



Lymphome de type MALT du tube digestif : aspects microbiologiques et immunologiques

Pr Philippe Lehours
INSERM U1053 UMR BaRITOn
Université de Bordeaux, France

Helicobacter pylori and gastric cancer

➤ Gram- bacterium, colonizes the human stomach (50% of the world)

➤ Isolated in 1982 (Warren & Marshall, Lancet 1984)

➤ 2005:



« for the role of *H. pylori* in gastritis and ulcer disease »

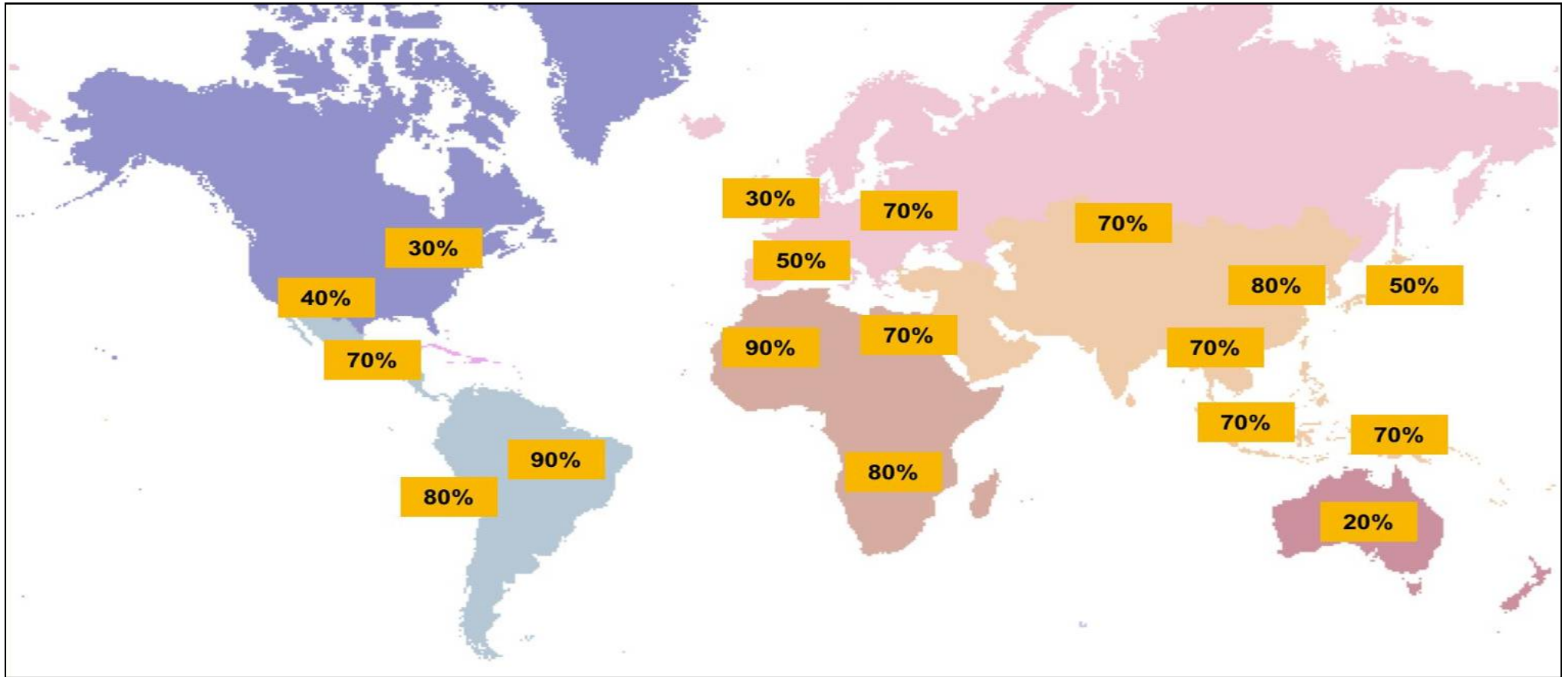


➤ 1994 : class I carcinogen by IARC/WHO



Epidemiology of *Helicobacter pylori* infection

H. pylori chronic infection: prevalence in the world



Its prevalence in developed countries has declined throughout the twentieth century, reflecting the gradual improvement of socio-economic conditions.

Possible vehicles of *H. pylori* transmission

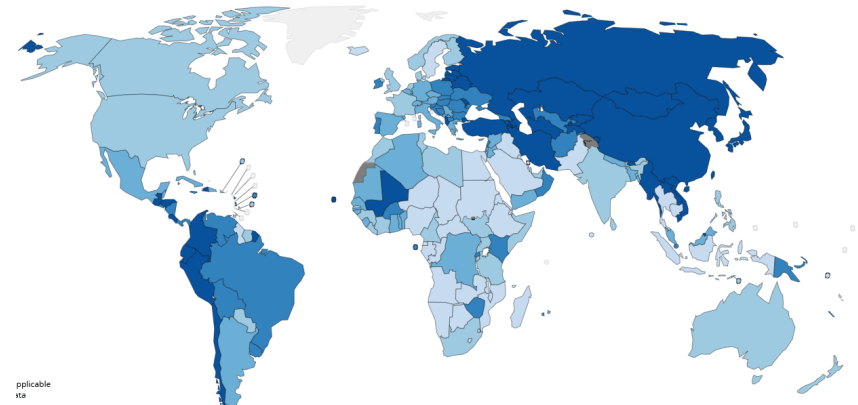
- Vomit
- Saliva
- Faeces

routes of transmission: faecal-oral, oral-oral or gastro-oral

Gastric adenocarcinoma

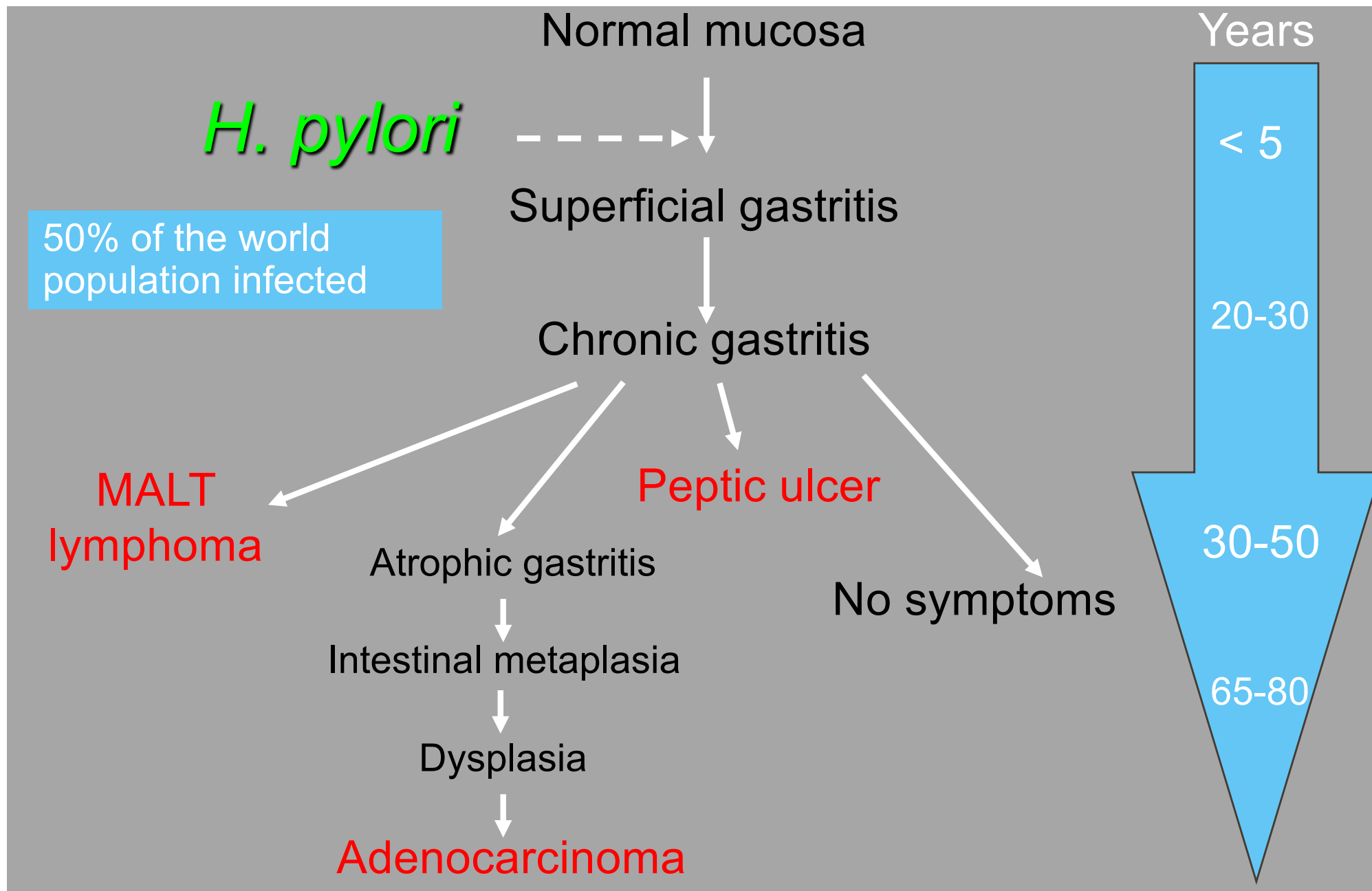
→ Epidemiological data:

- 3rd cause of mortality by cancer worldwide
- >600 000 new cases/year
- mean age: 70 years old



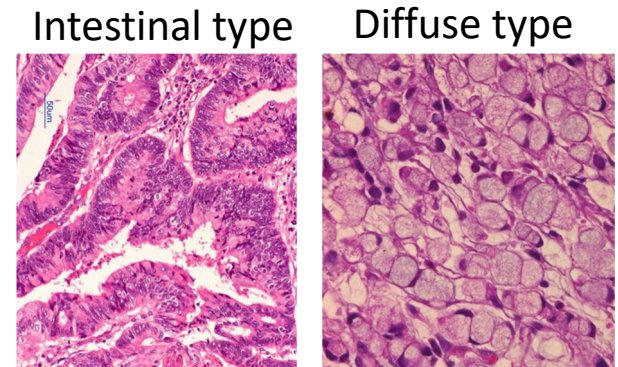
Age-standardized gastric cancer incidence rates
(Globocan, 2018)

Natural history of *H. pylori* infection



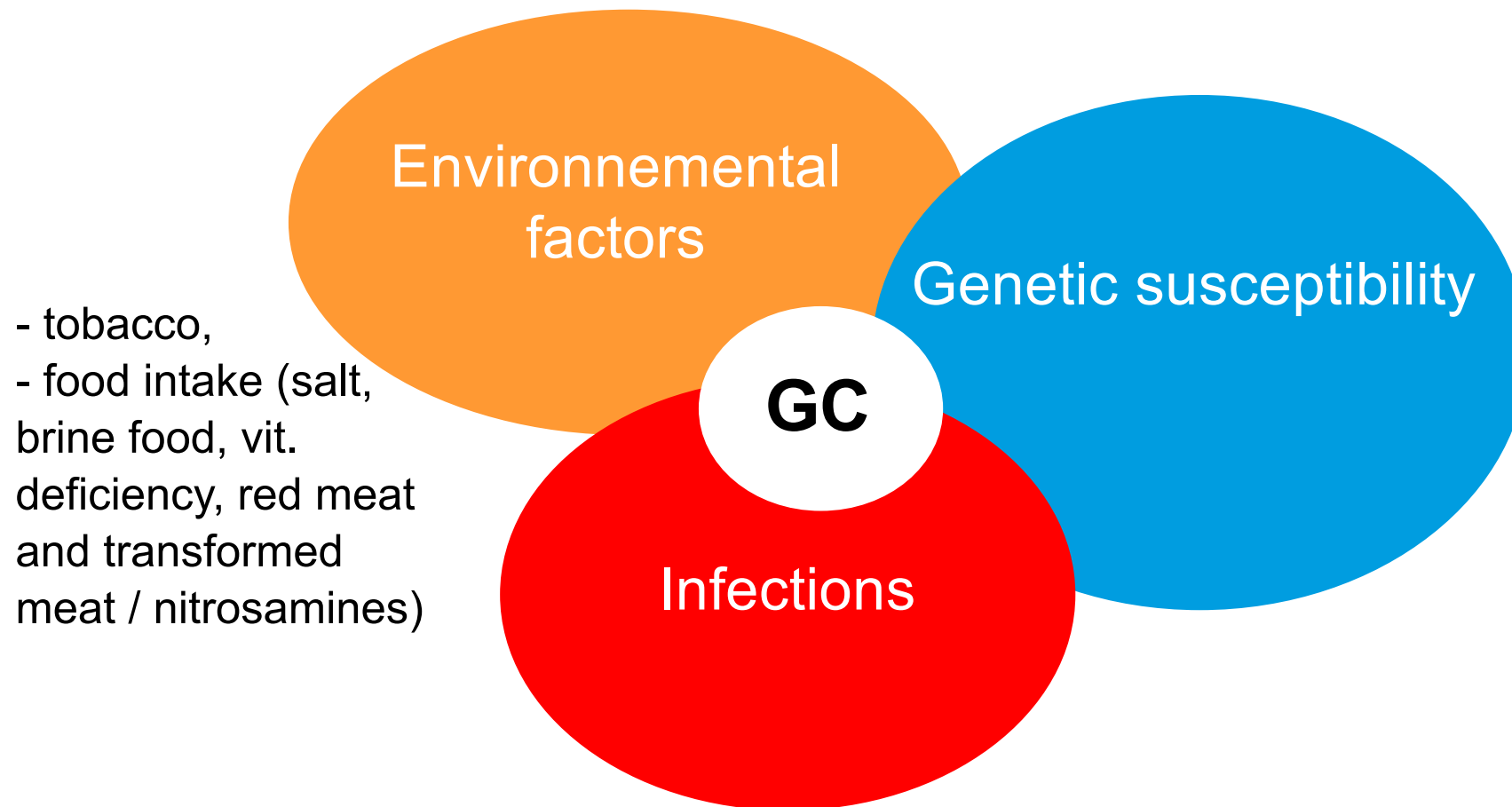
Gastric adenocarcinoma

- A cancer of bad prognosis:
 - survival rate after 5 years < 20%
 - histological (intestinal vs diffuse) and molecular (4 main classes, TCGA 2014) classifications
 - surgery, chemo/radiotherapy,
 - no specific therapy (except for Her2+ cases)
- Multifactorial disease:
 - *H. pylori* infection
 - environmental factors (tobacco, diet...),
 - genetic susceptibility (*cdh1* mutations, Lynch syndrome...)



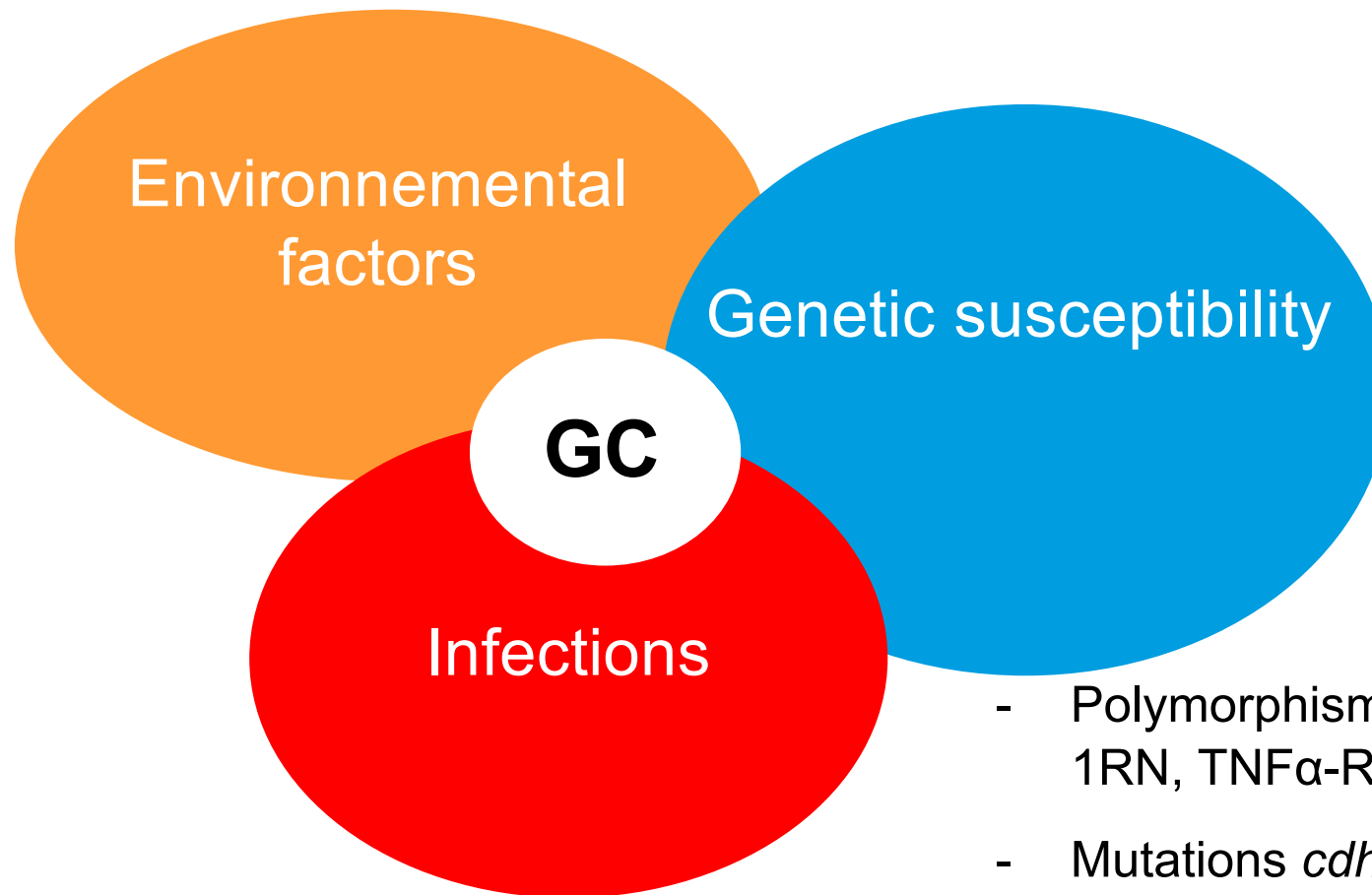
Gonzalez, Mégraud *et al.*
Ann. Oncol. 2012

Gastric Cancer is a multifactorial disease



Buiatti et al., Int J Cancer 1990, 45: 896-901

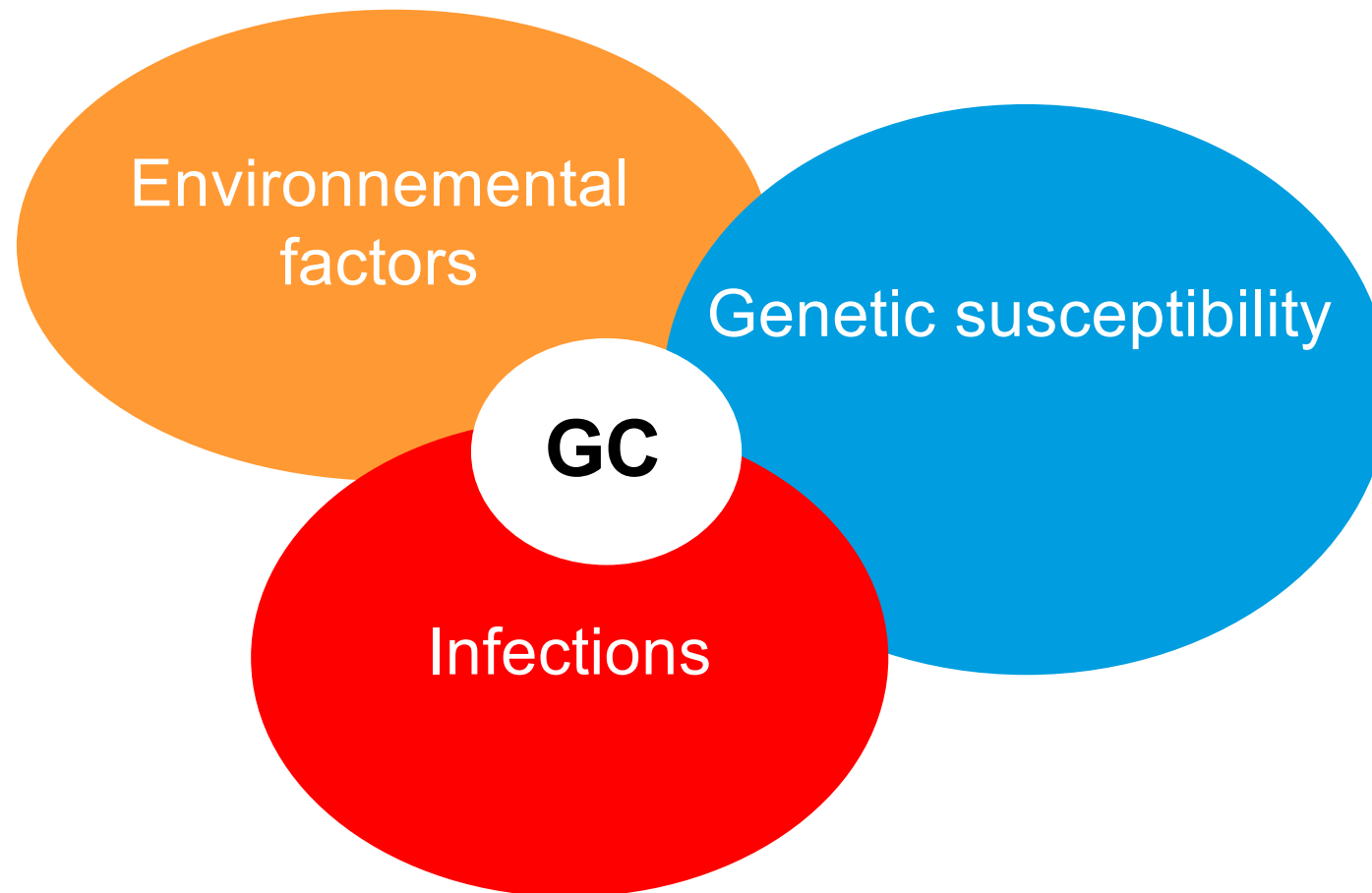
Gastric Cancer is a multifactorial disease



- Polymorphisms IL-1 β , IL-1RN, TNF α -R, IL-8, IL-10
- Mutations *cdh1*
- Lynch syndrome (MMR genes mutations)

Gastric Cancer is a multifactorial disease

→ Gastric Cancer and infections



- *H. pylori* infection associated to >93% of non-cardia GC (Gonzalez *et al.* Ann. Oncol. 2012)
- ~10 % of cases associated to EBV (Murphy *et al.*, Gastroenterology 2009)

Effect of an antibiotic eradication on gastric cancers

→ Gastric MALT lymphoma

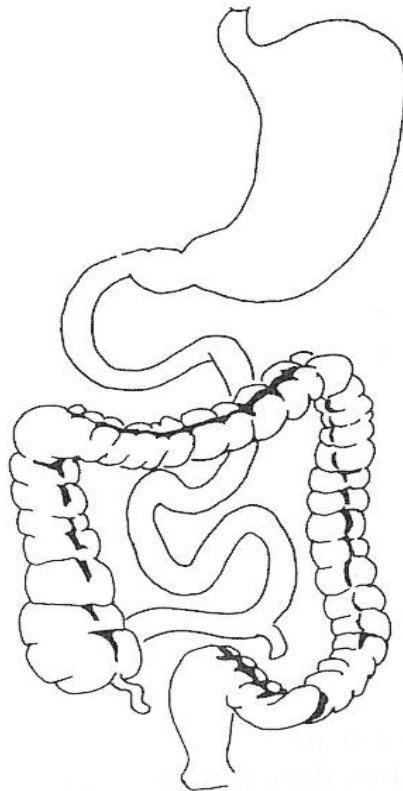
- › regression of lymphoma in 70 to 80% of patients treated for *H. pylori*
- › 1st cancer treated with antibiotic therapy!
- › appox. 600 cases/y in France

→ Adenocarcinoma = few data

- › decreased number of cancers in patients with pre-cancerous lesions
- › large-scale trial underway in China (Linqu Couty Trial)
- › appox. 6000 cases/y in France

Lymphomas

- Nodal lymphomas (75%)
- Extra-nodal lymphomas (25%)
 - Digestive localization (30-40%)



Stomach 79%

Small intestine 17%

Colon 4%

Gastric MALT (Mucosa Associated Lymphoid Tissue) lymphoma (GML)

- Incidence in Europe: 0.29 / 100 000
- Mainly after 50 years old, mostly in men
- Indolent disease, no diffusion to other organs
- Monoclonal B cell proliferation organized in a MALT-type tissue

Gastric MALT lymphoma (GML)

- Association and temporal relationship proved with *H. pylori* infection
- Tumor regression in 70 to 80% cases after *H. pylori* eradication

(Parsonnet *et al.*, 1994)

(Wotherspoon *et al.*, 1991 ; Nakamura *et al.*, 1997)

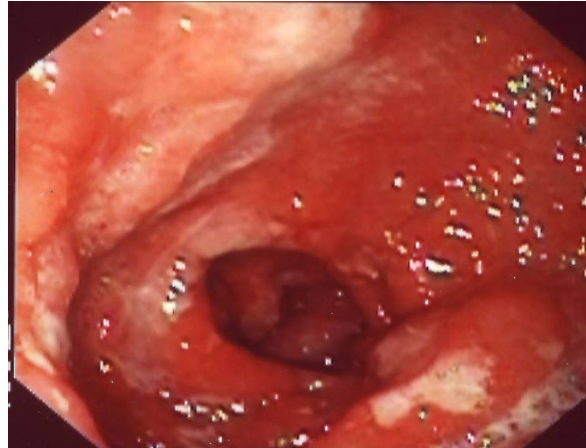


Gastric MALT lymphoma: clinic presentation

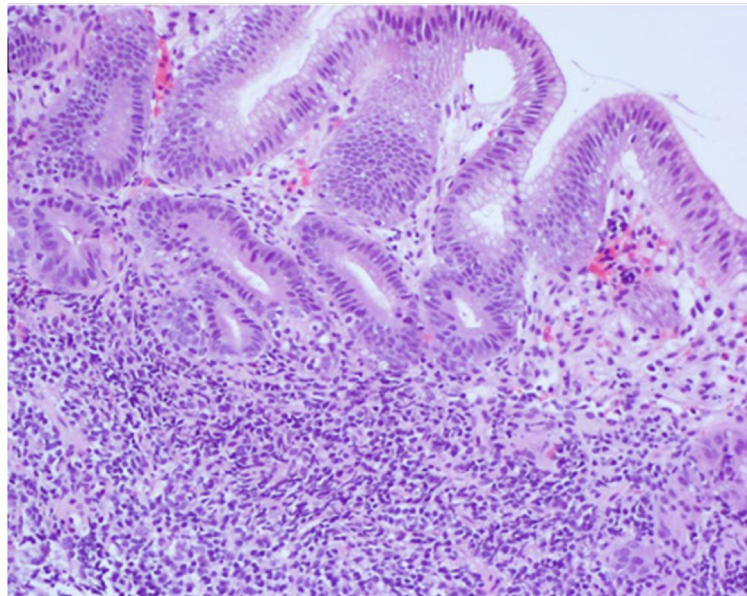
- Non specific epigastric pain (90%)
 - Dyspepsia, bleeding, anemia
 - Endoscopic aspect
 - › gastritis
 - › ulcer
 - › nodes
 - › non specific aspect
- ⇒ Histological diagnosis
(+ *H. pylori* status)

Endoscopic and histological aspect

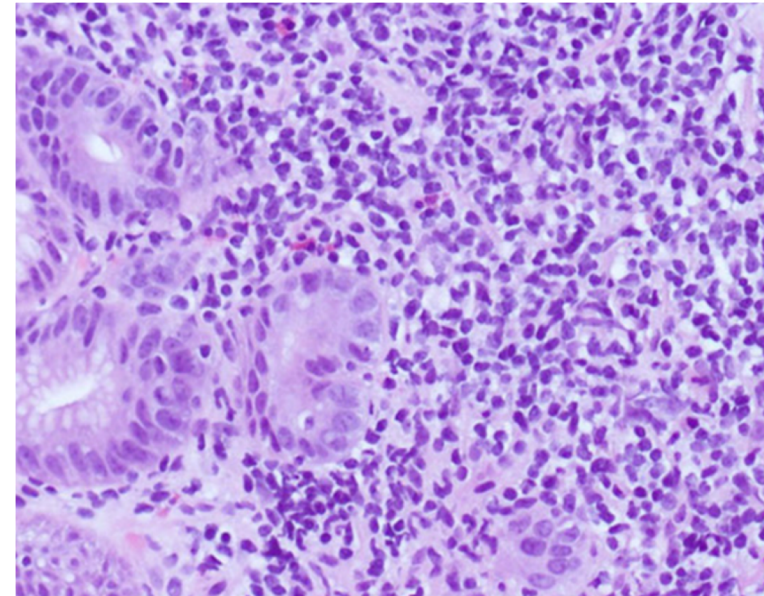
→ Endoscopic aspect



→ Histological aspect



Centrocyte-like cells infiltration

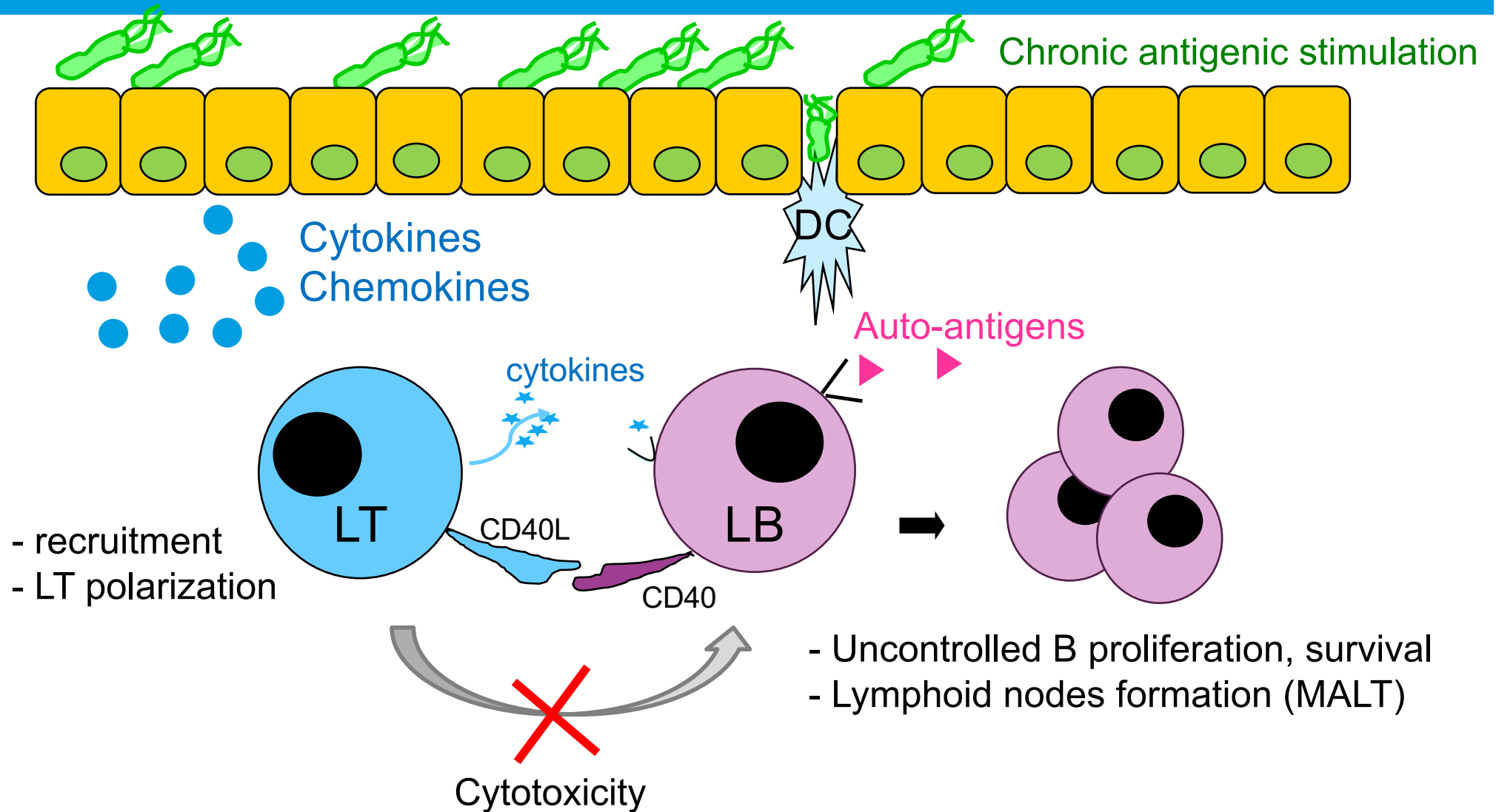


Lymphoepithelial lesions (LEL)

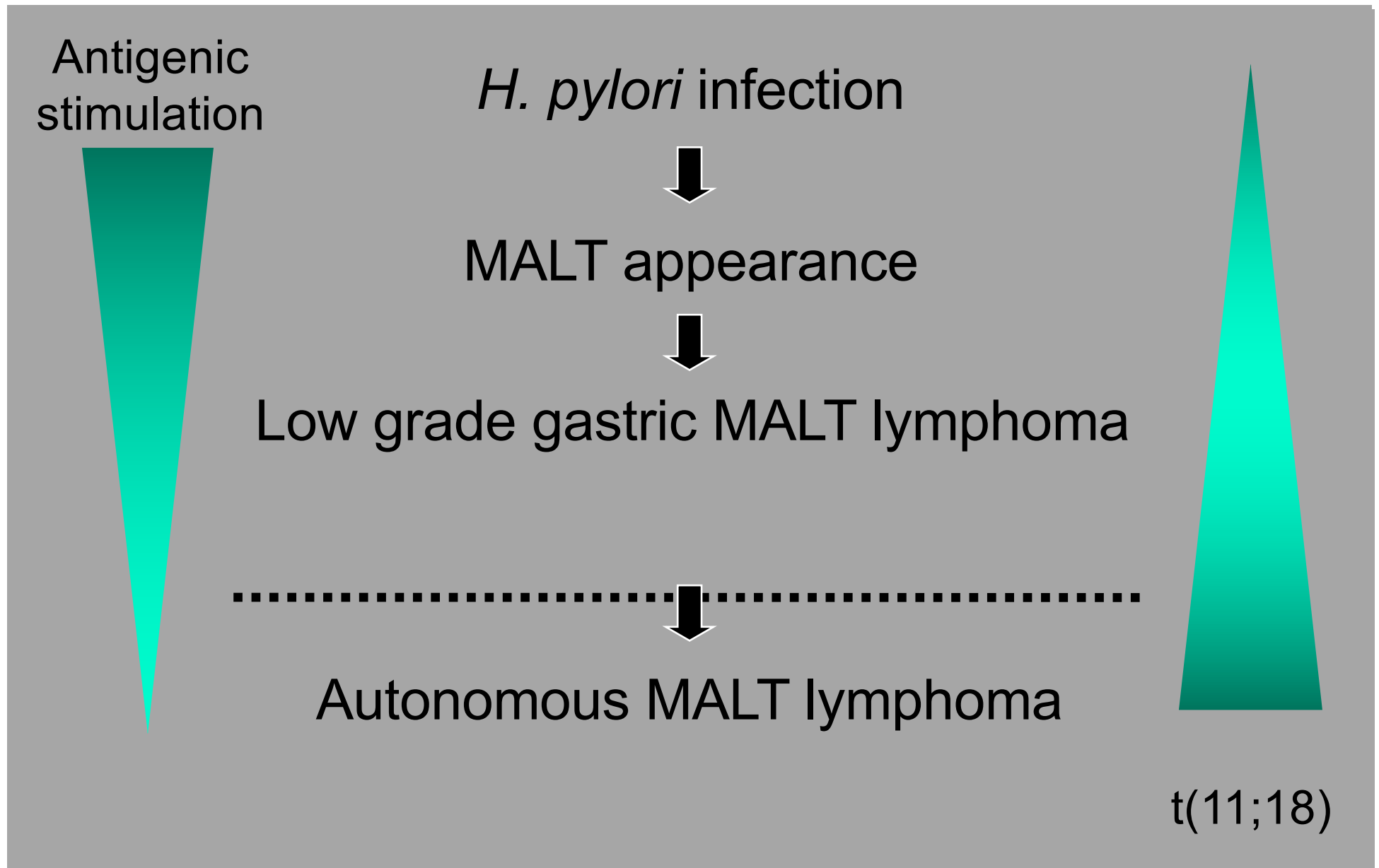
(Hu *et al.*, 2016)



Inflammatory response and GML



Gastric MALT lymphoma cascade



Gastric MALT lymphoma: a multifactorial disease

Environmental factors

Host factors



Gastric MALT lymphoma



Bacterial factors

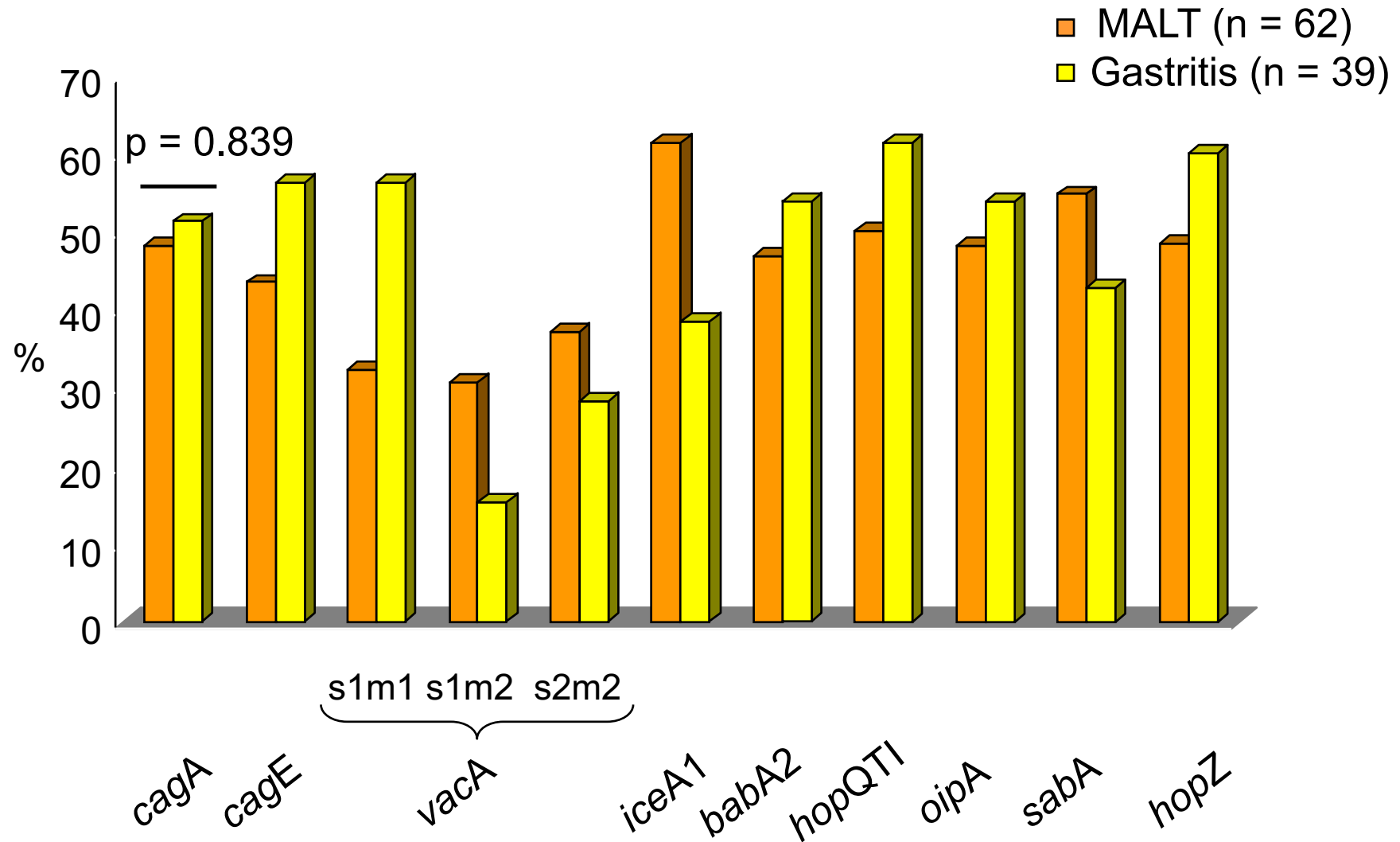
Do *H. pylori* strains with a high risk of
gastric MALT lymphoma exist ?

Gastric MALT lymphoma strain collection

- Collection of 62 *H. pylori* strains isolated from low grade gastric MALT lymphoma:
 - 43 obtained from two multicentre French studies: GELD (Dr. A. Ruskone) and GELA (Pr. J.C. Delchier)
 - 19 obtained from Germany (Dr. A. Morgner)

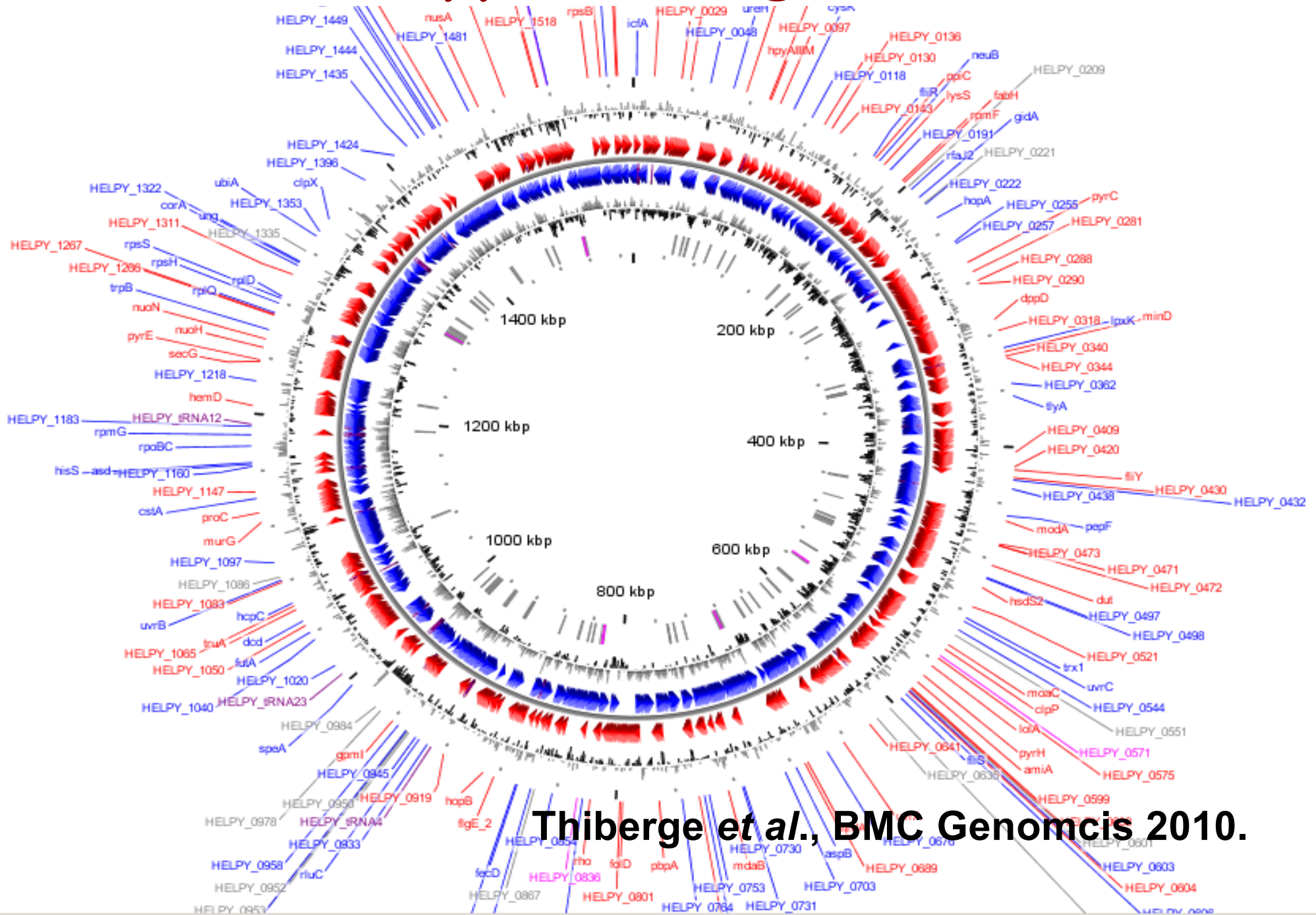
Virulence factors

Evaluation of the main *H. pylori* virulence factors in MALT strains



Genomics of MALT strains

Thiberge *et al.*, BMC Genomics 2010.



Sequencing of one gastric MALT lymphoma isolate: the B38 genome

(collaboration with the Pasteur Institute, Paris, France)

- Smallest *H. pylori* genome described to date (1, 576, 758 bp)
- Lacks the genes encoding major virulence factors: absence of *cagPAI*, *babB*, *babC*, *sabB*, and *homB*
- Remnant prophage sequences

Thiberge *et al.*, BMC Genomics 2010.

Immunology

Gastric MALT lymphoma: a multifactorial disease

Environmental factors

Host factors



Gastric MALT lymphoma



Bacterial factors

Inflammatory and immunological context?

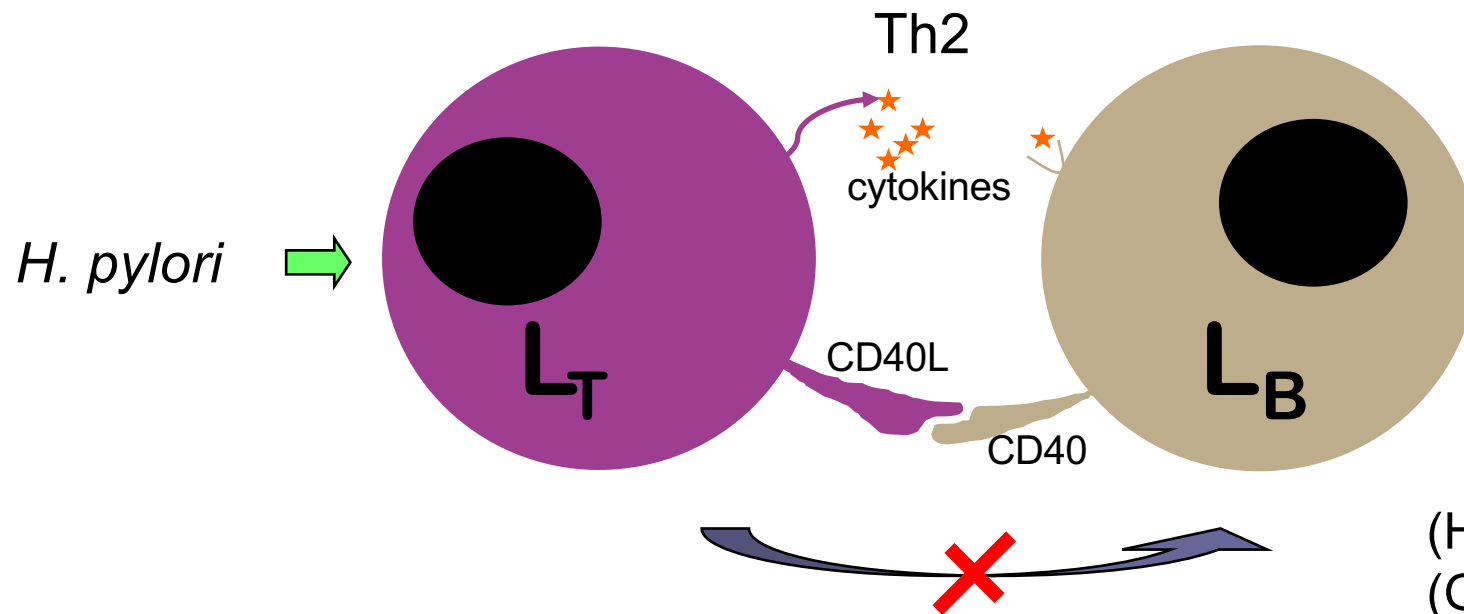
Cell response from low-grade B-cell gastric MALT lymphomas to *H. pylori*

Hussell T, Isaacson PG, Crabtree JE, Spencer J. The Lancet 1993

1. Lymphocytes only: no proliferation
2. Lymphocytes + heat killed *H. pylori*: B-cell proliferation
3. B lymphocytes only + heat killed *H. pylori*: no proliferation

⇒ lymphocyte proliferation was specific to *H. pylori*

Modulation of lymphocyte proliferation and lymphoma



Impaired cytotoxicity of T lymphocytes

(Hussel *et al.* 1996)
(Greiner *et al.*, 1997)
(D'Elis *et al.*, 1999)
(D'Elis *et al.*, 2004)
(Schmees *et al.*, 2007)
(Ferrand *et al.*, 2008)

Mouse models of gastric MALT lymphoma

Animal models for GML?

- Which species?
- Which strains?
- Which hosts?
- Do these models reproduce human disease?

Gastric MALT lymphoma can occur during natural infection in animals

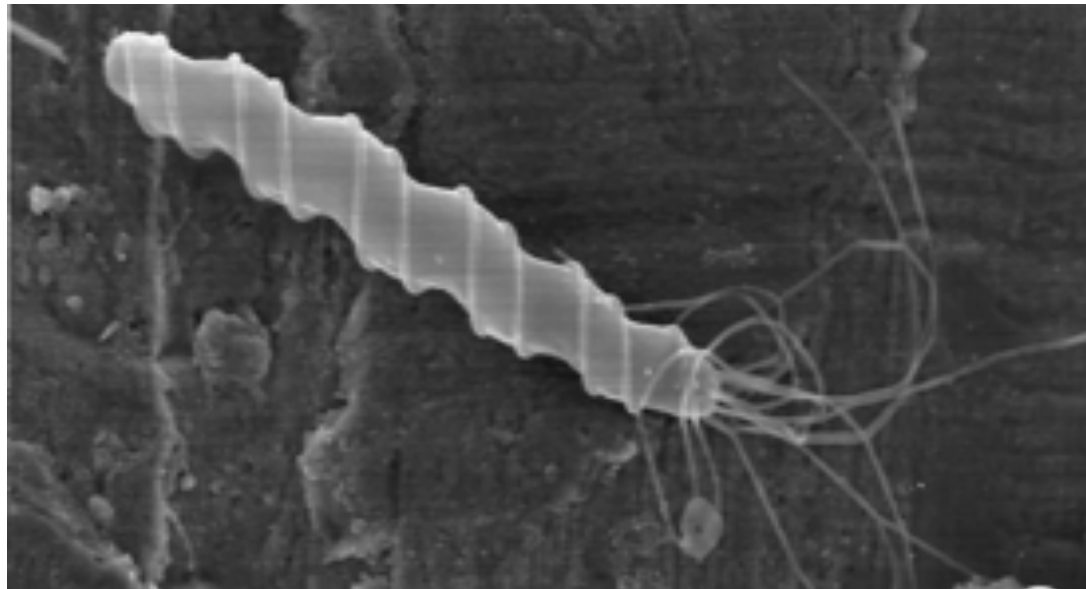
- *Helicobacter mustelae*-associated GML in ferrets (Erdman SE *et al.* AM J Pathol 1997)
 - 2 ferrets with low grade GML and 2 with high grade GML



- *Helicobacter sp* infection in 12-year old cat (Fry DR *et al.* Aust Vet Practit 2003)
 - gastric lymphoma with a partial remission after chemotherapy and antibiotic treatments
 - no species identification



Helicobacter felis



Animal models of gastric MALT lymphoma infected by *Helicobacter felis*

BALB/c



H. felis strain CS1(ATCC 49179)

- isolated from a cat stomach
(Lee *et al.* Infect Immun 1988)
- adapted to mouse stomach
(Lee *et al.* Gastroenterology 1990)

Enno A *et al.* Am J Pathol 1995

Enno A *et al.* Am J Pathol 1998

Mueller A *et al.* Int J Cancer 2001

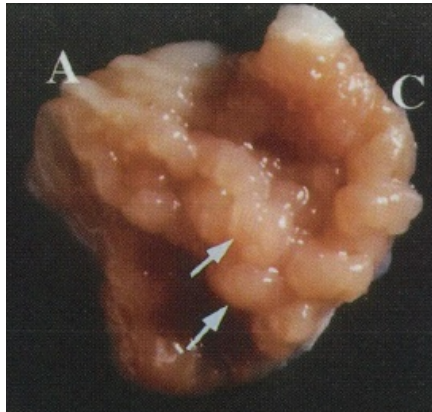
Mueller A *et al.* PNAS 2003

Mueller A *et al.* Am J Pathol 2005

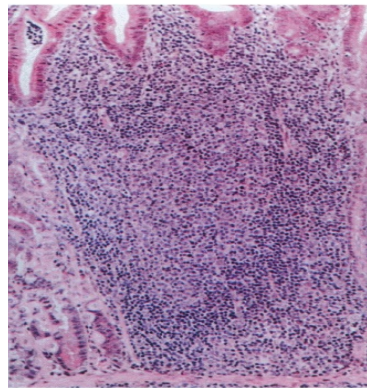
Craig VJ *et al.* Leukemia 2010

Animal models of gastric MALT lymphoma infected by *Helicobacter felis* (2)

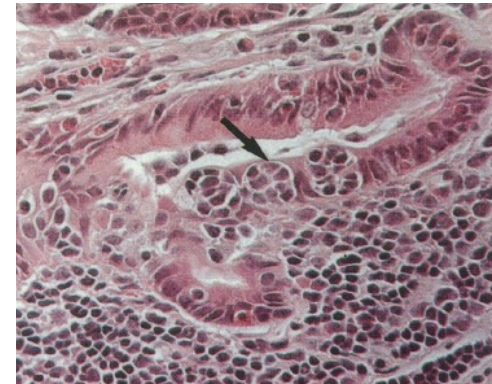
BALB/c mice infected up to 26 months



nodular aspect



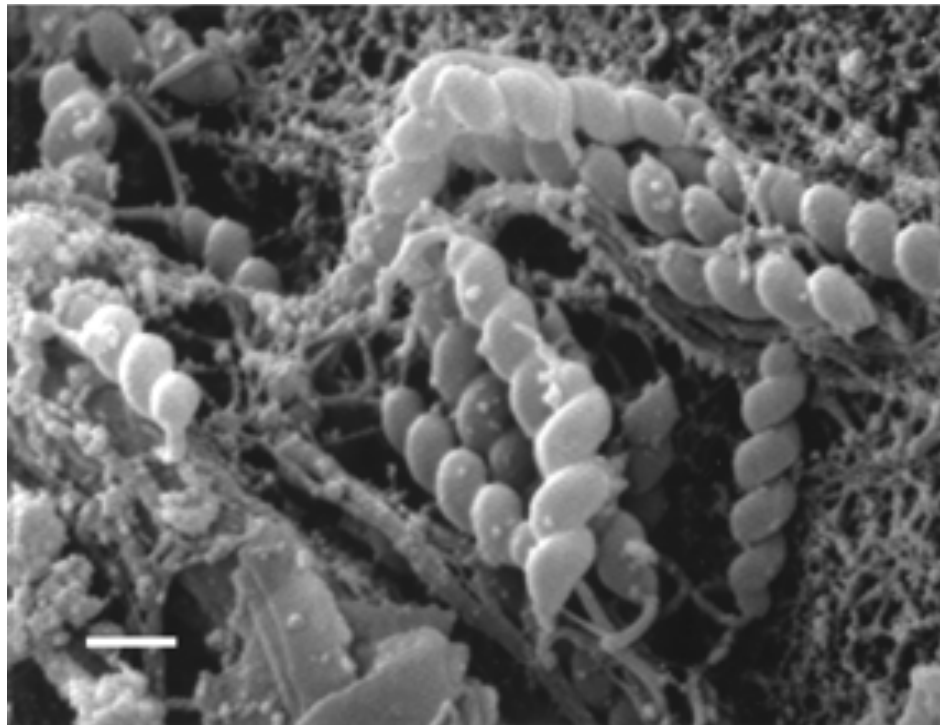
lymphoid aggregates
64%



lymphoepithelial lesions
25%

- earliest change in terms of lymphoid proliferation was seen at 22 months post-infection
- B-cell nature confirmed by IHC
- small number of T-cells (mainly CD4+)

Helicobacter heilmannii



Animal models of gastric MALT lymphoma infected by *Helicobacter suis*

H. heilmannii type 1 or *Helicobacter suis*

Mongolian gerbils



C57Bl6



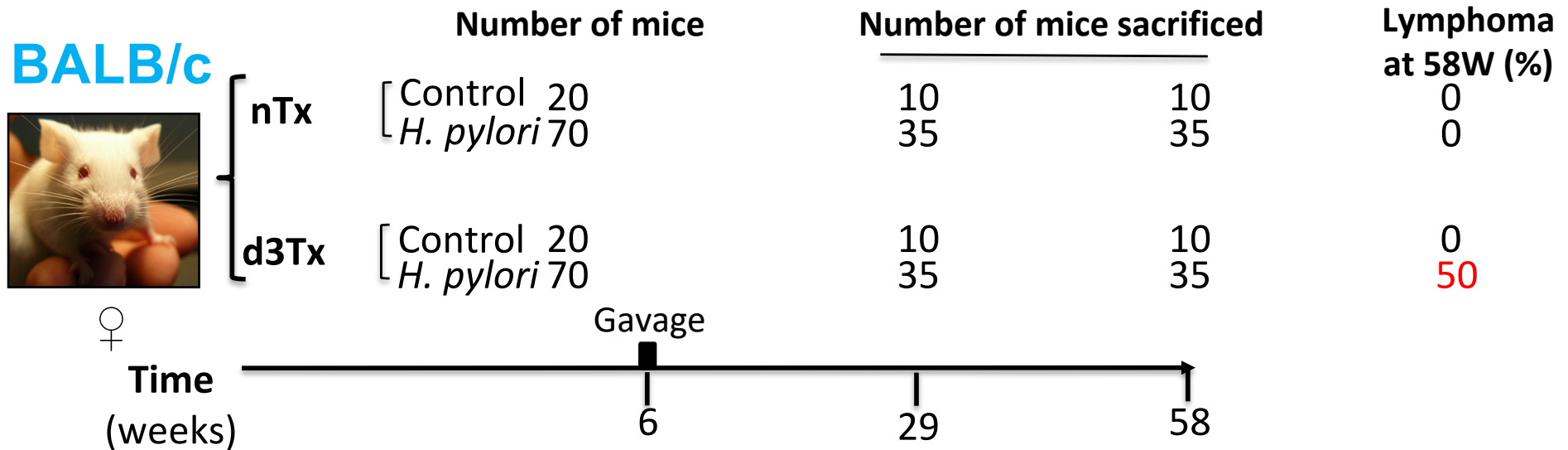
BALB/c



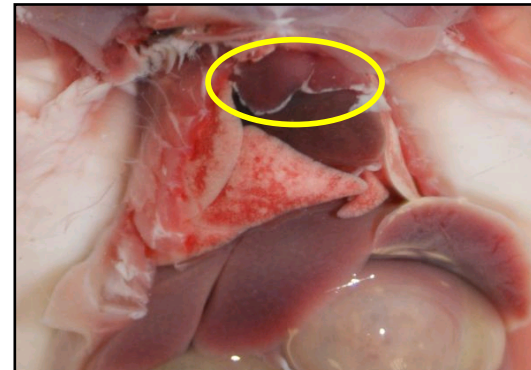
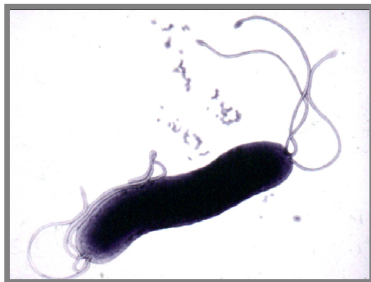
GML-like lesions were found 8 months post-infection in gerbils only

→ influence of the host and of the isolate

Animal models of gastric MALT lymphoma infected by *Helicobacter pylori*



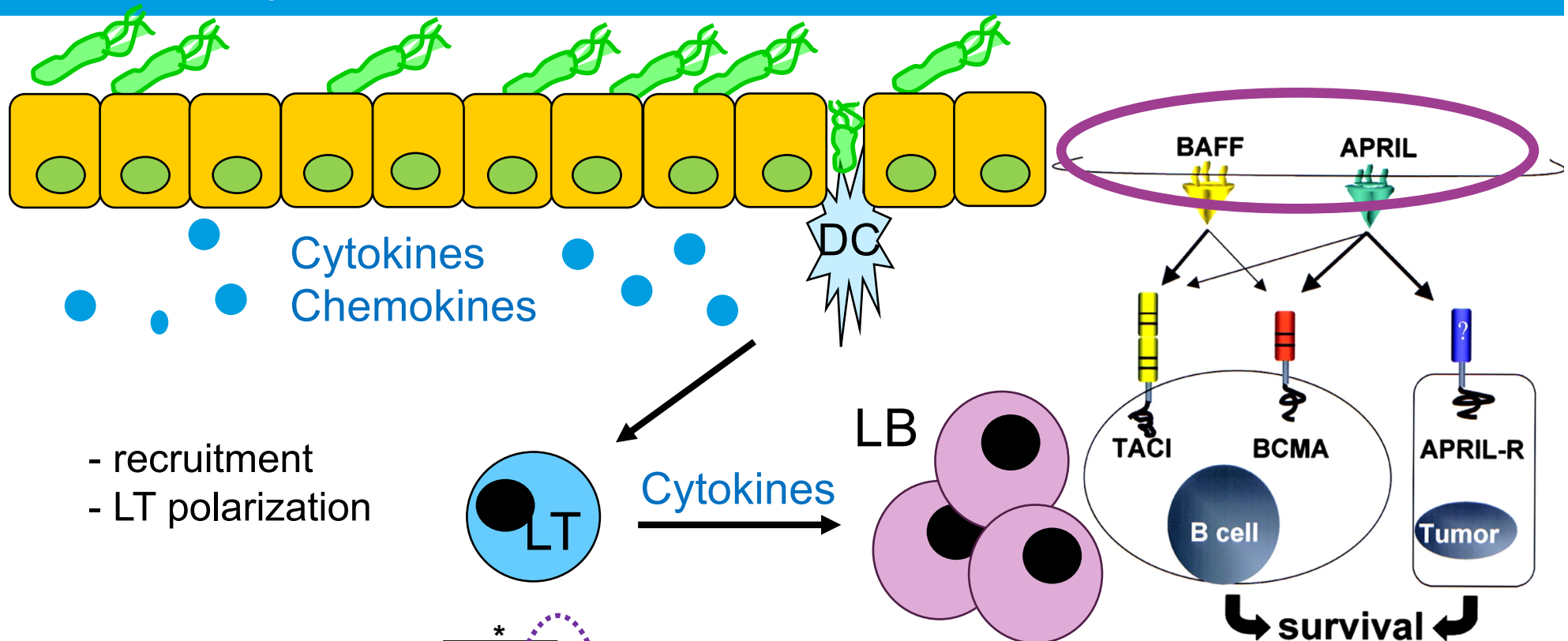
H. pylori strain B47 (GML-associated strain, *cagPAI* negative)



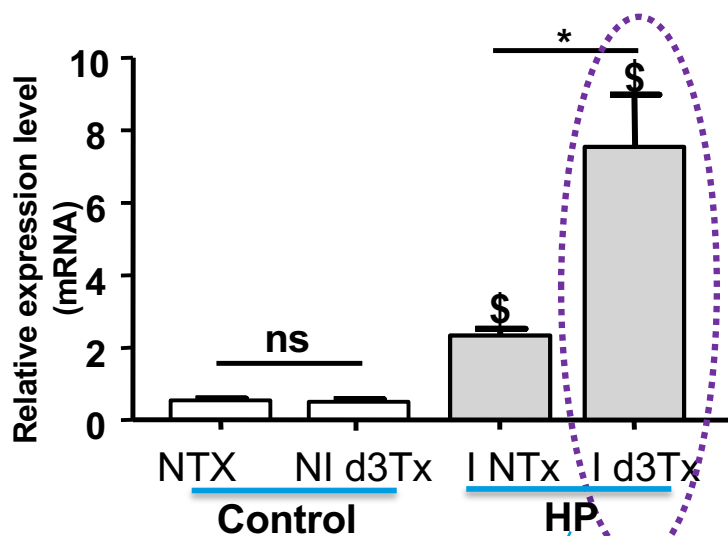
Chrismont *et al.*, Am J Pathol 2014

Réponse inflammatoire et LGM

Inflammatory response in GML-developing mice: TNF family members' implication in GML



- recruitment
- LT polarization



APRIL RT-qPCR
in GML vs. non GML
mice

CCL(1,19,20), CXCL13
Lymphotoxins α et β
CD40 (+L)
BAFF
CD27, GITR, TRANCE

APRIL transgenic mice expressing human APRIL cytokine as a transgene



(collaboration with Dr M Hahne, IGMM-UMR5535, University of Montpellier)

Stein *et al*, 2002

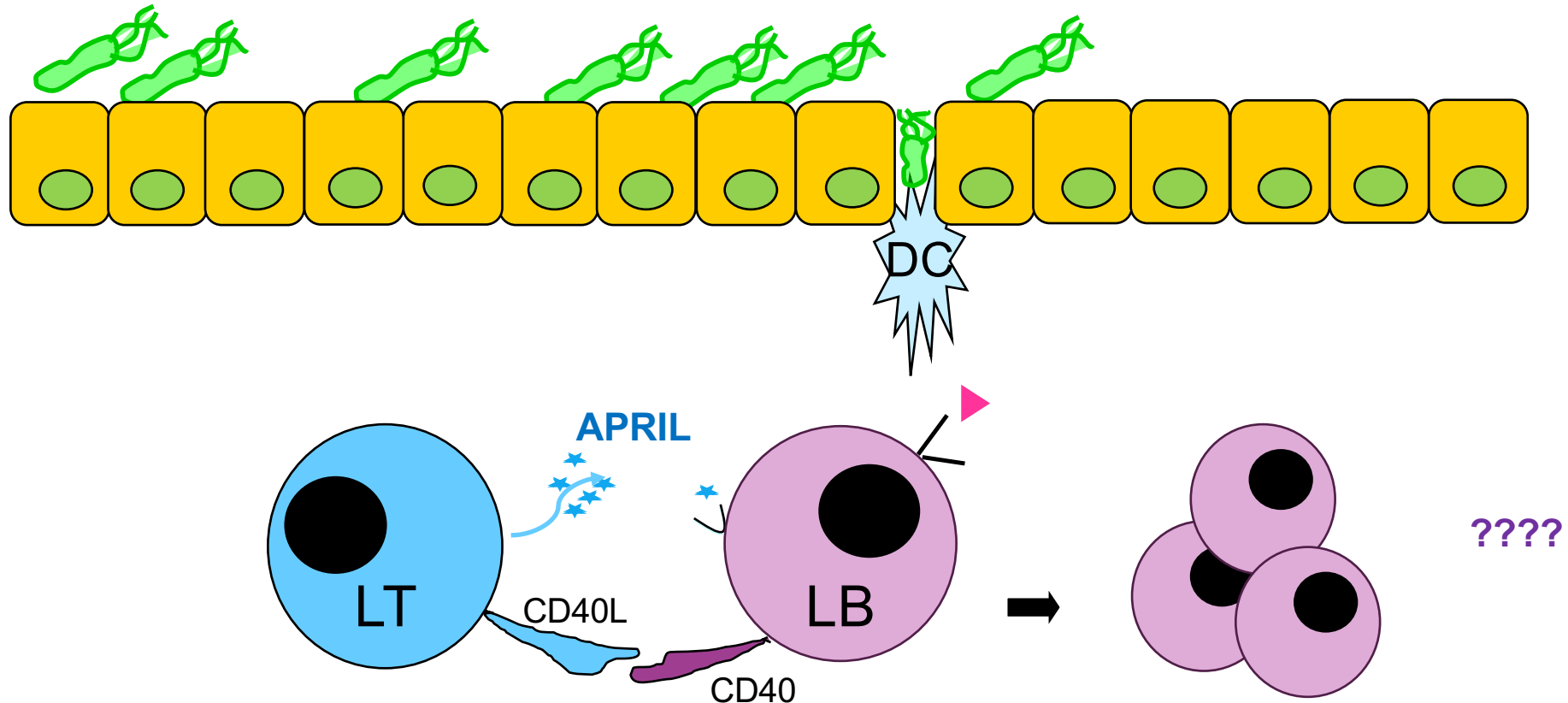
→ Heterozygote for APRIL

- hAPRIL addressed at T cells (promoter)
- APRIL can act systematically (secreted form)

→ Mice production in Bordeaux (animal facilities)

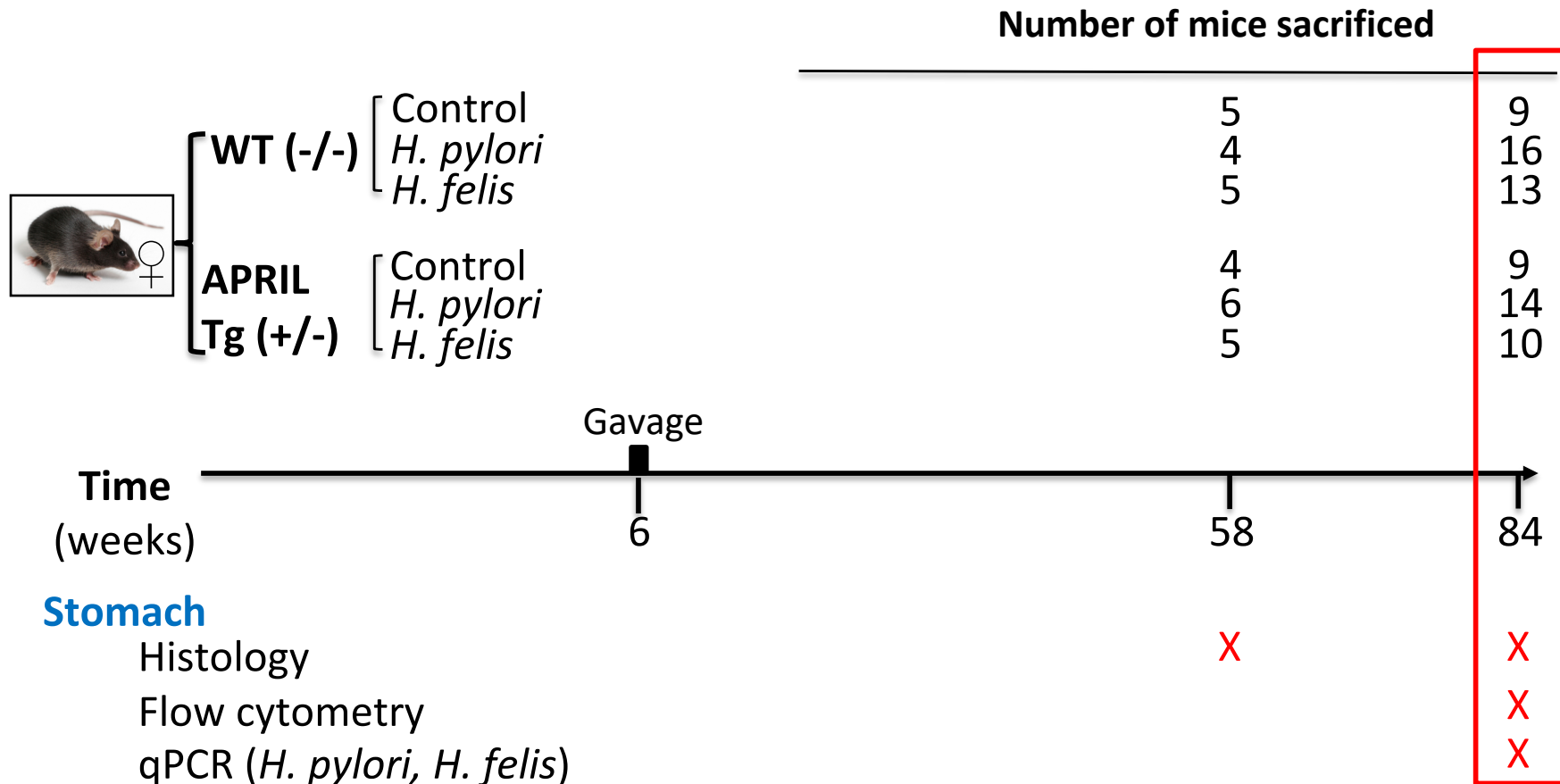
- produced by breeding of heterozygotes APRIL with WT mice (same genetic background)

Helicobacter/APRIL interaction study



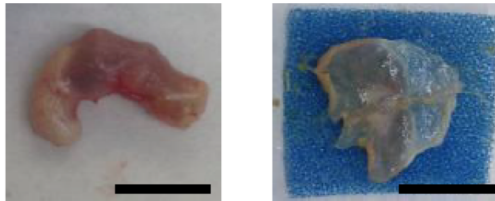
➡ What could be the consequences of *Helicobacter* infection in APRIL Tg mice?

Design of *Helicobacter*/APRIL interaction study



Macroscopic aspect of stomachs at 84W

APRILtg NI

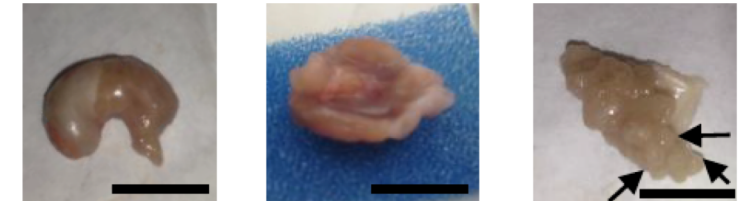


normal aspect

APRILtg *Hp*



APRILtg *Hf*



Abnormal aspects

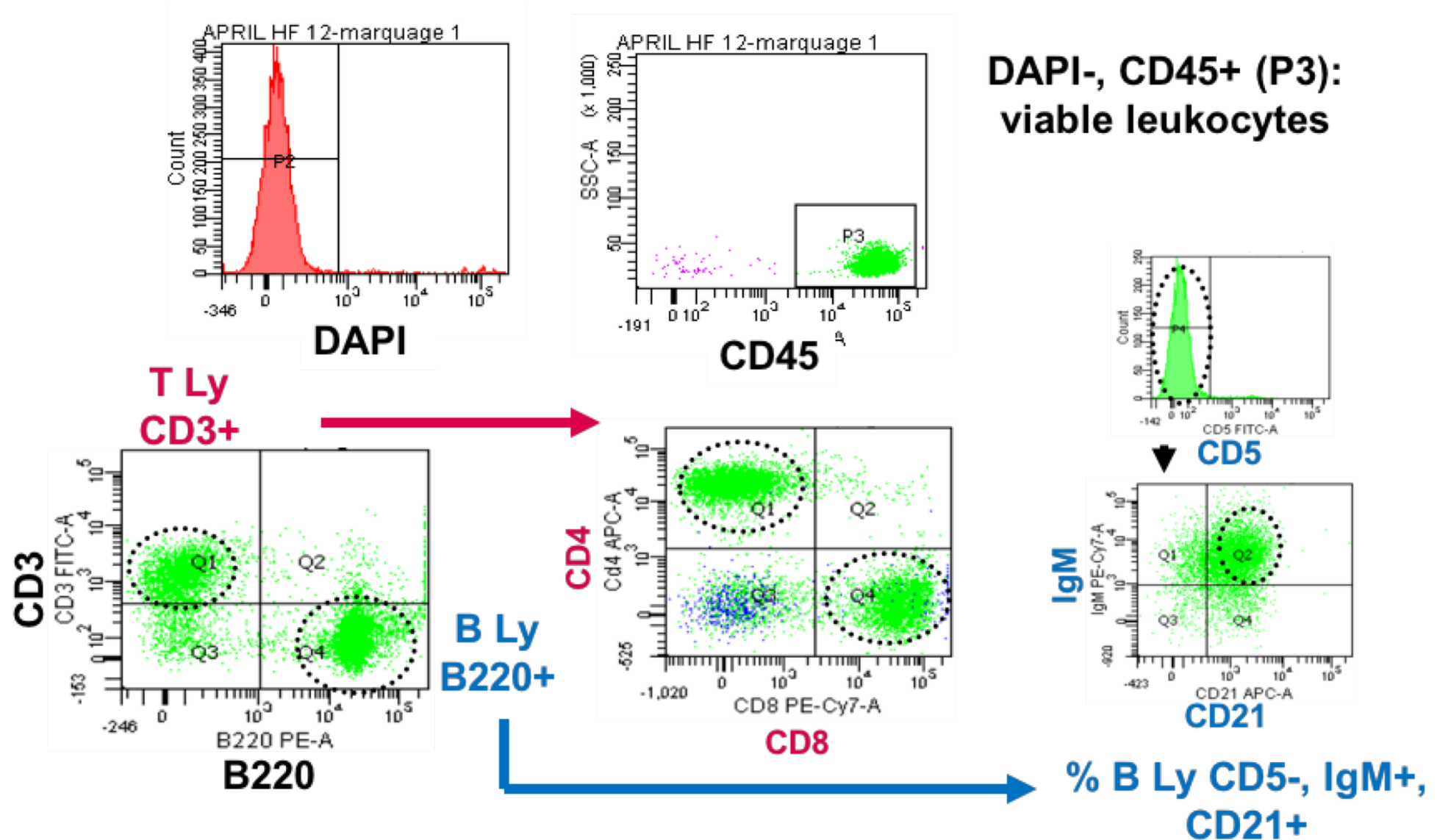
thicker and more rigid stomachs
with a nodular aspect

64.3% APRILtg *Hp*

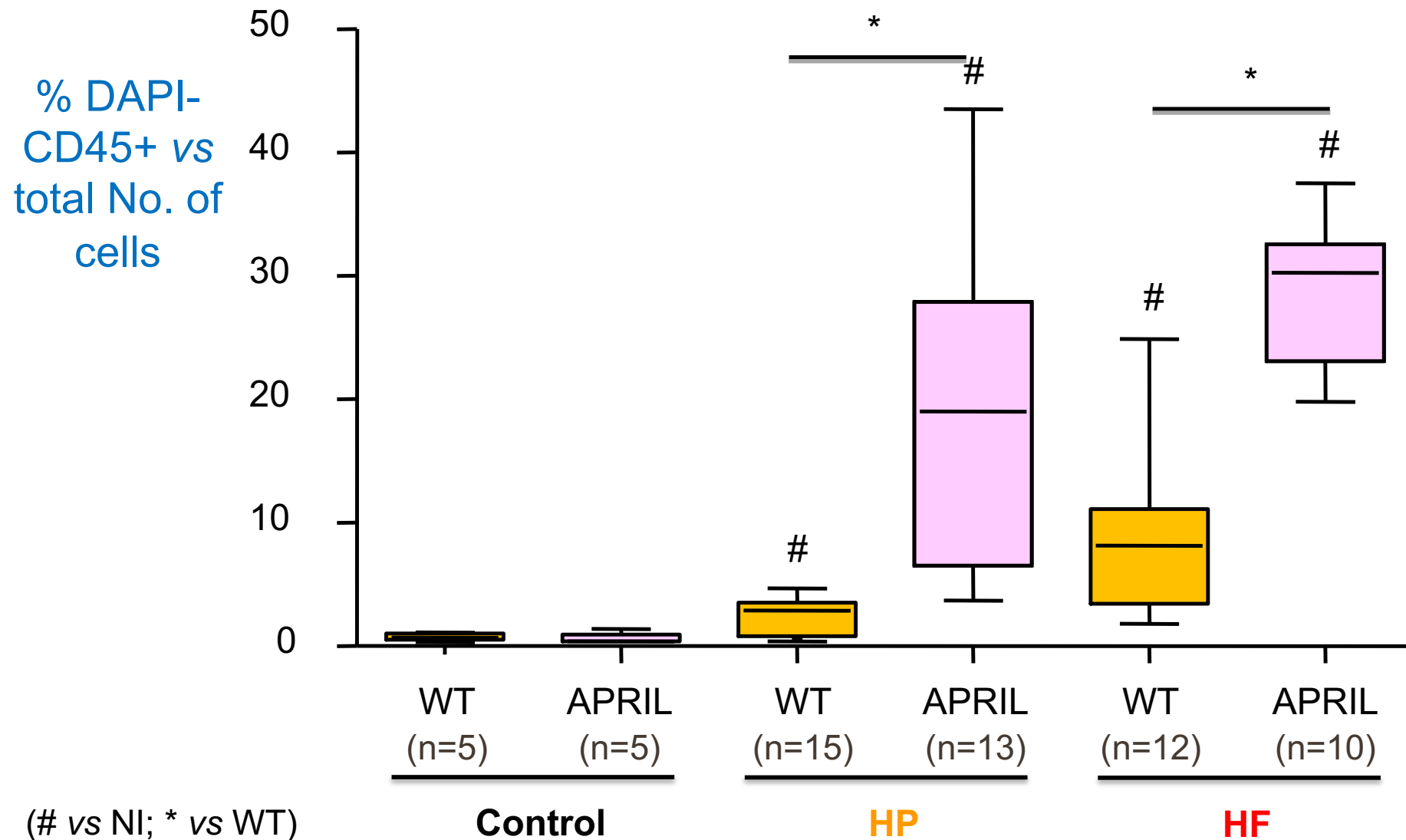
100% APRILtg *Hf*

— 1000µm

Flow cytometry analysis on gastric biopsies

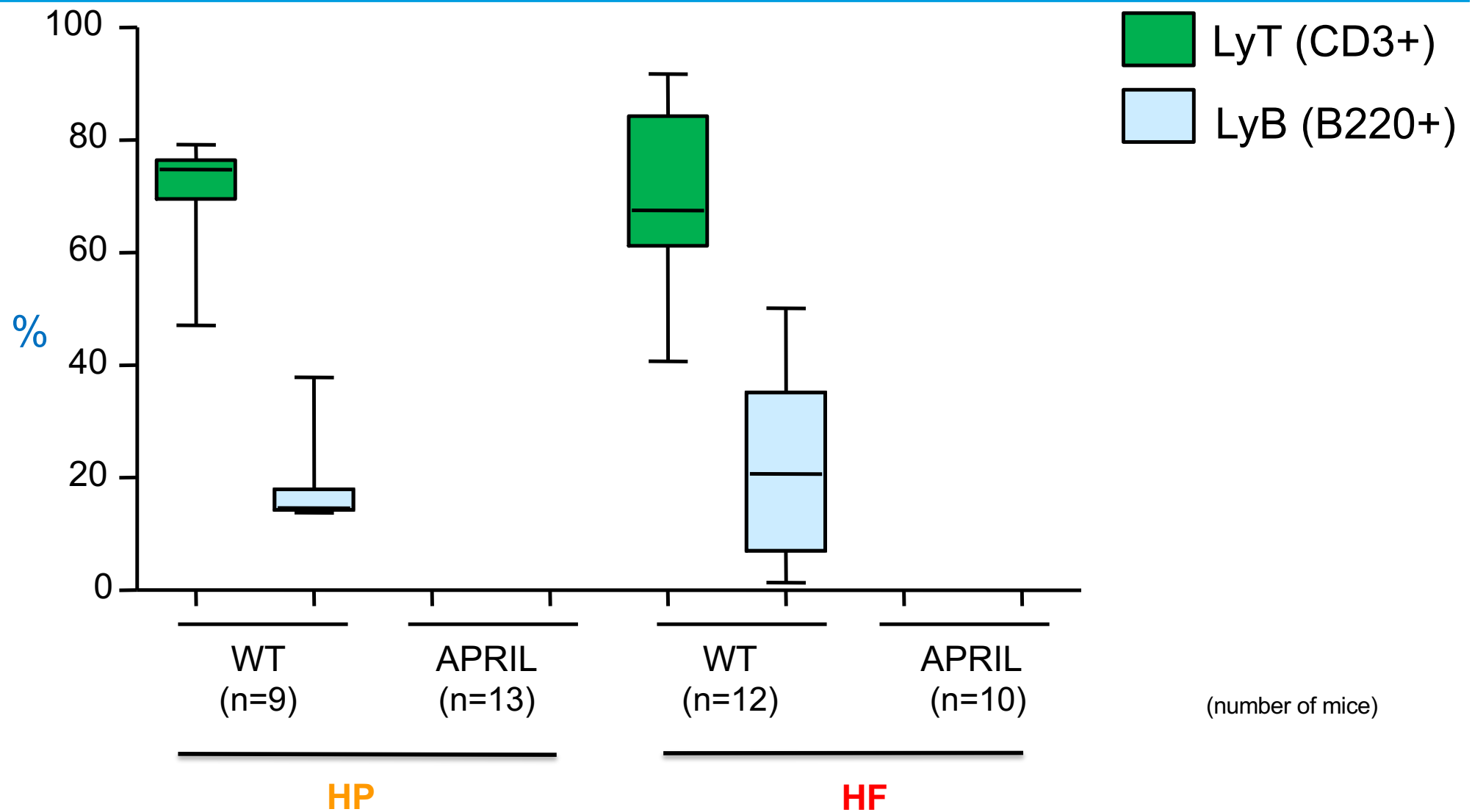


Flow cytometry: leukocyte infiltration



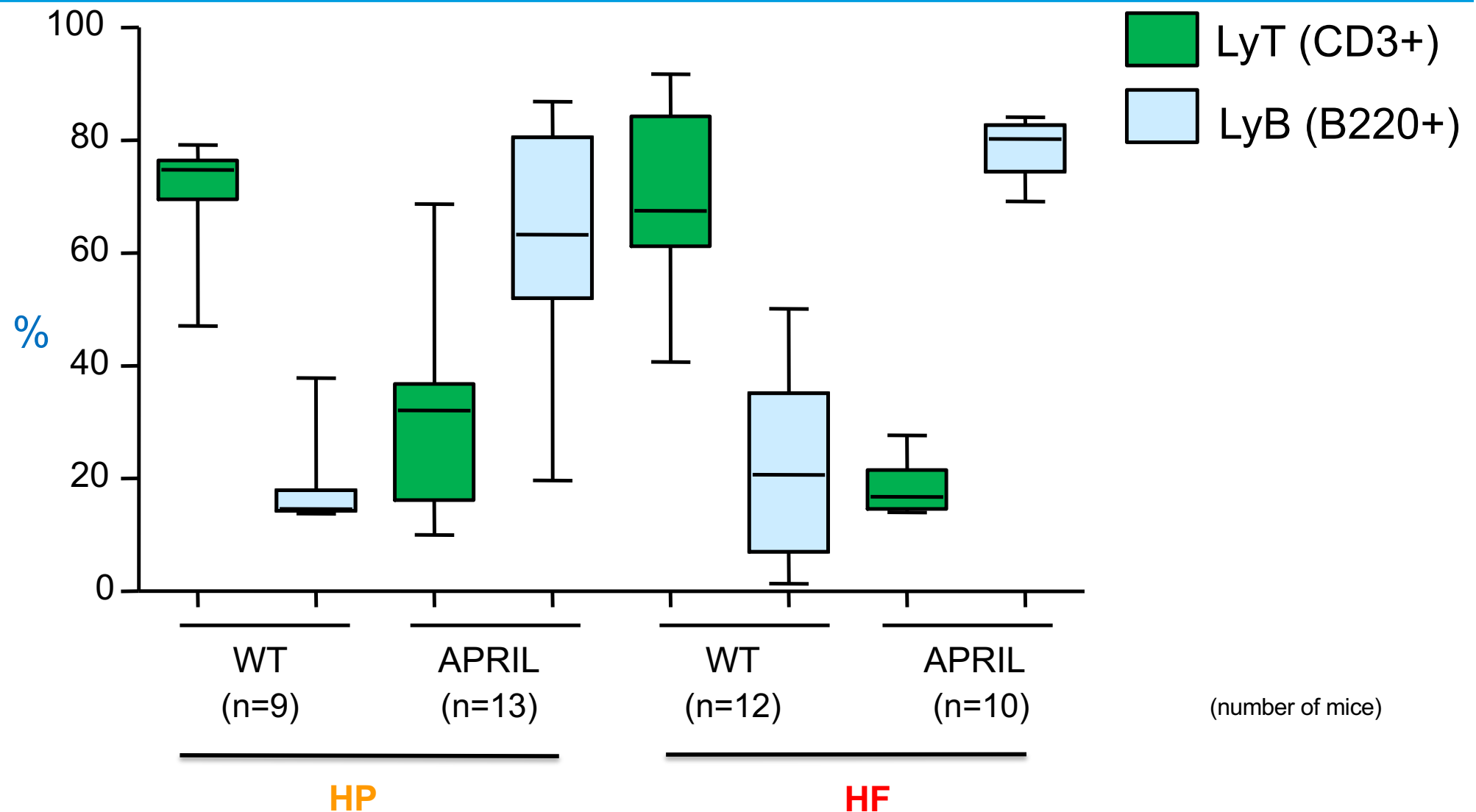
➡ higher leukocyte infiltration in infected APRIL mice *versus* WT

Flow cytometry: nature of the lymphocyte infiltration



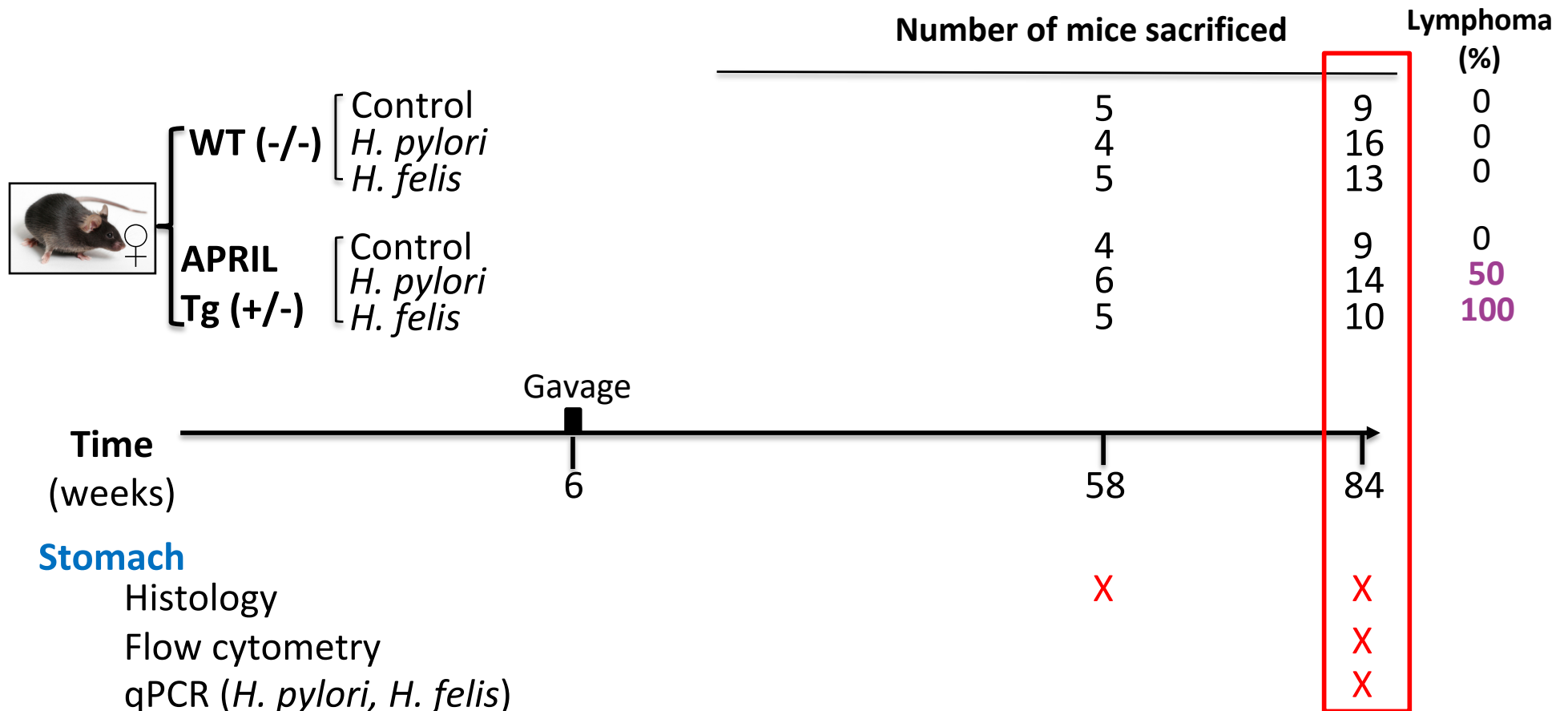
➡ Infiltrates mainly composed of T cells in HF and HP WT stomachs

Flow cytometry: nature of the lymphocyte infiltration

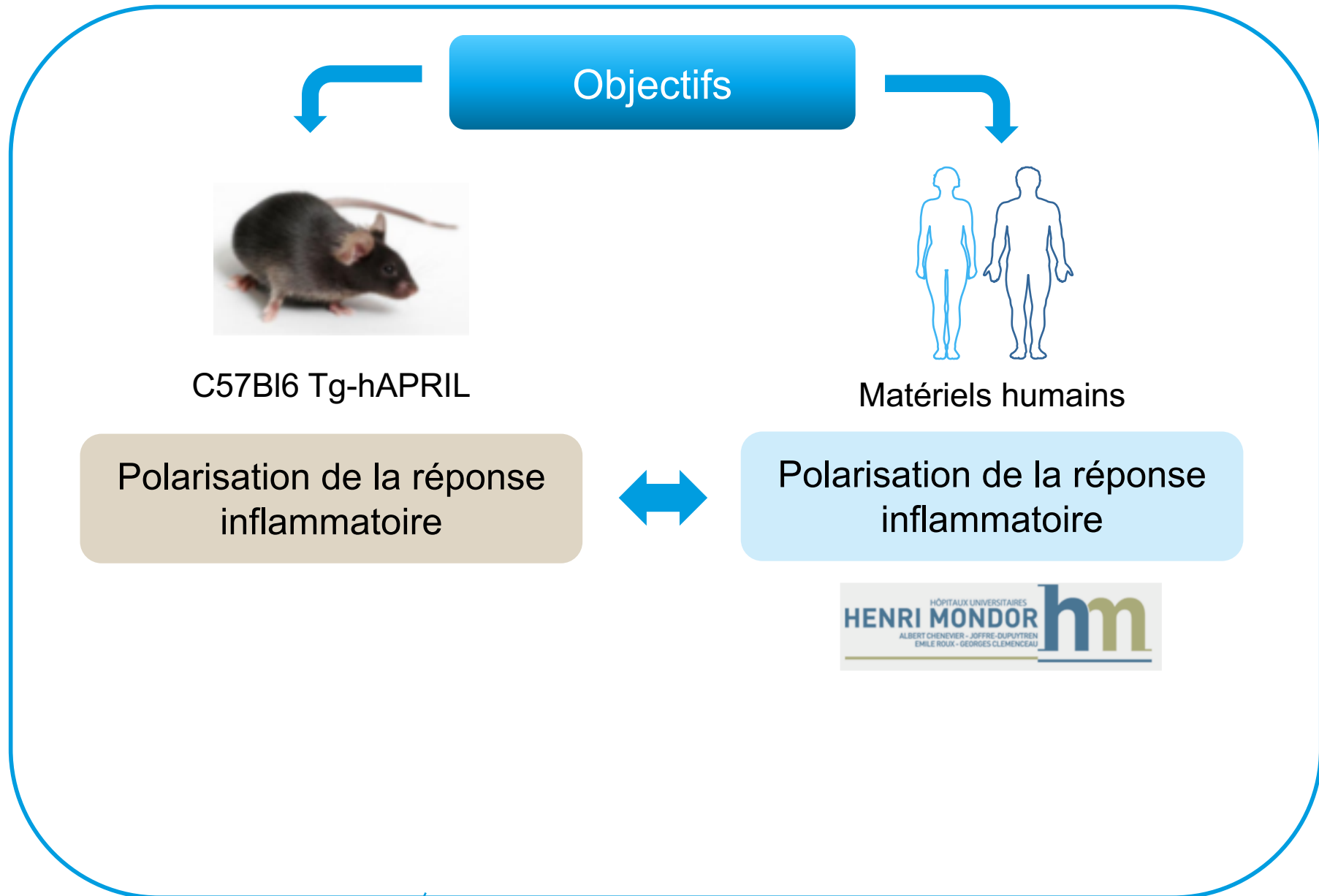


➡ Infiltrates mainly composed of B cells in HF and HP APRIL stomachs

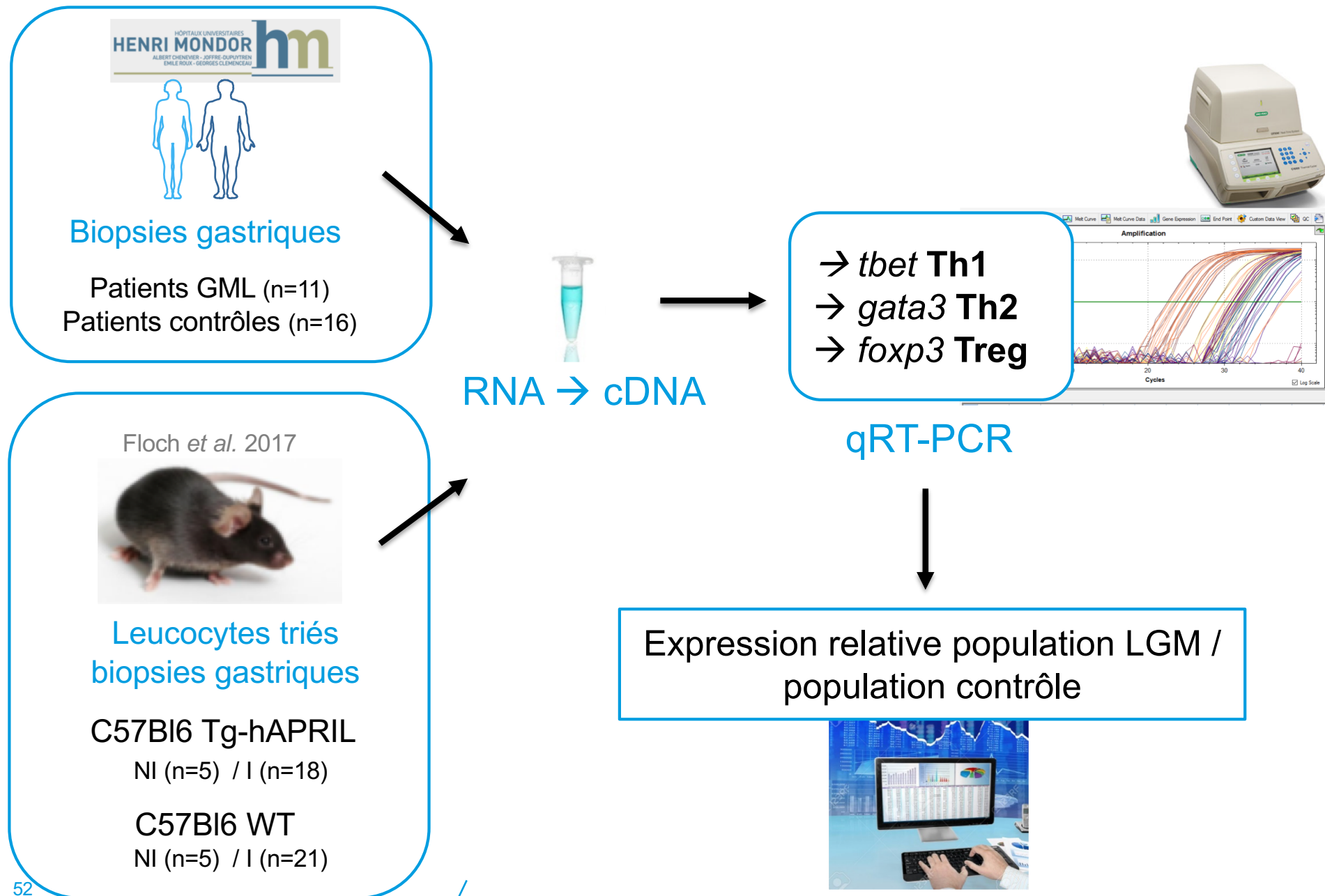
Design of *Helicobacter*/APRIL interaction study



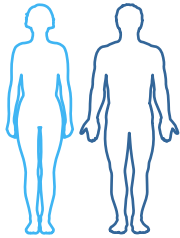
Lymphomagenèse gastrique et cytokine APRIL



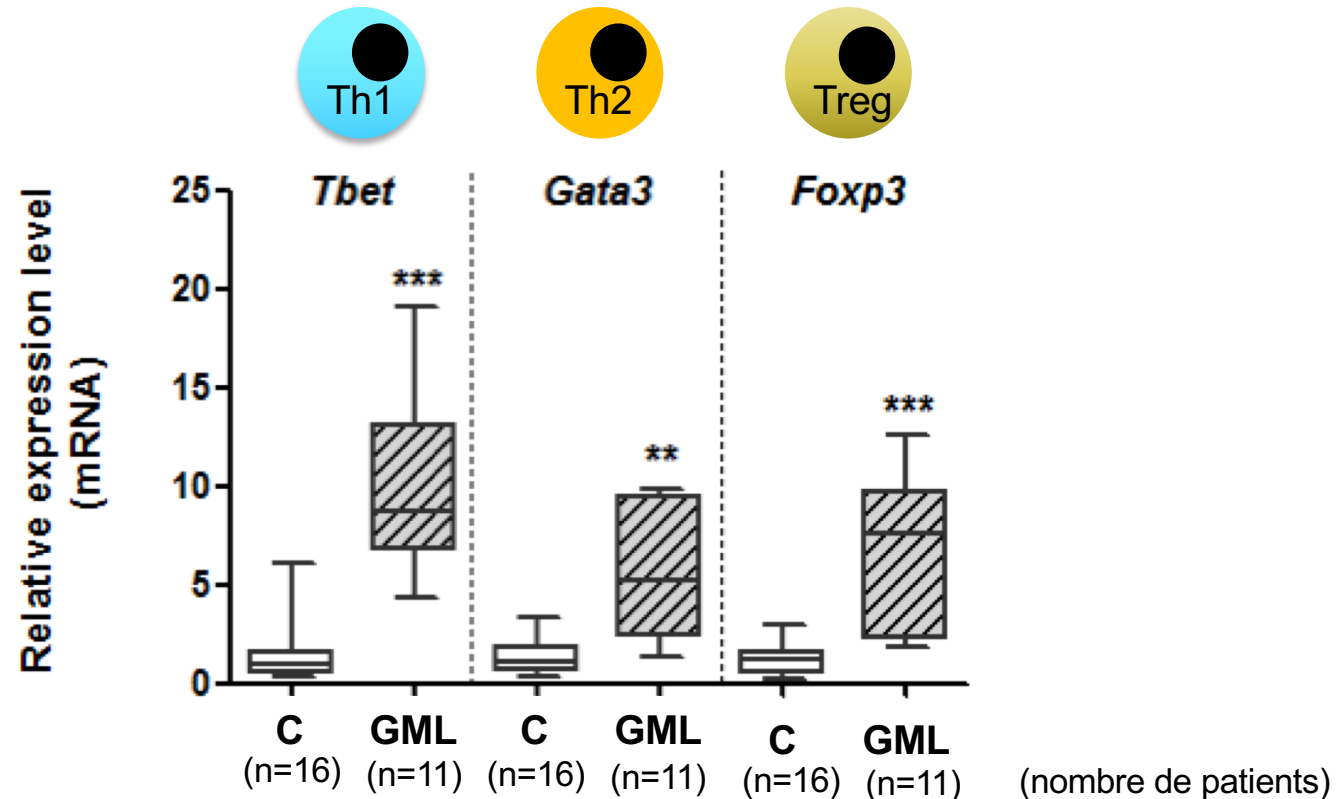
Réponse inflammatoire dans le LGM



Réponse inflammatoire dans le LGM



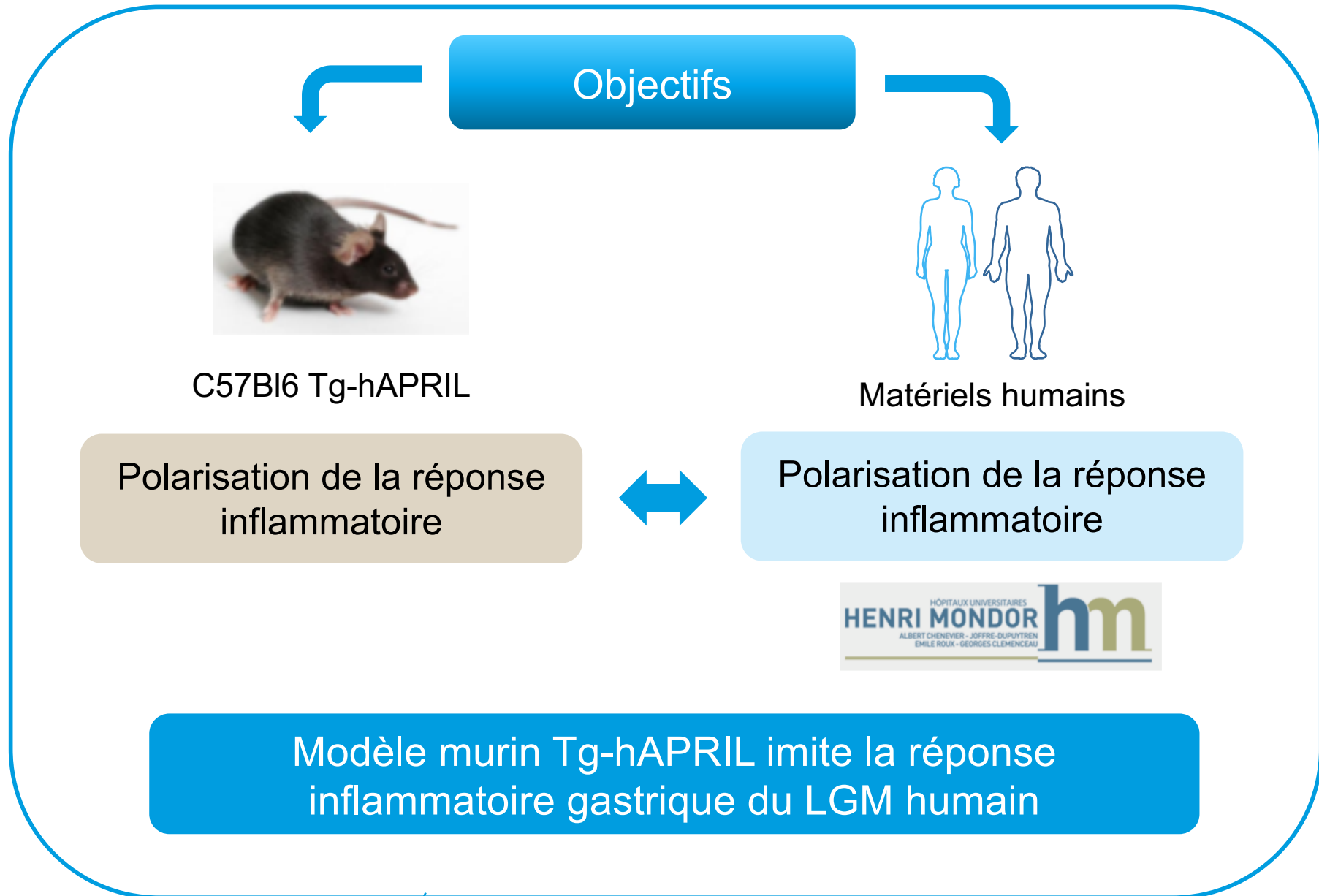
RT-qPCR sur les ARN extraits de biopsies gastriques



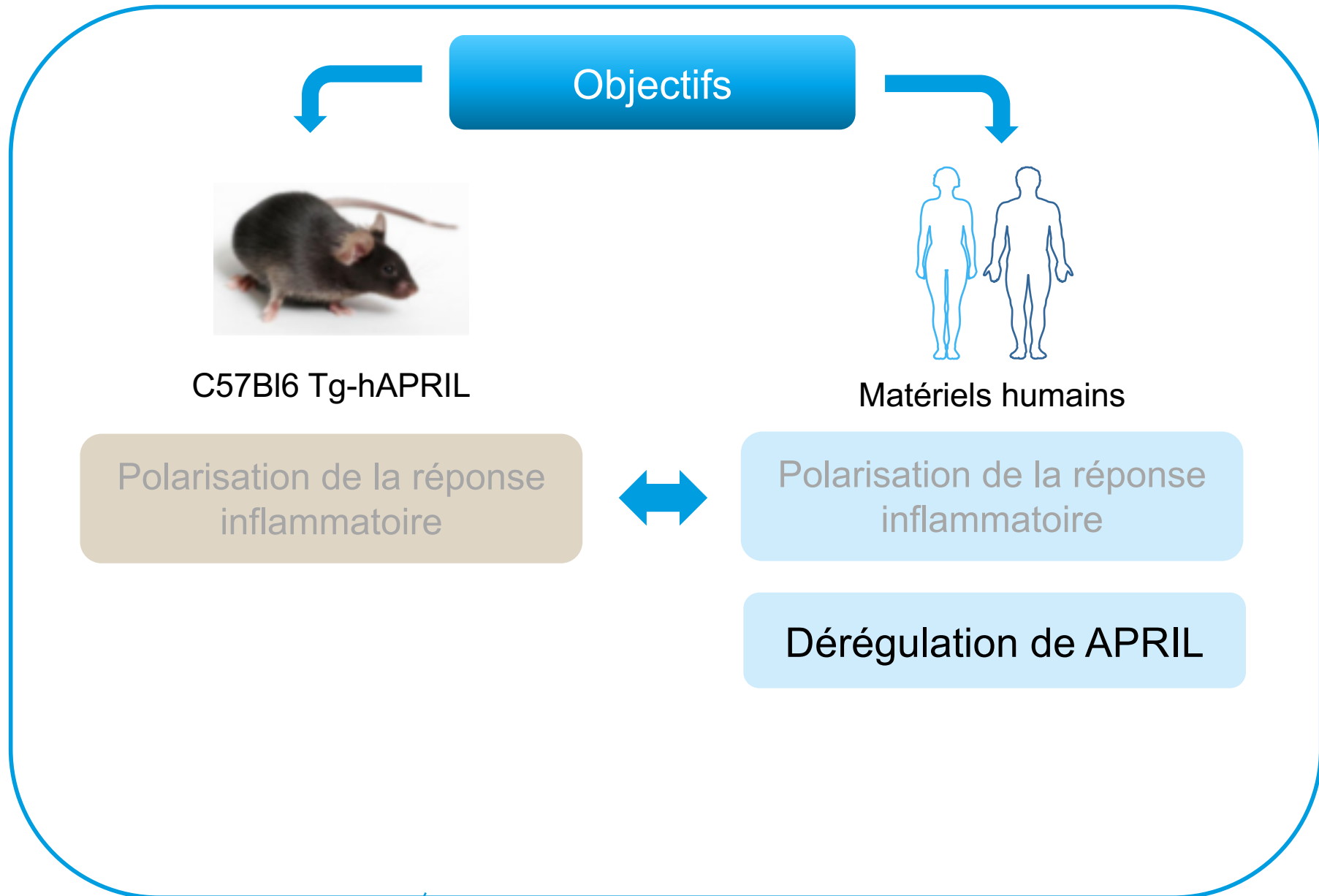
➔ Réponse inflammatoire équilibrée avec une réponse Th1, Th2 et Treg significativement plus importantes chez les patients LGM / population contrôle



Réponse inflammatoire dans le LGM

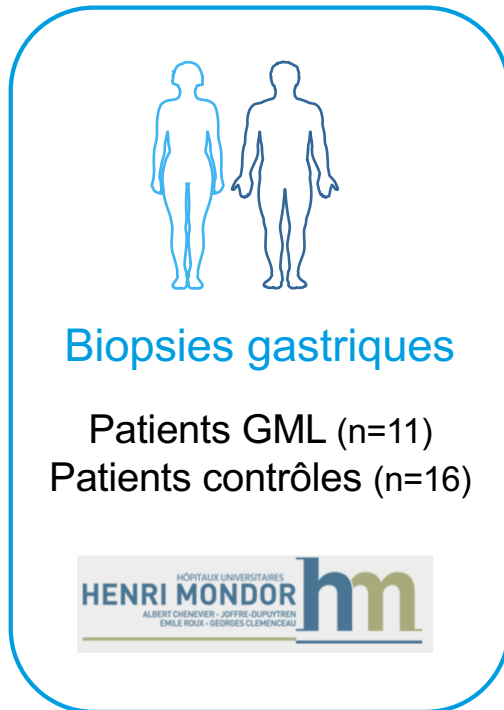


Question 2



Partie 2 – Dérégulation de APRIL dans le LGM

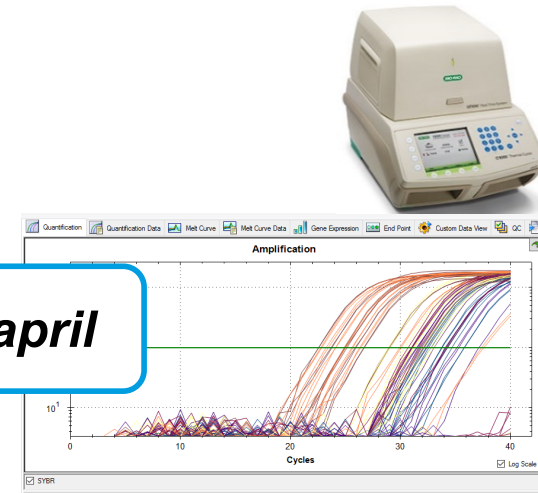
Même protocole...



ARN → ADNc



→ *april*



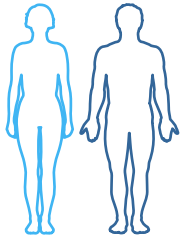
RT-qPCR



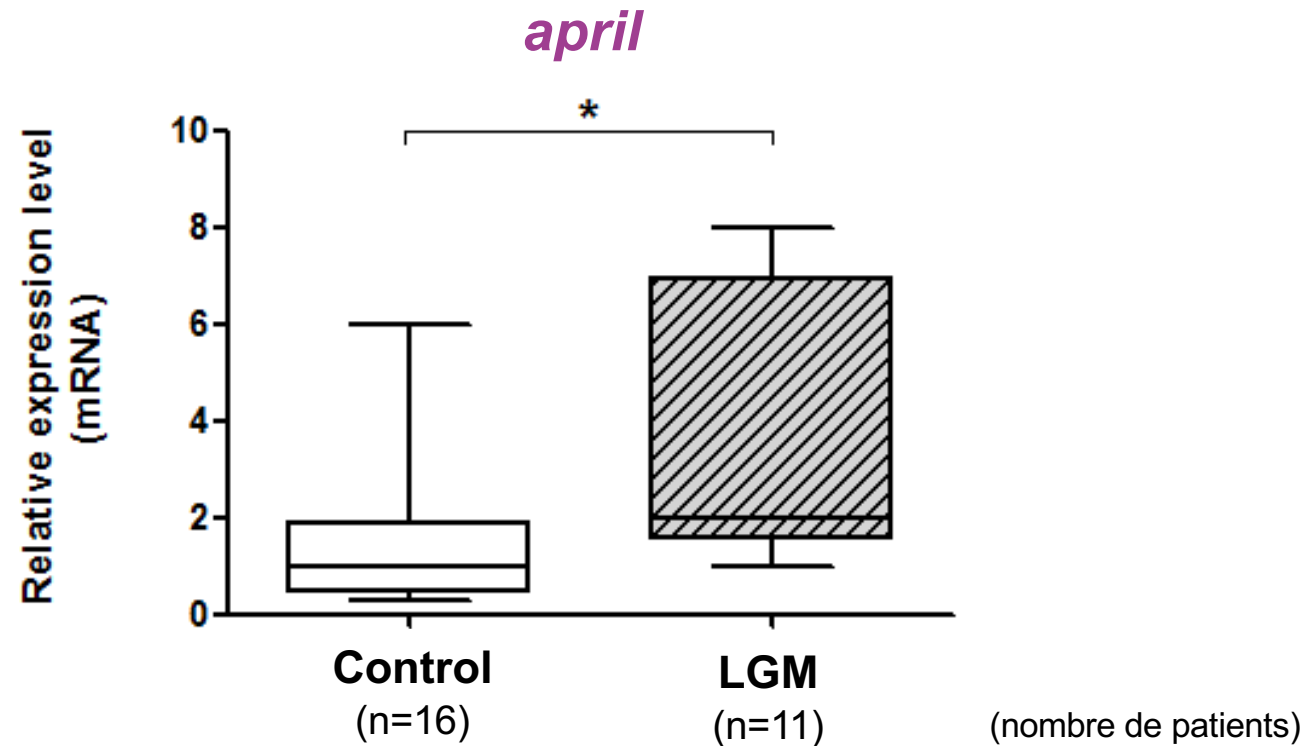
Expression relative de *april* population
LGM / population contrôle



Dérégulation de APRIL dans le LGM

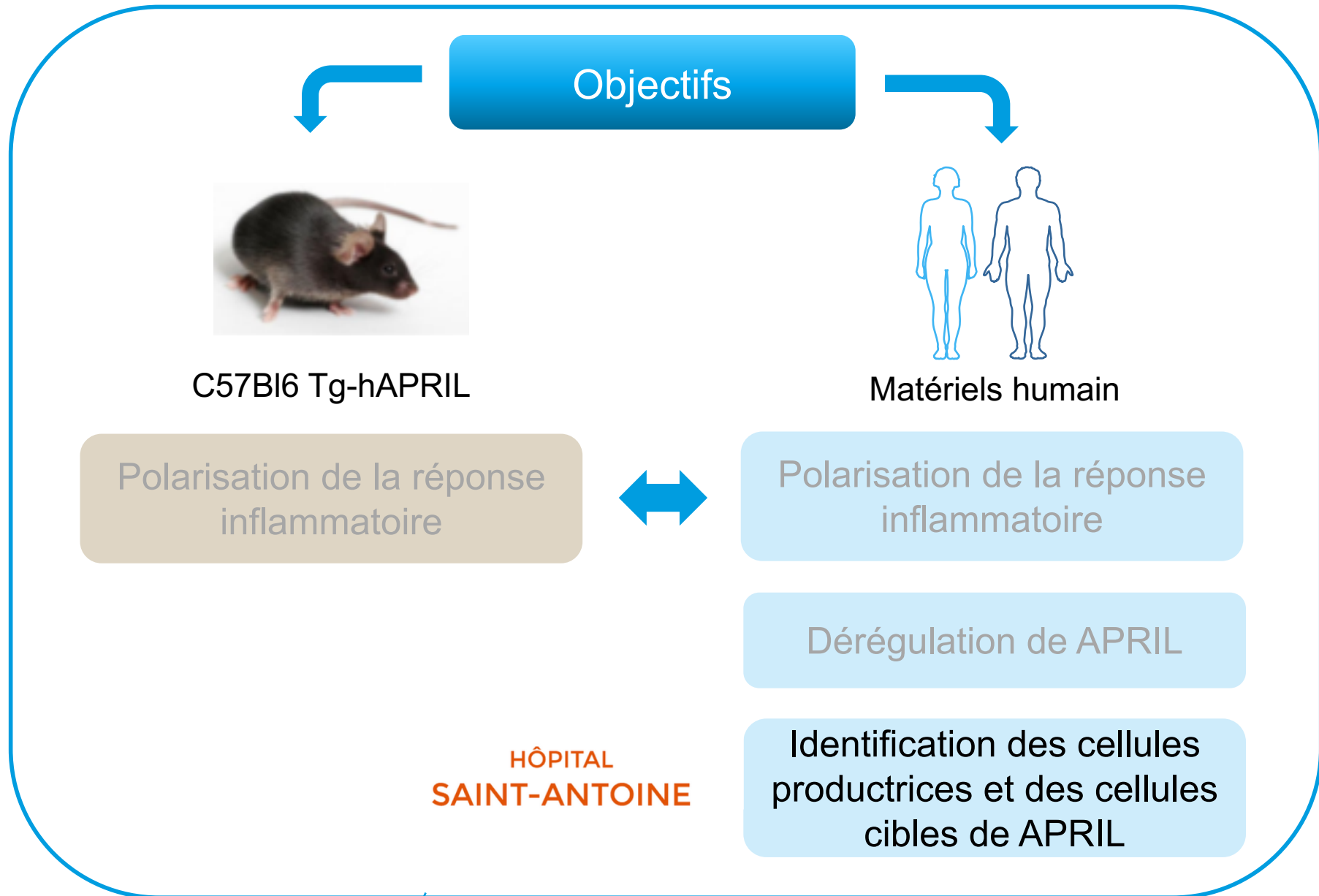


RT-qPCR sur les ARN extraits de biopsies gastriques

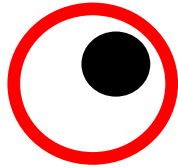
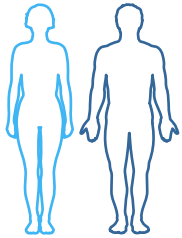


Augmentation significative de *april* au sein du microenvironnement gastrique des patients LGM

Question 3



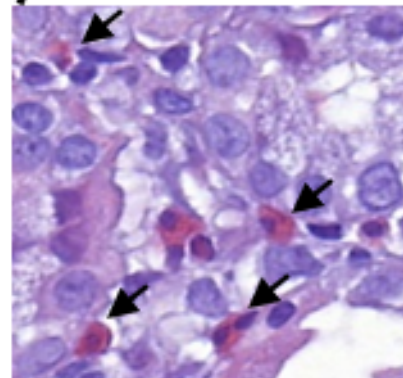
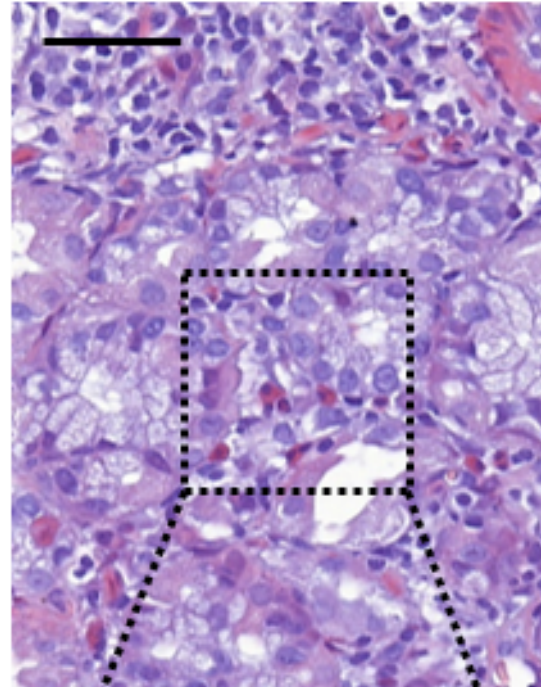
Cellules productrices de APRIL



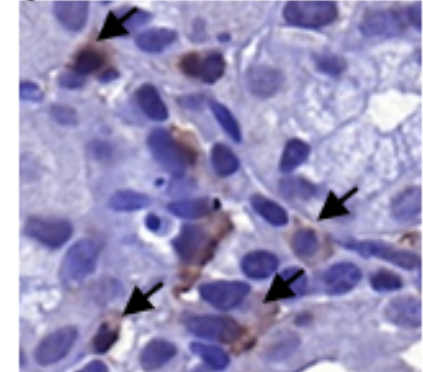
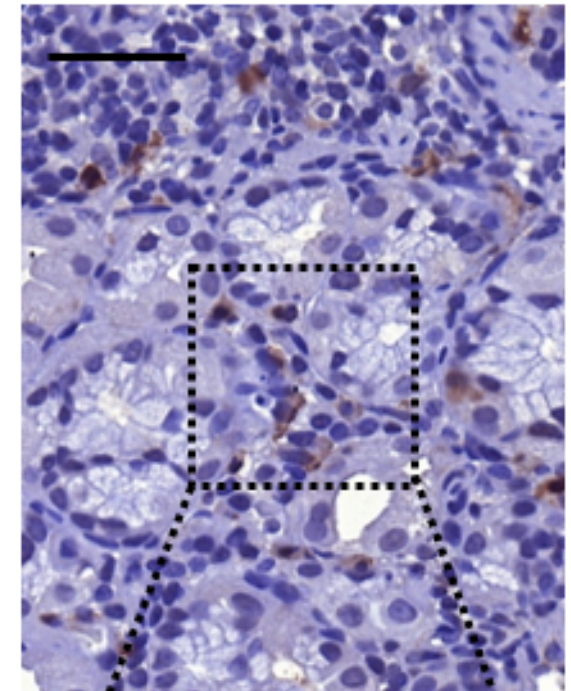
Cellules Stalk-1⁺

Forme liée à la membrane de APRIL

HE

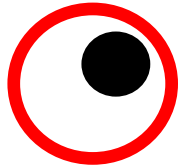
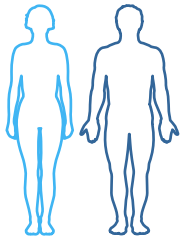


Stalk-1



Coloration H&E et marquage IHC Stalk-1

Cellules productrices de APRIL

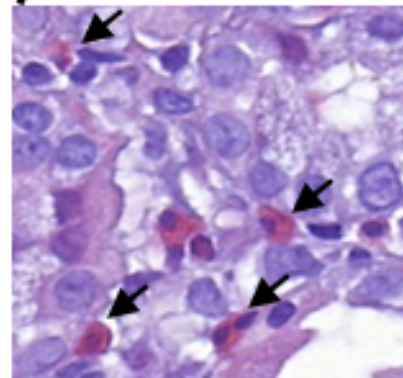
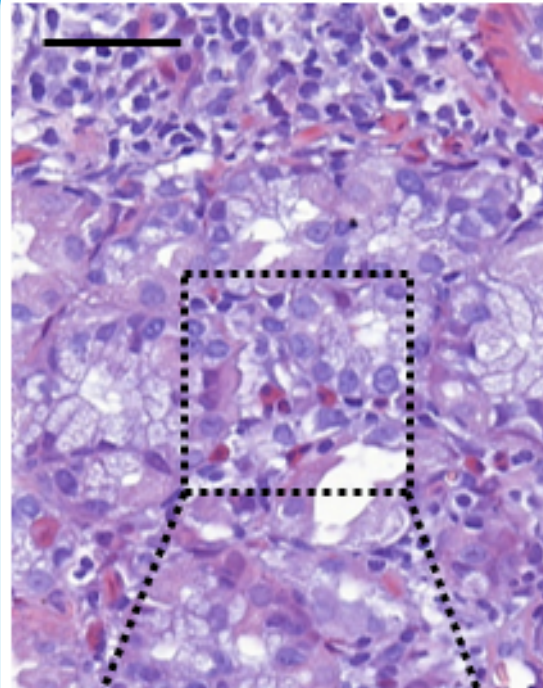


Cellules Stalk-1⁺

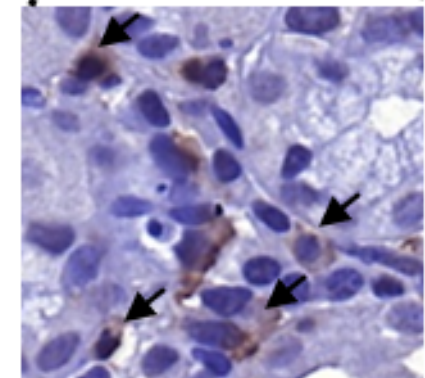
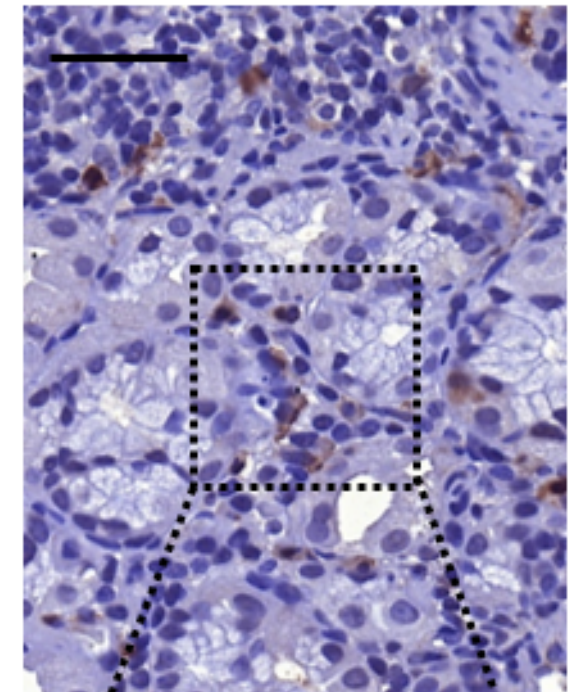
Forme liée à la membrane de APRIL

Polynucléaires éosinophiles ?

HE

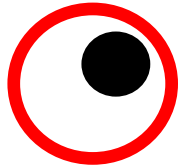
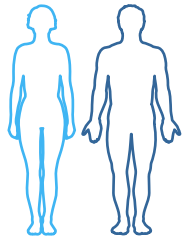


Stalk-1



Coloration H&E et marquage IHC Stalk-1

Cellules productrices de APRIL



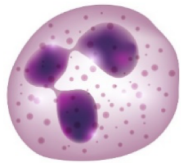
Cellules Stalk-1⁺

Forme liée à la membrane de APRIL

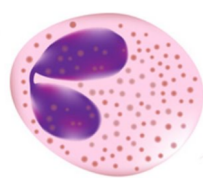
Polynucléaires éosinophiles ?

MUB40

Siglec8



PNN

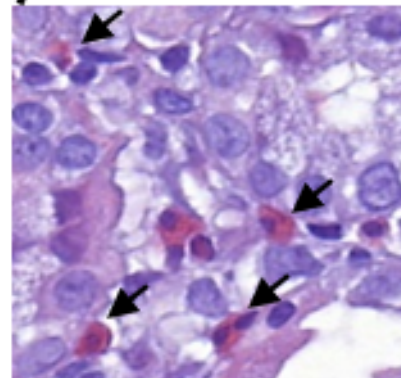
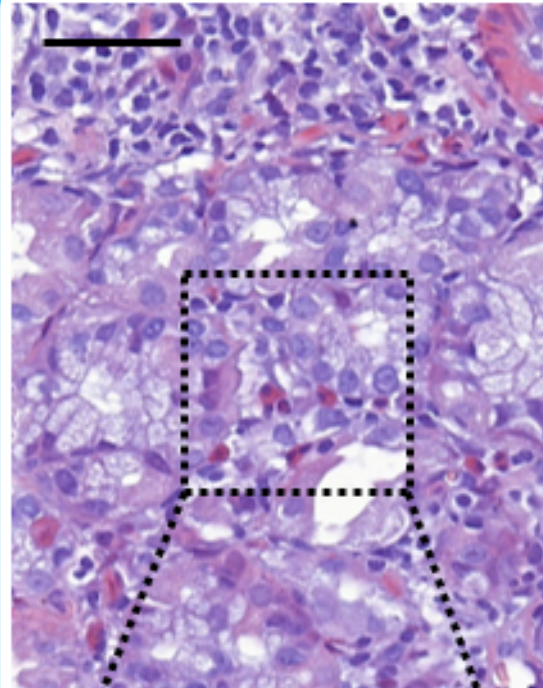


PNE

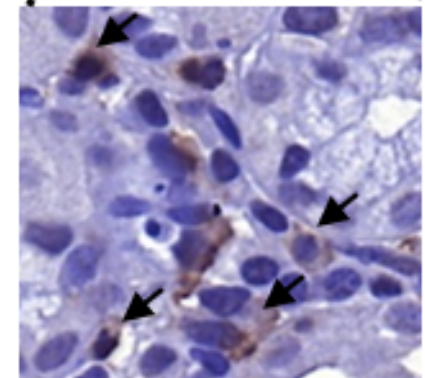
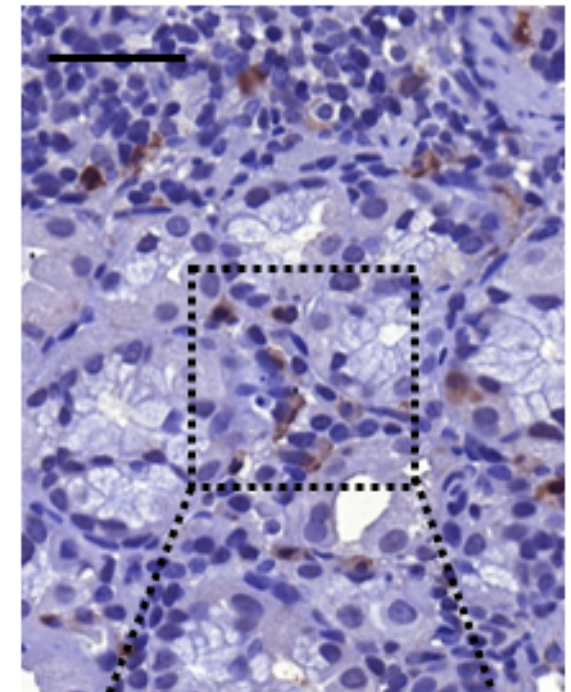
Anderson *et al.* 2018

Zhang *et al.* 2004

HE



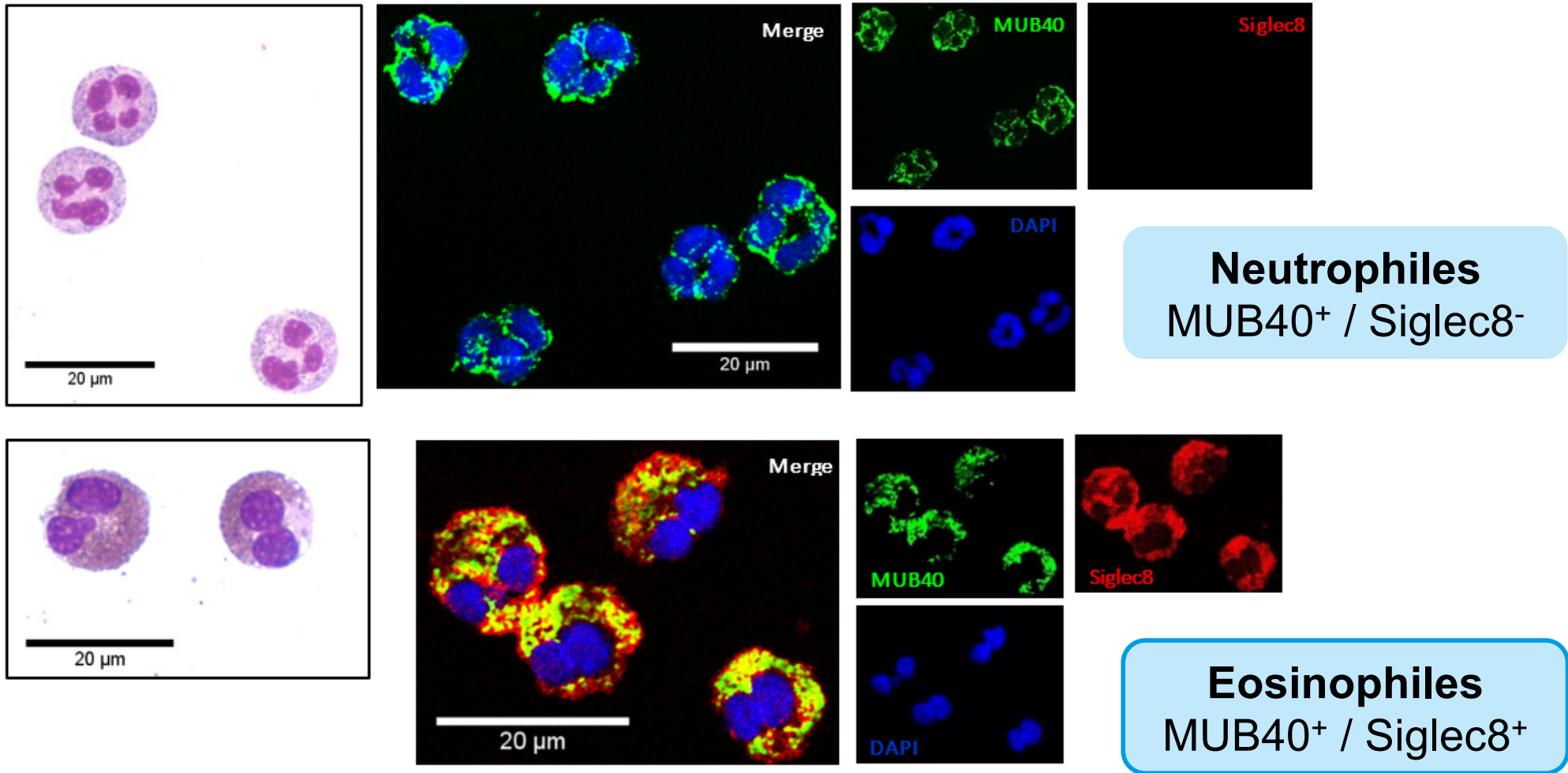
Stalk-1



Coloration H&E et marquage IHC Stalk-1

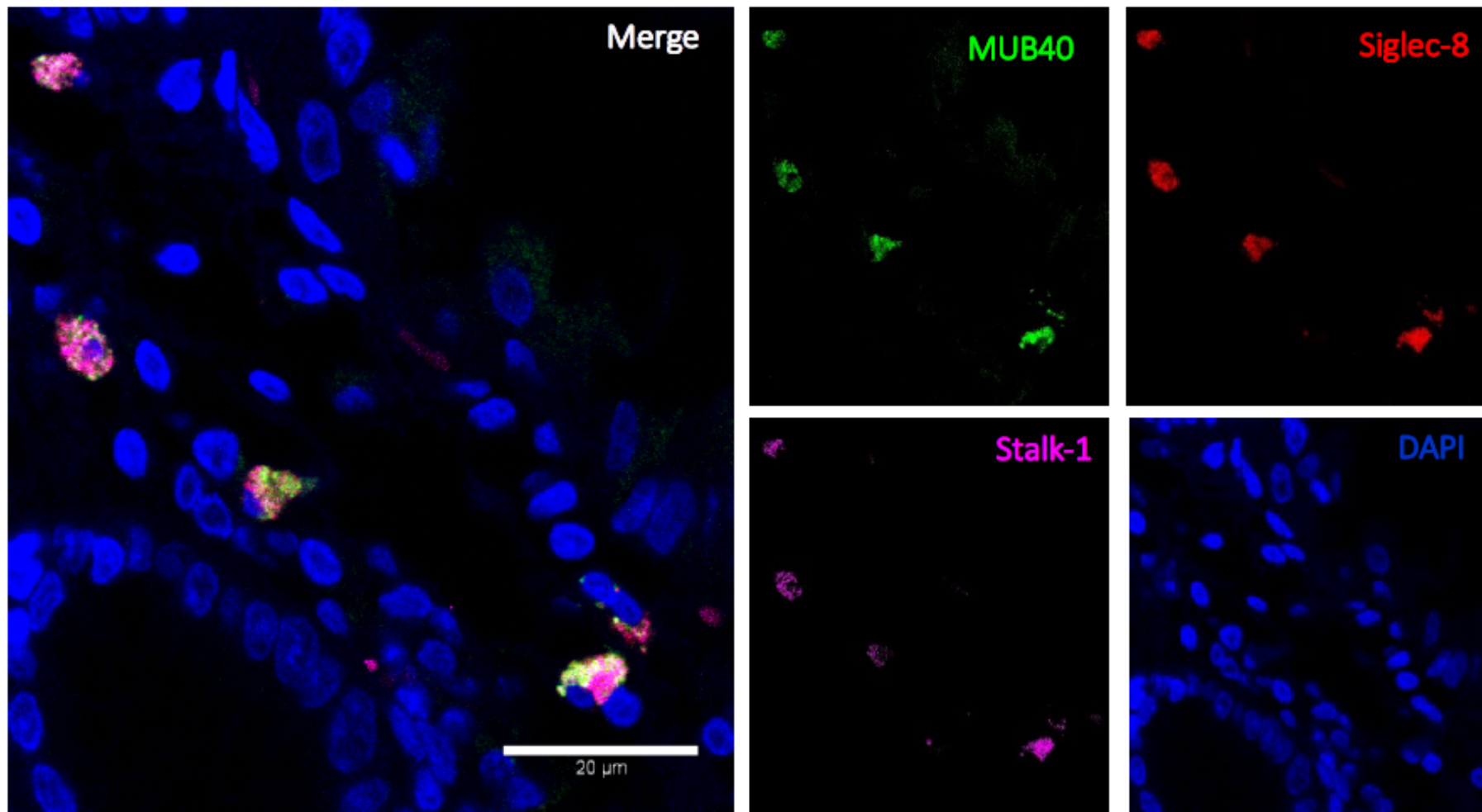
Cellules productrices de APRIL

Co-marquage IF MUB40 / Siglec8 sur des PNN et PNE humains purifiés

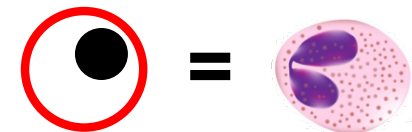


Cellules productrices de APRIL

Triple marquage IF Stalk-1 / MUB40 / Siglec8 sur des biopsies gastriques LGM



Cellules productrices d'APRIL = éosinophiles

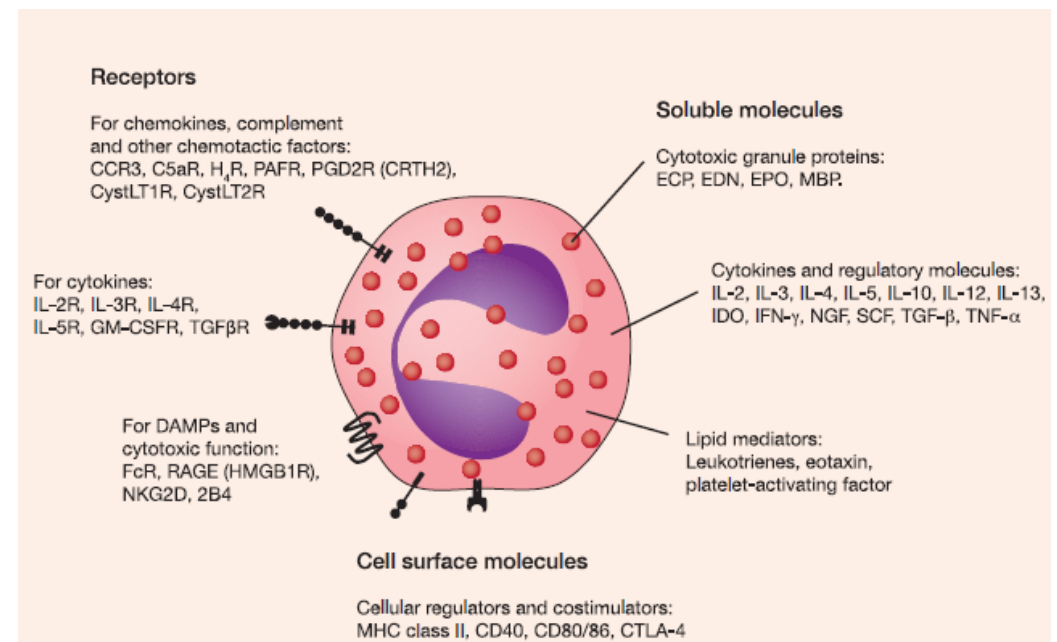


Rôle des éosinophiles dans le LGM ?

› Cellules résidentes du tractus gastro-intestinal Jung et Rothenberg *et al.* 2014

› Cellules multifonctionnelles :

- Allergies
- Infections parasitaires
- Effecteurs des réponses immunitaires innée et adaptative
- Sécrétions de nombreux médiateurs
- Immuno-régulateurs
- ...



Davis *et al.* 2014

Rôle des éosinophiles dans le LGM ?

- › Gastrites à *H. pylori* associées à une augmentation des éosinophiles infiltrés dans la muqueuse gastrique

McGovern *et al.* 1991

Aydemir *et al.* 2004

Bold A *et al.* 2013

- › Augmentation significative des éosinophiles infiltrés dans 70% des LGM étudiés

Herrera-Goepfert *et al.* 1999

- › Modèle murin d'infection à *H. pylori*:

- Rôle des éosinophiles dans la diminution de la réponse Th1 (*in vivo*)
- Activité antibactérienne des éosinophiles limitée sur *H. pylori* (*in vitro*)

→ les éosinophiles favoriseraient la persistance bactérienne

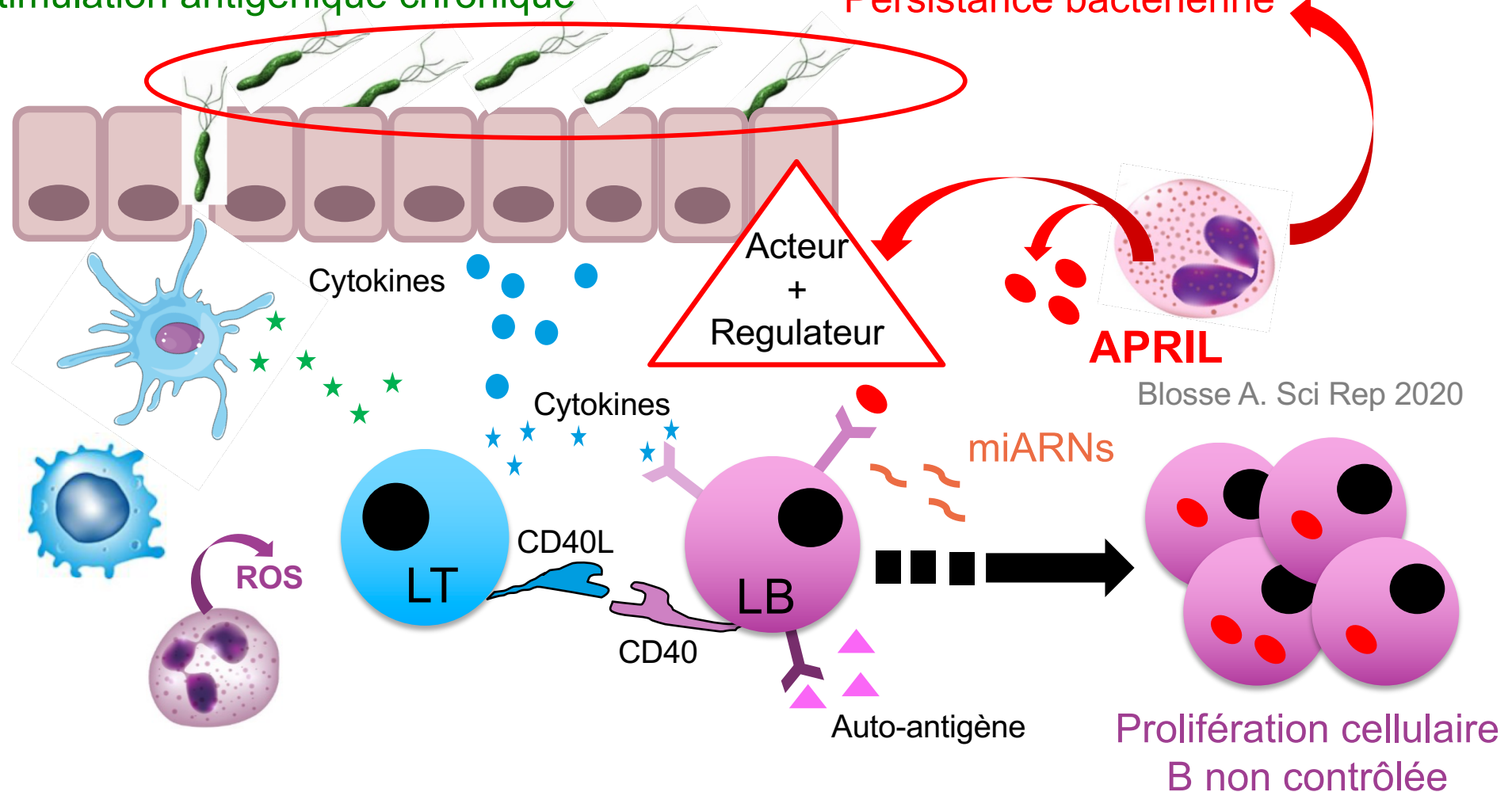
→ stimulation antigénique chronique

→ émerge du LGM

Arnold *et al.* 2018

Stimulation antigénique chronique

Persistance bactérienne



→ Rôle pro tumoral des éosinophiles dans le LGM

