
Dermatological uses

- Pemphigus foliaceus
- Cutaneous lupus erythematosis
- Pemphigus vulgaris
- Uveodermatological syndrome
- Canine immune-mediated perianal fistula
- Sebaceous adenitis
- Ischemic dermatopathies

Cyclosporine: *Adverse effects*

Table 2: Top 10 adverse clinical signs associated with ciclosporin treatment in dogs reported to pharmacovigilance between September 2002 and March 2012

Adverse clinical signs	Absolute incidence*
All suspected adverse events	71.81
Vomiting	27.57
Diarrhoea	13.46
Lethargy	9.58
Abnormal test result†	8.59
Pruritus	7.80
Anorexia	6.65
Hyperactivity	3.22
Gingival disorder	2.98
Tachypnoea	2.96
Polydipsia	2.58

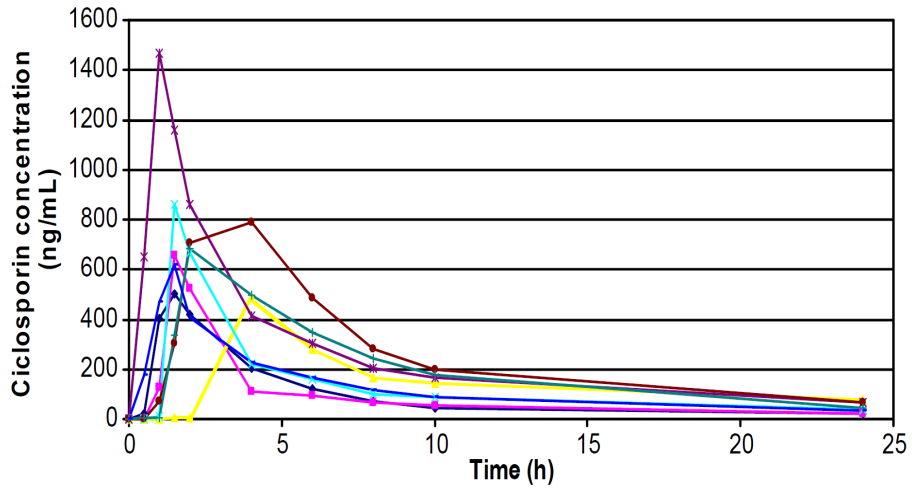
* Number of dogs affected/1 million capsules sold
† Includes various clinical pathology values outside of their normal range

(Nuttall, Vet Record 2014)

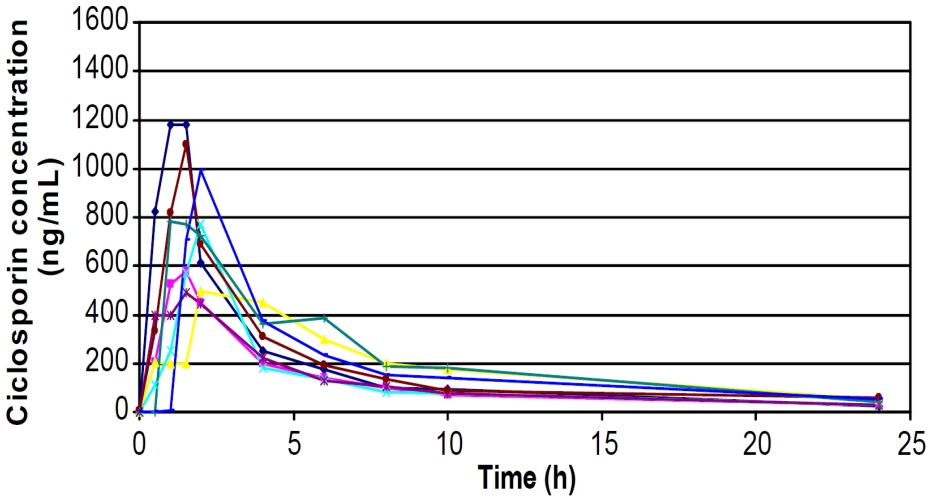
Stability and pharmacokinetics of modified cyclosporine stored at -20°C

Blinded, randomized cross-over study

8 healthy dogs, room temp/frozen CsA 5mg/mg once



Room Temperature



-20°C

Stability and absorption of modified CsA not affected by freezing

(Bachtel, Vet Dermatol 2015)

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Cyclosporine
Adverse effects

- Vomiting and diarrhea most common
- Gingival hyperplasia

Which of the following is one of the proposed pathogenesis of CsA-induced gingival hyperplasia?

A Reduction in the secretion of TGF

B Downregulation of salivary IL-6 and IL-8

C Increase in the expression of BAX/BCL2 ratio

D Inhibition of the secretion of matrix proteases

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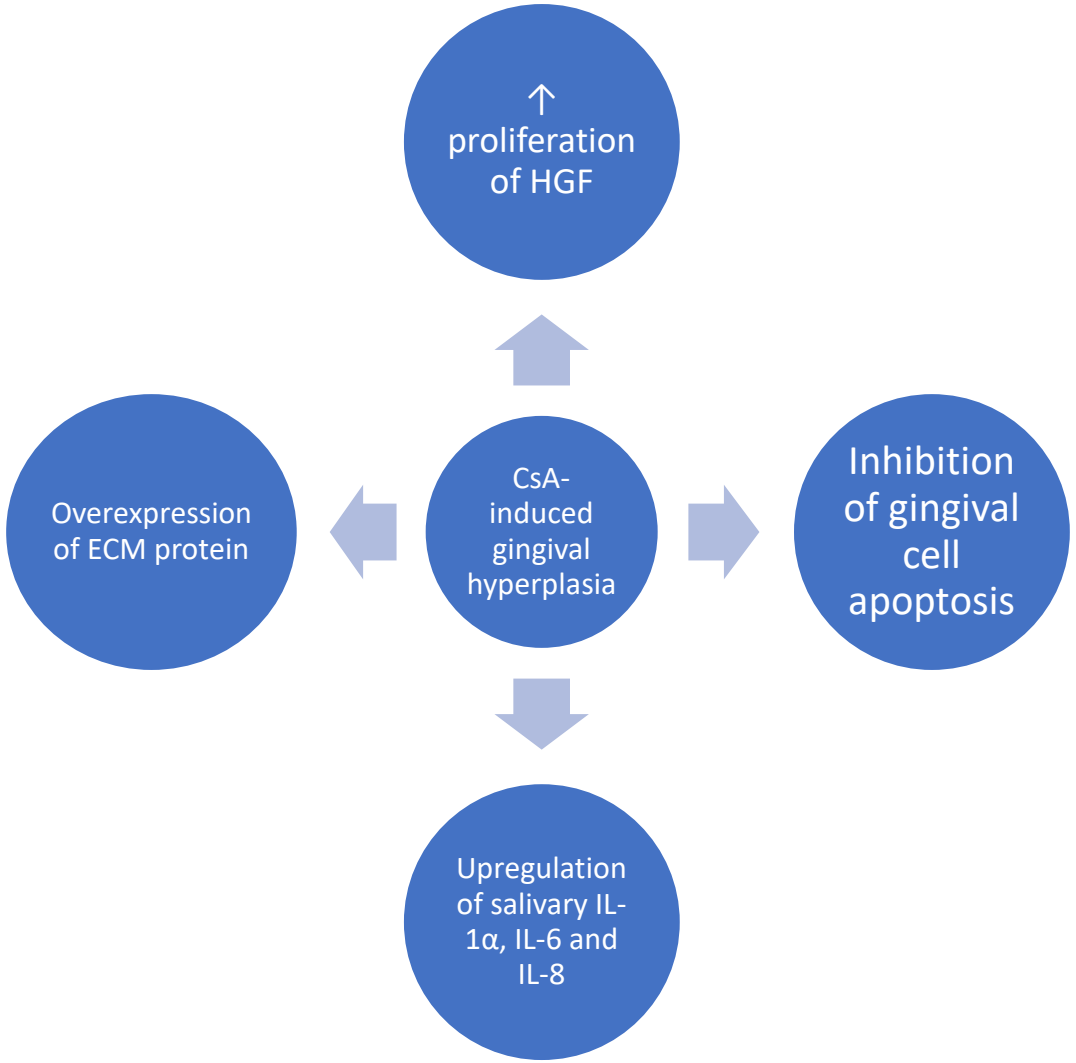
Cyclosporine & Gingival Hyperplasia

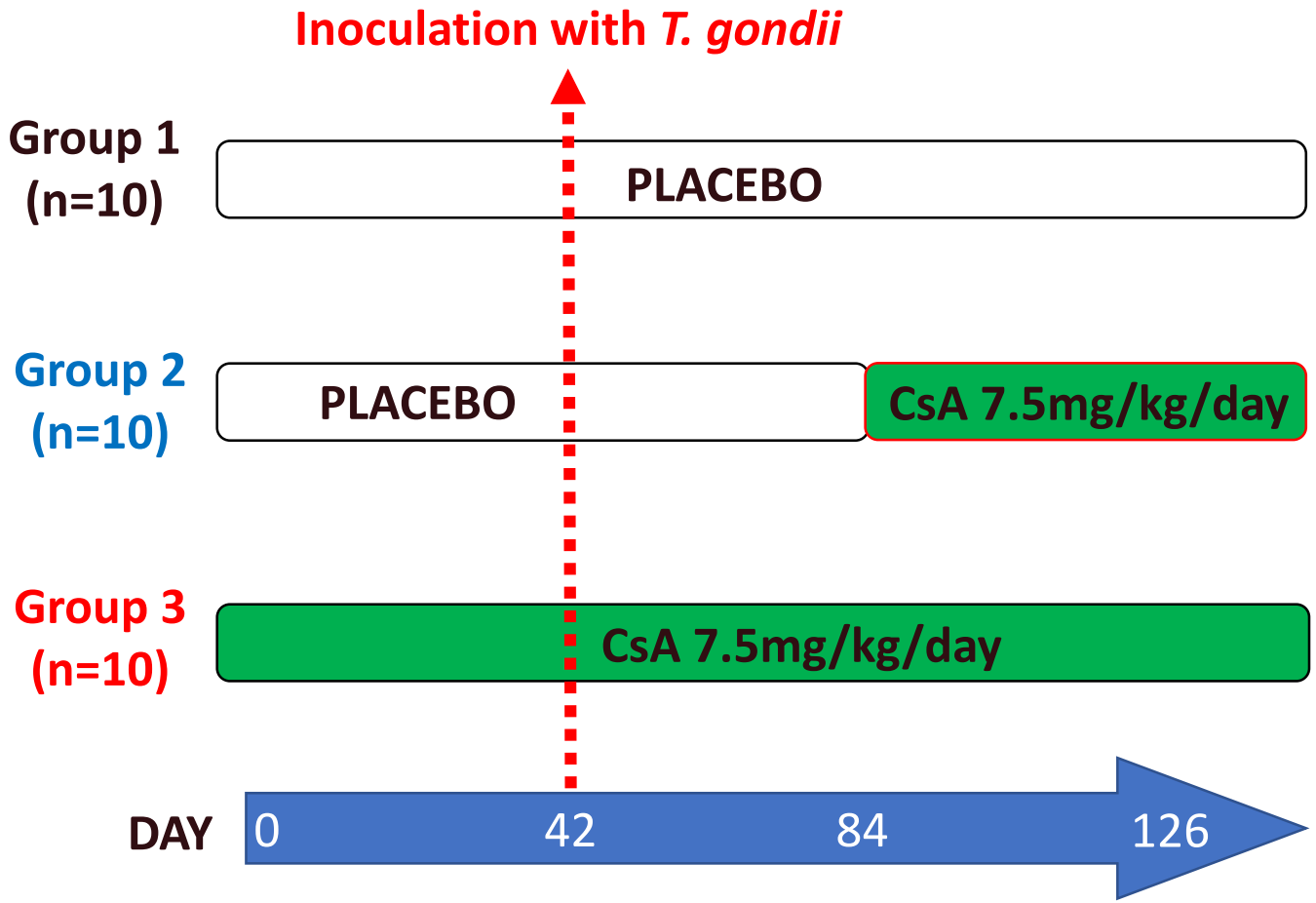


(Chojnacka-Purpurowicz, Dermatol Ther 2022)

Cyclosporine
Adverse effects

- Vomiting and diarrhea most common
- Gingival hyperplasia
- Cutaneous papillomatosis
- Hypertrichosis
- Psoriasiform-lichenoid dermatitis
- Opportunistic infections (especially when given with oral GC)
  - bacterial (*Nocardia spp*, *Burkholderia cepacian* complex)
  - fungal (*Alternaria spp*, *Curvularia spp*, *Aspergillus spp*.)

Effects of oral CsA on *Toxoplasma gondii* infection status of cats



27/30 cats developed self-limiting clinical signs; **resolved within 6 weeks**

CsA did not reactivate oocyst shedding and pre-existing subclinical disease

1 cat died on D64; CsA level D56: 1,690ng/ml
1 cat euthanized on Day 84, CsA level: 419ng/ml
1 cat found dead; mean CsA level >1000ng/ml higher than mean of Group 3
Clinical signs more severe

(Lappin, Am J Vet Res 2014)

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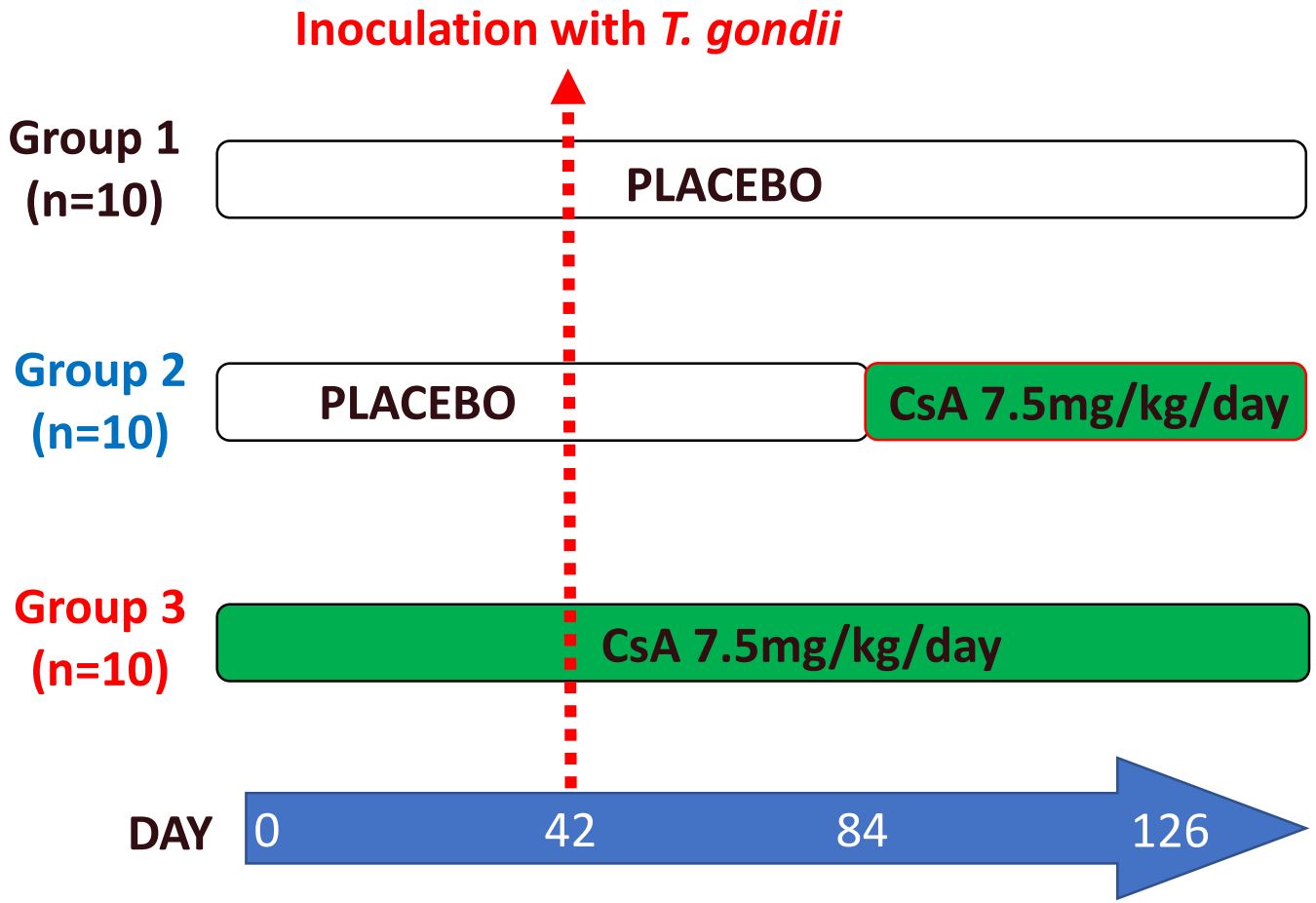
Effects of oral CsA on *Toxoplasma gondii* infection status of cats



Conclusions:

- More severe signs in naïve cats receiving CsA
- Naïve cats may be at risk of developing clinical toxoplasmosis while on CsA
- Treatment with CsA did not reactivate oocyst shedding or pre-existing subclinical disease
- **Avoid hunting and raw food** to reduce risk

(Lappin, Am J Vet Res 2014)

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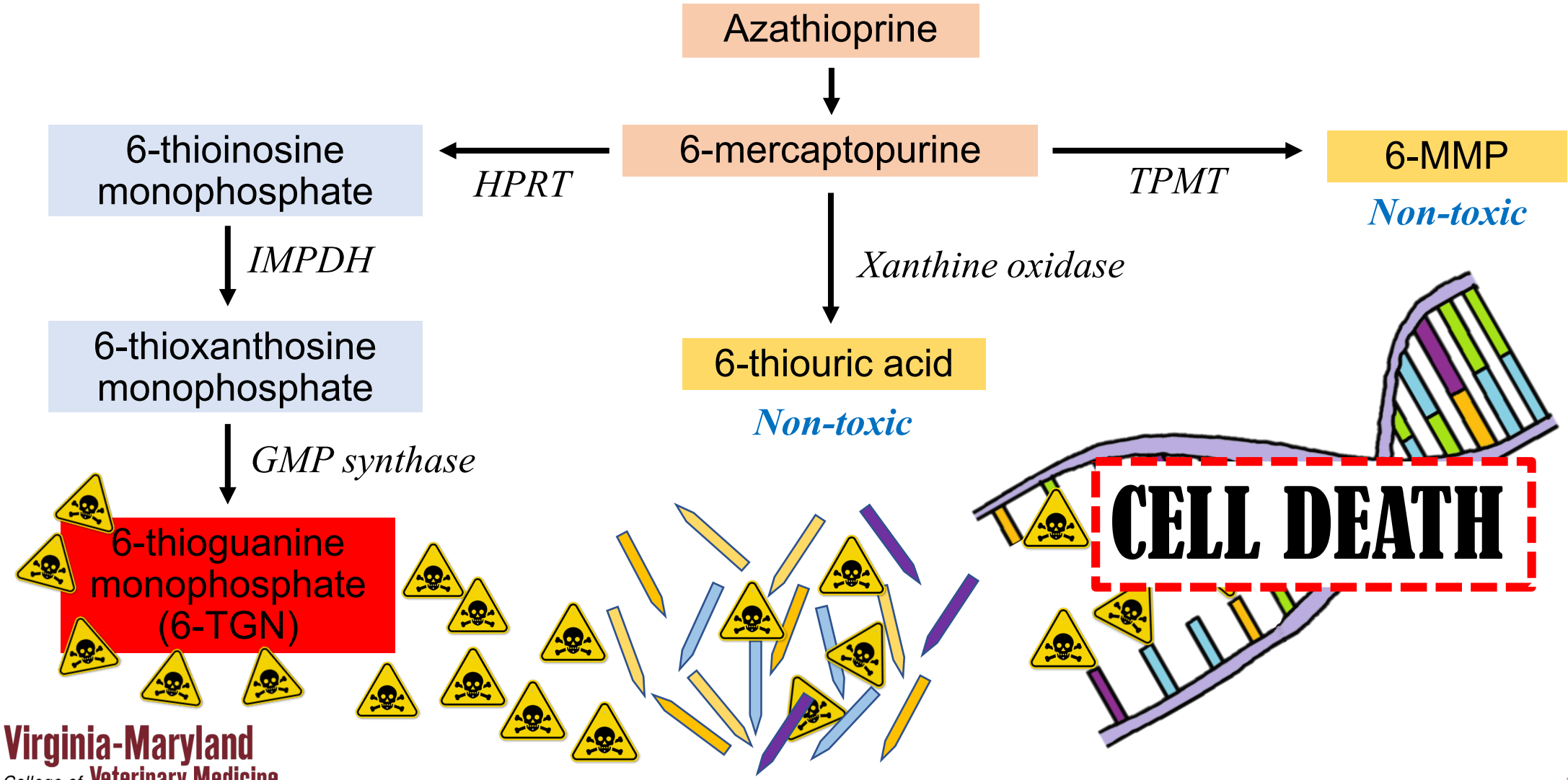
Azathioprine (AZA)

- Prodrug of 6-mercaptopurine (6-MP)
- Interferes with nucleotide synthesis

Azathioprine (AZA)
Mechanism of action



Azathioprine
Dermatological uses

- Steroid-sparing immunosuppressant
- PF, UDS, PV, VCLE, immune-mediated perianal fistula and symmetrical lupoid onychodystrophy
- AZA + oral GC combination most commonly used at time of disease control for:
  - Canine pemphigus foliaceus (Mueller, *JAAHA* 2006)
  - Uveodermatological syndrome (Tham, *BMC Vet Res* 2019)
  - Pemphigus vulgaris (Tham, *BMC Vet Res* 2020)

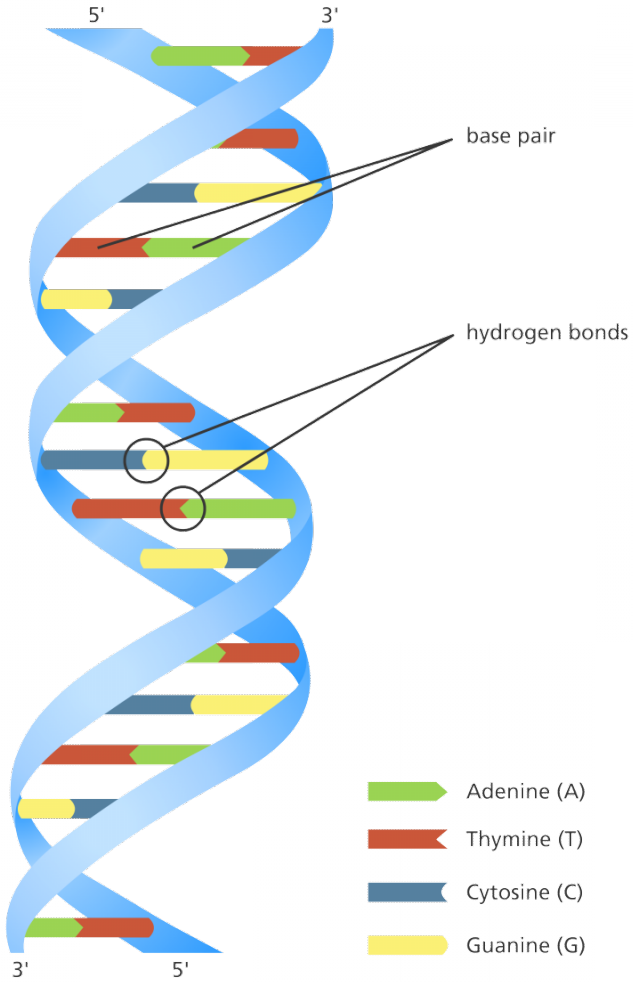
Azathioprine
Adverse effects

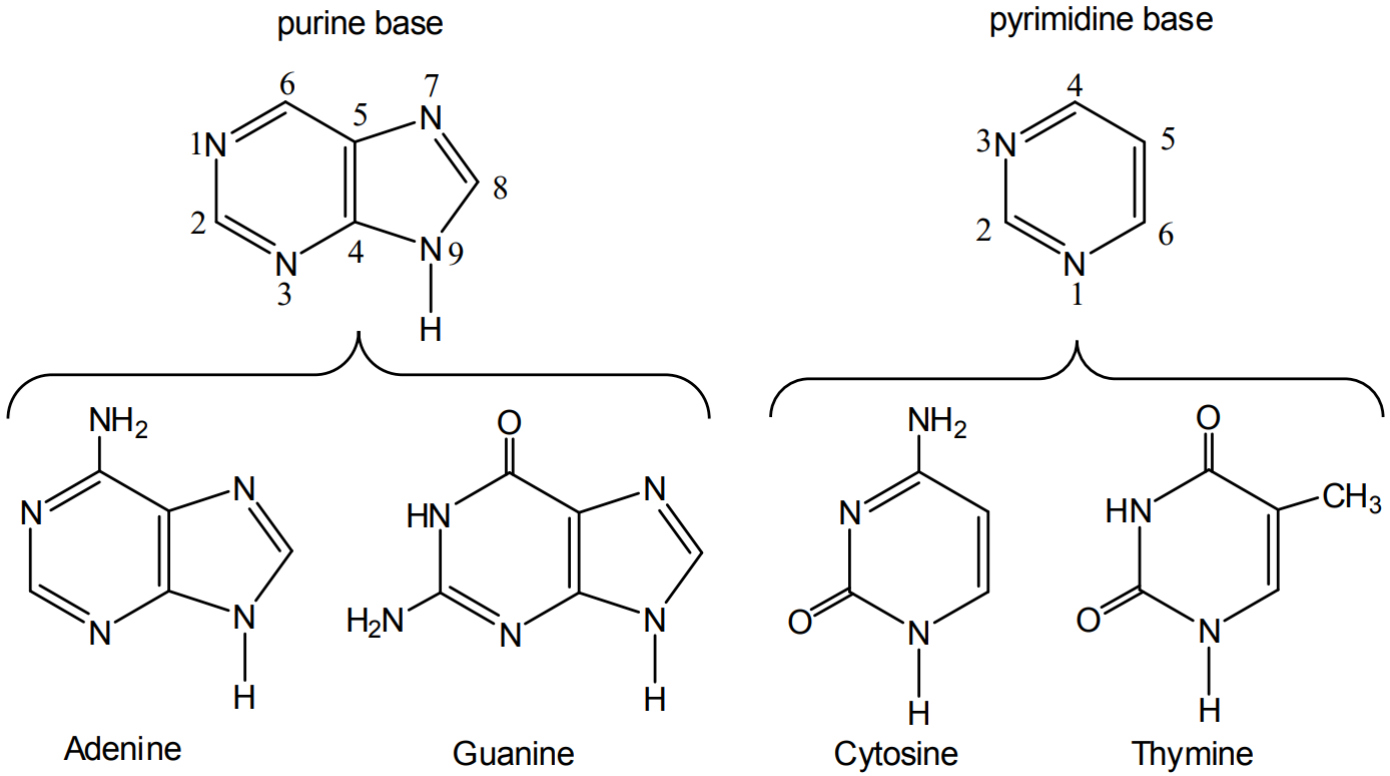
- Myelosuppression
  - TPMT levels not correlated with risk in dogs
  - TPMT level much lower in cats – higher risk of immunosuppression
- Hepatotoxicosis
  - Much lower incidence (5% vs 15%) when administered EOD along with tapering GC (Eberhardy, *Vet Dermatol* 2020)
  - Median onset: 14 days (13-22 days)

Chlorambucil (CLB)
Definition

- Alkylating agent: transfer of a reactive alkyl group from one molecule to another **forming covalent bonds**
- Nucleophile (“Donor”): provides a pair of electron to form covalent bond
- Electrophile (“Recipient”): accepts a pair of electron to form covalent bond
- **Chlorambucil is a reactive electrophile (Recipient)**
- **Guanine (N7 position)** is the most nucleophilic site (Most generous “donor”)
- Cytotoxic effects: alkylate the nucleophilic portion of a DNA molecule through the formation of covalent bonds.
- Causes “unwanted” cross-linking of DNA:
  - Intrastrand
  - Interstrand (most “devastating” effect)

Chlorambucil (CLB)
Mechanism of action





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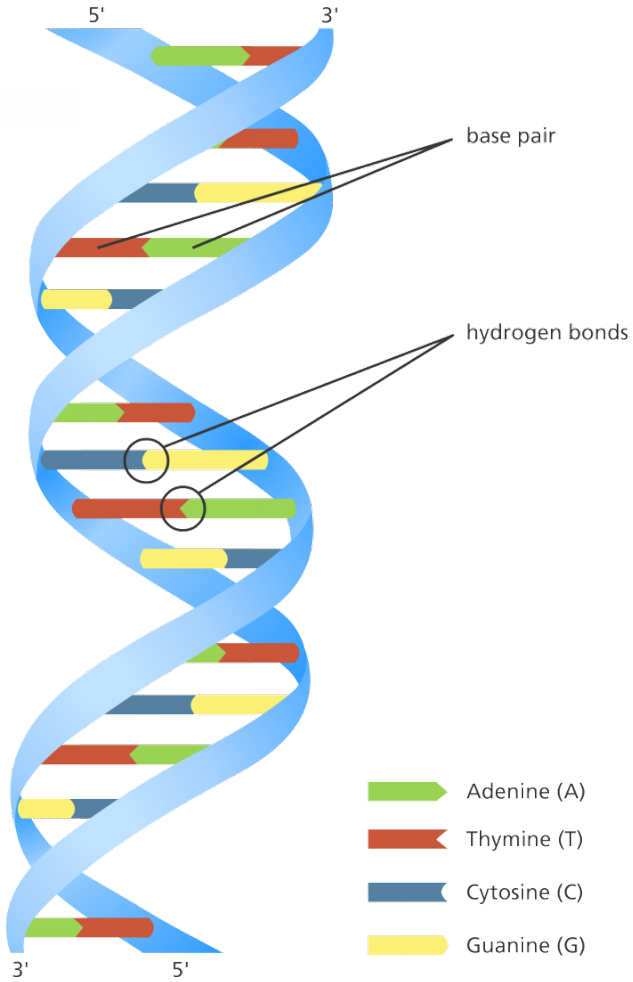
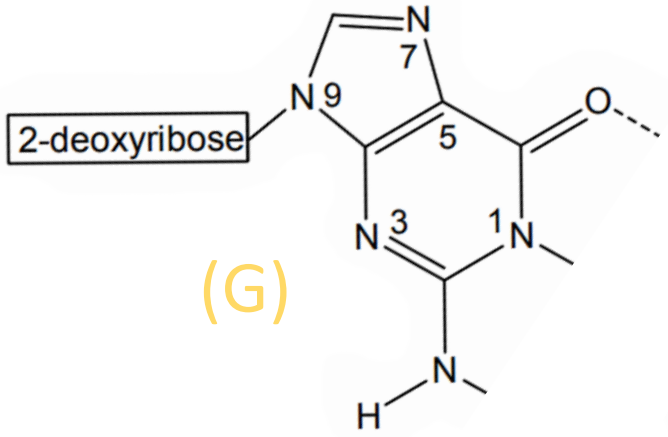
Chlorambucil (CLB)
Mechanism of action

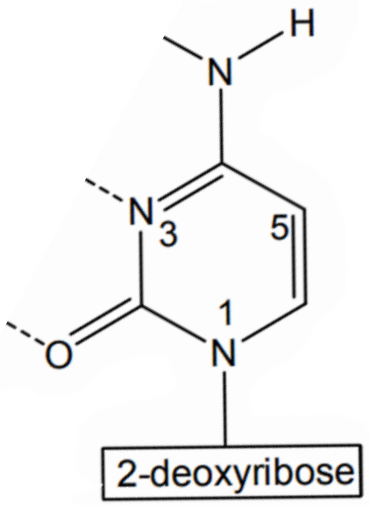


Adenine → Thymine

Guanine → Cytosine



(G)



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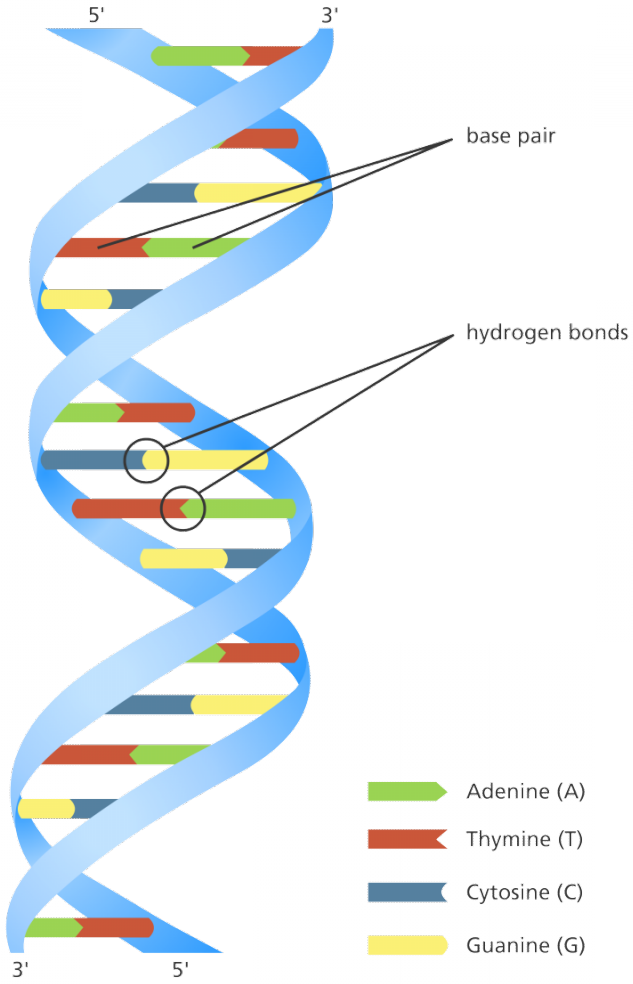
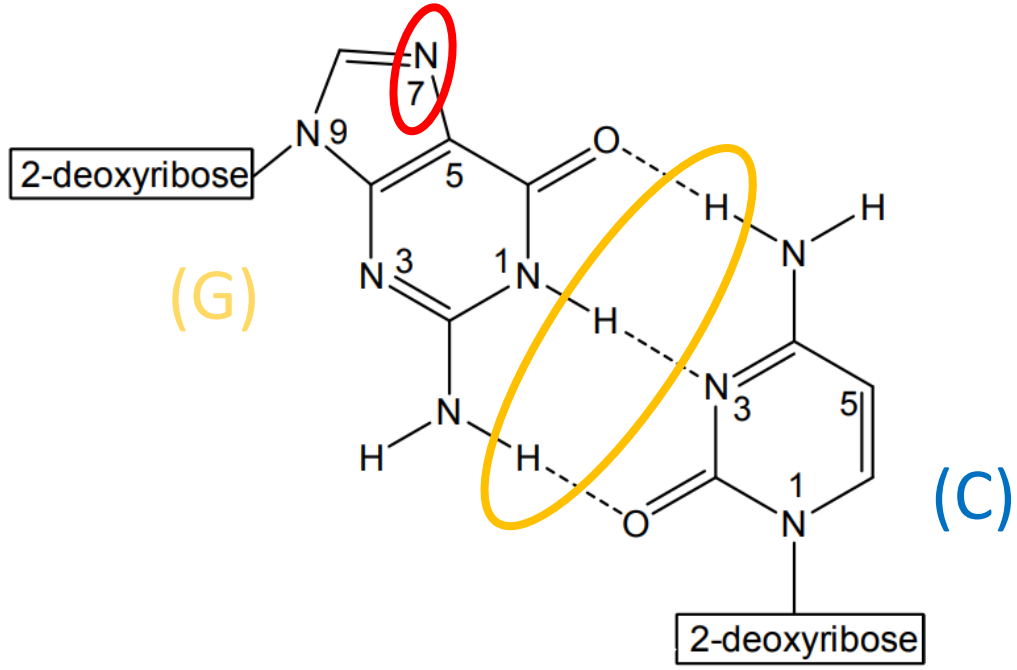
Chlorambucil (CLB)
Mechanism of action



Adenine → Thymine

Guanine → Cytosine



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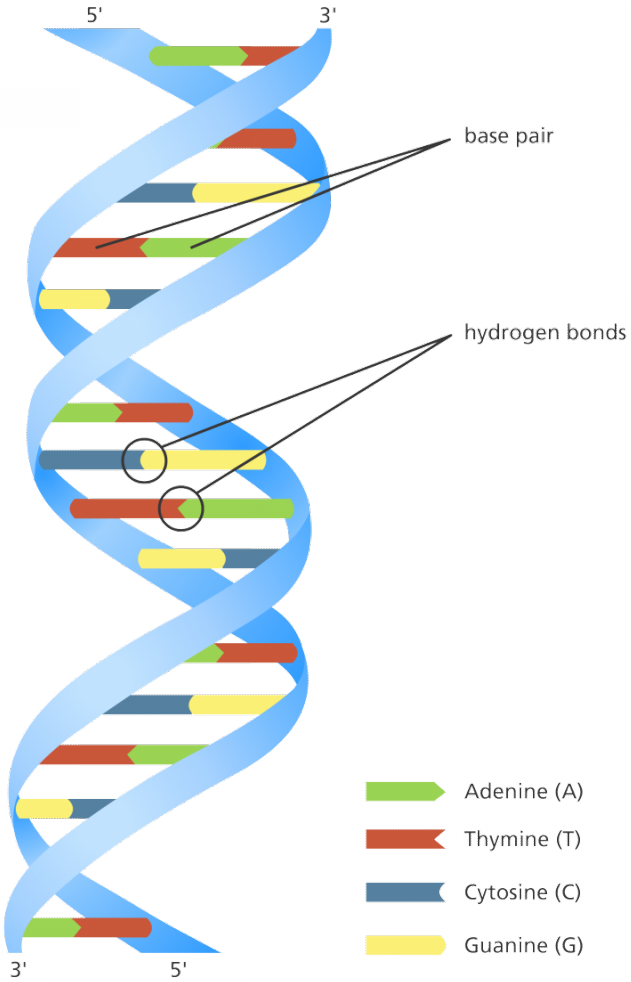
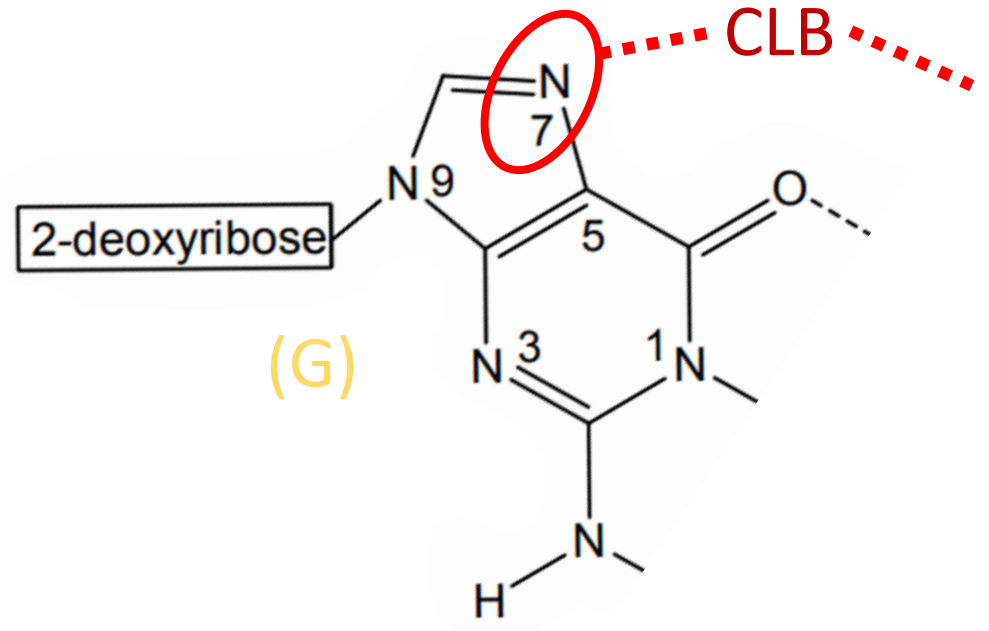
Chlorambucil (CLB)
Definition

- Alkylating agent: transfer of a reactive alkyl group from one molecule to another **forming covalent bonds**
- Nucleophile ("Donor"): provides a pair of electron to form covalent bond
- Electrophile ("Recipient"): accepts a pair of electron to form covalent bond
- Chlorambucil is a reactive electrophile (Recipient)
- **Guanine (N7 position)** is the most nucleophilic site (Most generous "donor")
- Cytotoxic effects: alkylate the nucleophilic portion of a DNA molecule through the formation of covalent bonds.
- Causes "unwanted" cross-linking of DNA:
  - Intrastrand
  - Interstrand (most "devastating" effect)

Chlorambucil (CLB): Bifunctional alkalyting agent
Mechanism of action



Guanine → Cytosine



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Chlorambucil (CLB): Bifunctional alkalyting agent

Mechanism of action

Intrastrand

CELL DEATH

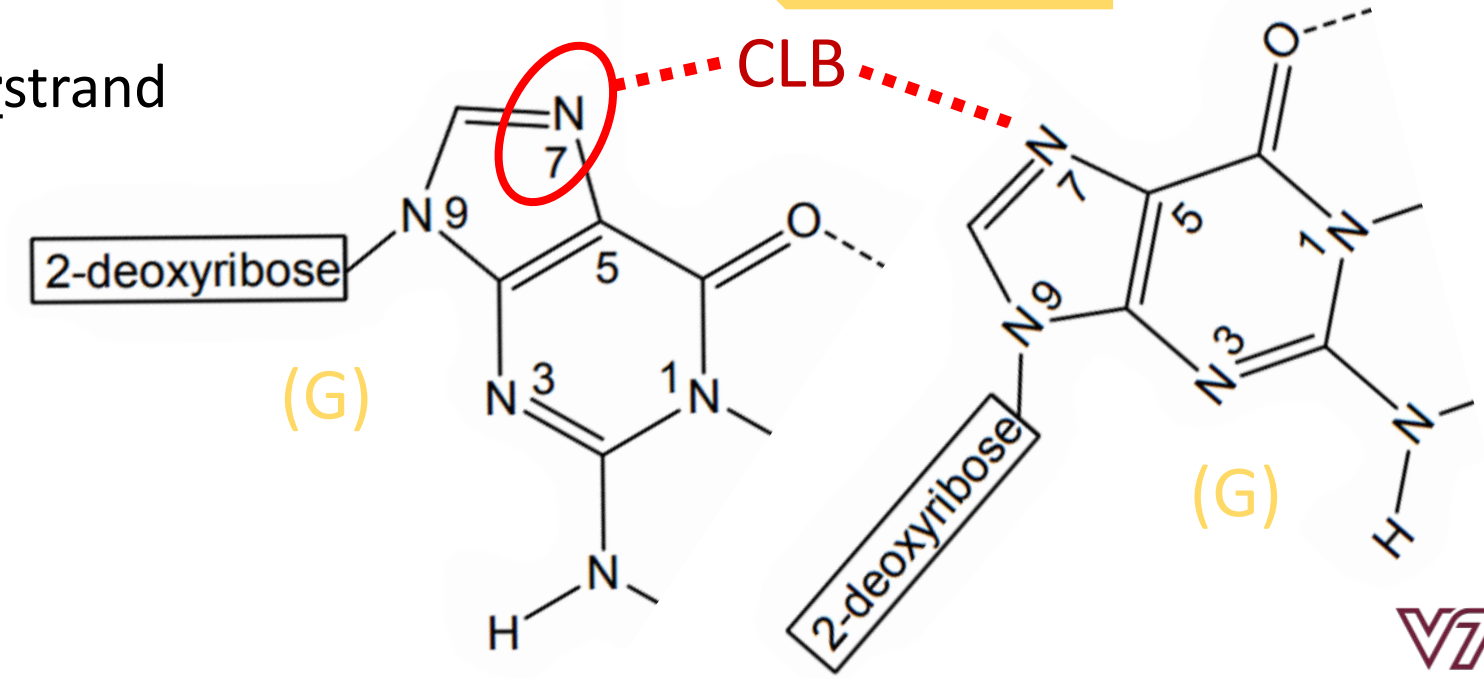
Interstrand

Guanine — Cytosine

Guanine — Cytosine

Guanine

CLB



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Chlorambucil (CLB)
Dermatologic uses

- In cats:
  - steroid-sparing agent in feline pemphigus foliaceus
- In dogs: (along with cyclosporine and prednisolone)
  - Vaccine-induced ischemic dermatopathy (Kim, *J Vet Med Sc* 2011)
  - Canine eosinophilic granuloma (Knight, *Vet Dermatol* 2016)

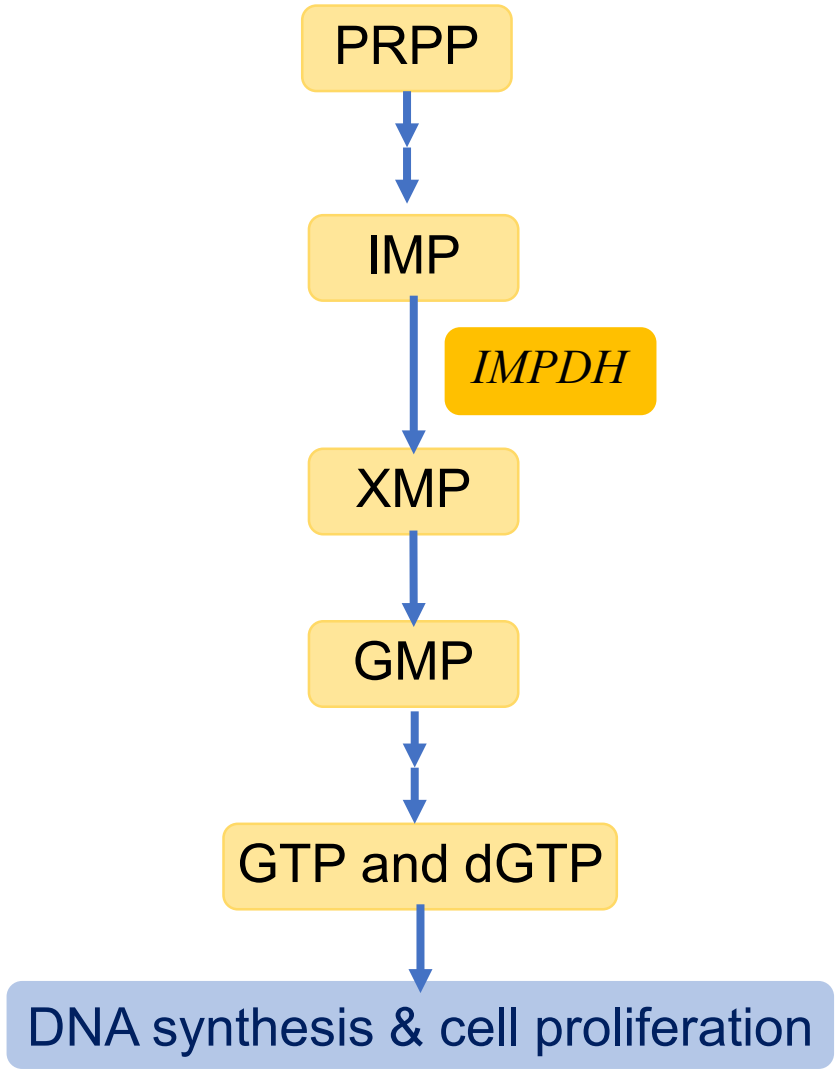
Chlorambucil (CLB)
Adverse effect

- Myelosuppression 7-14 days
- Reversible myoclonus (Benitah et al, *JAAHA* 2003)
- Fanconi syndrome (Reinert et al, *JFMS* 2016)

Mycophenolate mofetil (MMF)

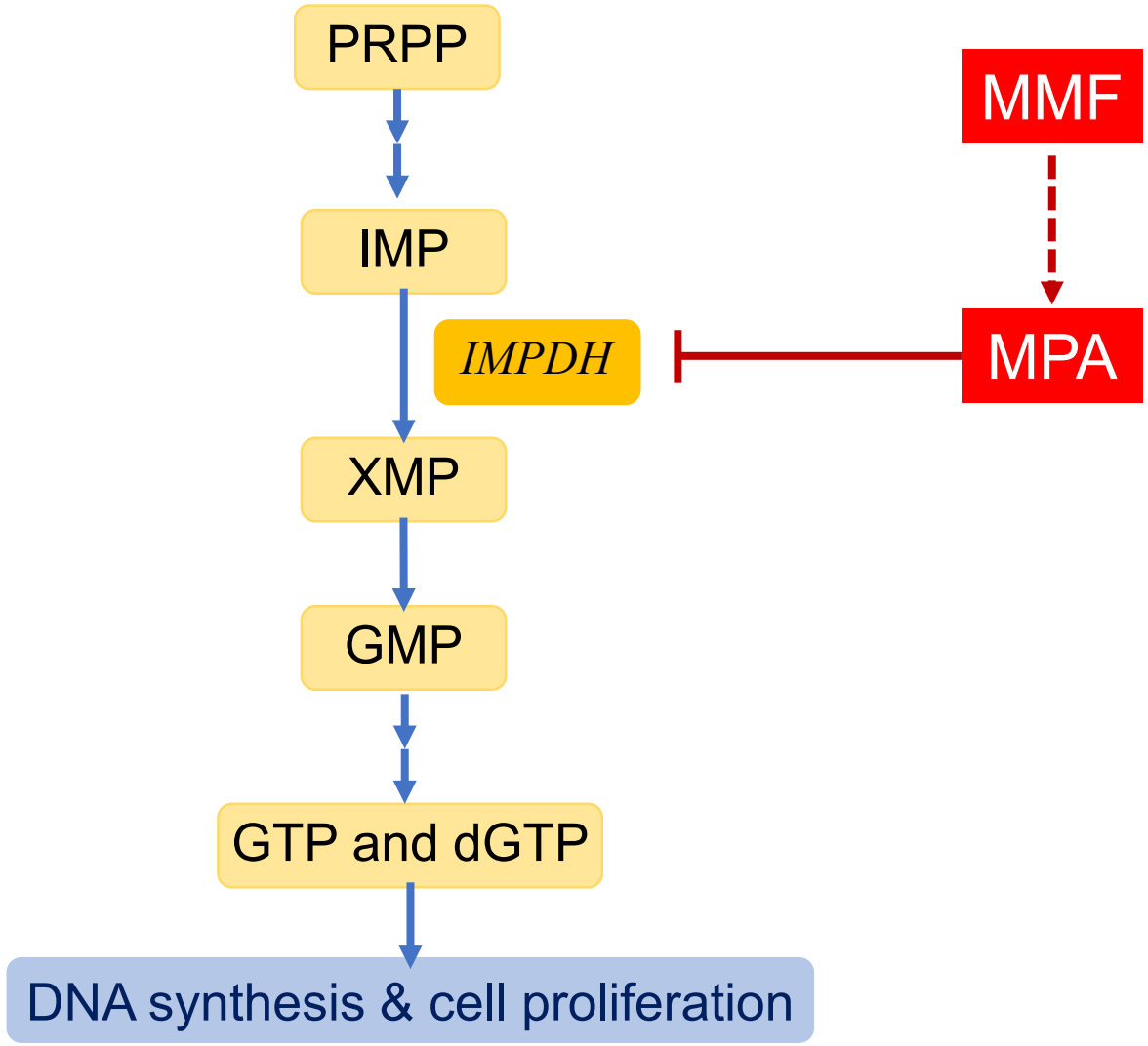
- Active metabolite: mycophenolic acid (MPA)
- Interferes with guanine synthesis via inhibition of inosine monophosphate dehydrogenase (IMPDH) enzyme

Guanine nucleotide and *de novo* pathway



PRPP: 5-**p**hospho**r**ibosyl-1-**p**yro**p**hosphate
IMP: **i**nosine **m**ono**p**hosphate
XMP: **x**anthine **m**ono**p**hosphate
GMP: **g**uanosine **m**ono**p**hosphate
GTP: **g**uanosine **t**ri**p**hosphate
dGTP: **d**eoxy**g**uanosine **t**ri**p**hosphate

Mycophenolate mofetil: *Mechanism of action*



MMF: **m**ycophenolate **mof**etil
MPA: **m**ycop**h**enolic **a**cid

Mycophenolate mofetil (MMF)
Dermatologic uses

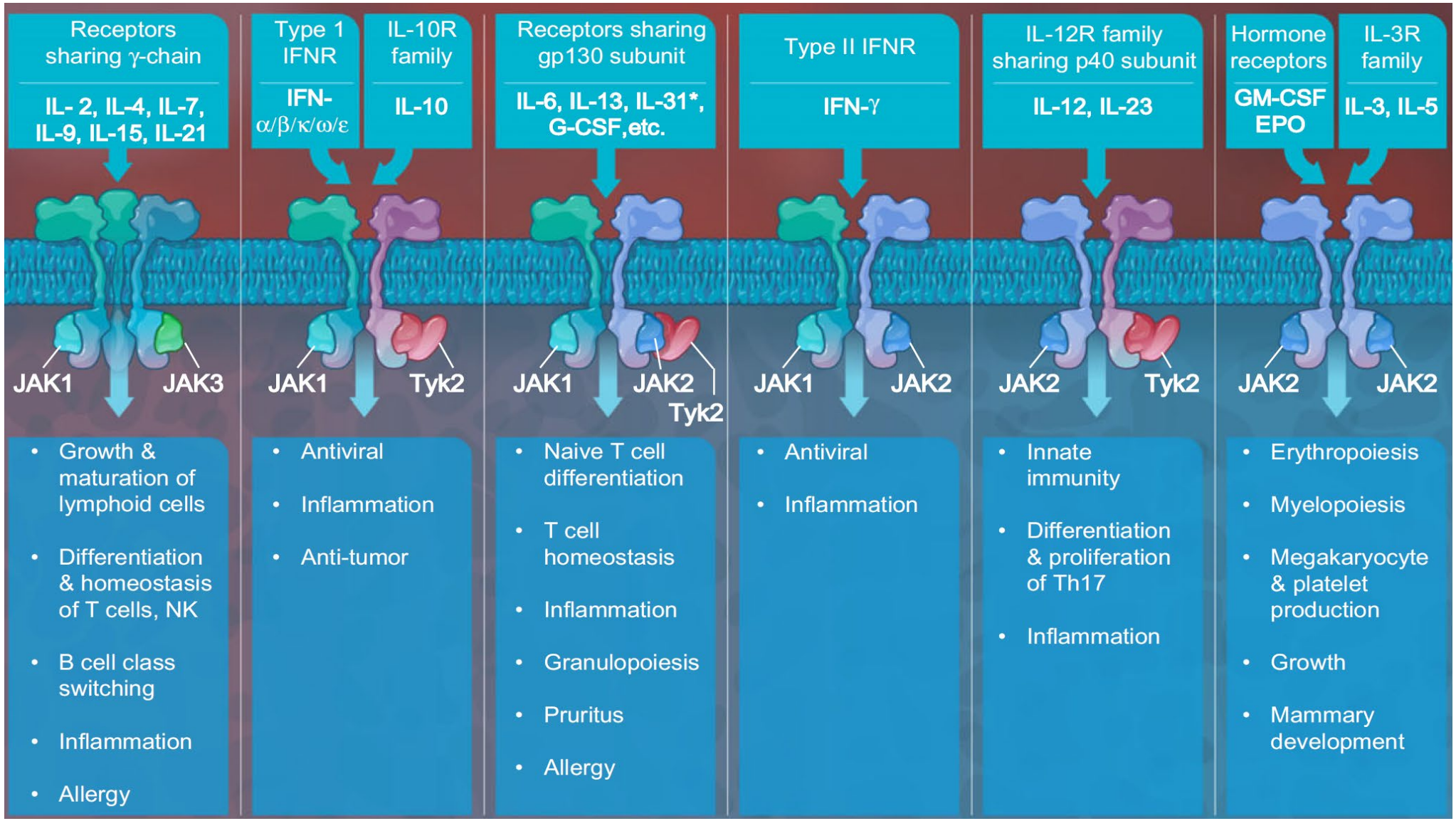
- Canine pemphigus foliacues
- Exfoliative cutaneous lupus erythematosus (ECLE)
- Vesicular cutaneous lupus erythematosus (VCLE)
- Mucocutaneous cutaneous lupus erythematosus (MCLE)
- Epidermolysis bullosa acquisita (EBA)
- **Except** for the dog with **ECLE** (Ferigno, *Vet Dermatol* 2019), MMF was used as an adjunct therapy along with GC, CsA and/or topical tacrolimus

Mycophenolate mofetil (MMF)
Adverse effect

- Diarrhea most common
- In one study, 23/127 dogs (18%) had diarrhea (Fukushima, *J Vet Int Med* 2021)
- Resolved with dose reduction or discontinuation of MMF

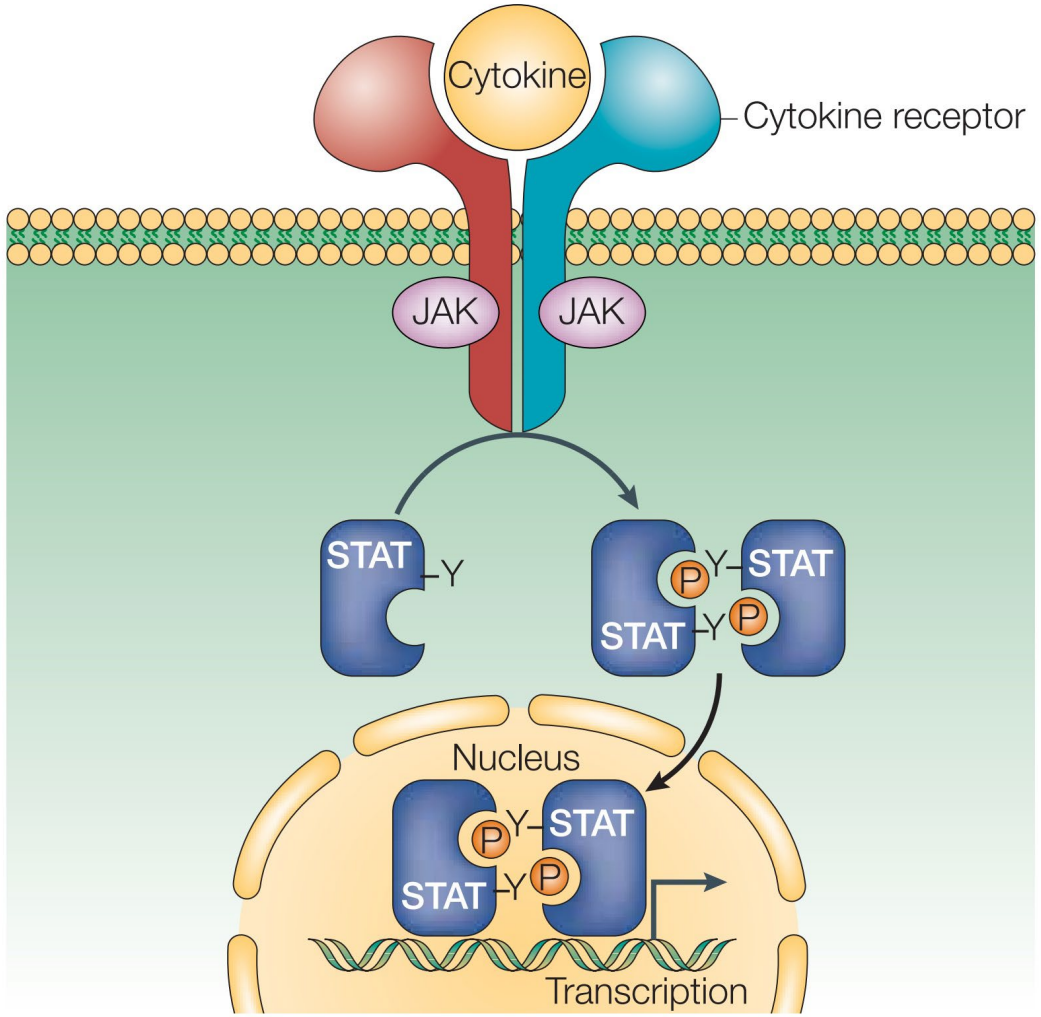
Oclacitinib

- Janus kinase inhibitor (JAKi)
- Four members: JAK1, JAK2, JAK3 and TYK2



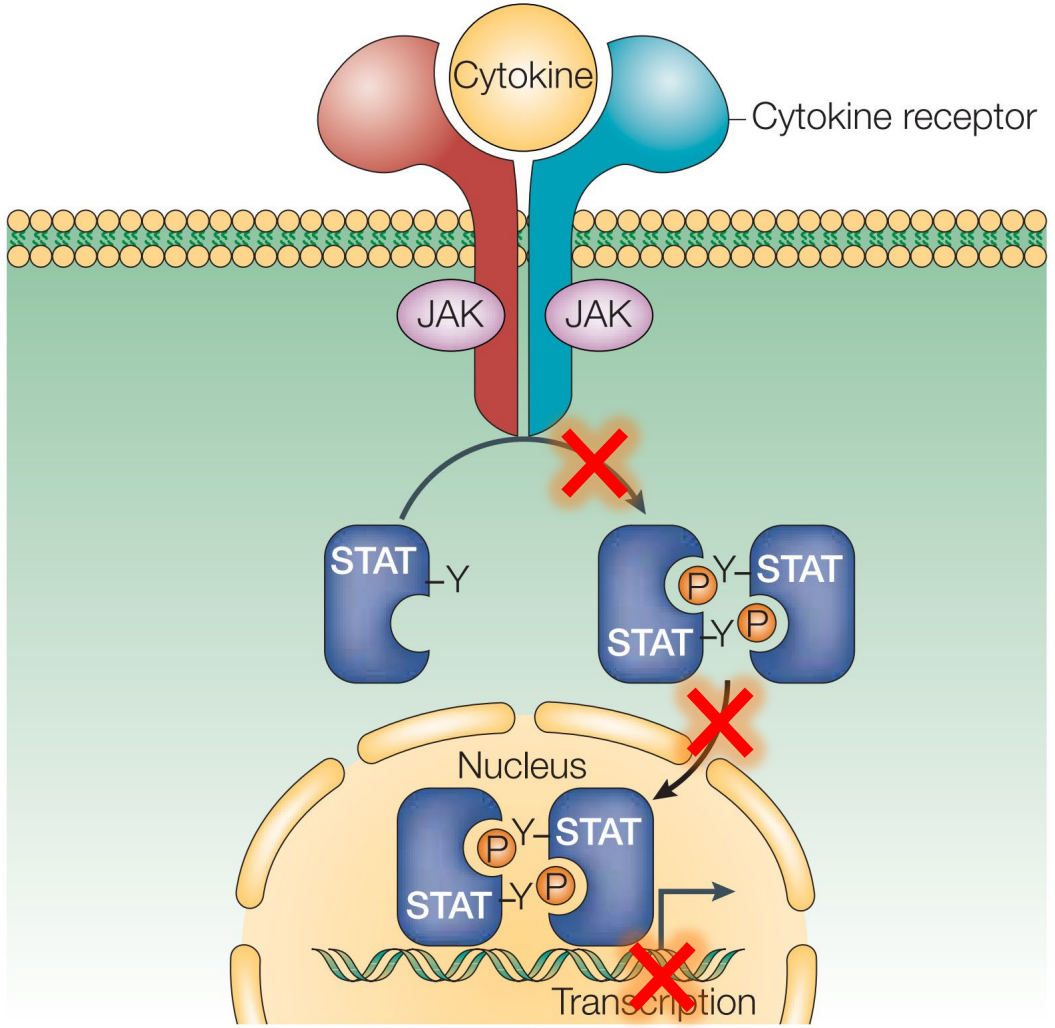
(Gonzales, J Vet Pharmacol Ther 2014)

Cytokines and JAKs



(Shuai, Nature Reviews Immunology 2003)

Oclacitinib: Mechanism of action



✕JAK inhibitor

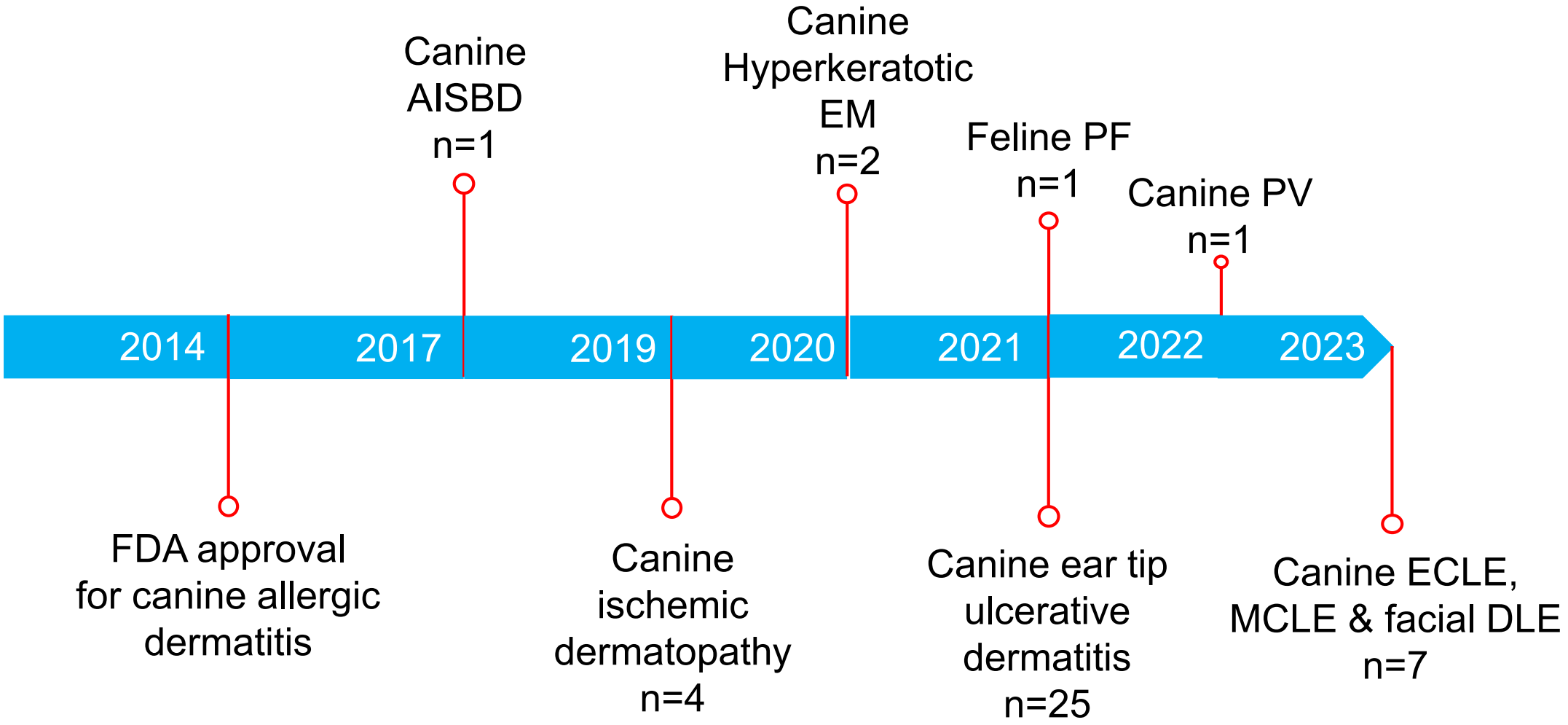
(Modified from Nature Reviews Immunology 2003)

Oclacitinib and immunosuppression

Higher doses result in:

- Reduction in IL-2, IL-15, IL-18 and IFN-γ (Banovic, *Vet Dermatol* 2019)
- Induced apoptosis of canine CD4+ and CD8+ T cells *in vitro* (Jasiecka-Mikołajczyk, *Res Vet Sci* 2018)

Oclacitinib
Dermatologic uses



Oclacitinib
Adverse effect

- Cutaneous papilloma
- Demodicosis
- Bacterial pneumonia in several 6- and 12-month-old dogs

(Zoetis safety studies)

Oclacitinib
Adverse effect

Vet Dermatol 2022; **33**: 149–e42 DOI: 10.1111/vde.13053

Prolonged twice-daily administration of oclacitinib for the control of canine atopic dermatitis: a retrospective study of 53 client-owned atopic dogs

Daria Denti*, Marco Caldin†, Laura Ventura‡ and Michela De Lucia*

- Median treatment duration: 113 days (Range: 21-1277 days)
- Pyoderma, gastrointestinal upset and otitis externa
- Mild neutropenia (n=3), eosinopenia (n=6) and leukopenia (n=2)
- Hypercholesterolemia in 3 dogs

Oclacitinib
Adverse effect

Veterinary Dermatology

CASE REPORT

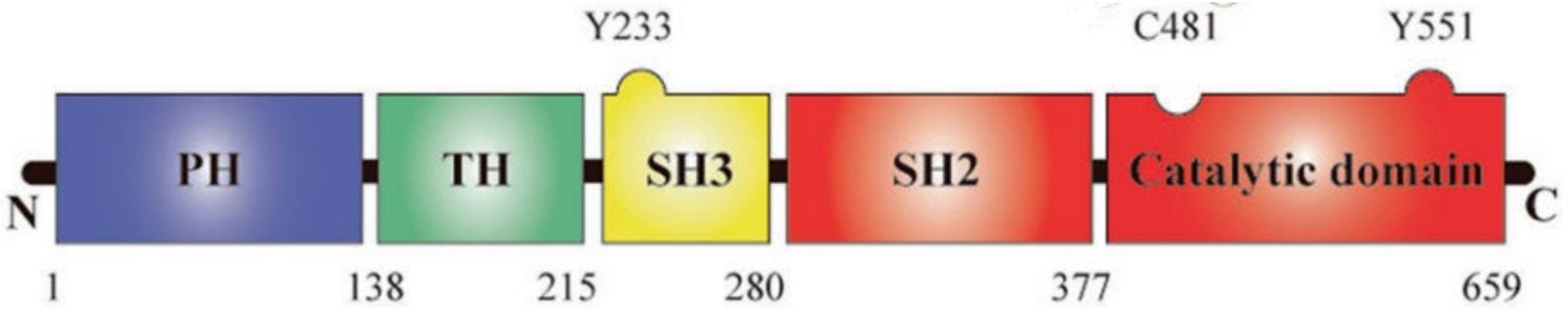
Fatal disseminated toxoplasmosis in a feline immunodeficiency virus-positive cat receiving oclacitinib for feline atopic skin syndrome

Alexandra Moore[1] | Amanda K. Burrows[1] | Richard Malik[2] | Rudayna M. Ghubash[3] | Robert D. Last[4] | Benjamin Remaj[4]

(Vet Dermatol 2022)

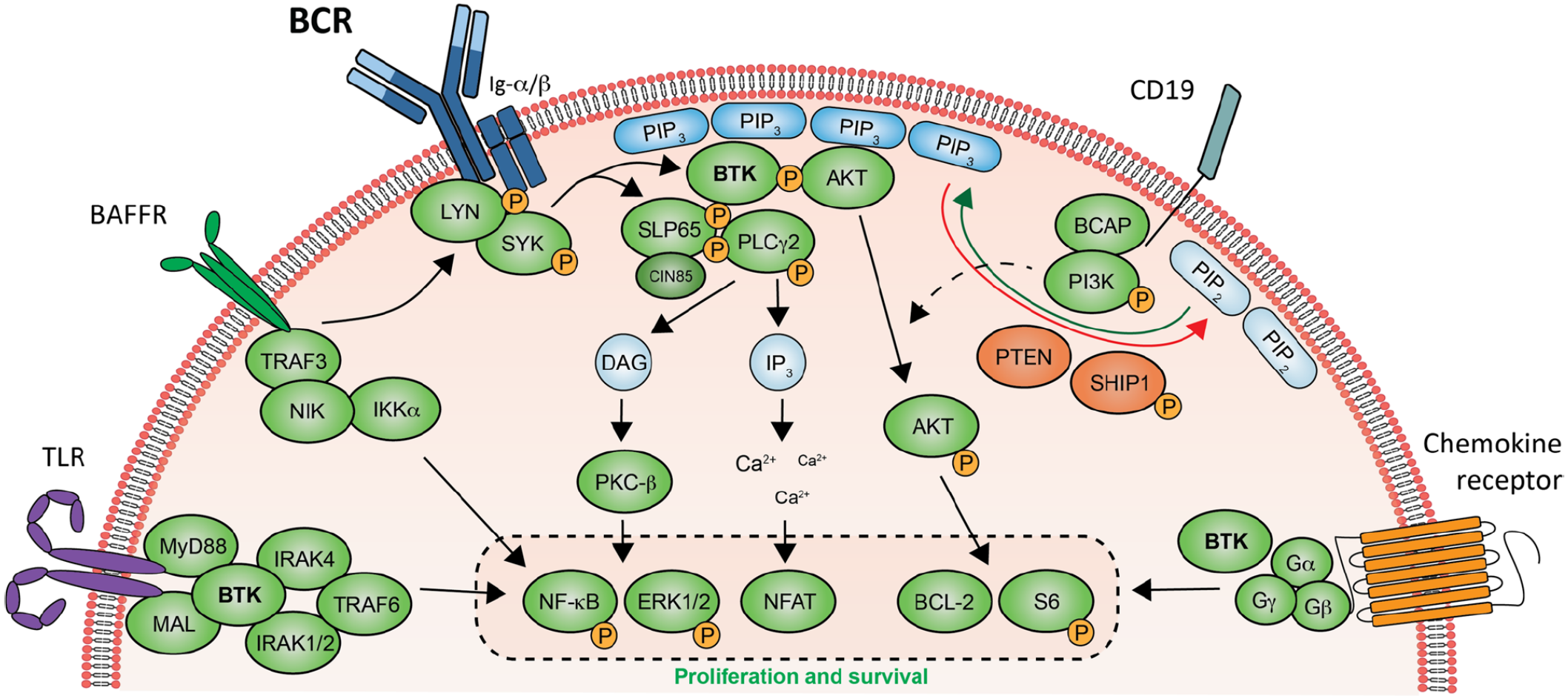
Bruton's tyrosine kinase (BTK)

- Signaling protein
- Links the B-cell receptor (BCR) with B cell proliferation and survival
- Also present in mast cells, monocytes, macrophages, neutrophils and platelets.
- **NOT present in T cells.**



(Modified from Tingyu, Leukemia 2021)

Bruton's tyrosine kinase (BTK)



(Neys, Drugs 2021)

BTK *associated diseases in humans*

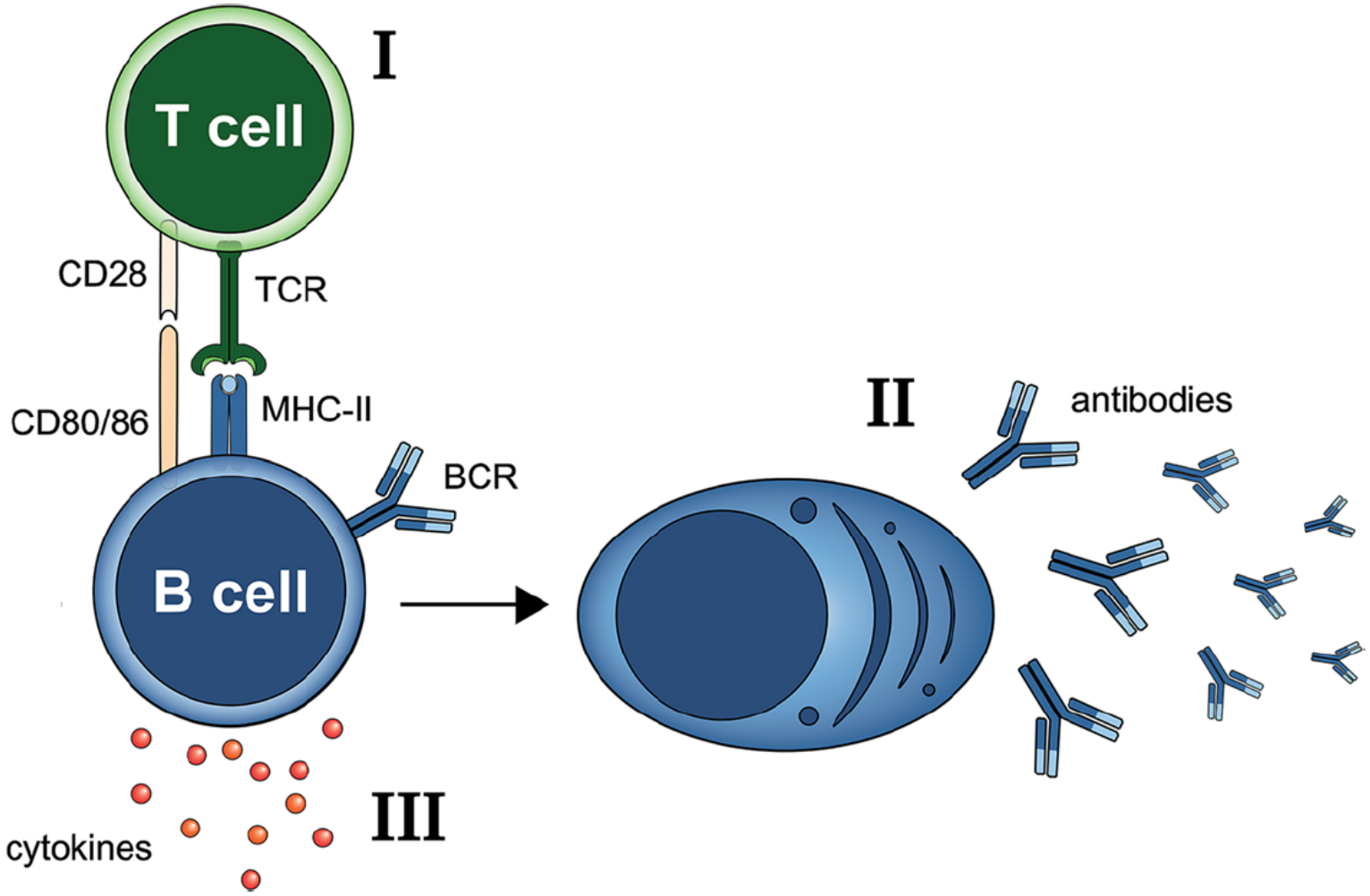
Lymphoproliferative disorders

- Chronic lymphocytic leukemia
- Mantle cell lymphoma
- Waldenström's macroglobulinemia
- Diffuse larger B cell lymphoma

Autoimmunity

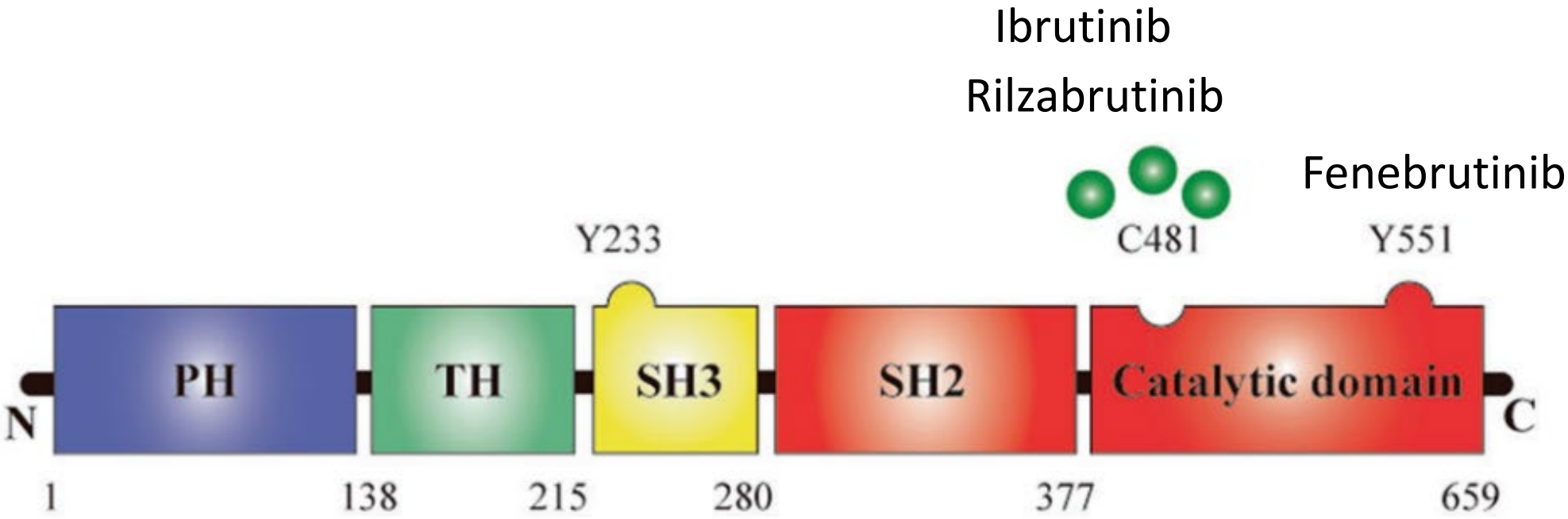
- Systemic lupus erythematosus
- Rheumatoid arthritis
- Multiple sclerosis
- Pemphigus vulgaris

B cells and autoimmunity



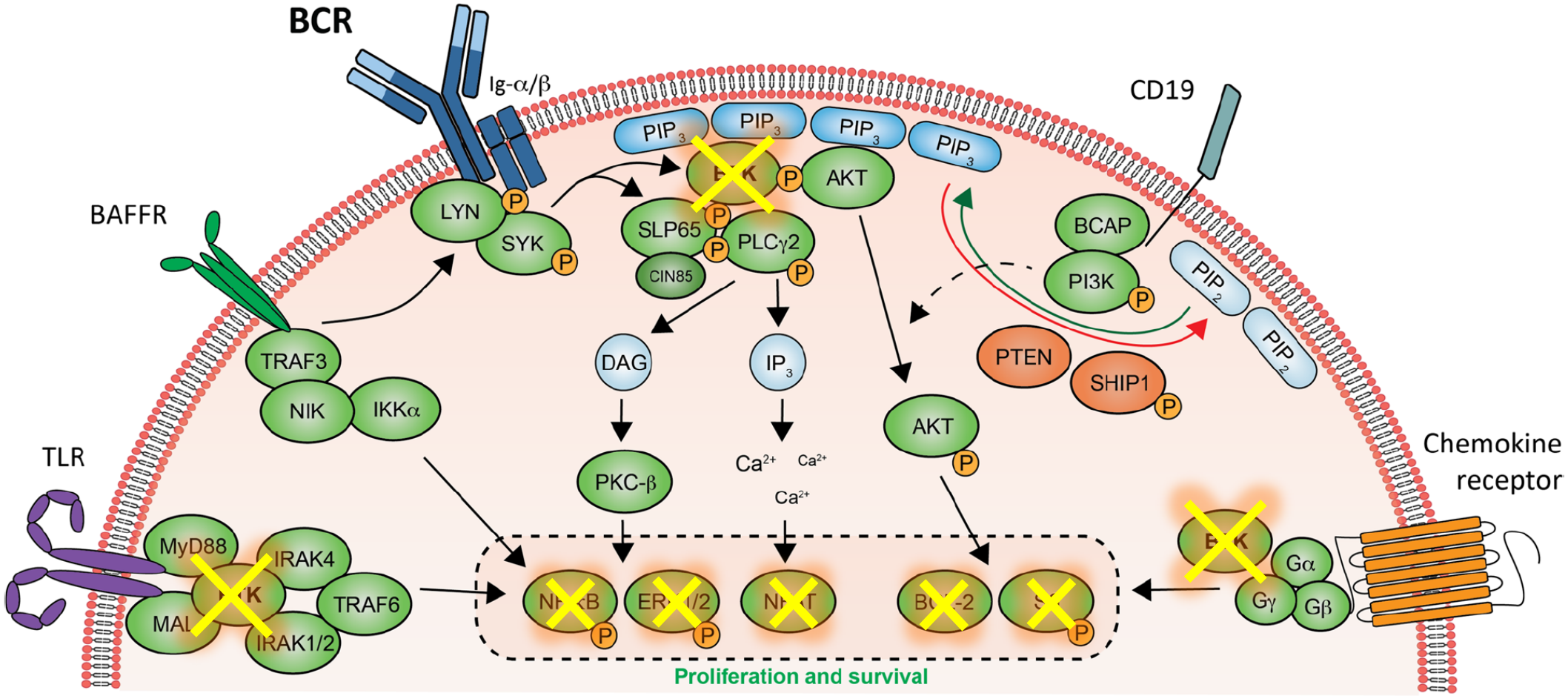
(Modified from Neys, Drugs 2021)

BTK inhibitors
Mechanism of action



(Modified from Tingyu, Leukemia 2021)

BTK inhibitor
Mechanism of action



(Neys, Drugs 2021)

BTK inhibitor and canine pemphigus foliaceus

PRN 473

- 9 dogs
- Dosage: 15mg/kg SID for 20wks
- Outcome:
  - Good: 4 dogs
  - Fair: 2 dogs
  - Poor: 2 dogs
  - Withdrew: 1 dog (relapse of mast cell tumor)

*3 achieved near CR by week 20

PRN1008

- 4 dogs
- Dosage: 15mg/kg SID for 20wks
- Outcome:
  - Good: 3 dogs
  - Fair: 1 dog

(Goodale et al, *Vet Dermatol* 2020)

BTK inhibitor and canine pemphigus foliaceus

PRN 473 (n=9)

Adverse effects:

- Immune-mediated polyarthritis (n=1)
- Peripheral lymphadenopathy (n=1)
- Diarrhea and inappetence (n=1; diagnosed with chronic pancreatitis)
- Mast cell tumor?? (n=1)

PRN1008 (n=4)

Adverse effects:

- Pyometra?? (n=1)

(Goodale et al, *Vet Dermatol* 2020)

Pharmacology of immunosuppressants used in cats and dogs

DR BEN THAM, DVM
DIPLOMATE, ACVD

NAVDF RESIDENT EDUCATION FORUM
8TH MAY, 2023

Virginia-Maryland College of Veterinary Medicine

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