Gut Function and Microbiota

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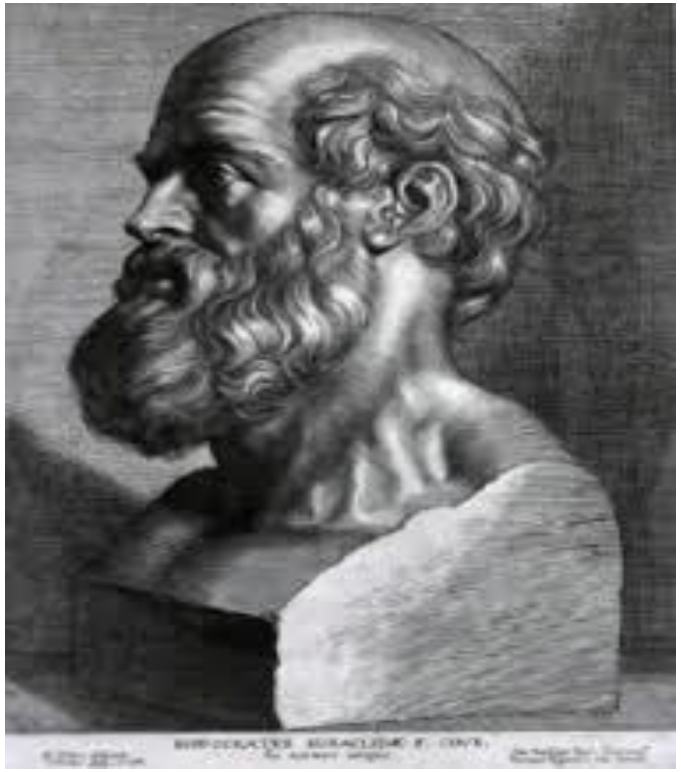
TUMS

All disease begins in the gut

Hippocrates



The newborn gut is still developing

Examples of structural maturation

Stomach size after birth

Day 1	Day 3	Day 7	Day 30
Size of a cherry 5 - 7 ml 0.1 - 0.2 oz	Size of a walnut 22 - 27 ml 0.8 - 1 oz	Size of an apricot 45 - 60 ml 1.5 - 2 oz	Size of a large egg 80 - 150 ml 2.5 - 5 oz

Small intestinal length after birth

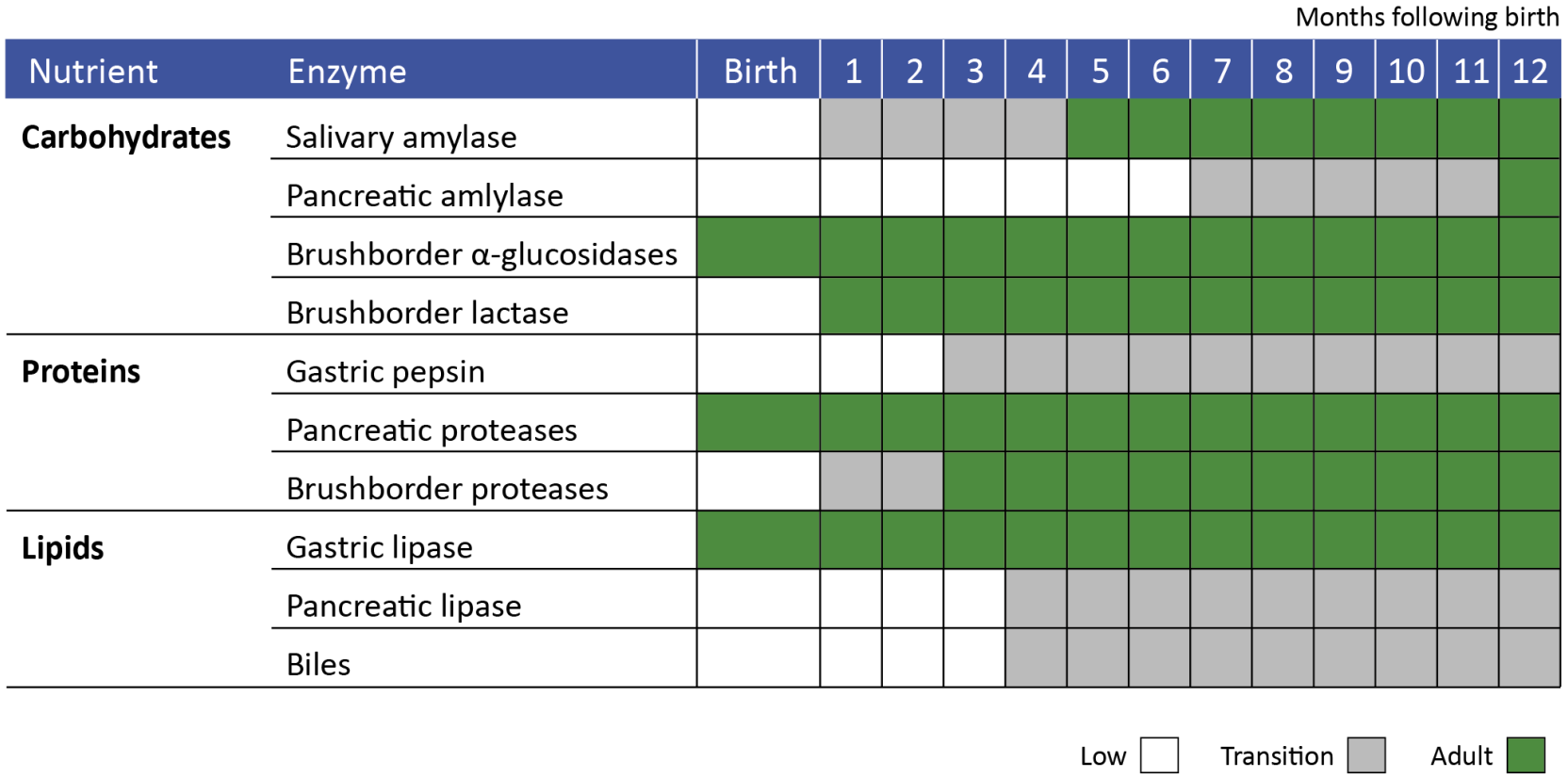
Age	Average length (cm)
Term	275
1 year	380
5 years	450

Weaver LT, et al. Gut 1991; 32:1321-3. Health & Parenting. Preparing for Breastfeeding: How much milk will my newborn need? - www.health-and-parenting.com/preparing-for-breastfeeding-how-much-milk-will-my-newborn-need/. Accessed on: 20 May, 2015.

The newborn gut is still developing

Example of physiological maturation

Development of digestive enzymes from low levels (white) to adult levels (green) over 12 months



Vandenplas Y, Ludwig T, Szajewska H. Gut Health In Early Life: Implications and Management of Gastrointestinal Disorders. Essential Knowledge Briefing. Wiley, Chichester (2015).

Definition of Gut Health

Gut health can be defined in accordance to the WHO definition of health.

“State of physical and mental wellbeing”

- in the absence of gastrointestinal complaints that require the consultation of a doctor,
- in the absence of indications or risks of bowel disease,
- and in the absence of confirmed bowel disease.

Bischoff SC. *BMC Medicine* 2011;9:24

Potential Indicators for a Healthy Gut (General)

Effective digestion and absorption of food and nutrition

- Good nutritional status
- Regular bowel movement
- Normal stool consistency

Normal, stable gut microbiota

- Lack of bacterial overgrowth
- Normal gut microbiota composition and viability
- No gut infection or antibiotic-associated diarrhea

Wellbeing

- Good quality of life
- Positive mood
- Balanced serotonin production
- Normal function of the enteric nervous system

Absence of GI illness

- No acid reflux disease or other gastric inflammatory disease
- Absence of enzyme deficiencies or nutrient intolerances
- No inflammatory intestinal diseases
- Absence of cancer

Effective immune function

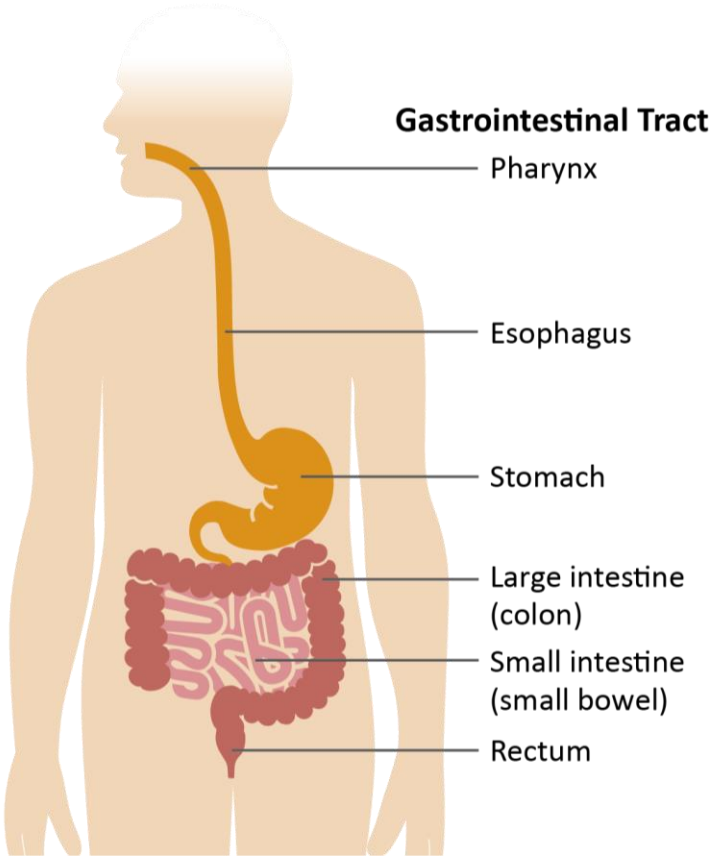
- Normal gut barrier function, normal mucus production
- Normal levels of IgA and essential immune cells
- Appropriate oral tolerance
- No allergy or mucosal hypersensitivity

IgA, immunoglobulin A.

Shamir R, van Elburg R, Knol J, Dupont C. Gut Health in Early Life: Significance of the Gut Microbiota and Nutrition for Development and Future Health. Essential Knowledge Briefing, Wiley, Chichester (2015)

The GUT (Gastrointestinal Tract)

The gastrointestinal tract is part of the gastrointestinal system, which has the primary function of digesting food and the eventual absorption of nutrients contained within. Corollary to that would be the excretion of waste from undigested materials. The gut extends from the mouth to the anus, and has a length of 9 to 10 m in healthy adults. However, it has a total surface area of around 32 $m.^2$ This makes it the largest interface of the body to the external environment. In contrast, the skin has an area of 1.5 to 2 m^2 in adults. Initially, it was thought that the mucosal surface area was in the order of 300 m^2. This was corrected when Helander and Fandriks used morphometric data to show that the true surface area was only 32 m^2.



From mouth to anus

Length (adult): 9 to 10 m

Surface area: 32 m^2

The largest interface of the body to the outside world

- Larger than skin (1.5 to 2 m^2)

American Society for Gastrointestinal Endoscopy. Quick Anatomy esson: Human Digestive System. Available at: www.asge.org/press/press.aspx?id=546. Accessed on: 17 June 2015. Helander HF, Fändriks L. Scand J *Gastroenterol* 2014;49:681-689.

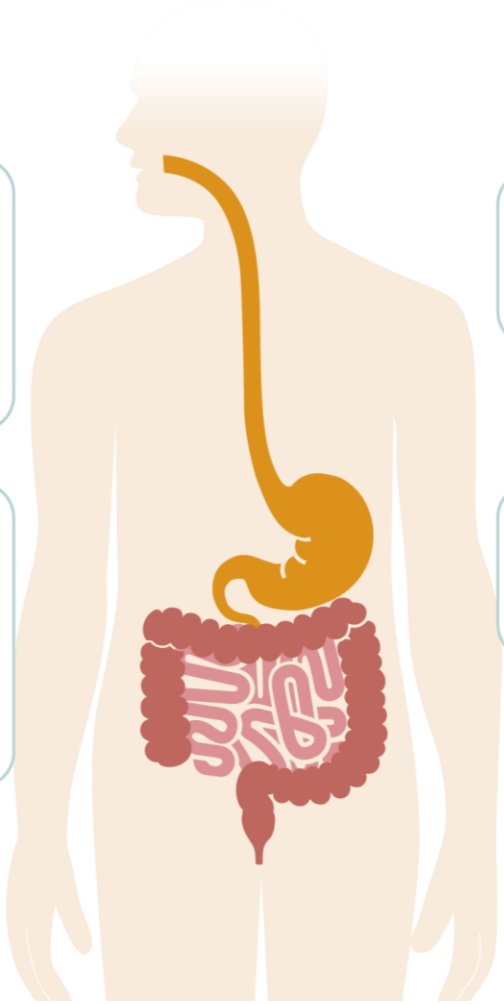
However, the gut is not simplistically a tube and an absorptive surface. Being the largest point of contact to the external environment, it can be exposed to external hazards. Thus, it has its own immune system, which accounts for up to 80% of the body's total immune cells. Furthermore, it has 100 million neurons, a significant number controlling gastrointestinal motility, but also has neurosecretory function that could influence mood, memory, sleep, appetite and satiety. Serotonin is one of the neurotransmitters in the gut, and 95% of the body's total serotonin can be found here. Lastly, the gut is home to 100 trillion bacteria which have developed a symbiotic relationship with us, their hosts.

70% to 80% of the body's immune cells

- Forming a gut-specific immune system[1]

100 million neurons

- Secreting various neurotransmitters regulating mood & satiety[2]



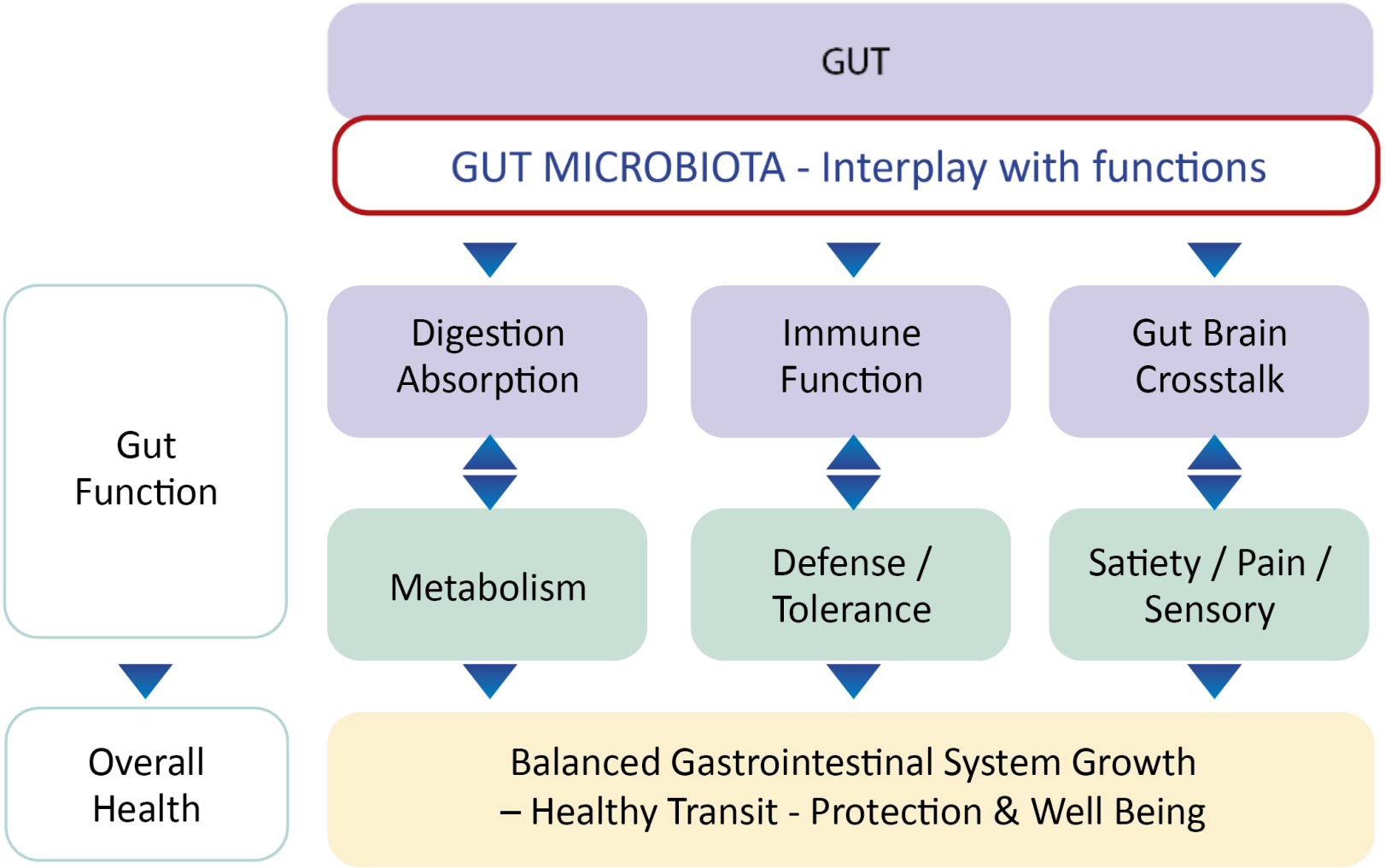
95% of the body's total serotonin[3]

About 100 trillion bacteria[4]

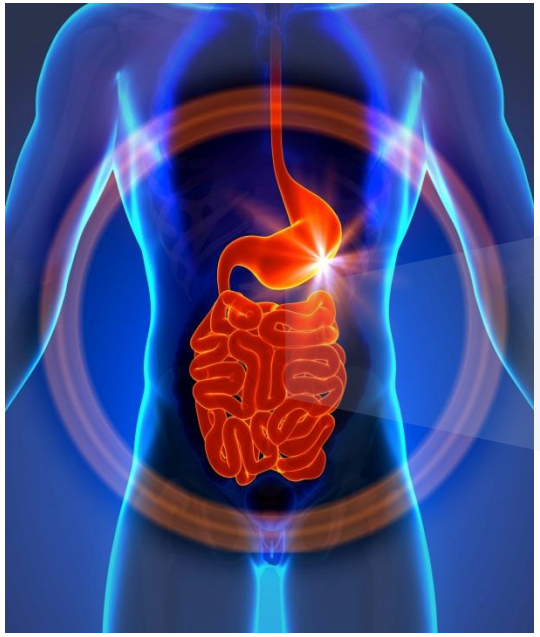
References:

1. Furness JB, Kunze WA, Clerc N. Nutrient tasting and signaling mechanisms in the gut. II. The intestine as a sensory organ: neural, endocrine, and immune responses. *Am J Physiol.* 1999;277:G922–G928.
2. Goyal R, Hirano I. The enteric nervous system. *N Engl J Med*. 1996;344:1106–1115.
3. Baganz NL, Blakely RD. A dialogue between the immune system and brain, spoken in the language of serotonin. *ACS Chem Neurosci*. 2013;4:48–63.
4. Mitsuoka, T. Intestinal flora and aging. *Nutr Rev.* 1992;50: 438–446.

Summary of Gut Functions



Distribution of microbial species along the gut

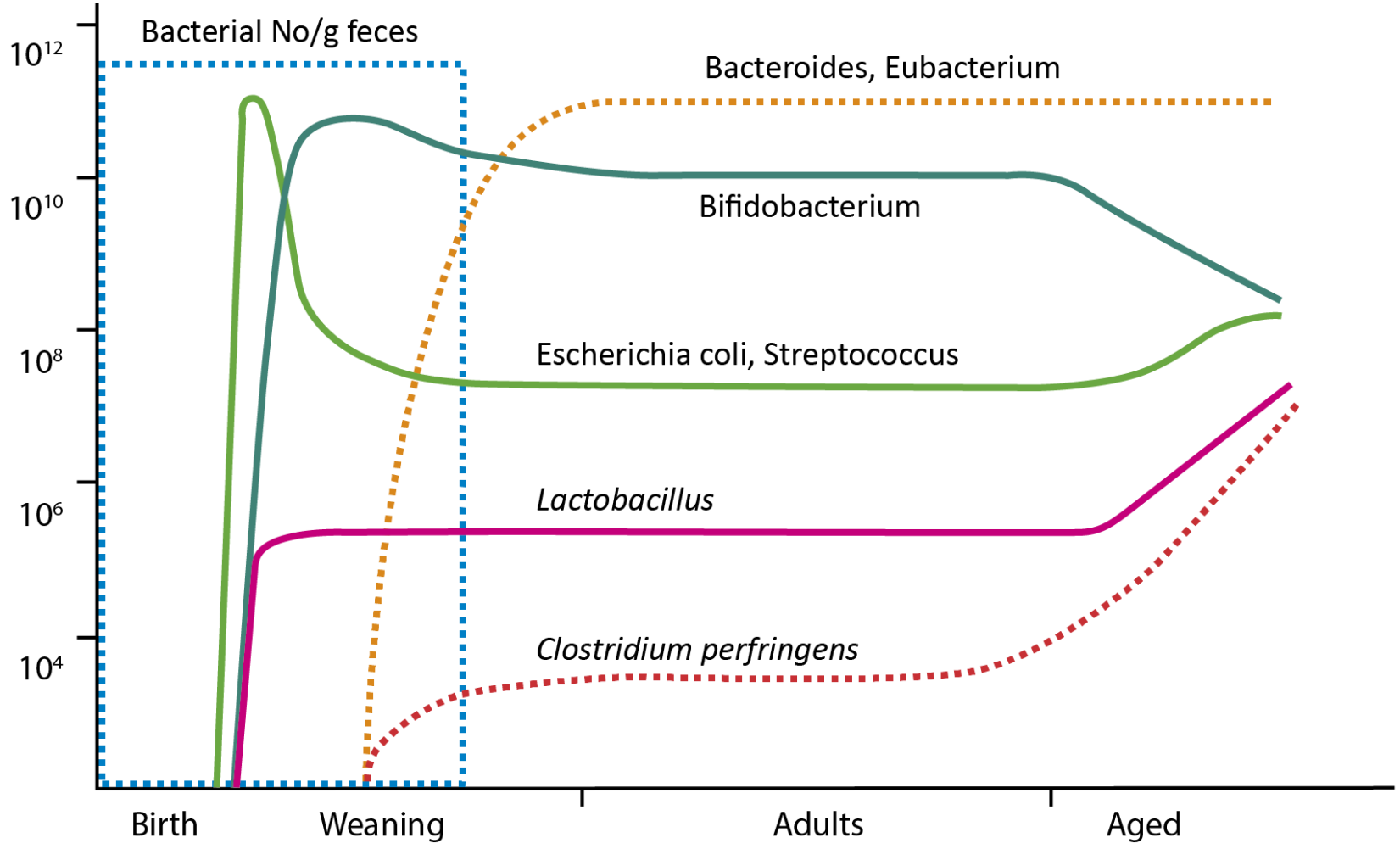


	Cfu / ml
Stomach	10^{1} to 10^{2}
Small intestine	10^{1} to 10^{9}
Large intestine	10^{11} to 10^{12}

Korecka A, Arulampalam V. *J Oral Microbiol* 2012;4. doi: 10.3402/jom.v4i0.9367.

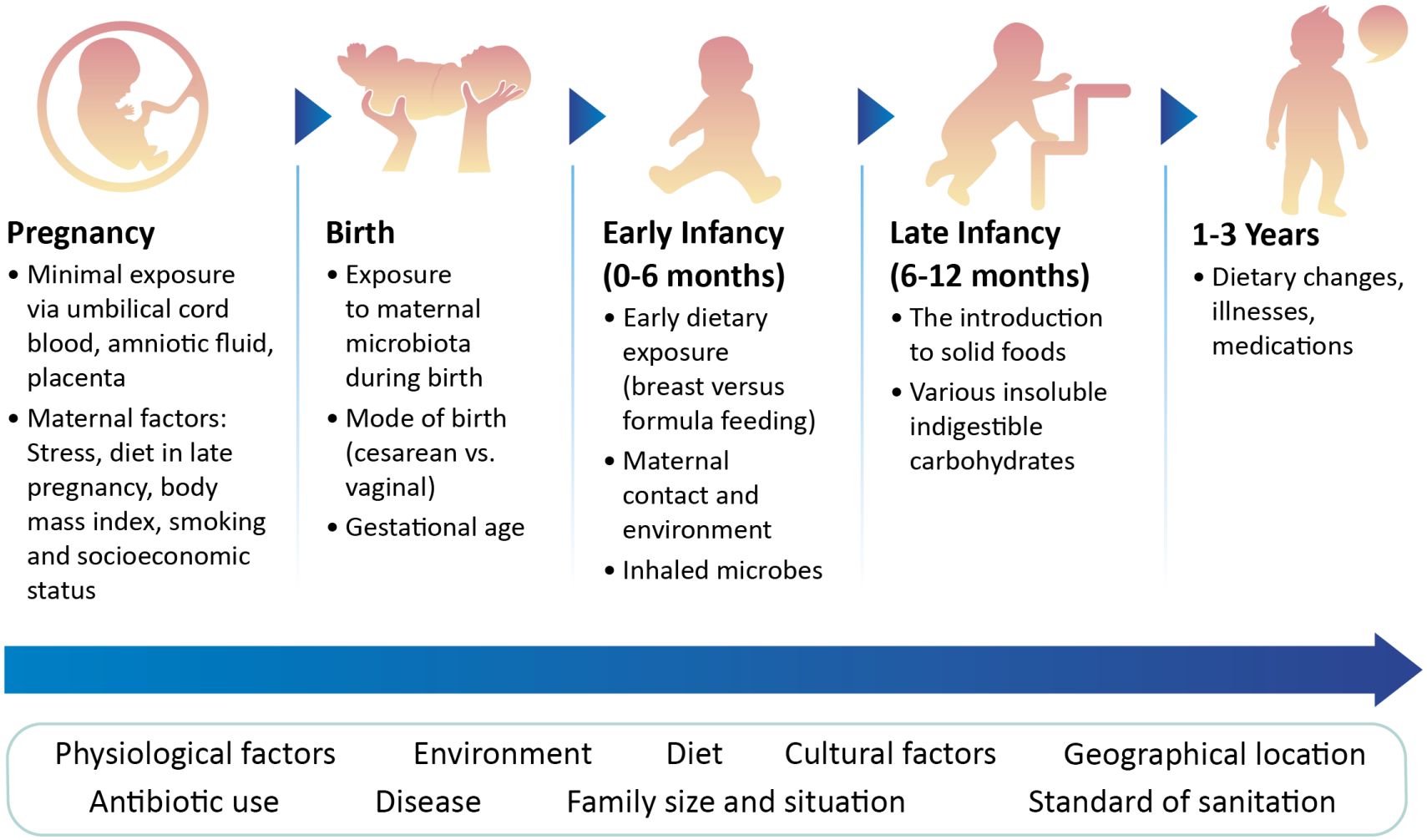
Gut microbiota evolves throughout life

The first 1,000 days is a “window of opportunity”



Mitsuoka T. *Biosci Microbiota Food Health* 2014;33:99–116.

Sources and factors of gut colonization in early life



Shamir R, van Elburg R, Knol J, Dupont C. Gut Health in Early Life: Significance of the Gut Microbiota and Nutrition for Development and Future Health. Essential Knowledge Briefing, Wiley, Chichester (2015)

Gut Microbiota

There is significant variability in microbial composition at different body sites, with a vast difference between health and disease.

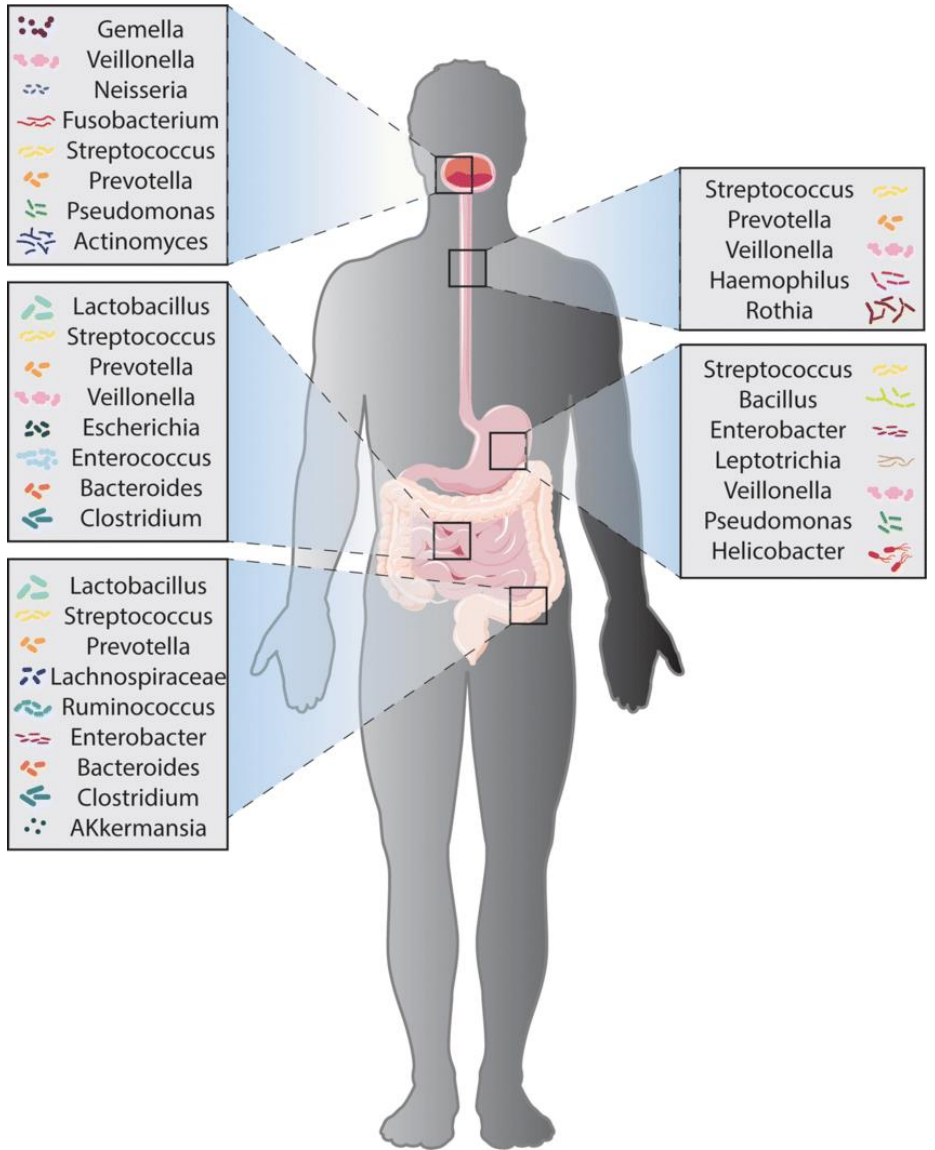
Although the term microbiota is sometimes interchangeably used with the term microbiome, microbiota refers to the organisms living in a specific environment, and microbiome refers to the microorganisms and their genome in a particular environment

The microbial microbiome has a set of genes of approximately 3.3 million active genes compared to 22000 human genes.

The gut microbiota is the organisms that inhabit the gut, forming about 60% of the dry faces; 99% are anaerobic bacteria

Though bacteria form the main bulk of the microbiome, viruses, archaea, and eukaryotes are present in fewer numbers, but we should not ignore their presence

Microbial colonization with more than 1000 species plays an essential role in gut development and maturation.



Gut Microbiota And Digestive And Nutritional Function

Improve digestion with enzymatic capacity to degrade

- Indigestible fiber & oligosaccharides, producing short chain fatty acids (SCFA)[1]
- Phytic acid in grains, releasing minerals[2]
- etc.

Synthesize a variety of essential micronutrients[1]

- Vitamin B12
- Vitamin K
- Folate
- etc.

Metabolize bile acid

- Essential step in bile acid recycling & homeostasis[3]

Microbiota also has an essential metabolic function in the biosynthesis of vitamins (vitamin K, biotin, folic acid, vitamin B12, and pantothenic acid) and amino acids from urea or ammonia. It also plays a role in xenobiotics and drug metabolism

Gut microbiota-derived enzymes can metabolize the bile acids produced by the liver, a critically crucial process to maintain a healthy gut microbiota, enhance lipid and carbohydrate metabolism, increase insulin sensitivity, and enhance innate immunity

1. Wopereis H, et al. *Pediatr Allergy Immunol* 2014;25:428-438. 2. Famularo G, et al. *Med. Hypotheses* 2005;65: 1132–1135.
3. Tremaroli V, Backhed F. *Nature*. 2012;489: 242-249.

Short Chain Fatty Acid (SCFA)

Other microbiota strains can ferment and digest nondigestible carbohydrates, fibers, and endogenous intestinal mucus, producing gases and short-chain fatty acids (SCFAs) such as acetate (the most abundant), propionate, and butyrate. These SCFAs can modulate the various activities in the gastrointestinal tract, including cell proliferation and differentiation, water and electrolytes absorption, hormonal secretion, and immune system activation

Nutrition and Metabolism[1]

- Source of energy (acetate and propionate can account for 10% of daily energy requirement)
- Gut integrity maintenance (60% energy of enterocytes)
- Lipid, glucose and cholesterol metabolism

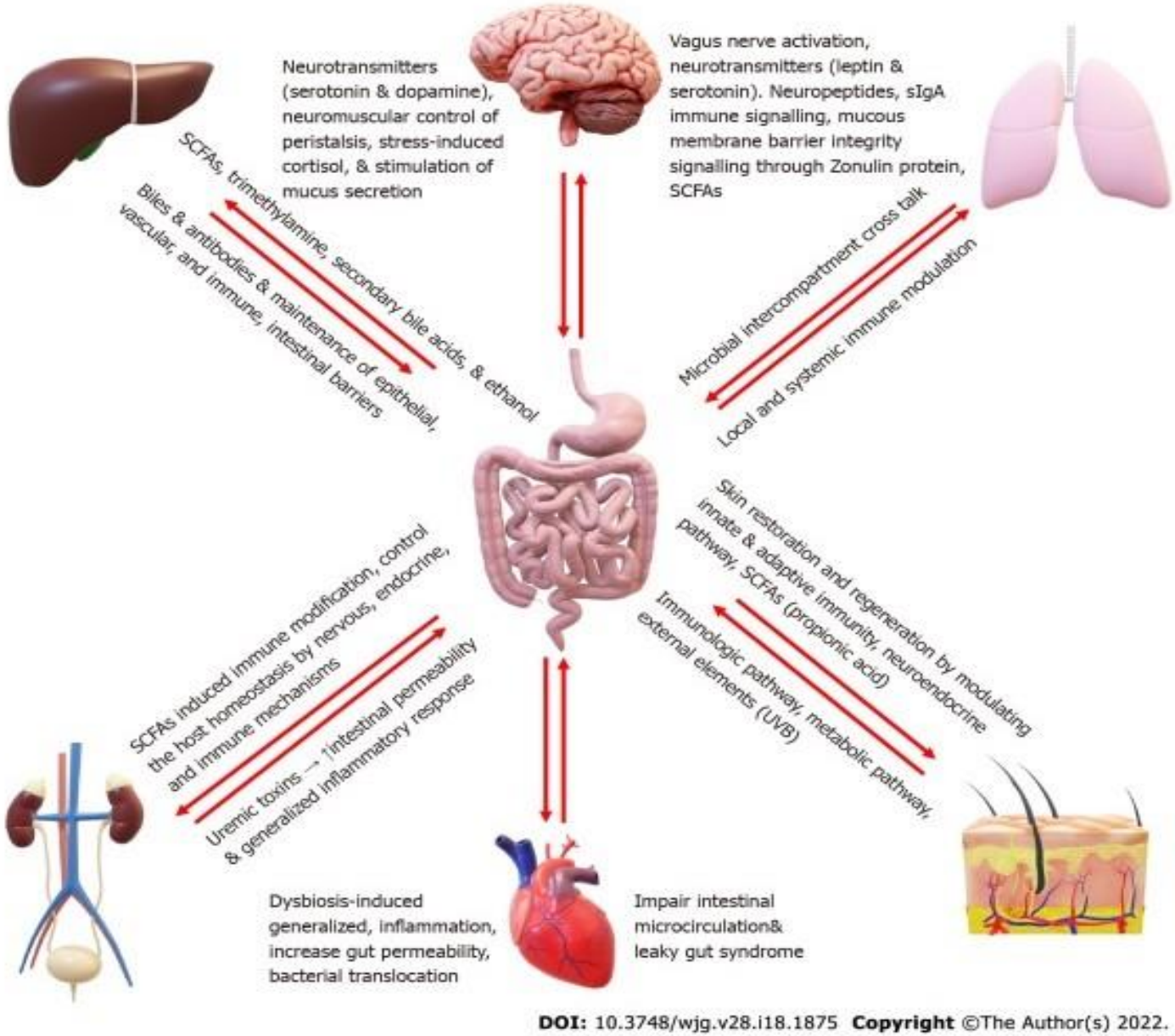
Protection and immunity[2]

- Signaling and precursor role in immune pathways (direct interaction with immune cells)
- Absorption of water & electrolytes (can help minimizing diarrhea)
- Increases colonic blood flow and enhances ileal motility
- Acetate/propionate creates an acidic environment - not favorable for pathogen growth

Gut brain connection[3]

- Signaling and precursor role in neuronal pathways

1. den Besten G, et al. *J Lipid Res* 2013;54:2325-2340. 2. Roy CC, et al. *Nutr Clin Pract* 2006;21:351-366.
3. Nankova BB, et al. *PLoS One* 2014;9:e103740.



DOI: 10.3748/wjg.v28.i18.1875

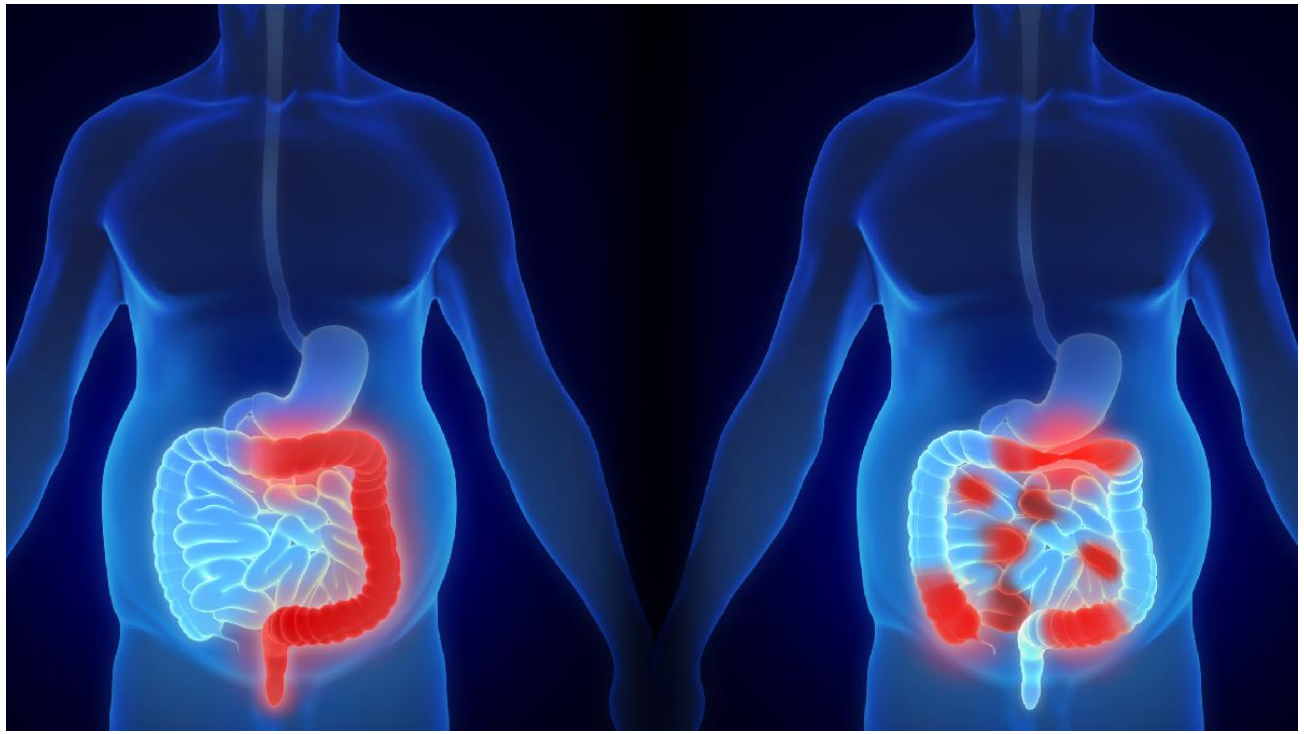
The different gut-microbiota-axes (brain-gut-microbiota axis, liver- gut-microbiota axis, skin- gut-microbiota axis, kidney- gut-microbiota axis, lung- gut-microbiota axis).

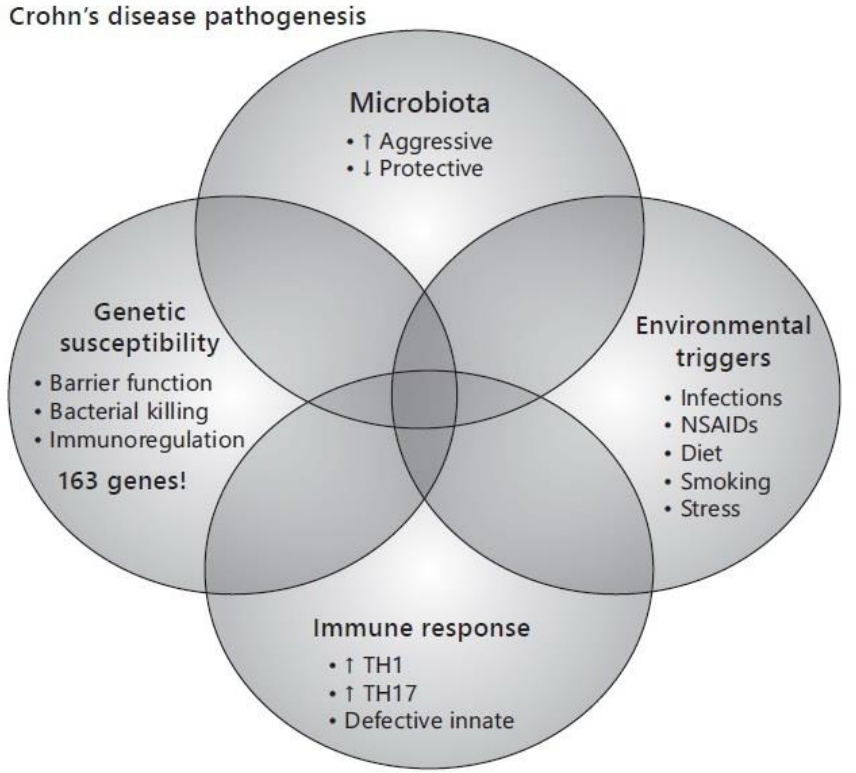
IBD

Currently, IBD affects an estimated 1.4 million individuals in the USA

Ulcerative colitis (UC)

Crohn's disease (CD)

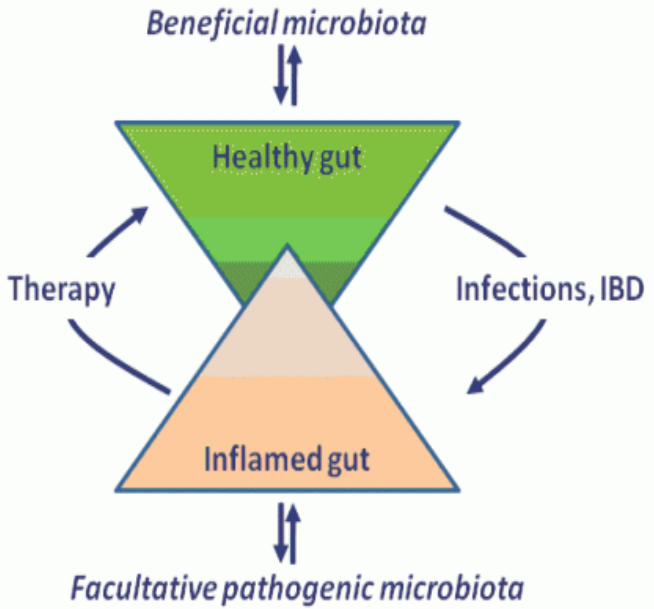




The gut microbiota plays a key role in the pathogenesis of IBD.

Observations supporting a role for the gut microbiota in IBD

- The effectiveness of faecal diversion as a treatment for Crohn's disease
- IBD can respond to antibiotic treatment
- Predisposition of inflammation for anatomical regions with relative faecal stasis (terminal ileum and rectum)
- Germ-free animals do not develop colitis



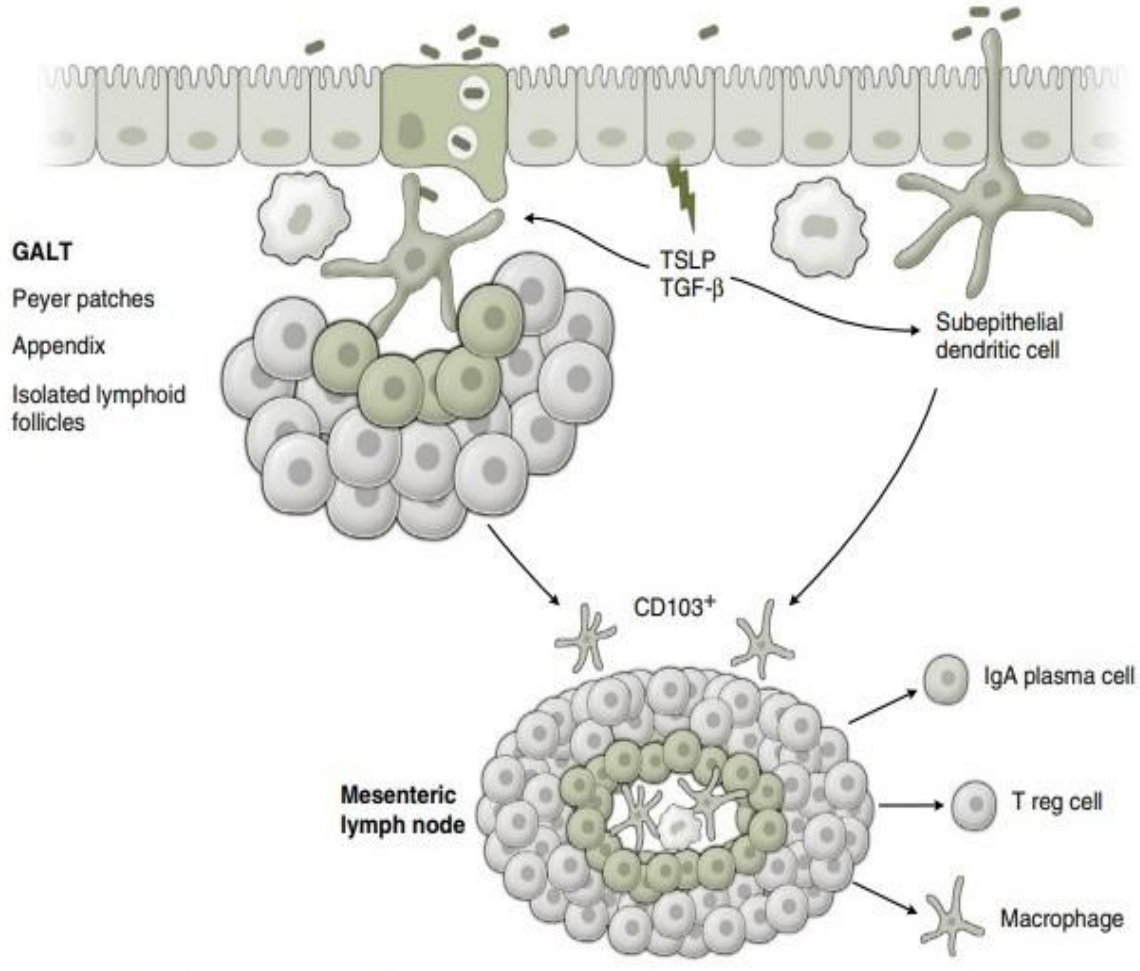
The Mucosal Immune System

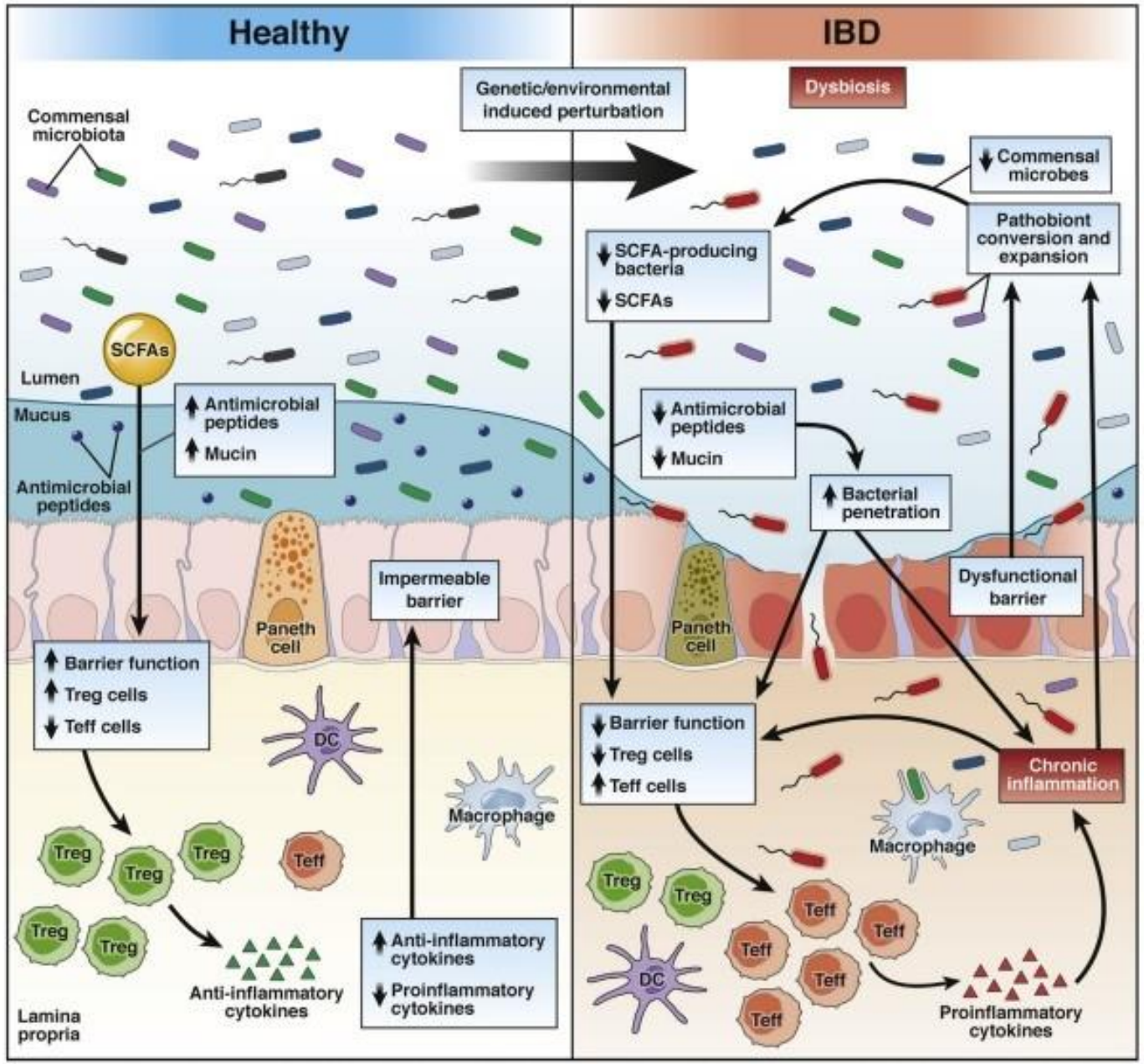
The GALT is organized within three compartments

1. The diffuse lymphoid tissue of the intestinal lamina propria

2. The intraepithelial compartment contains populations of lymphocytes that are uncommon elsewhere in the immune system.

3. Organized lymphoid follicles occur throughout the intestine.




Healthy
IBD
Genetic/environmental induced perturbation
Dysbiosis
Commensal microbiota
Commensal microbes
Pathobiont conversion and expansion
SCFA-producing bacteria
SCFAs
SCFAs
Lumen
Mucus
Antimicrobial peptides
Mucin
Antimicrobial peptides
Mucin
Antimicrobial peptides
Bacterial penetration
Paneth cell
Impermeable barrier
Paneth cell
Dysfunctional barrier
Barrier function
Treg cells
Teff cells
DC
Macrophage
Barrier function
Treg cells
Teff cells
Chronic inflammation
Macrophage
Treg
Treg
Treg
Teff
Treg
Treg
Treg
Treg
Teff
Teff
Teff
Teff
Teff
DC
Anti-inflammatory cytokines
Proinflammatory cytokines
Anti-inflammatory cytokines
Proinflammatory cytokines
Lamina propria

What Determines the Development of IBD Dysbiosis?

Genetic Susceptibility and Immune Regulation

2 of the major IBD susceptibility genes include NOD2 and ATG16L1. These genes are involved in the autophagy pathway, a major component in pathogen sensing and clearance to ensure only the correct microbes remain in the host.

Mutations in these IBD-associated genes may lead to defects in immune responses including T-cell differentiation (IL10, IL21), TH17 cell maintenance (IL23R, JAK2, PTPN2), and nuclear factor–kB activation (TNF-signaling genes)

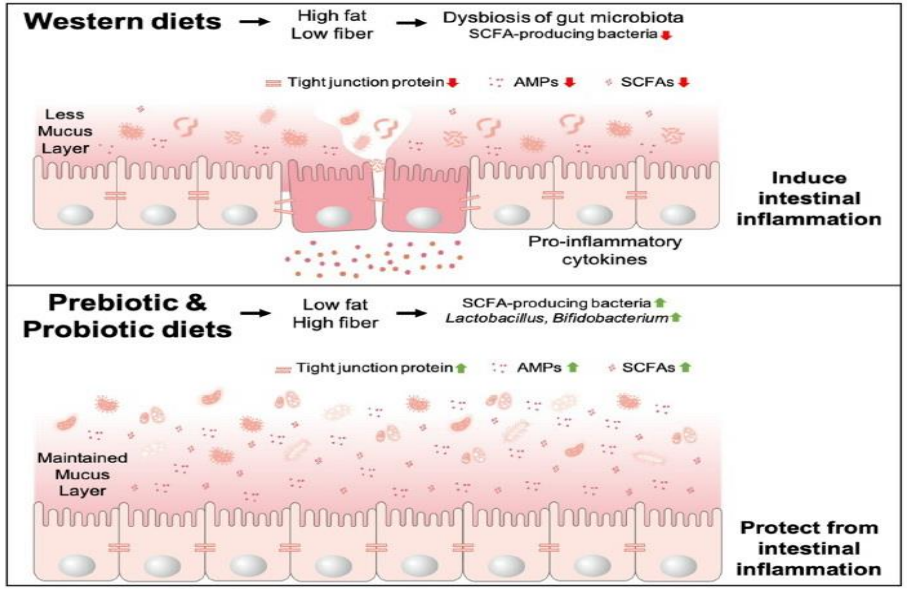
Aside from impaired microbial sensing ability and bacterial clearance, IBD gene variants also may alter intestinal immune homeostasis by disrupting the intestinal epithelial barrier.

These genes could act as genetic biomarkers to identify individuals at higher risk of developing IBD to potentially treat the disease before onset of symptoms.

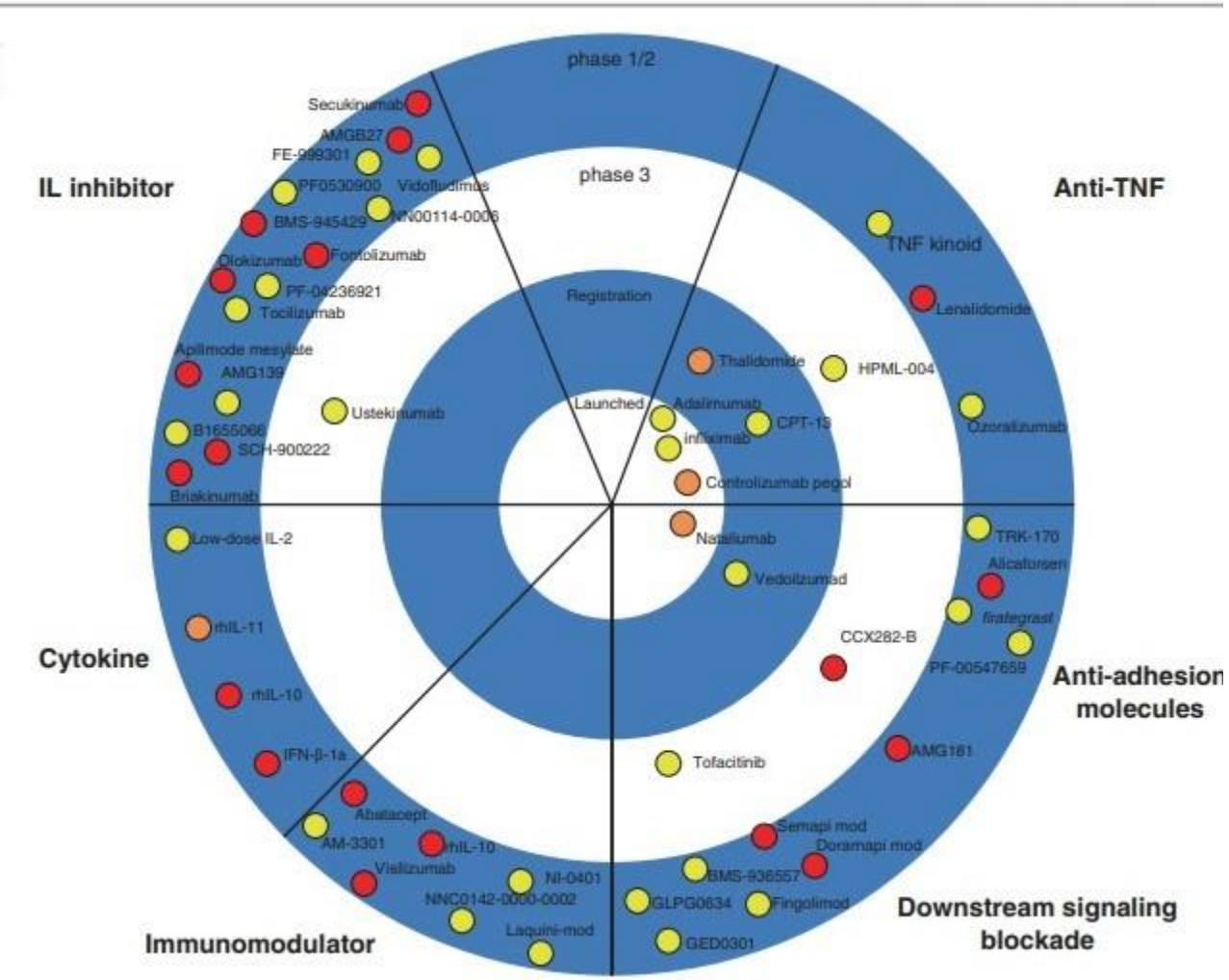
Diet

Western diets containing
high fat and lack of fiber changes gut microbiota population leading to
1. **Bacteroides** often found at relatively low abundances, while **Firmicutes** and **Proteobacteria** are found at high abundances in patients consuming a high-fat diet
2. Decrease of bacterial SCFAs, host AMPs, and mucus production as well as tight junction protein expression.
3. It disrupts intestinal barrier, leads to bacterial translocation and increases pro-inflammatory cytokine production—resulting in intestinal inflammation.

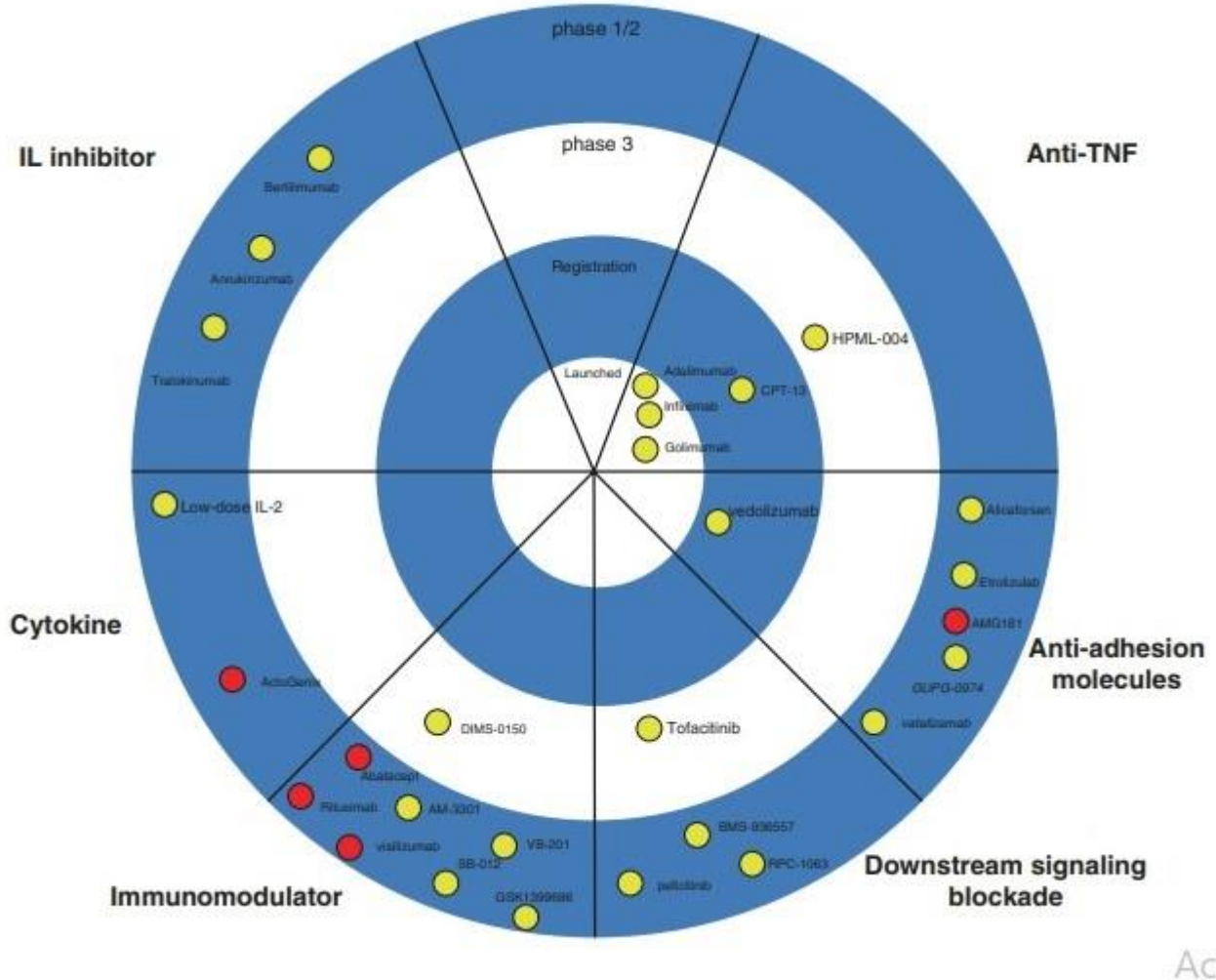
Prebiotic and probiotic diets provide a high fiber content, which increases the production of SCFAs, AMPs, mucus, and tight junction protein expression resulting in intact intestinal barrier and prevent from intestinal inflammation.



Drugs in pipeline for CD

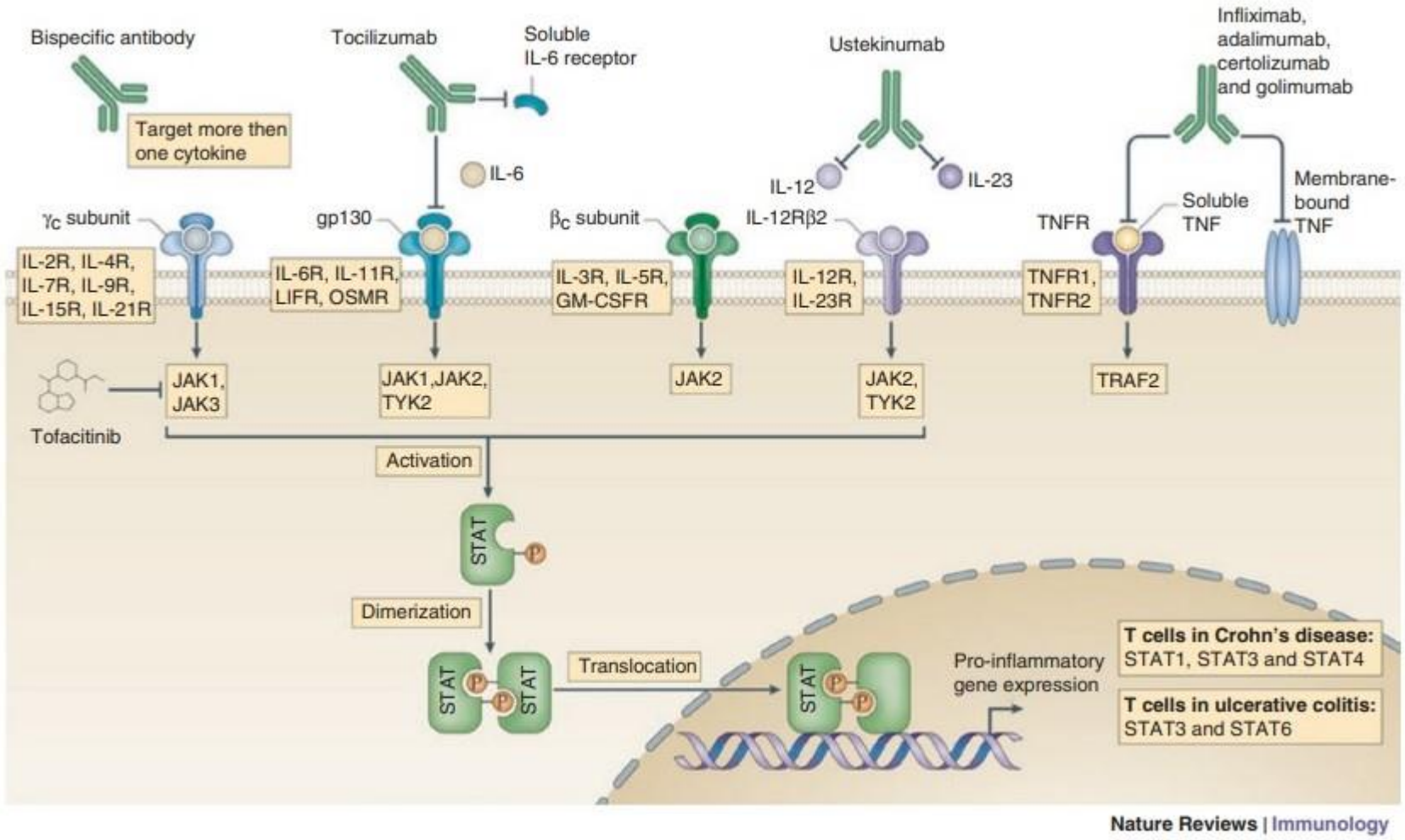


Drugs in pipeline for UC



New Non-anti-TNF-α Biological Therapies

Anti-TNF Biologic Therapies

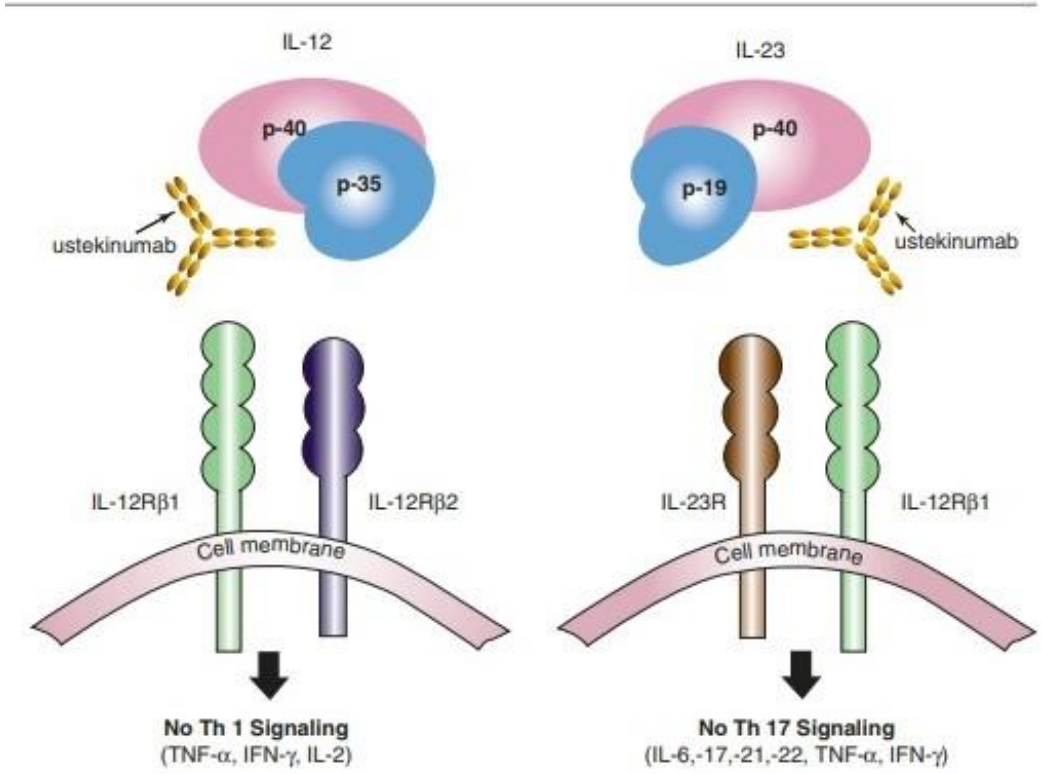


Ustekinumab

Ustekinumab, a monoclonal antibody targeting interleukin 12 and 23, has demonstrated efficacy for induction and maintenance of clinical remission in randomised placebo-controlled trials

Data on ustekinumab efficacy in paediatric CD are still limited. Dayan et al. retrospectively reviewed outcomes with ustekinumab therapy administered similarly to the UNITI trials in 52 patients with median age 16.8 years , 42 of whom had CD. Steroid-free clinical remission was achieved in 40% at Week 52

The first dose of ustekinumab is usually administered intravenously and is 6 mg/kg rounded to 130 mg [maximum 520 mg]. SC dosing starts at Week 8; adult patients receive a 90-mg injection. Children should receive a body surface area [BSA]-adjusted dose [considering a standard adult of 1.73 m2] every 8 weeks. Clinical benefit can be observed from 8 weeks following intravenous induction.



Vedolizumab

Vedolizumab is a gut-selective humanized monoclonal antibody targeting the α4β7 integrin that is effective in patients with IBD who are refractory or intolerant to systemic steroids, immunomodulators, or anti-TNF agents.

Vedolizumab downregulates intestinal inflammation by specifically inhibiting intestinal T-lymphocyte migration into the tissue

Vedolizumab is effective in both CD and ulcerative colitis [UC], but is likely more effective in UC

Higher rates of clinical response are observed when vedolizumab is given as a first-line biologic treatment [ie, no previous anti-TNF therapy]

Vedolizumab use is not associated with increased risk of opportunistic infections or malignancy

Standard vedolizumab dosing in adults has been adapted in pediatric studies (5 mg/kg up to 300 mg per dose at weeks 0, 2, 6 followed by every 8 weeks thereafter).

The effect of vedolizumab in UC has been described to occur by week 6 of treatment, but complete response may not be apparent until week 14. Shortening of interval between infusions to 4 weekly may be required during maintenance in partial responders

Tofacitinib

G-O068 Topic: AS01 GASTROENTEROLOGY / AS01i Inflammatory bowel disease

TOFACITINIB IN PEDIATRIC ULCERATIVE COLITIS: A RETROSPECTIVE MULTI-CENTER EXPERIENCE FROM THE PAEDIATRIC IBD PORTO GROUP OF ESPGHAN

Oren Ledder, Michael Dolinger, Marla Dubinsky, Ronen Stein, Siddhi Savla, Ayesha Fatima, David Suskind, Jarrad Scarlett, Dennis Roeser, Dror Shouval, Gabriele Meyer, Zarela Molle Rios8, Gemma Pujol, Ana Lozano Ruf, Kaija-Leena Kolho, Pejman Rohani, Seamus Hussey, Tim De Meij, Travis Ayers, Víctor Manuel Navas López, Dan Turner, Christos Tzivinikos

Objectives and Study: Tofacitinib, a Janus kinase (JAK) inhibitor, has recently been approved for treatment of moderate to severe active ulcerative colitis (UC) in adults. Data on efficacy and safety in pediatrics are limited. In this multicenter study from the Paediatric IBD Porto group of ESPGHAN, we describe the short-term effectiveness and safety of tofacitinib in an international pediatric IBD cohort.

Methods: Retrospective review of children (2-18 years) diagnosed with UC treated with tofacitinib from 15 pediatric centers internationally. Primary outcome was corticosteroid-free clinical remission (PUCAI <10) at week 8, with secondary outcomes including clinical response (≥20 point decrease in PUCAI), colectomy rate and safety. Primary outcome was calculated utilizing non-response imputation (NRI), whereby drug cessation for any reason was considered treatment failure

Results: 78 patients (43 (55%) female, mean age at diagnosis 12.5 (±2.7) years, median disease duration 20 months (IQR 10.3-38.8)), all with previous biologic failure, including 20/78 (26%) with previous failure of three biologic classes. 15/78 (19%) patients achieved corticosteroid-free clinical remission at week 8 with a further 18/78 (23%) demonstrating clinical response. 9/78 (12%) underwent colectomy by week 8, and 21/78 (27%) by week 24. Twelve adverse events were reported including five infective (three of which deemed possibly related to treatment – zoster, HSV-2 cheilitis and septic arthritis), one case of pancreatitis, and abnormal blood test results in 5 children (anemia, lymphopenia, elevated hepatic transaminases and hypercholesterolemia)

Conclusions: In this largest real-life cohort of tofacitinib in pediatric UC to date, tofacitinib seemed effective in at least 19% of highly refractory patients by week 8. Adverse reactions and safety were largely consistent with adult data.

Upatacitinib

The NEW ENGLAND JOURNAL of MEDICINE

RESEARCH SUMMARY

Upadacitinib Induction and Maintenance Therapy for Crohn's Disease

Loftus EV Jr. et al. DOI: 10.1056/NEJMoa2212728

CLINICAL PROBLEM

Treatment options with new mechanisms of action are needed for patients with moderate-to-severe Crohn's disease. Upadacitinib — an oral, reversible Janus kinase (JAK) inhibitor — showed promise for treatment of Crohn's disease in a phase 2 trial.

CLINICAL TRIALS

Design: Two multinational, phase 3, double-blind, randomized, placebo-controlled induction trials (U-EXCEL and U-EXCEED) and one maintenance trial (U-ENDURE) evaluated the efficacy and safety of upadacitinib in adults with moderate-to-severe Crohn's disease.

Intervention: 1021 patients were assigned to receive induction therapy with upadacitinib (45 mg) or placebo (2:1 ratio) once daily for 12 weeks; 502 who had a clinical response at week 12 were then assigned to receive maintenance therapy with upadacitinib (15 mg or 30 mg) or placebo (1:1:1 ratio) once daily for 52 weeks. The primary end points — clinical remission and endoscopic response — were evaluated at week 12 of induction treatment and week 52 of maintenance treatment.

RESULTS

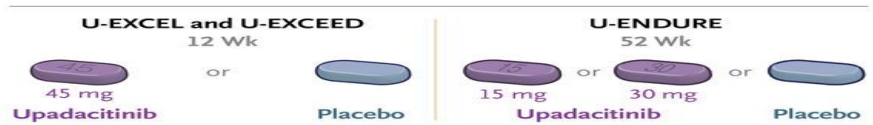
Efficacy: Upadacitinib was superior to placebo with respect to clinical remission and endoscopic response in both induction trials and in the maintenance trial.

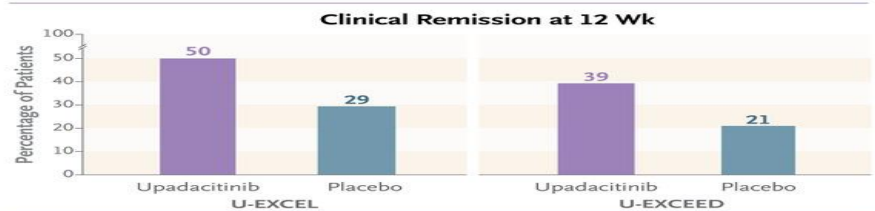
Safety: The frequencies of any, serious, and severe adverse events were similar across the groups at week 12 of induction and week 52 of maintenance. Herpes zoster, hepatic disorders, and neutropenia were more common with some doses of upadacitinib than with placebo.

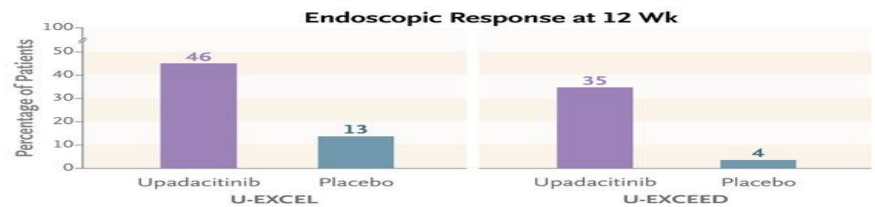
LIMITATIONS AND REMAINING QUESTIONS

- The trials could not identify adverse events that were rare or had a long latency. The ongoing extension study of U-ENDURE will continue to evaluate safety for up to 5 years.

Links: Full Article | NEJM Quick Take | Science behind the Study







CONCLUSIONS

In patients with moderate-to-severe Crohn's disease, induction and maintenance treatment with the JAK inhibitor upadacitinib was associated with higher percentages of patients with clinical remission and endoscopic response than receipt of placebo.

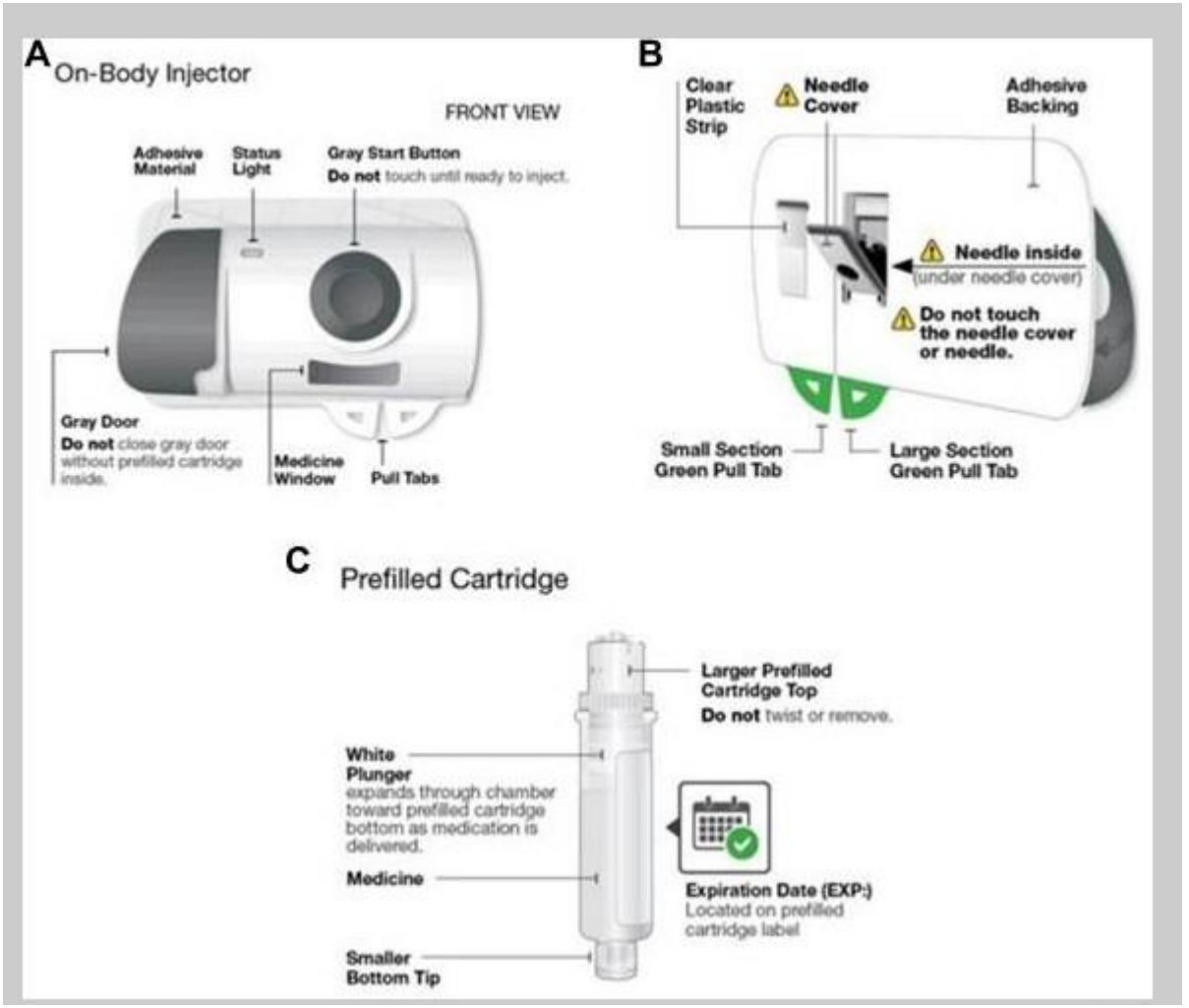
Risankizumab

FDA grants orphan drug designation to risankizumab for pediatric Crohn's disease

The FDA has issued orphan drug designation to AbbVie's investigational interleukin-23 inhibitor risankizumab for children with Crohn's disease, the company announced.

Risankizumab (ABBV-066; formerly BI 655066) selectively blocks IL-23, and is currently being evaluated in Crohn's disease, psoriasis, psoriatic arthritis and asthma, according to a press release.

Risankizumab is approved for treatment of moderately to severely active Crohn's disease in adults. Patients receive three intravenous induction doses of 600 mg over at least one hour at weeks 0, 4, and 8. They then start 180 mg/1.2 mL or 360 mg/2.4 mL injections at week 12 and continue every 8 weeks with an on-body injector device.



Ozanimod

Sphingosine-1-phosphate (S1P) is a membrane-derived phospholipid molecule.

Sphingomyelin, a major component in the mammalian cell membrane, is first broken down to ceramide and phosphorylcholine via acid sphingomyelinase. Ceramide is then converted to sphingosine and a fatty acid residue chain via ceramidase and sphingosine is then phosphorylated by sphingosine kinase to yield S1P

The current model of understanding lymphocyte and other leukocyte migration throughout their life cycle includes this S1P gradient and the ability for S1P to bind S1P1

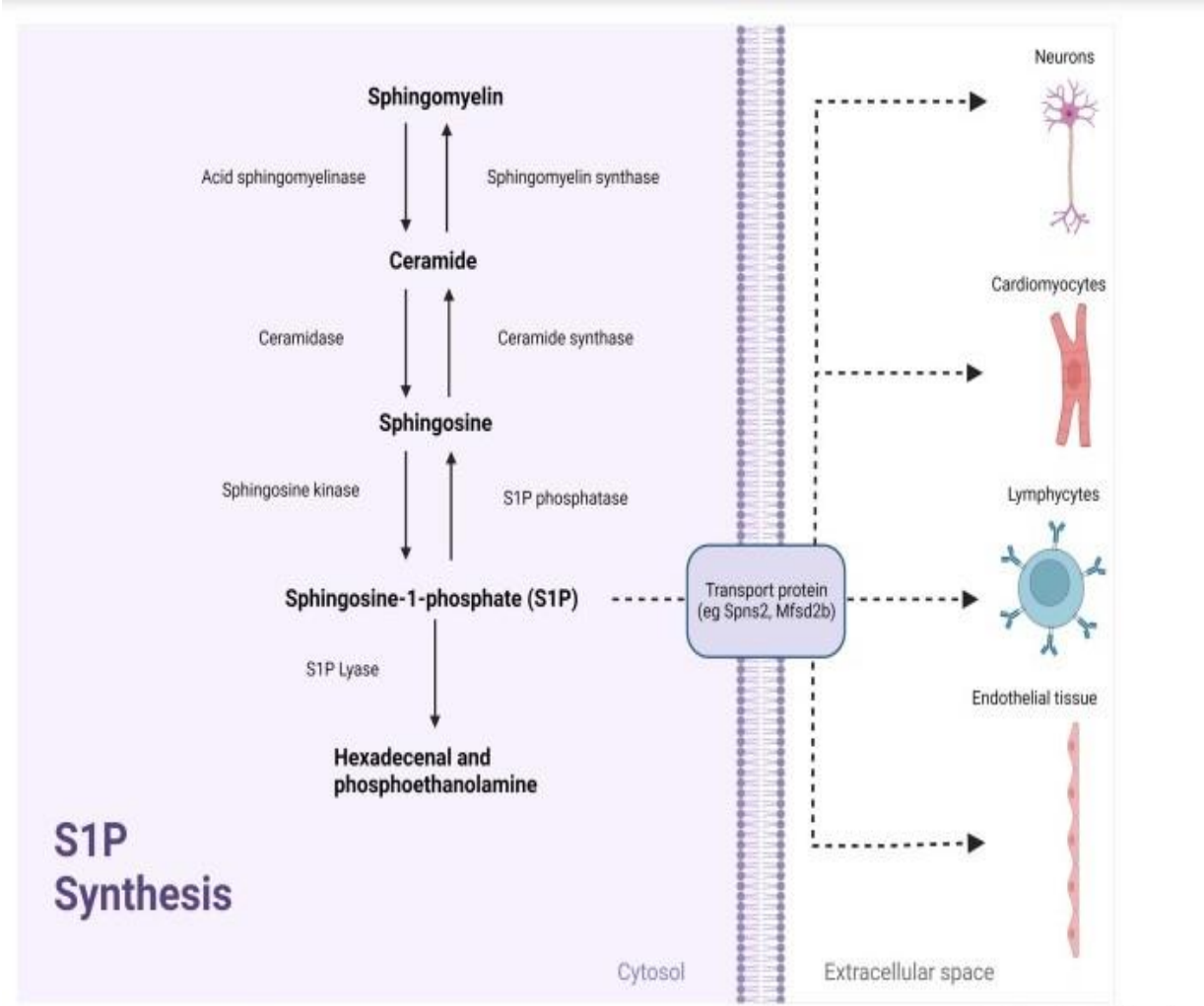
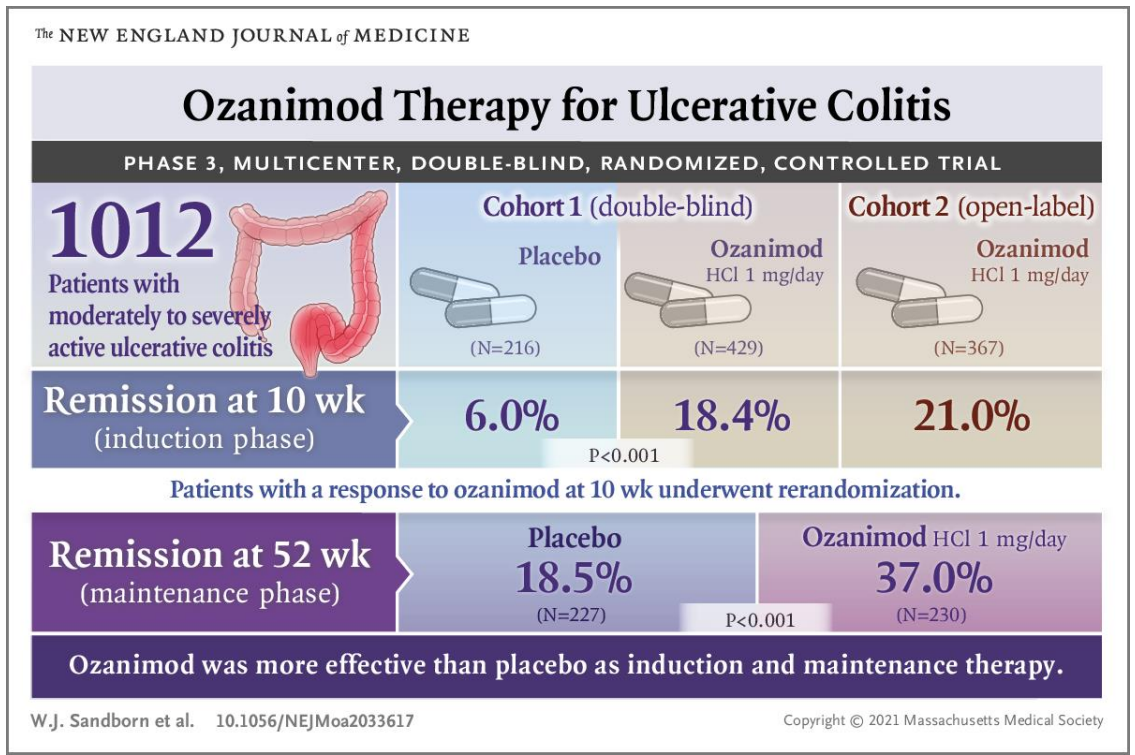


Figure 1 S1P Synthesis. Adapted from Nitric Oxide Synthesis 1, by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates.

Small molecule therapy with ozanimod, a first in class S1P modulator for IBD, offers an oral treatment option for the treatment of UC. Ozanimod demonstrated efficacy and a favorable side effect profile, providing a unique option among the currently available therapies. **Though there are no restrictions to ozanimod as a first-line therapy, its correct positioning in the therapeutic algorithm for the treatment of UC remains to be defined**



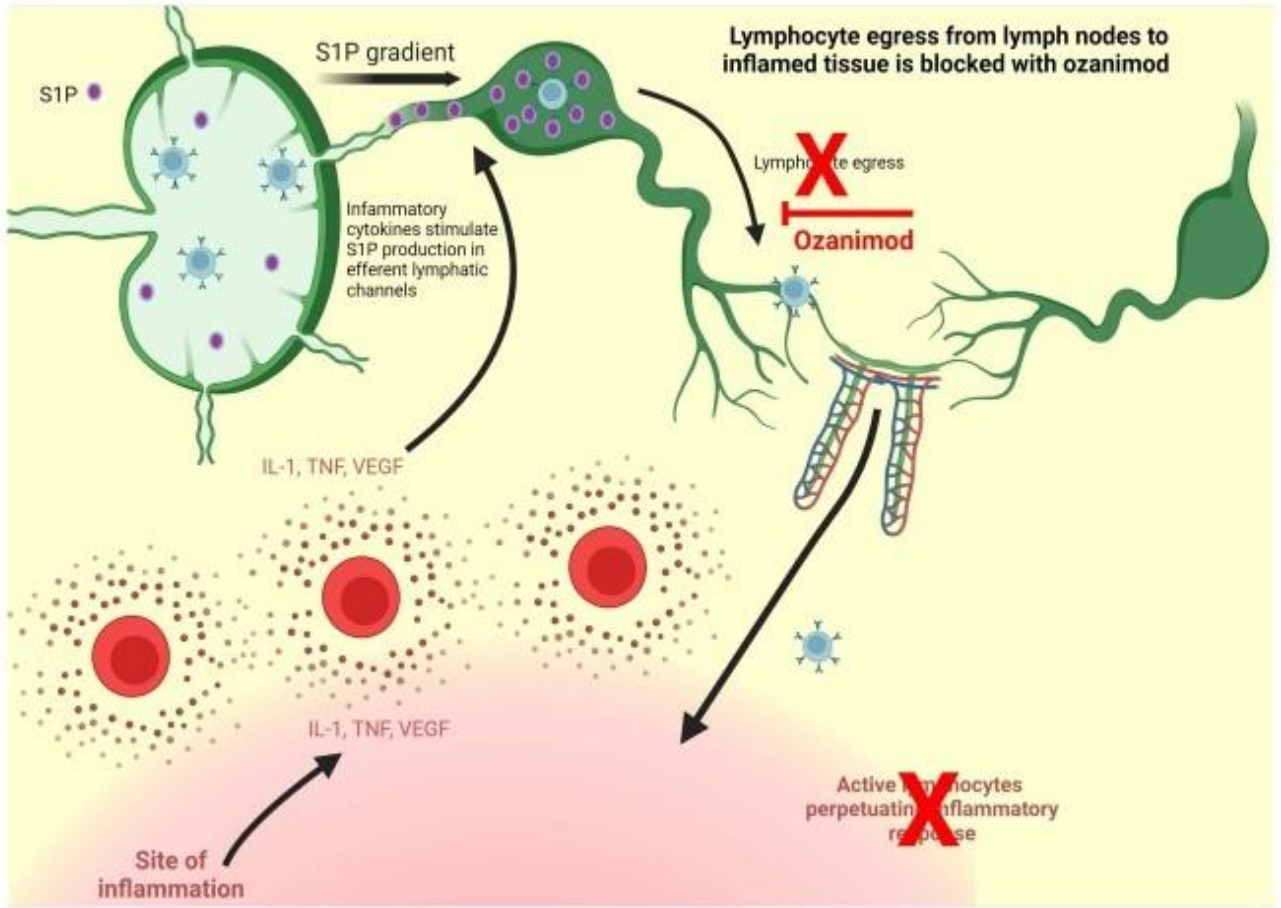


Figure 3 Ozanimod mechanism of action. Adapted from Stimulated T Cells Migrate Out of Lymph Nodes and Enter Inflamed Tissue, by BioRender.com; 2022. Retrieved from: https://app.biorender.com/biorender-templates.[32]