


## Gastroenteropatie nel paziente emopatico

# LA GVHD INTESTINALE

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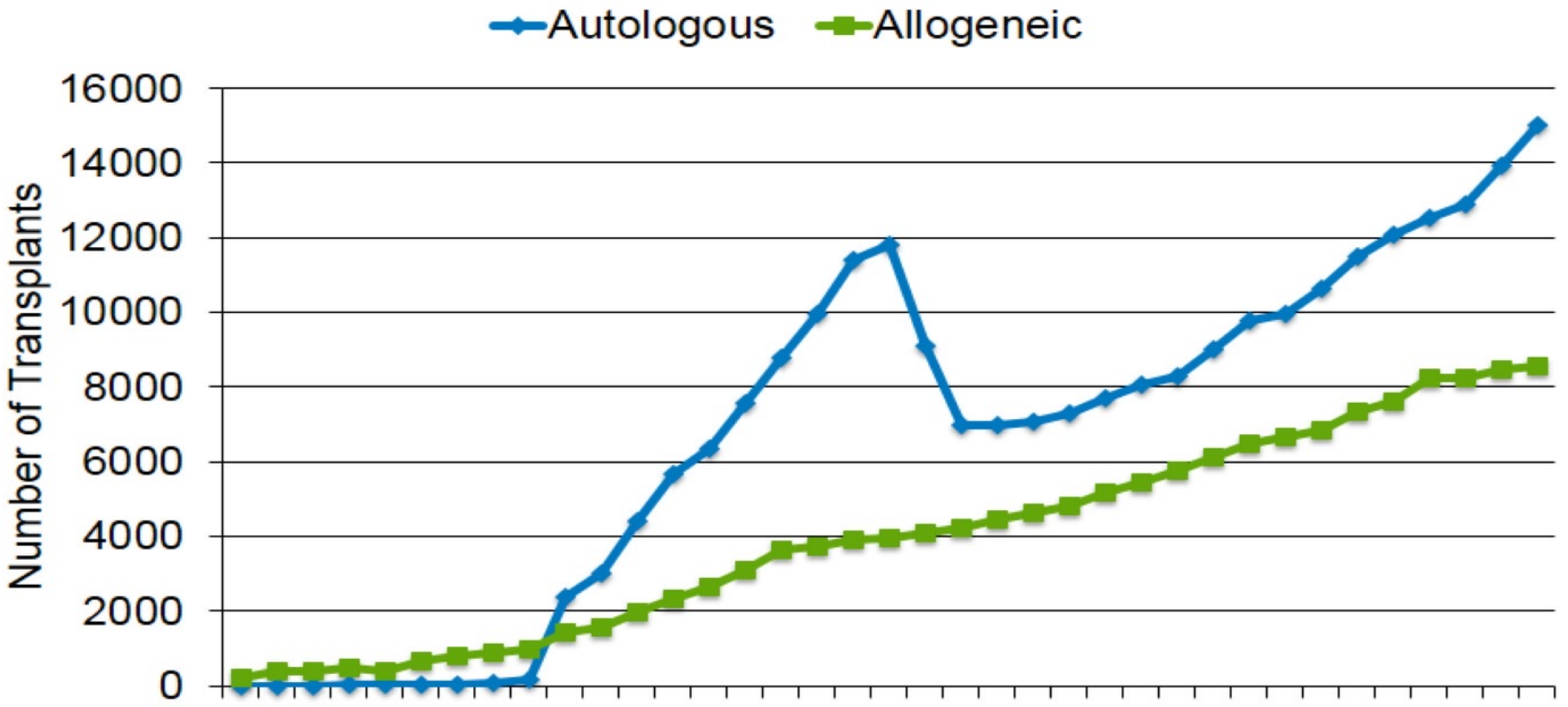
# 20 ANNI DI EMATOLOGIA A TREVISO
# Treviso, Auditorium Fondazione Cassamarca
# 18-20 Novembre 2021

## DICHIARAZIONE

**Come da nuova regolamentazione della Commissione Nazionale per la Formazione Continua del Ministero della Salute, è richiesta la trasparenza delle fonti di finanziamento e dei rapporti con soggetti portatori di interessi commerciali in campo sanitario.**

- **Posizione di dipendente in aziende con interessi commerciali in campo sanitario (NIENTE DA DICHIARARE)**
- **Consulenza ad aziende con interessi commerciali in campo sanitario (NIENTE DA DICHIARARE)**
- **Fondi per la ricerca da aziende con interessi commerciali in campo sanitario (NIENTE DA DICHIARARE)**
- **Partecipazione ad Advisory Board (NIENTE DA DICHIARARE)**
- **Titolarità di brevetti in compartecipazione ad aziende con interessi commerciali in campo sanitario (NIENTE DA DICHIARARE)**
- **Partecipazioni azionarie in aziende con interessi commerciali in campo sanitario (NIENTE DA DICHIARARE)**
- **Altro**

# Annual Number of HCT Recipients in the US by Transplant Type



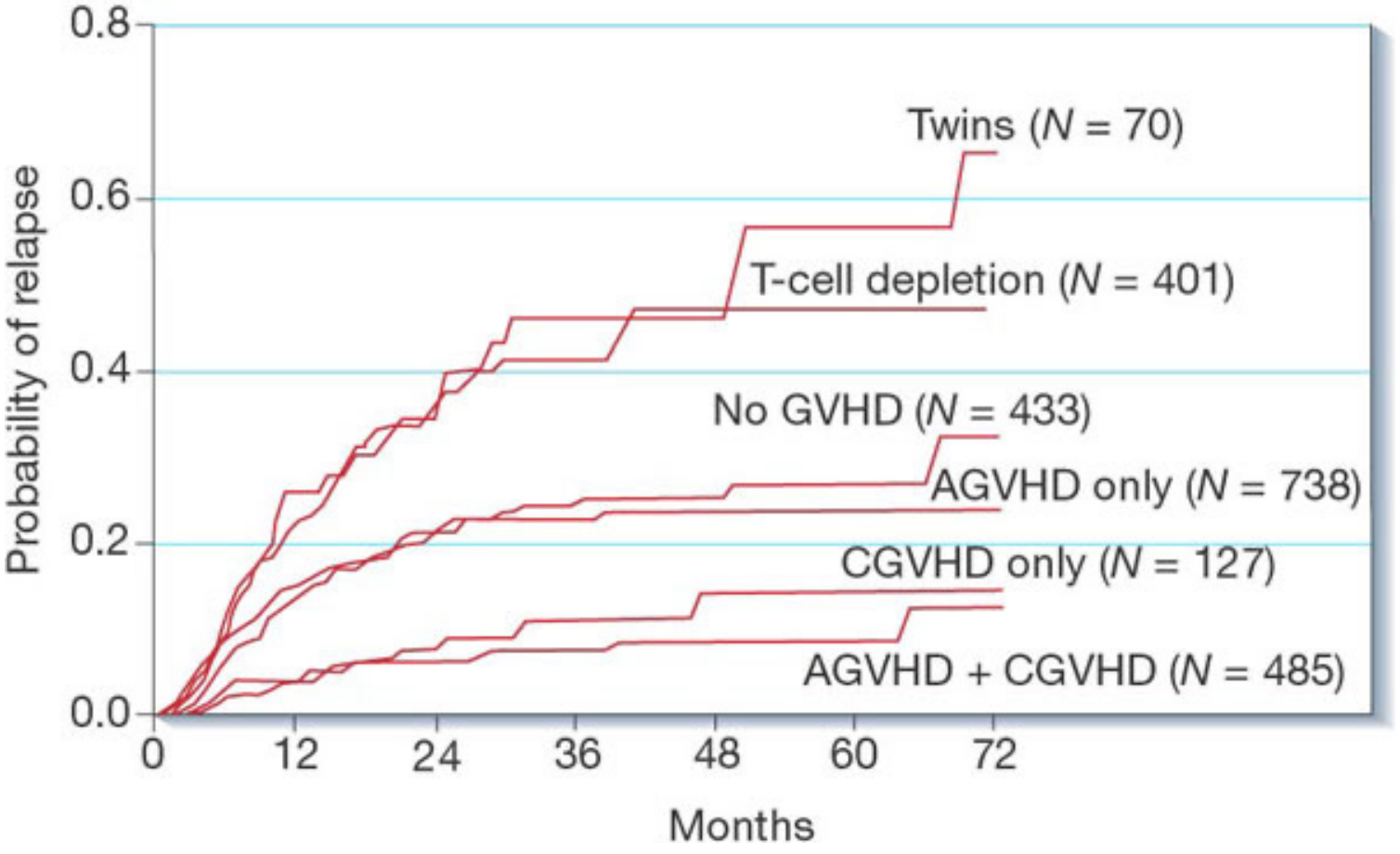

CIBMTR
CENTER FOR INTERNATIONAL BLOOD & MARROW TRANSPLANT RESEARCH

# GRAFT-VERSUS-LEUKEMIA REACTIONS AFTER BONE MARROW TRANSPLANTATION

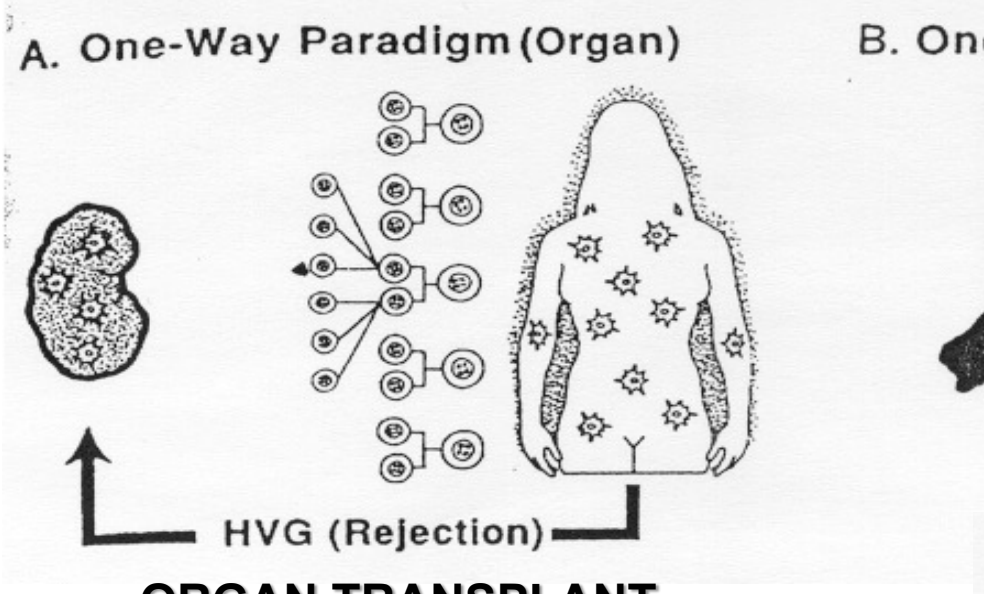
- *Horowitz MM, Blood '90; 75: 555 - 562*

- **2254 patients;**
- **AL- 1CR**
- **CML – CP**

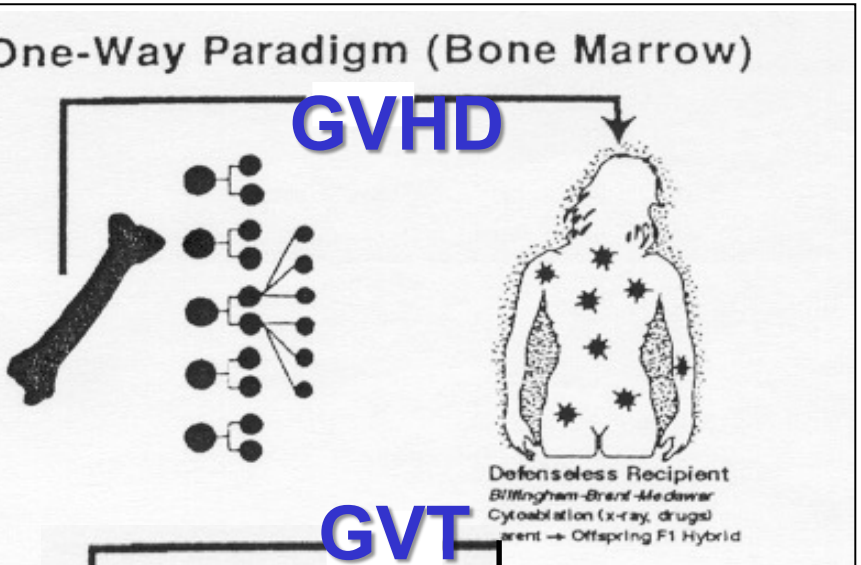
- **TCD**
- **no TCD**
- **Twins**

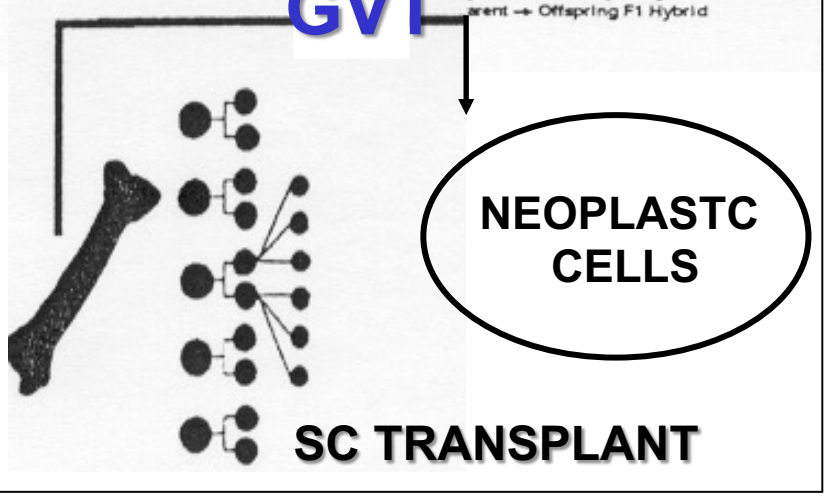


# TRAPIANTO DI CELLULE STAMINALI ALLOGENICHE: BARRIERA IMMUNOLOGICA




ORGAN TRANSPLANT

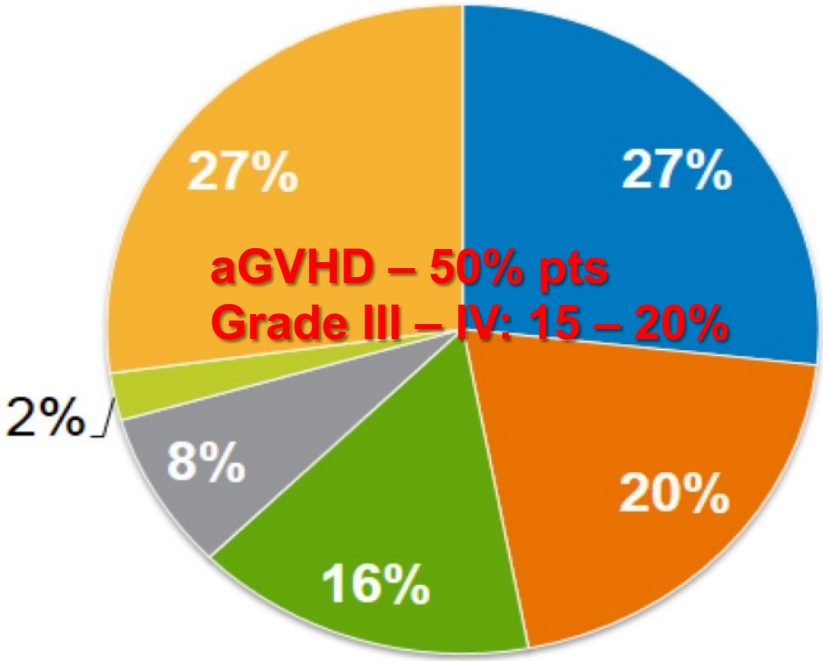


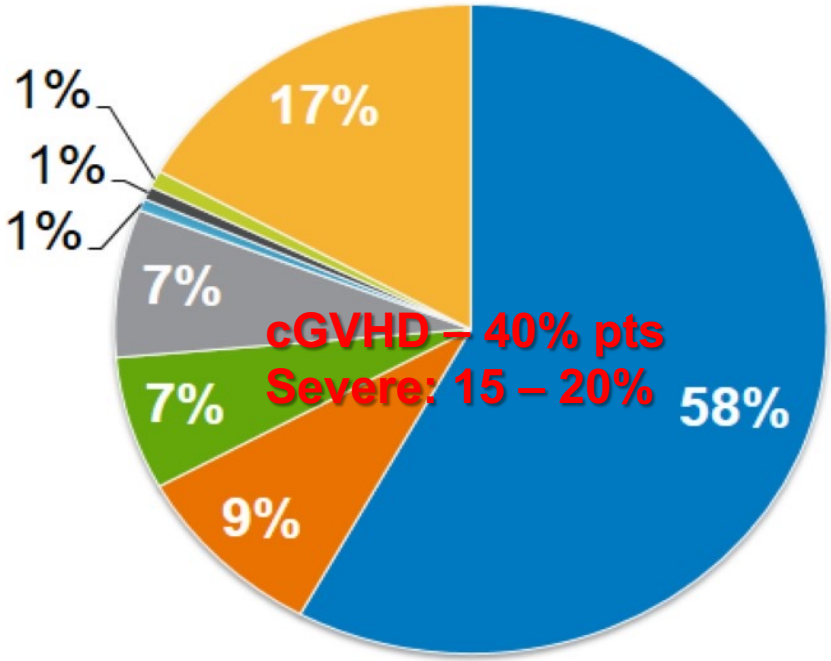

**GOAL**
**SEPARAZIONE GVHD - GVL**

# Causes of death after allogeneic stem cell transplantation

Died within 100 days post-transplant




Died at or beyond 100 days post-transplant*




*Data reflects 3-year mortality

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# CLINICAL STAGE AND GRADE OF ACUTE GVHD

| Stage | Skin | Liver bilirubin | GI diarrhoea |
|---|---|---|---|
| 1 | Maculopapular rash <25% body surface | 2 - 3mg/dl | 500 - 999ml/d |
| 2 | Maculopapular rash 25-50% body surface | 3.1- 6mg/dl | 1000 – 1500ml/d |
| 3 | Maculopapular rsh >50% body surface | 6.1 – 15mg/dl | >1500ml/d |
| 4 | Generalized erythroderma + bullous formation and desquamation | >15mg/dl | severe abdominal pain - ileus |

**Grade**

| | |
|---|---|
| I | stage 1-2 without liver, GI |
| II | stage 3 skin and/or stage 1 liver and/or stage 1 GI |
| III | stage 2-3 liver and/or stage 2-3 GI with stage 0-3 skin and or stage 0-1 GI |
| IV | stage 4 skin, liver, or GI |

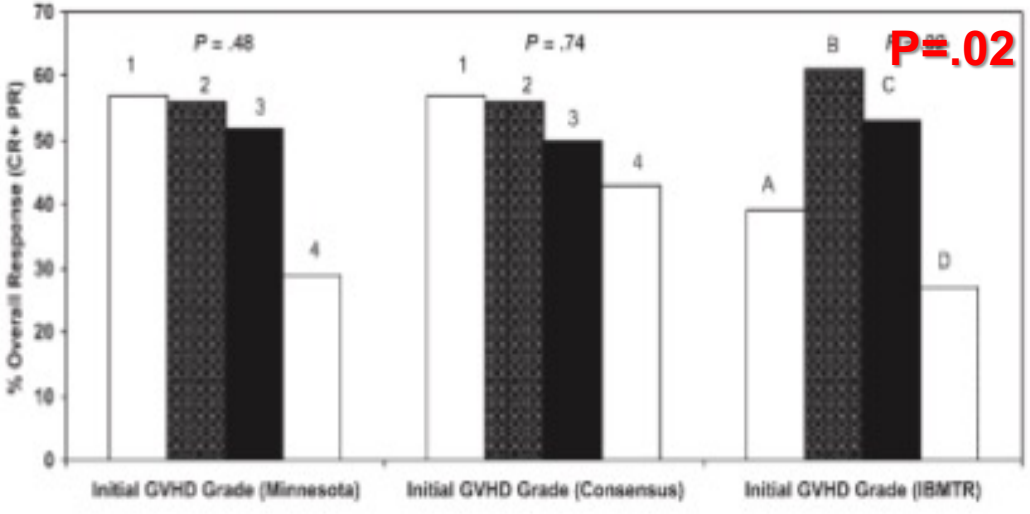
1. Skin
1. Intestine
1. liver

# Response of 443 patients to steroids as primary therapy for acute GVHD: comparison of grading systems

Biol Blood Marrow Transplant '02; 8(7): 387

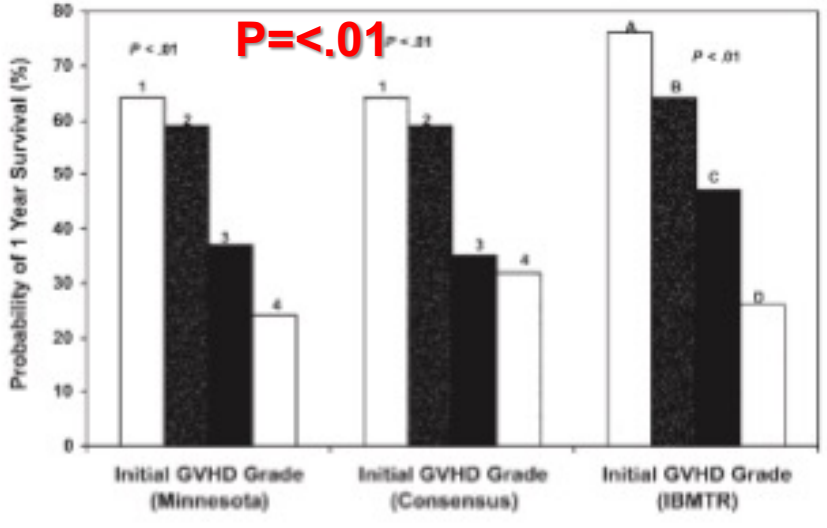
- 1181 pts (10yrs)
- 741 aGVHD (63.0%)
- 443 (60.0%) steroid therapy
- MAC transplant;
- MUD and sibling

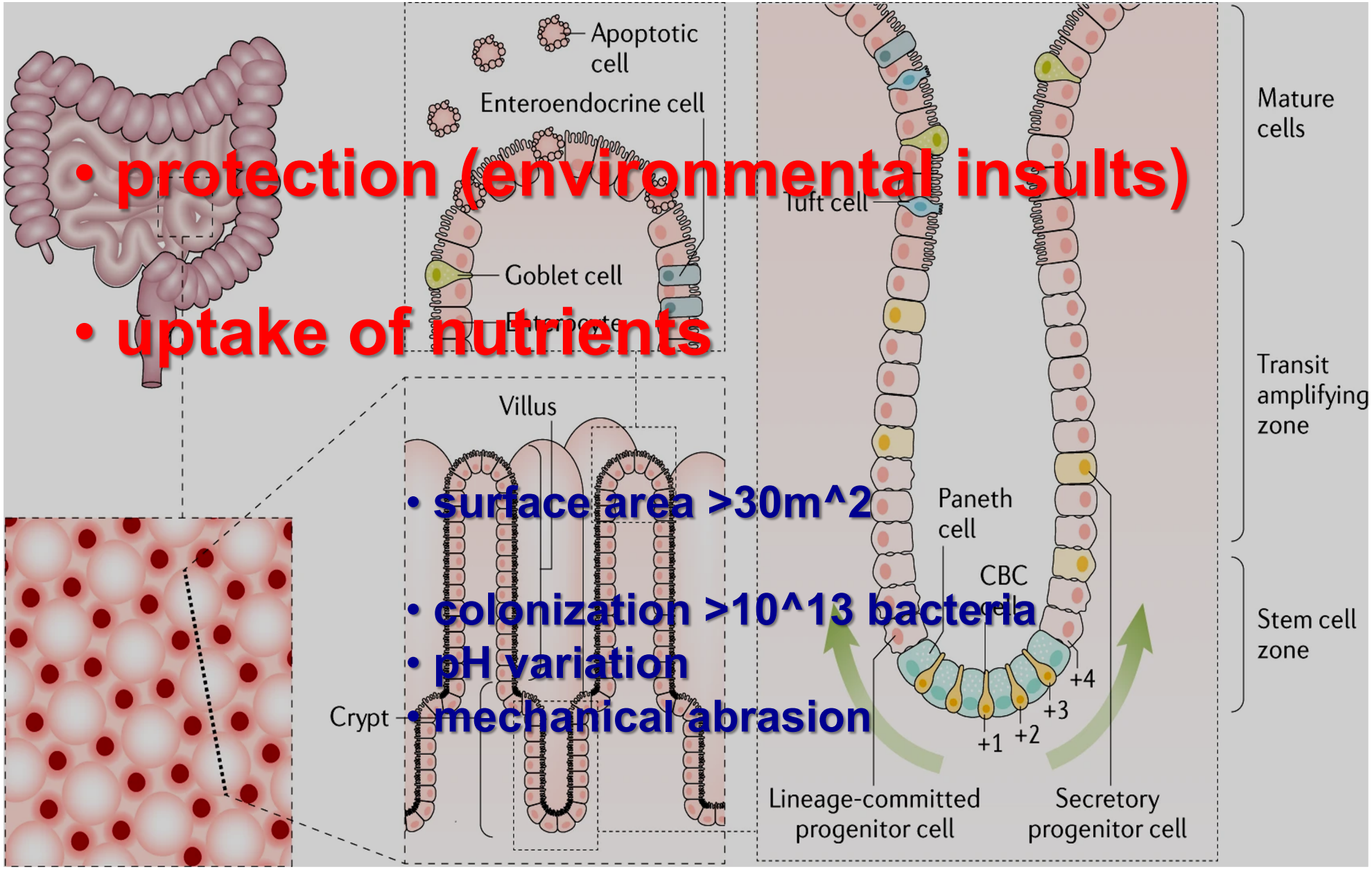
## Initial aGVHD grade and overall response to steroid




1yr Mortality – multivariate analysis
- mismatched URD
- III – IV grade aGVHD
- lower GI aGVHD

## Initial aGVHD grade and probability of survival 1yr after steroid therapy

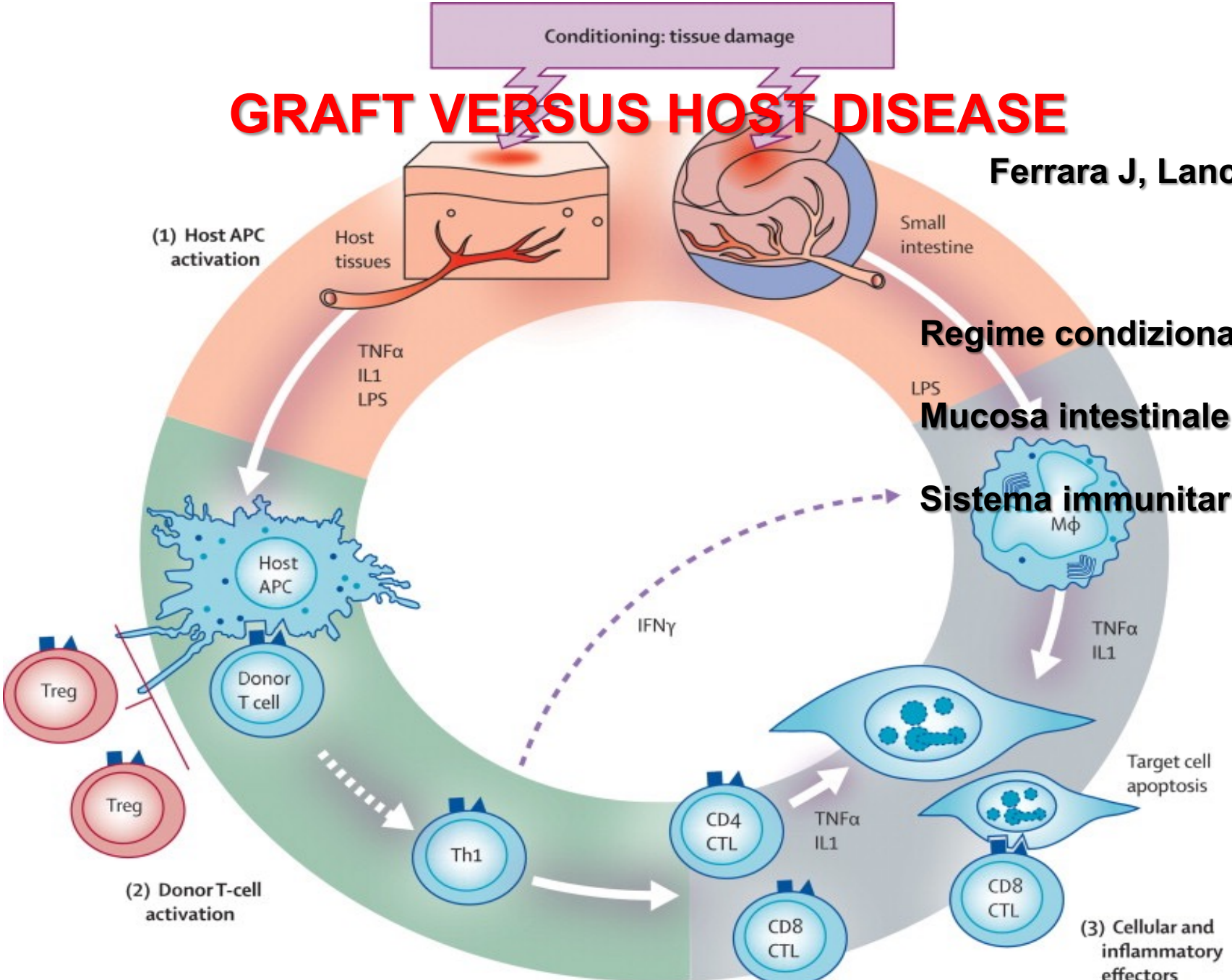





- protection (environmental insults)
- uptake of nutrients
- surface area >30m^2
- colonization >10^13 bacteria
- pH variation
- mechanical abrasion

# GRAFT VERSUS HOST DISEASE

Ferrara J, Lancet '09




**Regime condizionamento**

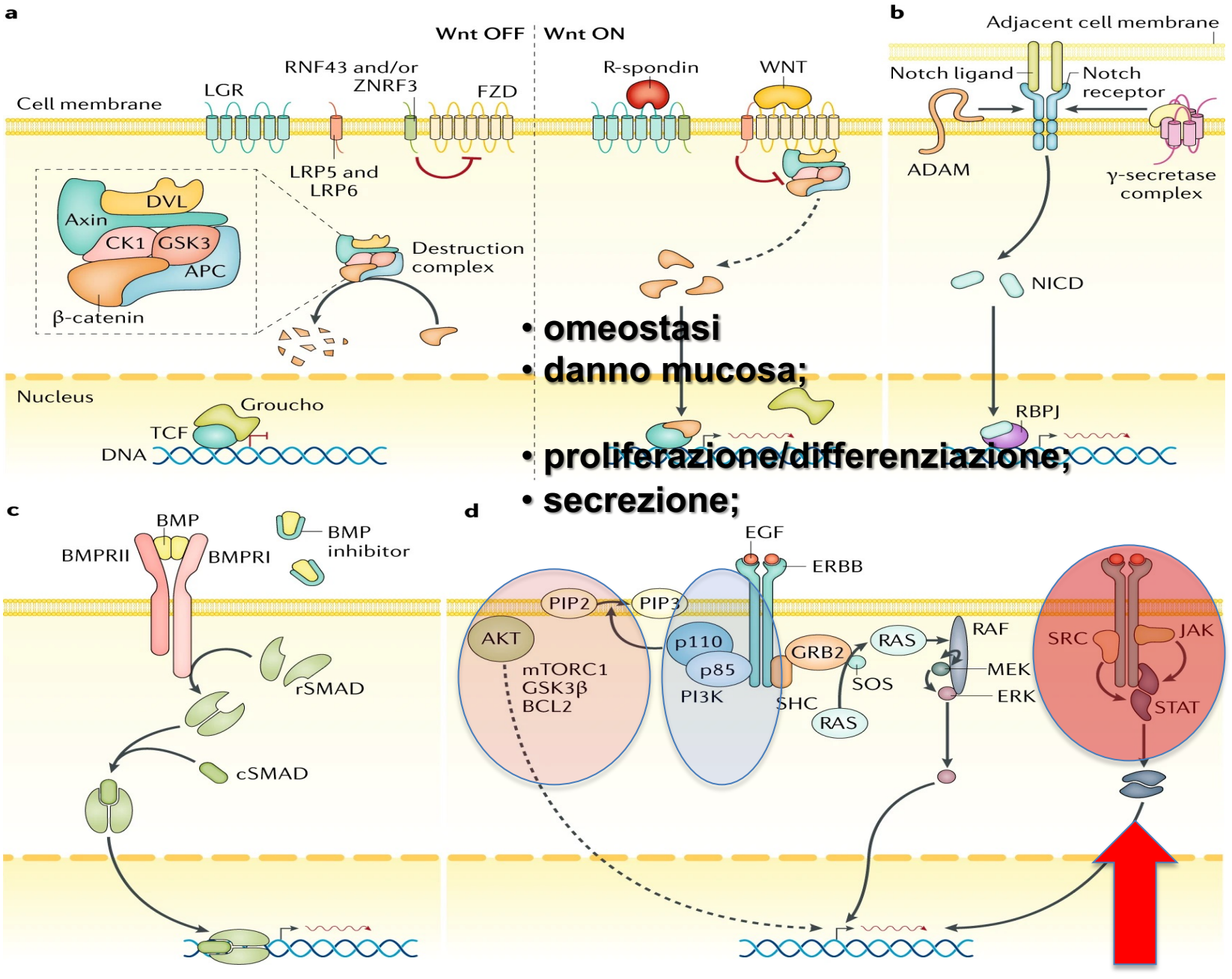
**Mucosa intestinale**

**Sistema immunitario**

- **IDENTIFICATION AND CULTURE OF ISCs**
- **PANETH CELLs**
- **interaction MICROBIOMA ↔ IMMUNE SYSTEM**

**Intestinal GVHD**

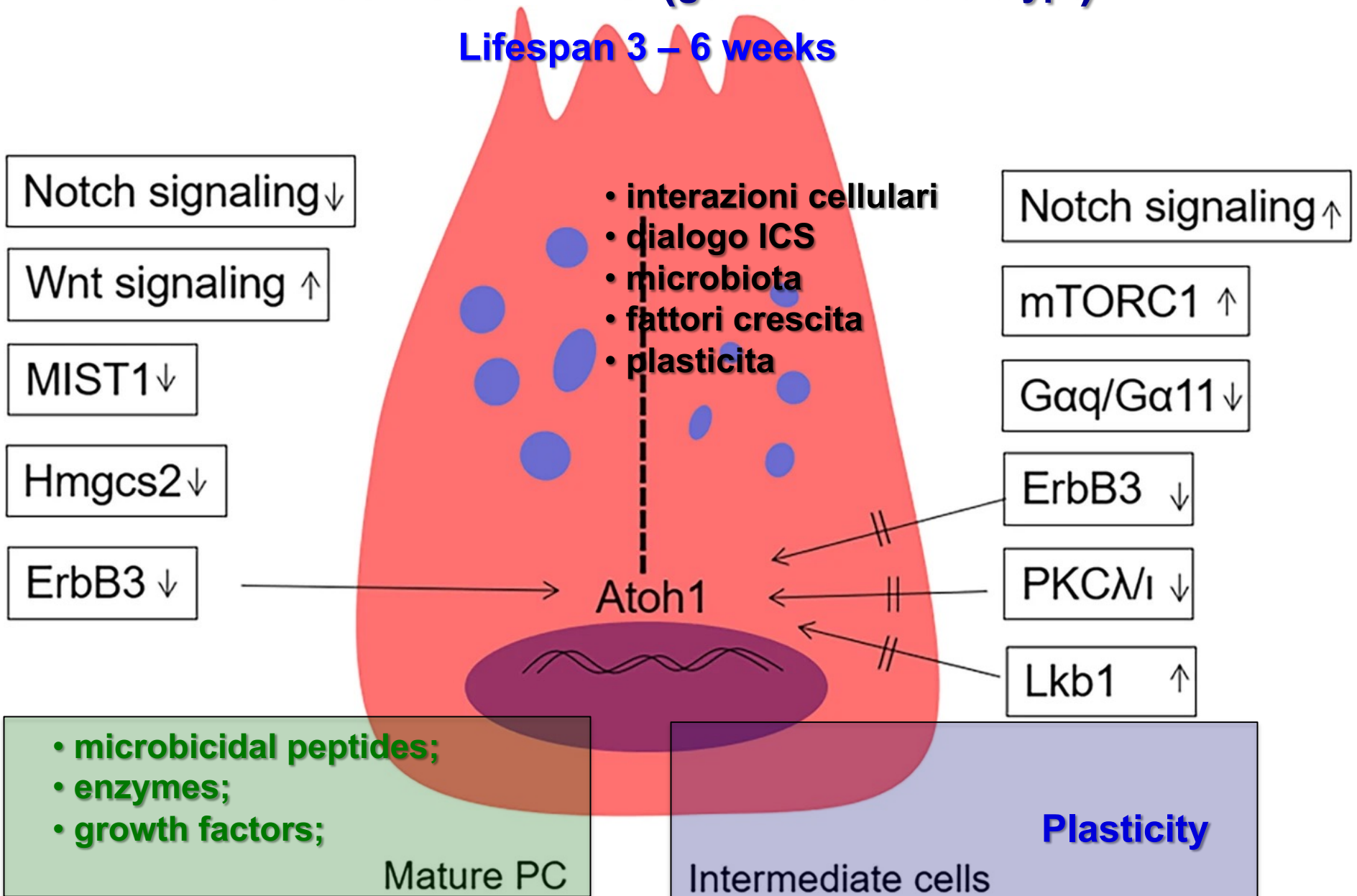
- **IDENTIFICATION OF SERUM BIOMARKERS**
- **NEW TREATMENT (TARGET THERAPY)**




- **omeostasi**
- **danno mucosa;**
- **proliferazione/differenziazione;**
- **secrezione;**

# Paneth cell – PC (guardians of the crypt)

**Lifespan 3 – 6 weeks**



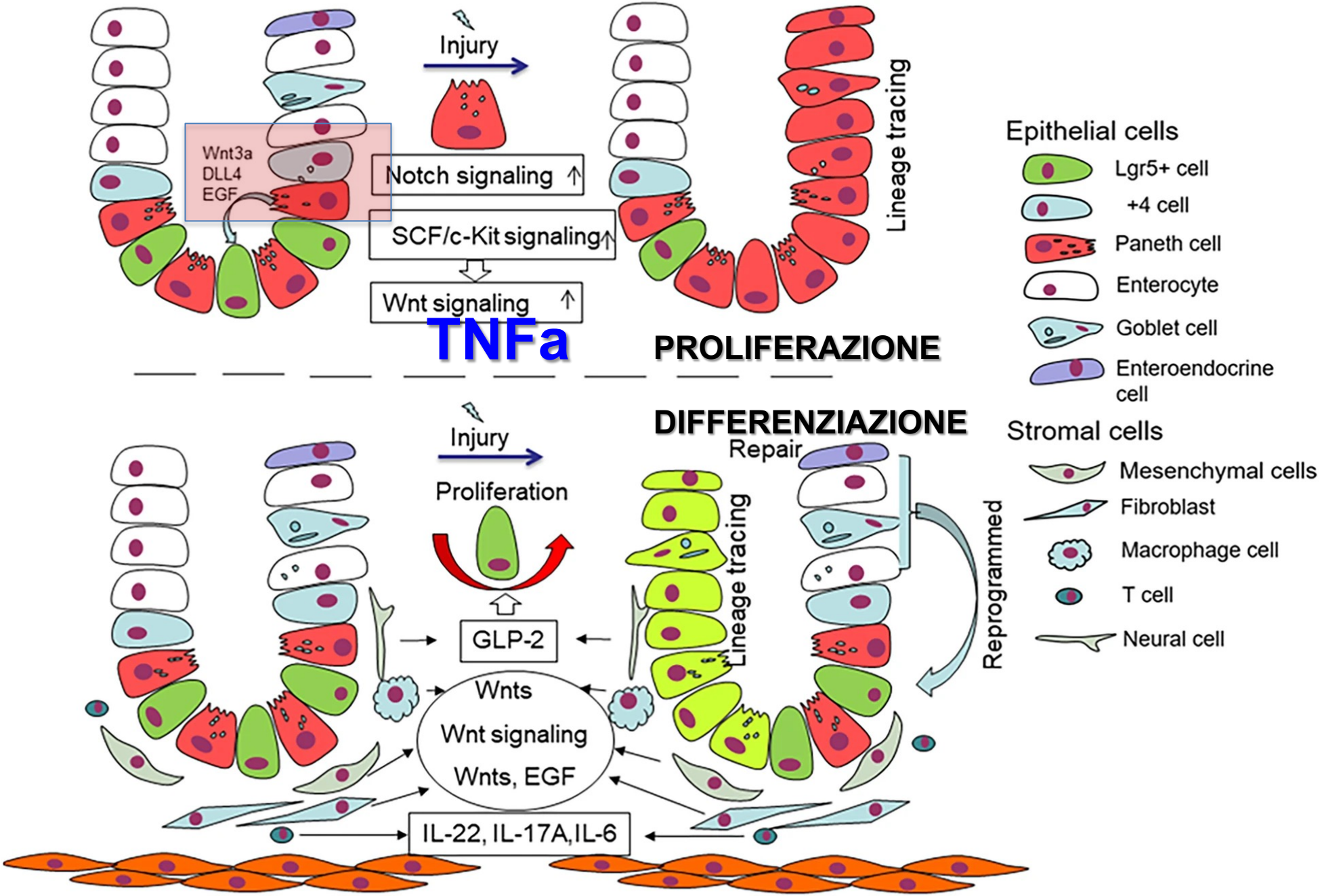

Injury
Wnt3a
DLL4
EGF
Notch signaling ↑
SCF/c-Kit signaling ↑
Wnt signaling ↑
Lineage tracing
TNFa
PROLIFERAZIONE
DIFFERENZIAZIONE
Injury
Proliferation
Repair
Lineage tracing
Reprogrammed
GLP-2
Wnts
Wnt signaling
Wnts, EGF
IL-22, IL-17A,IL-6
Epithelial cells
Lgr5+ cell
+4 cell
Paneth cell
Enterocyte
Goblet cell
Enteroendocrine cell
Stromal cells
Mesenchymal cells
Fibroblast
Macrophage cell
T cell
Neural cell

Table 1 | **Grading endoscopic severity in gastrointestinal acute GVHD**

| Grade | Freiburg Classification for endoscopic findings[64] |
|---|---|
| 1 | Normal mucosa or the absence of higher-grade findings |
| 2 | Spotted erythema or initial aphthous lesion |
| 3 | Aphthous lesions or focal erosions |
| 4 | Confluent defects, ulcerations and/or complete denudation of the mucosa |

Table from REF. 64, Macmillan Publishers Limited.

Table 2 | **Grading histological severity in gastrointestinal acute GVHD**

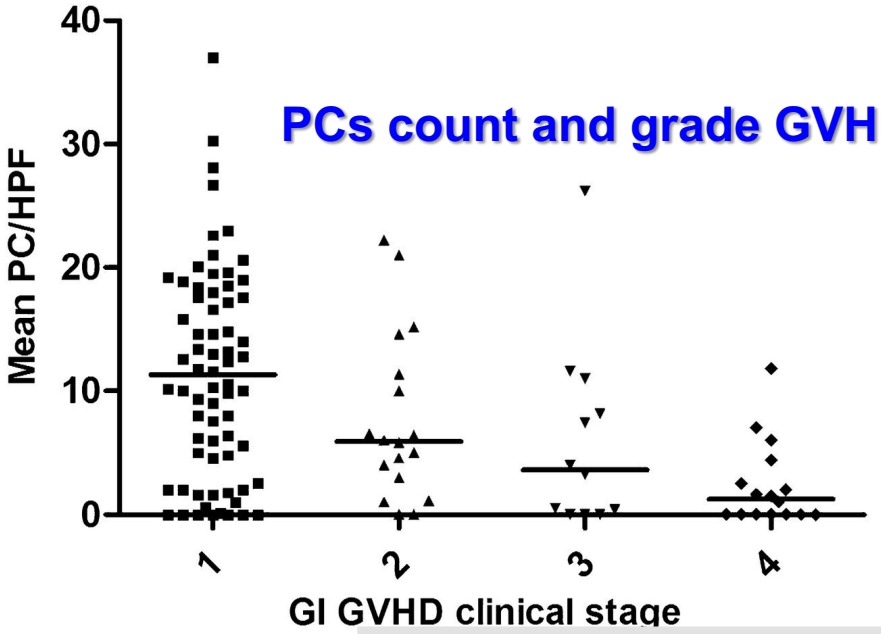
| Grade | Histological classification |
|---|---|
| 1 | Isolated apoptotic epithelial cells without crypt loss |
| 2 | Crypt necrosis, withering and individual crypt loss |
| 3 | Contiguous areas of multiple crypt loss |
| 4 | Extensive crypt dropout with denudation of the epithelium |

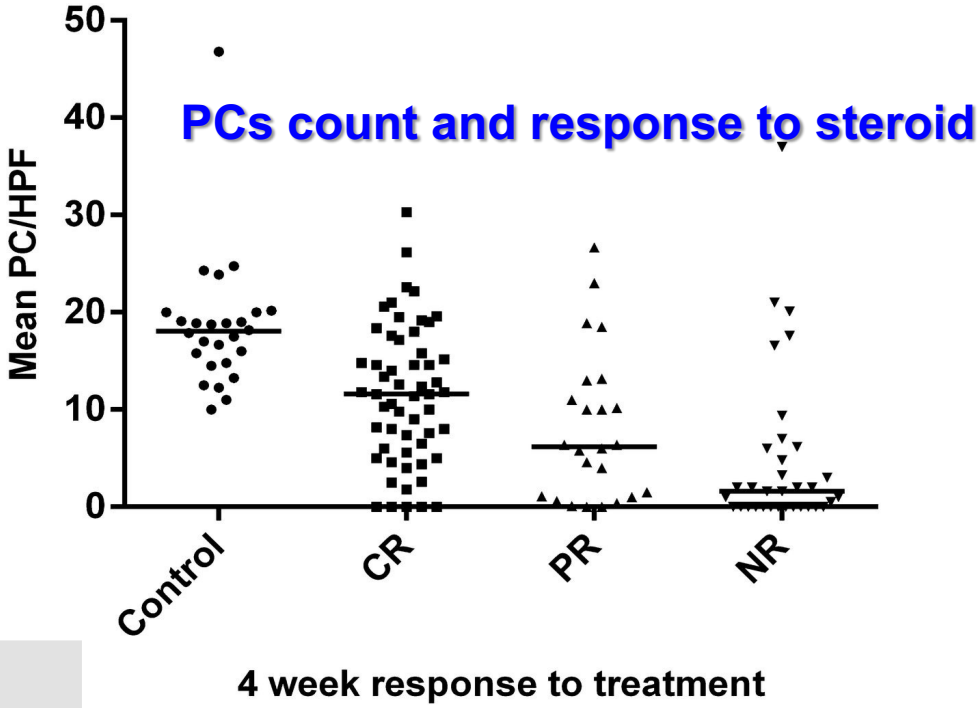
Data from REF. 99 and REF. 104.

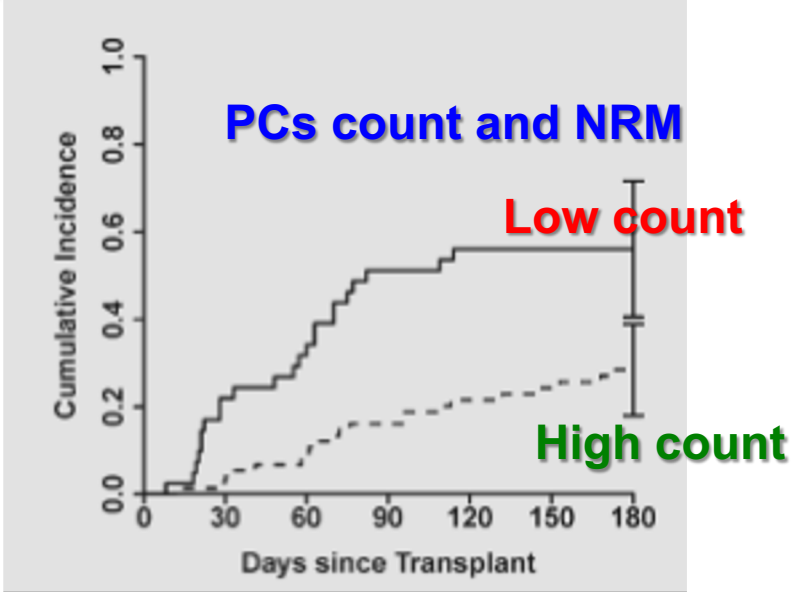
**VALUTAZIONE**
- **QUANTITATIVA**
- **QUALITATIVA**
- **NON PREDITTIVA**

# Low Paneth cell numbers at onset of gastrointestinal GVHD identify patients at high risk of NRM

Levine JE et al; Blood '13; 122 (8) 1505

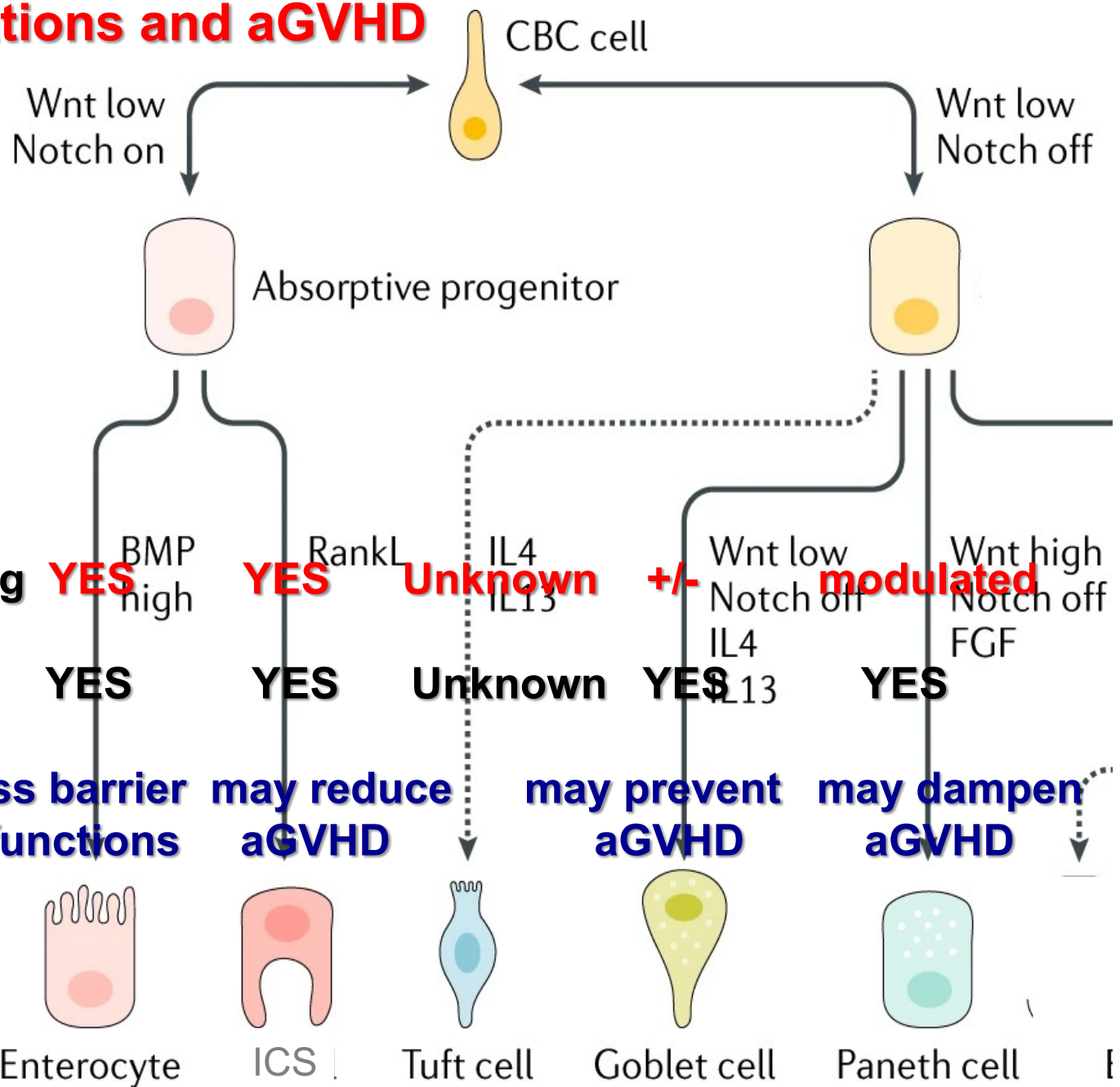



**116 pts – aGVHD Intestinal biopsies;**

# Epithelial cell populations and aGVHD



# Innate lymphoid cells (ILCs)

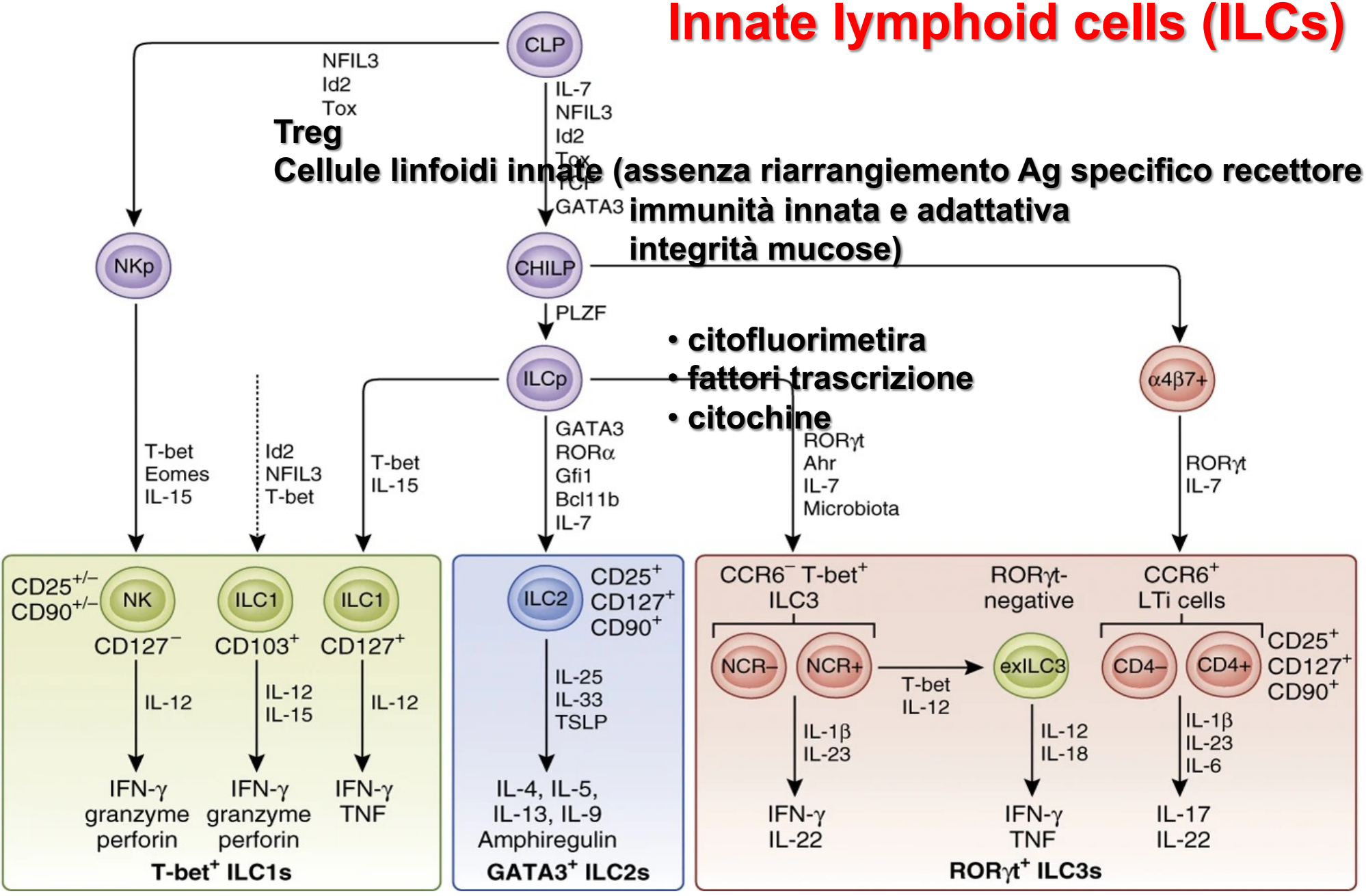
**Treg**

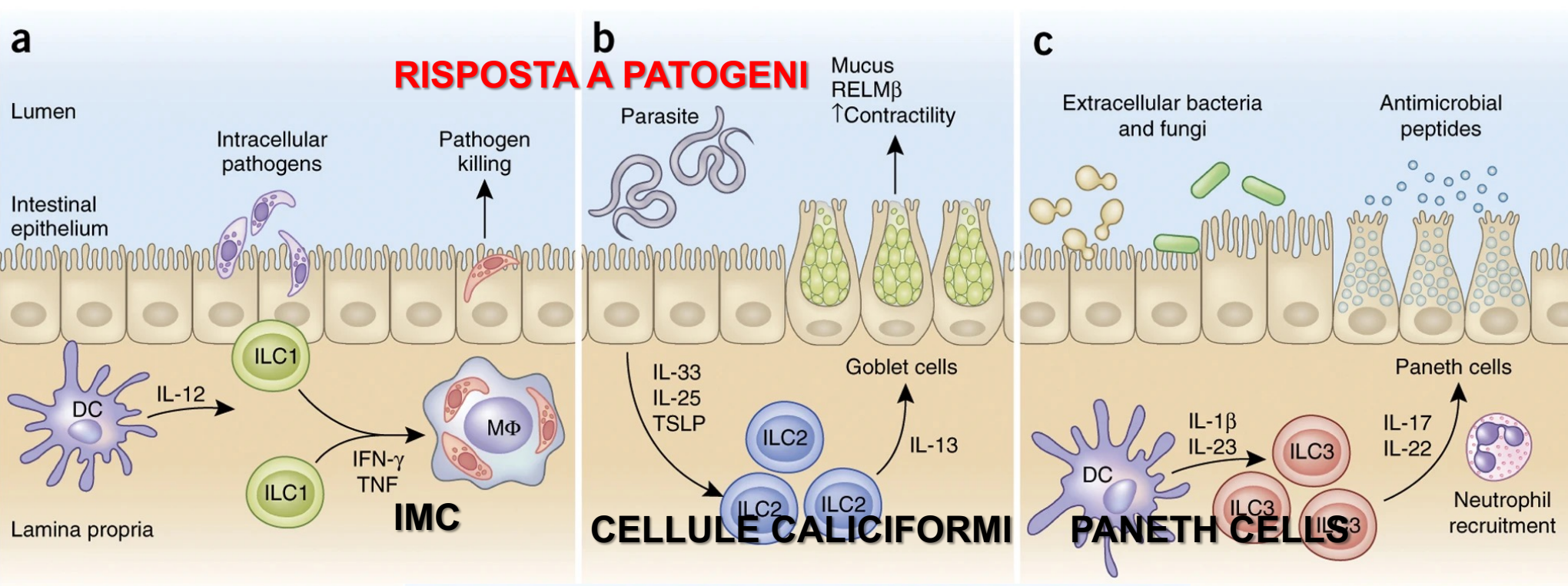
**Cellule linfoidi innate (assenza riarrangiemento Ag specifico recettore**

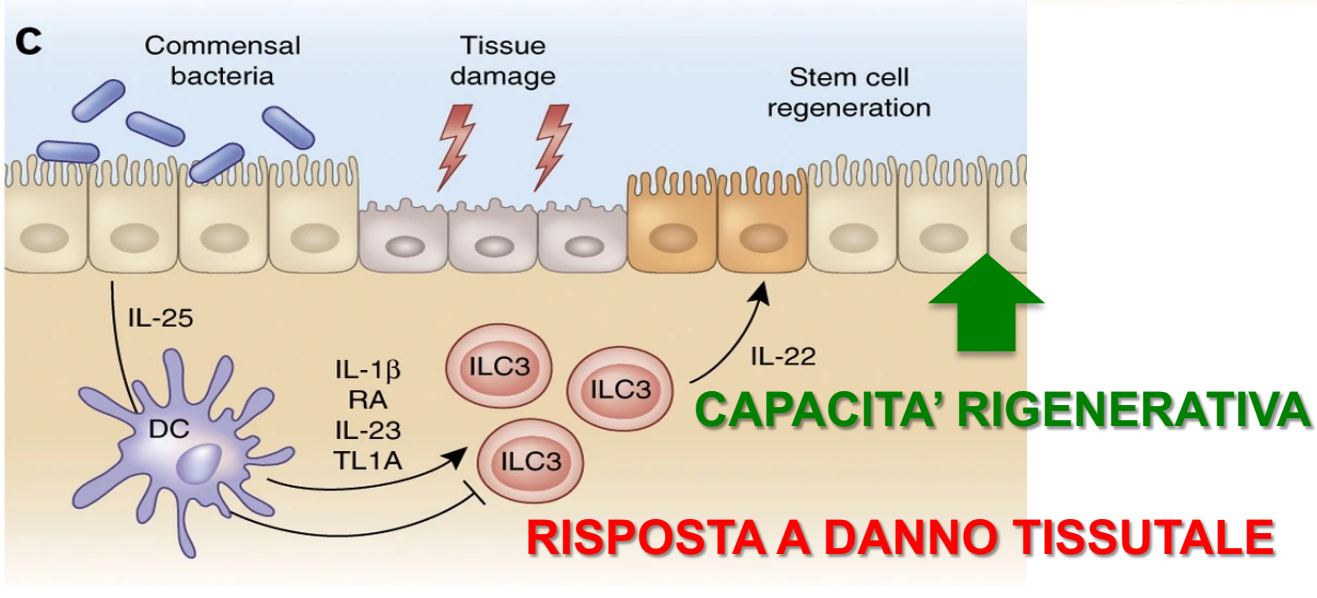
**immunità innata e adattativa**

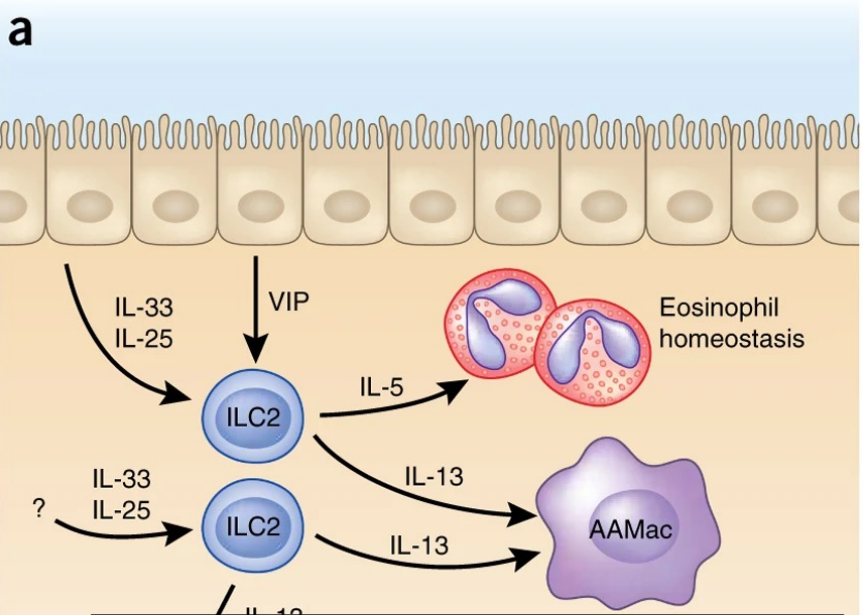
**integrità mucose)**

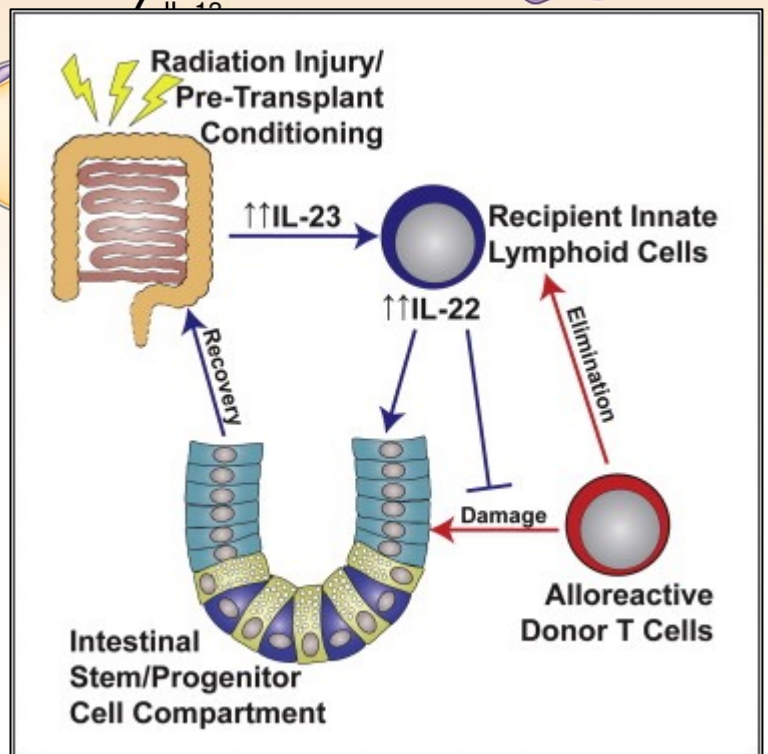
- **citofluorimetira**
- **fattori trascrizione**
- **citochine**

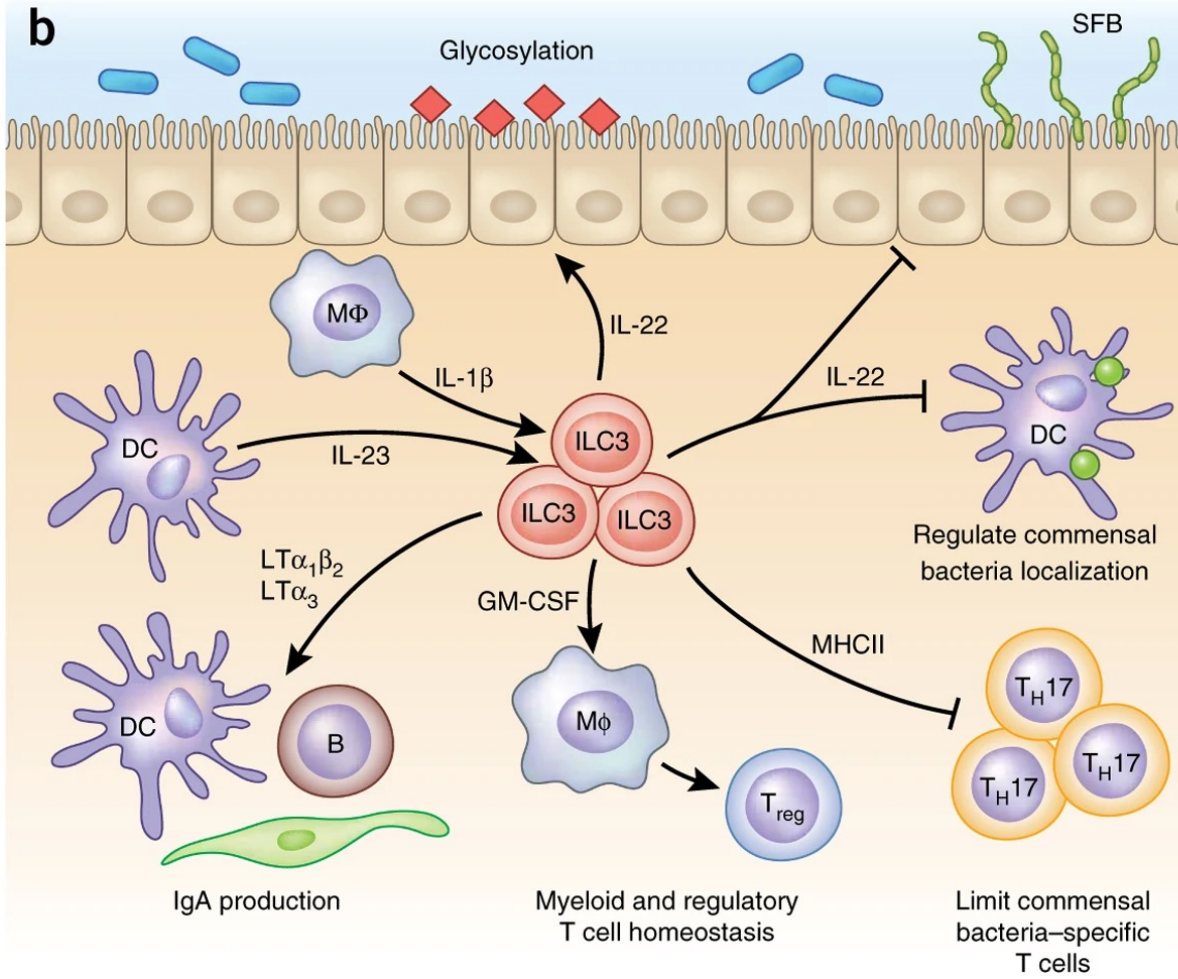



a
RISPOSTA A PATOGENI
Lumen
Intracellular pathogens
Pathogen killing
Intestinal epithelium
ILC1
DC
IL-12
MΦ
ILC1
IFN-γ
TNF
IMC
Lamina propria
b
Parasite
Mucus
RELMβ
↑Contractility
IL-33
IL-25
TSLP
Goblet cells
ILC2
ILC2
ILC2
IL-13
CELLULE CALICIFORMI
c
Extracellular bacteria and fungi
Antimicrobial peptides
Paneth cells
IL-1β
IL-23
ILC3
IL-17
IL-22
DC
ILC3
ILC3
Neutrophil recruitment
PANETH CELLS


c
Commensal bacteria
Tissue damage
Stem cell regeneration
IL-25
DC
IL-1β
RA
IL-23
TL1A
ILC3
ILC3
ILC3
IL-22
CAPACITA' RIGENERATIVA
RISPOSTA A DANNO TISSUTALE



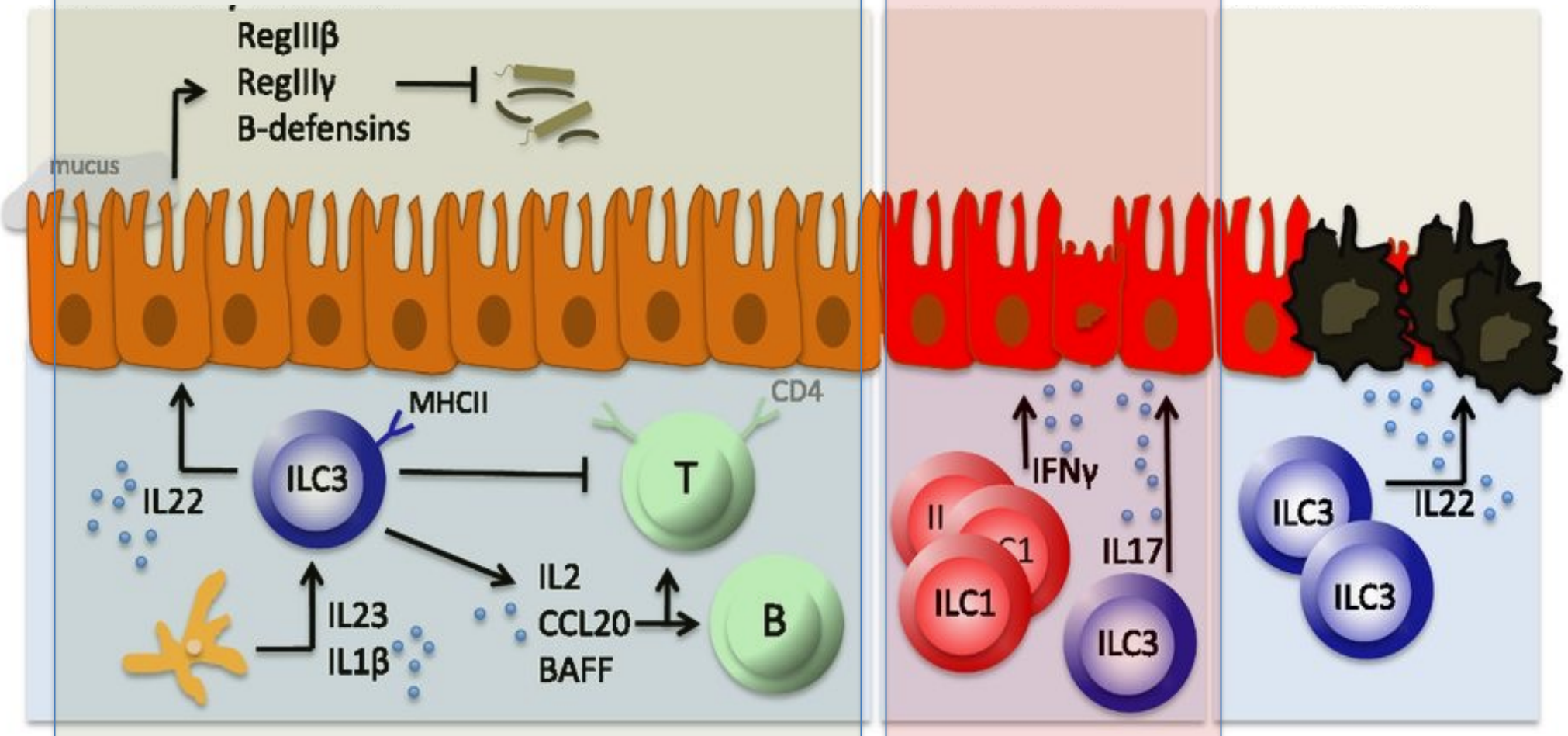




**Down – regolazione infiammazione**

# Healthy situation

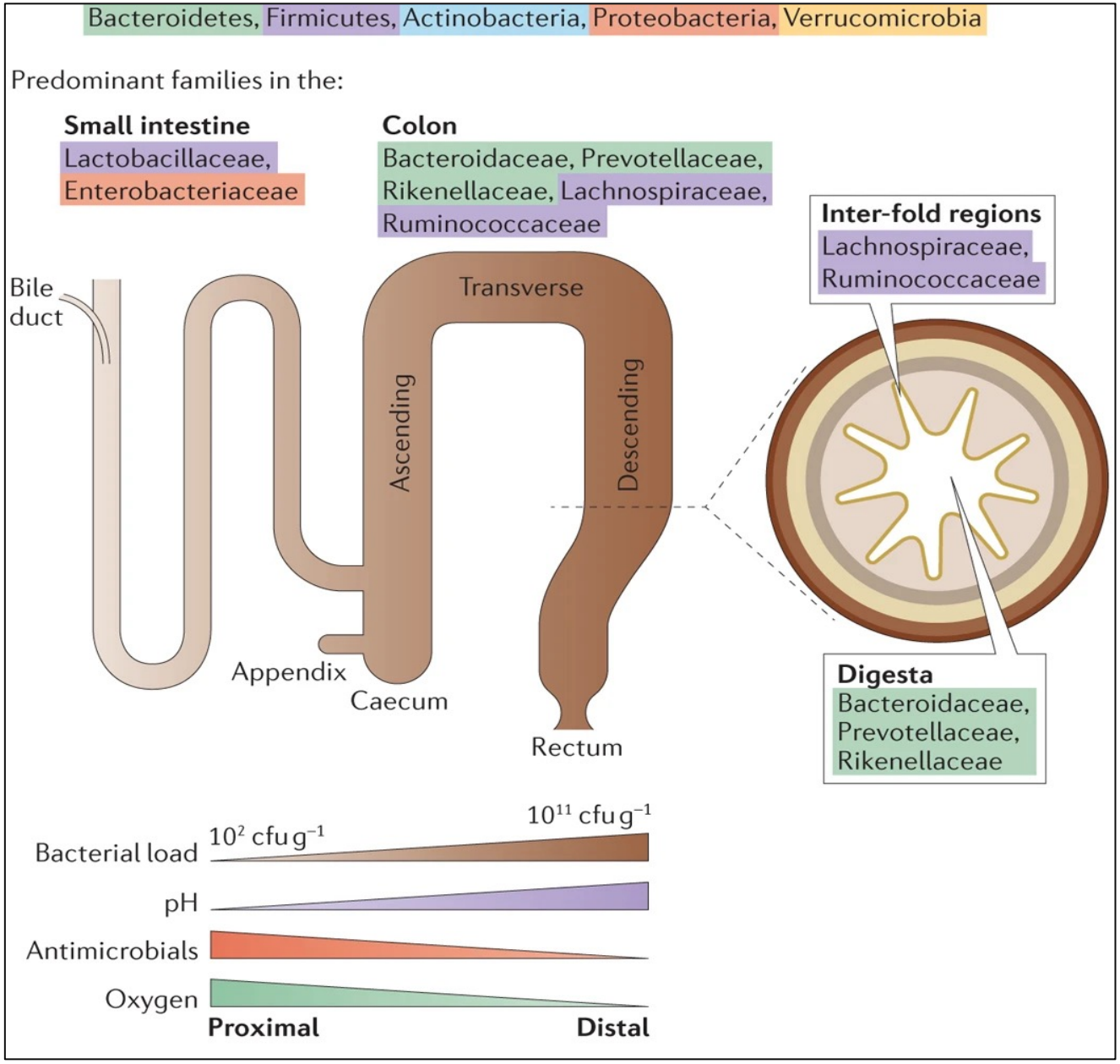
# GVHD



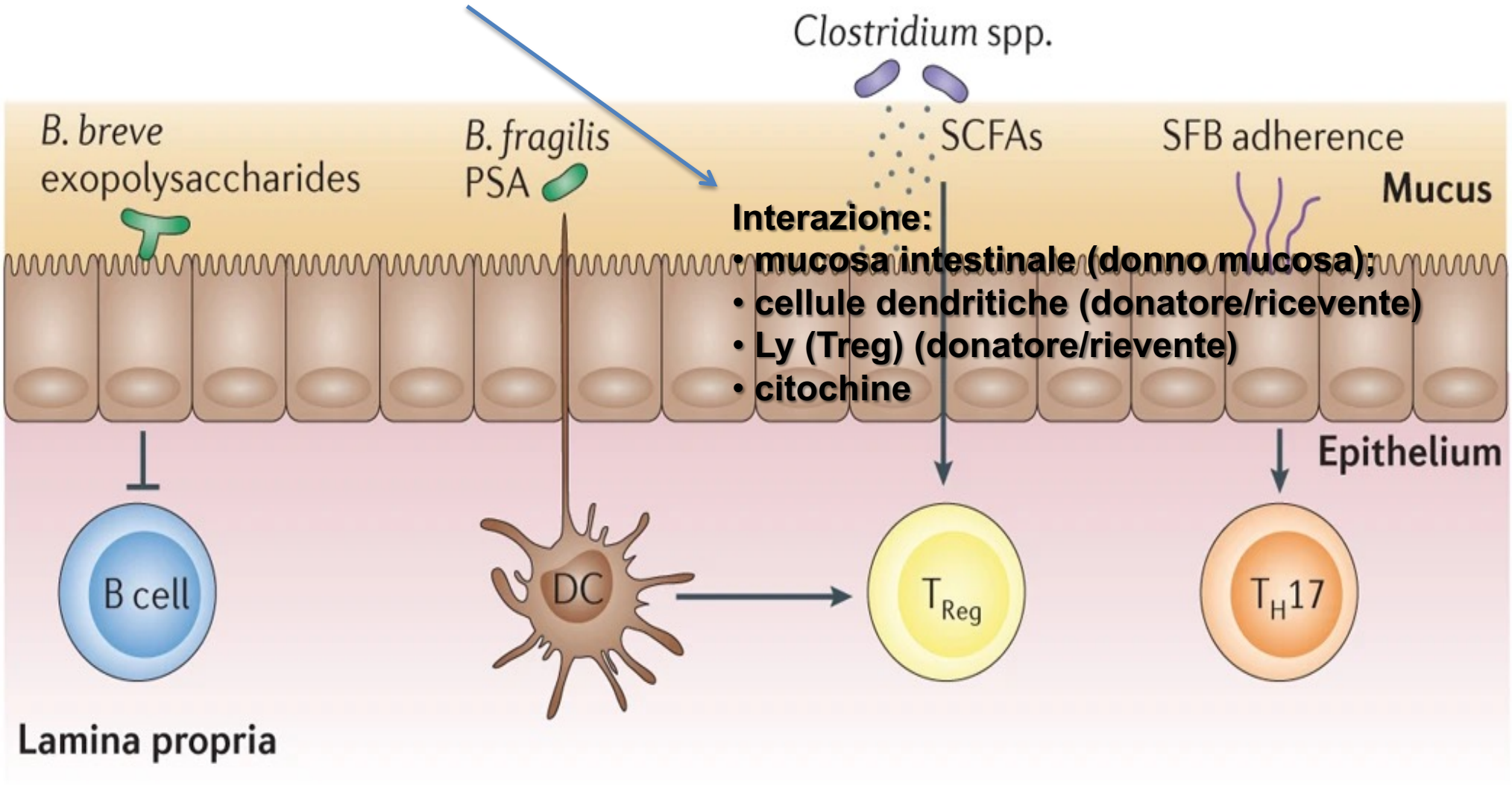

**ILC3 ->**
- **IL22: normal epithelial barrier**
- **antimicrobial products**
- **regulate T and B cell reactivity**

**ILC1 ILC3**
**Intestinal inflammation:**
**No swithced off of autoregenerative function**

# Gut biogeography of the bacterial microbiota



# Immunomodulation by commensal gut bacteria




Nature Reviews | Microbiology

**Regulation of intestinal inflammation by microbiota following allogeneicBMT; JEM '12;**

- GVHD intestinal inflammation: major shifts of microbiota;
- Microbiota: can modulate the severity of intestinal GVHD;
- GVHD: loss of overall microbiota diversity;
- Increased microbial chaos: increased risk of aGVHD;
- Flora manipulation: may reduce intestinal inflammation and improve BMT outcome

**GVHD disrupts intestinal microbial ecology by inhibiting Paneth cell production of α-defensins; Blood '12**

- α-defensins (PCs): antimicrobial peptides;
- α-defensins: kill noncommensals; preserving commensls;
- GVHD: reduction of - α-defensins
- molecular profiling (GVHD): overhelming expansion E. coli;

**The effects of intestinal tract bacterial diversity on mortality following allogeneicHSCT; Blood '14;**

- highly diverse bacterial population: modulate host inflammation;
- highly diverse bacterial population: promoto immune tolerance;
- lower vs intermediate vs high intestinal diversity: OS 36% vs 60% vs 67% (p=.019)
- multivariate analysys (TRM): lower intestinal diversity p=.014

- microbiota
- metaboliti
- profilassi/terapia

- Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells; Nature ’13; 504 (7480): 446 – 450;

- microbial metabolites → mucosal immunity
- T cell →Tregs
- butyrate: suppress T cell – medited intestinal damage
- GVHD → loss of butyrate
- tryptophan metabolites -> ILC3; IL22; ICS growth

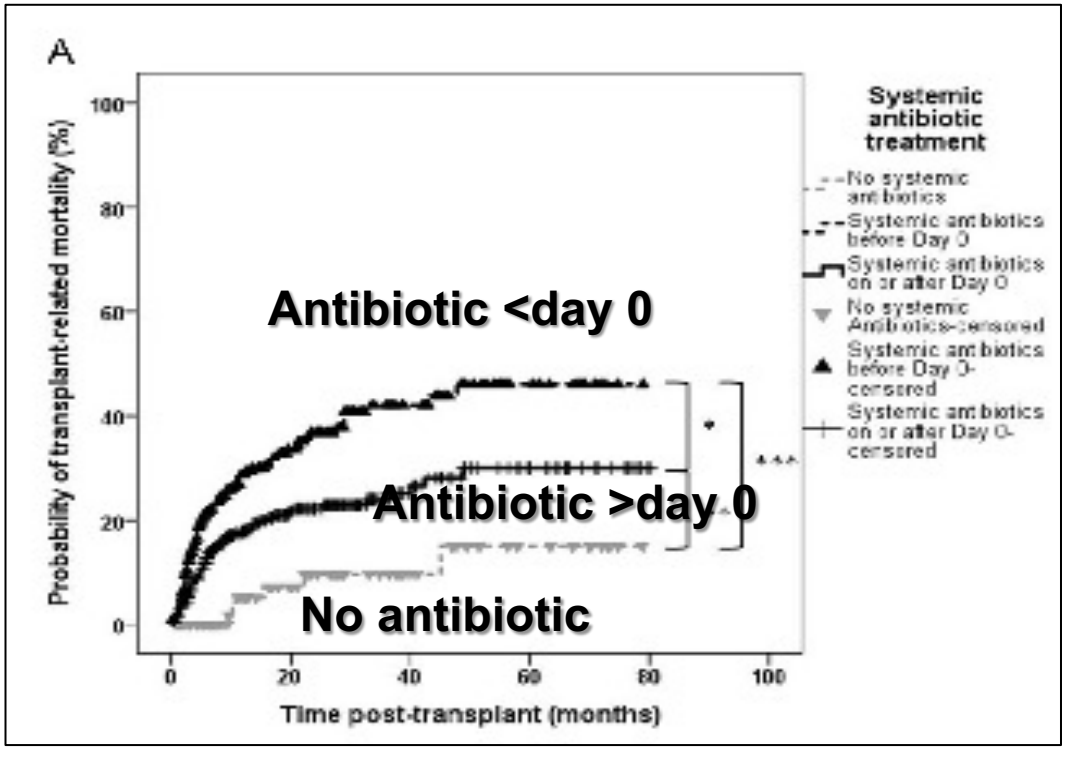
- Treg induction by a rationally selected mixture of Clostridia strians from the human microbiota; Nature ’13; 500 (7461): 232 – 236;

- Gut microbiome – derived metabolites modulate intestinal epithelial cell damage and mitigate GVHD; Nat Immunol ‘16; 17 (5): 505 – 13;

# Microbiota disruption induced by early use of broad – spectrum antibiotics is an independent risk factor of outcome after allogeneicSCT;

Webe D et al; BBMT '17;

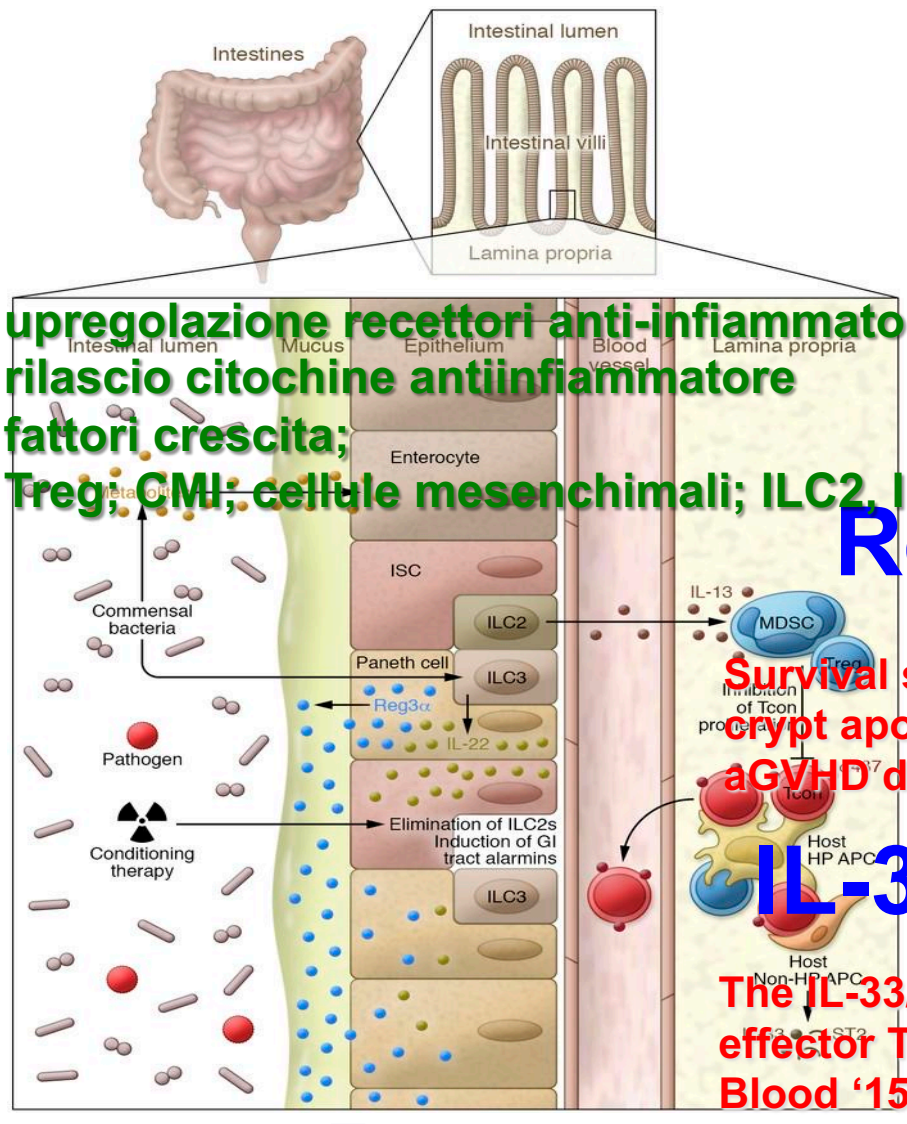
- 621 pts;
- non TCD;
- early antibiotic treatment:day -7 -> 0
- late antibiotic treatment: day 0
- EAT: 236 (38.0%)
- LAT: 297 (48.0%)
- no antibiotic: 88 (14.0%)



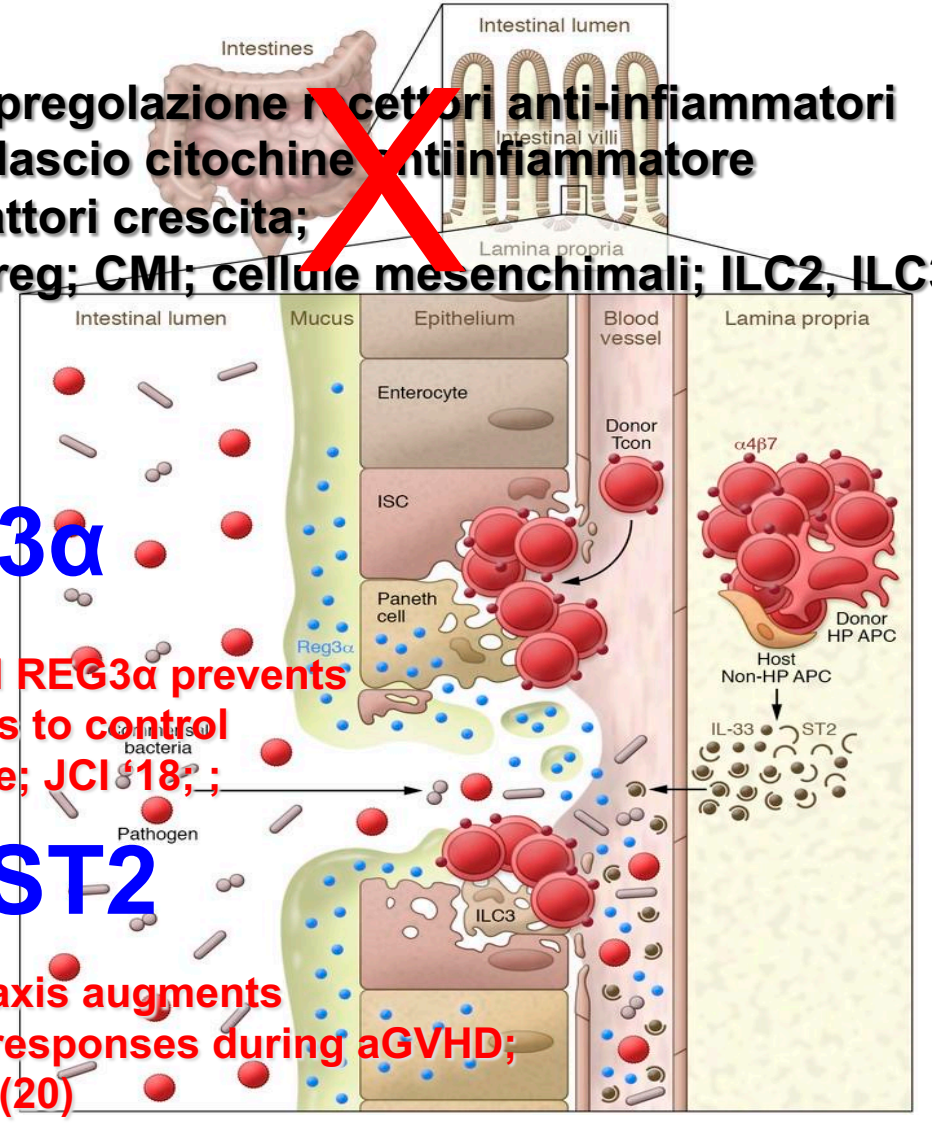

| Multivariate analysis | TRM | aGVHD |
|---|---|---|
| EAT | <.001 | .004 |
| Age | .004 | NS |
| PS | .03 | NS |
| Female donor | .02 | NS |

# GVHD pathophysiology

- upregolazione recettori anti-infiammatori
- rilascio citochine antiinfiammatore
- fattori crescita;
- Treg; CMI; cellule mesenchimali; ILC2, ILC3;




- upregolazione recettori anti-infiammatori
- rilascio citochine antiinfiammatore
- fattori crescita;
- Treg; CMI; cellule mesenchimali; ILC2, ILC3;




## Reg3α

Survival signal REG3α prevents crypt apoptosis to control aGVHD disease; JCI '18; ;

## IL-33/ST2

The IL-33/ST2 axis augments effector T-cell responses during aGVHD; Blood '15; 125 (20)

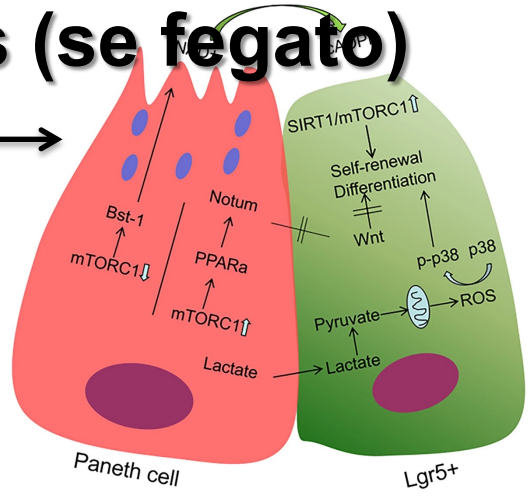
**Early** **Late**

# Sperimental approaches to enhancing homeostatic mechanisms in the GI tract during GVHD

| Cell process/mechanism | Function | Therapeutic approach |
|---|---|---|
| P2X receptors (50) | Binding of ATP enhances APC activation and proinflammatory donor T cells | P2R inhibitors |
| $\alpha_1$-Antitrypsin (72, 73) | Modulates APCs to increase Tregs and decrease effector T cells; reduces IL-32 generation | $\alpha_1$-Antitrypsin systemic delivery |
| $\beta_7$ Integrin (146–148) | Promotes trafficking of T cells to the colon and small bowel | $\beta_7$-Specific mAbs, including vedolizumab, etrolizumab, and AMG181 |
| MAdCAM-1 (149) | Addressin that binds to $\alpha_4\beta_7$ integrins | MAdCAM-1–specific mAb PF-00547659 |
| IL-6 (111, 113) | Decreased Treg numbers; increased proinflammatory donor T cells | IL-6 receptor–targeting mAb tocilizumab |
| IL-23 (125, 150, 151) | Enhances proinflammatory cytokine production by donor T cells | Ustekinumab and briakinumab are specific for the p40 subunit of IL-23 and IL-12 |
| IL-18 generation via inflammasome induction (152) | Reduces Th1 generation; induces Th2 polarization | IL-18 cytokine therapy |
| IL-1β generation via inflammasome induction (153–155) | Induces Th17 polarization; inhibits MDSCs and Tregs | IL-1 receptor antagonist anakinra |
| Enhanced microbial diversity (95, 98, 156–158) | Promotes persistence of Tregs, decreases donor proinflammatory T cells | Donor stool transplant; delivery of bacterial strains that induce Tregs |
| Short-chain fatty acids (104, 159) | Enhances numbers of Tregs | Butyrate or propionate infusions |
| Antimicrobial peptides | Mediate antimicrobial activity; promote barrier repair; activate immunosuppressive immune cells | REG3 infusion |
| ISC maintenance (133, 135, 137, 160) | Promotes persistence of ISCs; enhances activity of Paneth cells | IL-22 fusion protein; R-spondin-1 administration |
| Donor/third-party ILC2 cells (138) | Enhances numbers of MDSCs and GI tract barrier protection | Ex vivo administration of ILC2 cells |
| Donor/third-party MDSCs (161–163) | Reduces number/function of proinflammatory donor T cells | Ex vivo administration of MDSCs |
| Donor/third-party Tregs (164–167) | Enhances number of Tregs in the GI tract | Ex vivo administration of donor/third-party Tregs |
| Donor/third-party MSCs (168, 169) | Induces APC production of IL-10 and prostaglandin $E_2$ and decreases proinflammatory T cells | Ex vivo administration of third-party MSCs |

MDSC, myeloid-derived suppressor cell; MSC, mesenchymal stem cell.

**Steroide 2mg/kg per 5 giorni poi a scalare**
**Ciclosporina → tacrolimus (se fegato)**
**Digiuno** ⟶



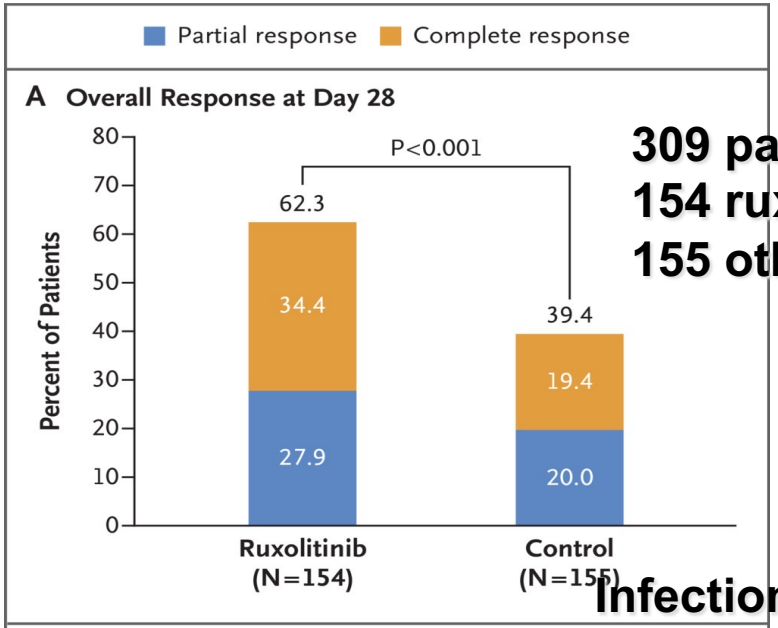

**SECONDA LINEA:**

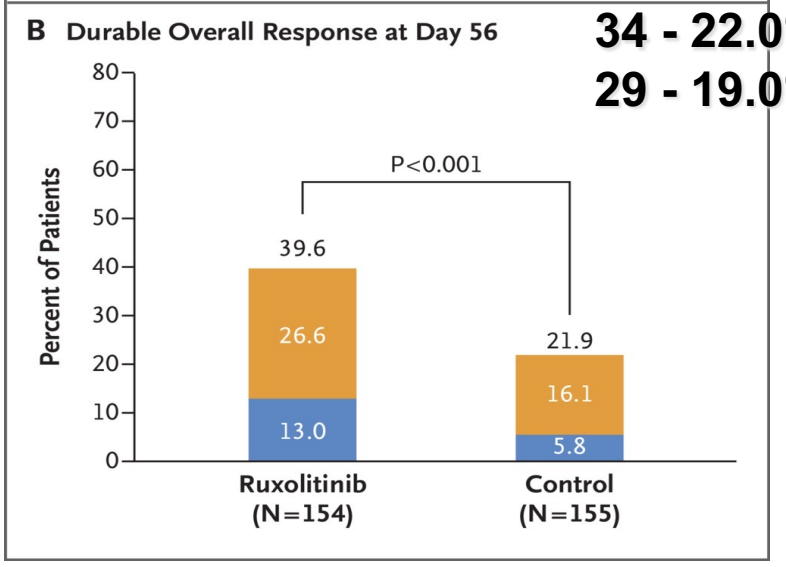
- **ECP (se anche cute)**
- **INFLIXIMAB**
- **ETANERCEPT**

**RUXOLITINIB**

# Ruxolitinib for Glucocorticoid-Refractory Acute Graft-versus-Host Disease

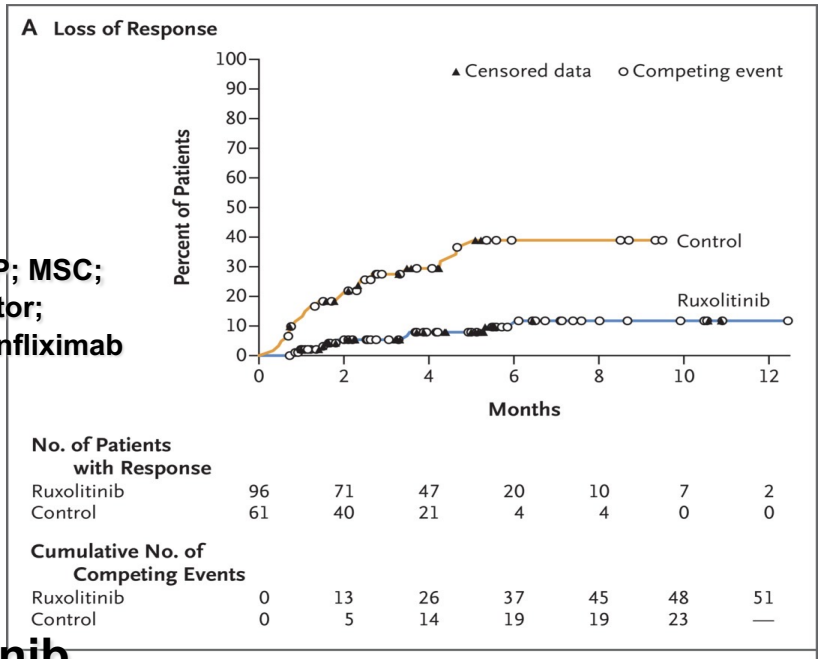
Zeiser R et al. NEJM '20;

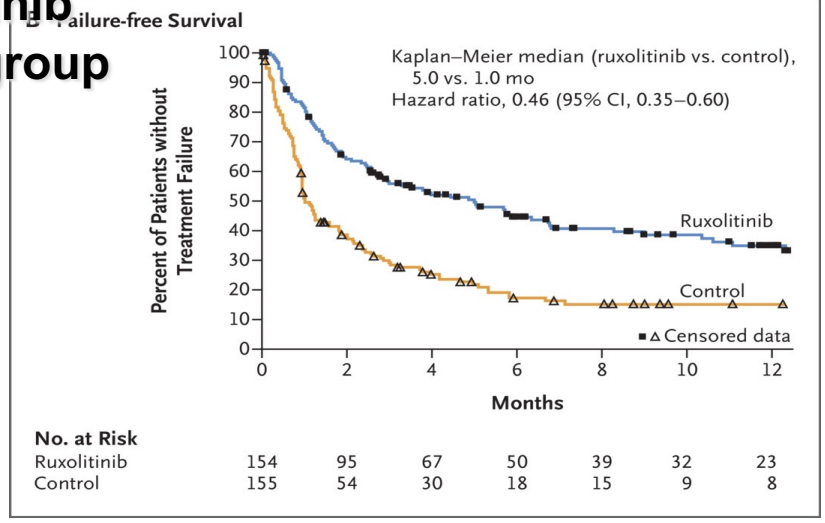



**309 patients**
**154 ruxolitinib**
**155 other (ATG, ECP; MSC; mTOR inhibitor; etanercept; infliximab**

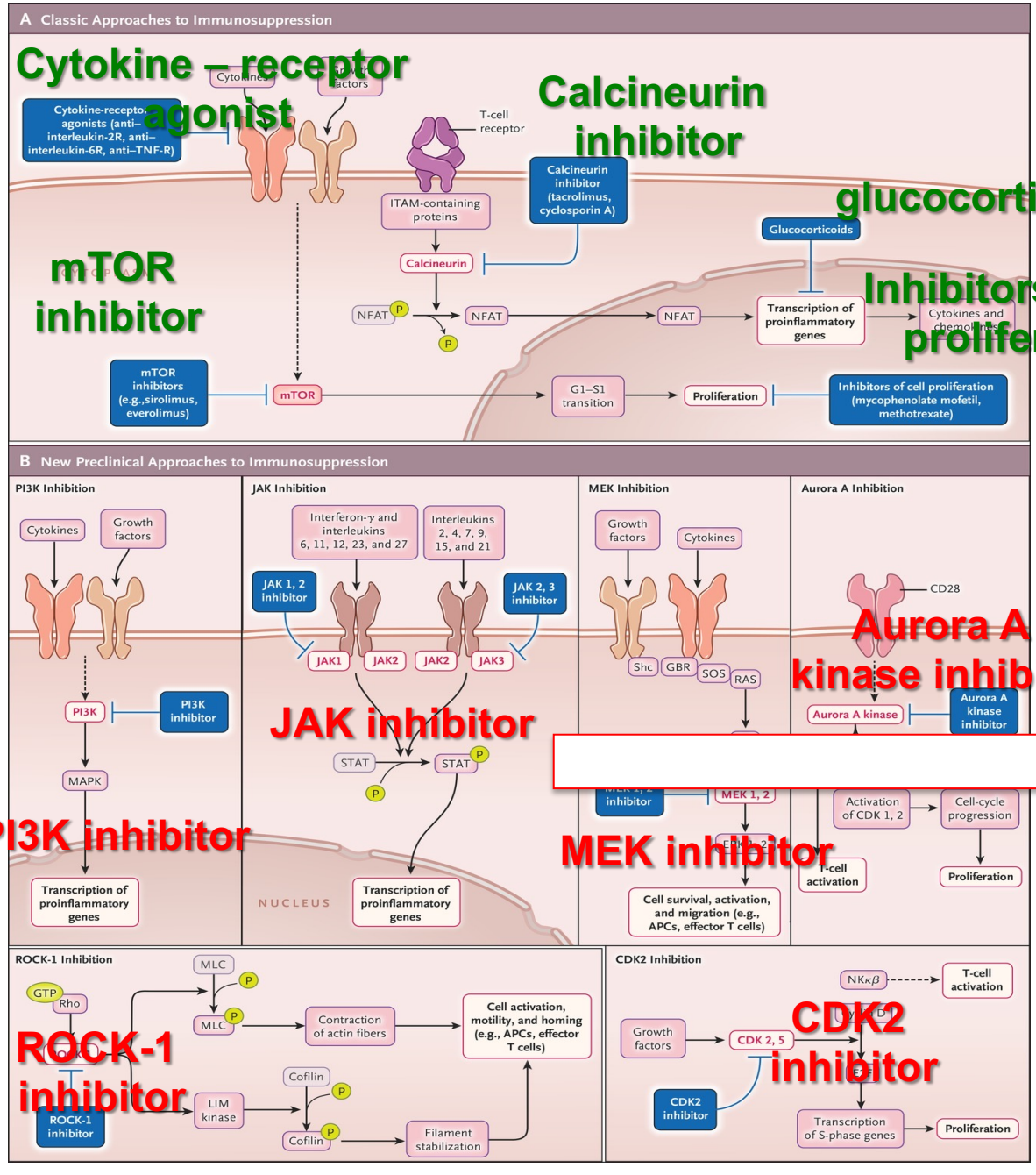
**Infections**
**34 - 22.0% ruxolitinib**
**29 - 19.0% other group**



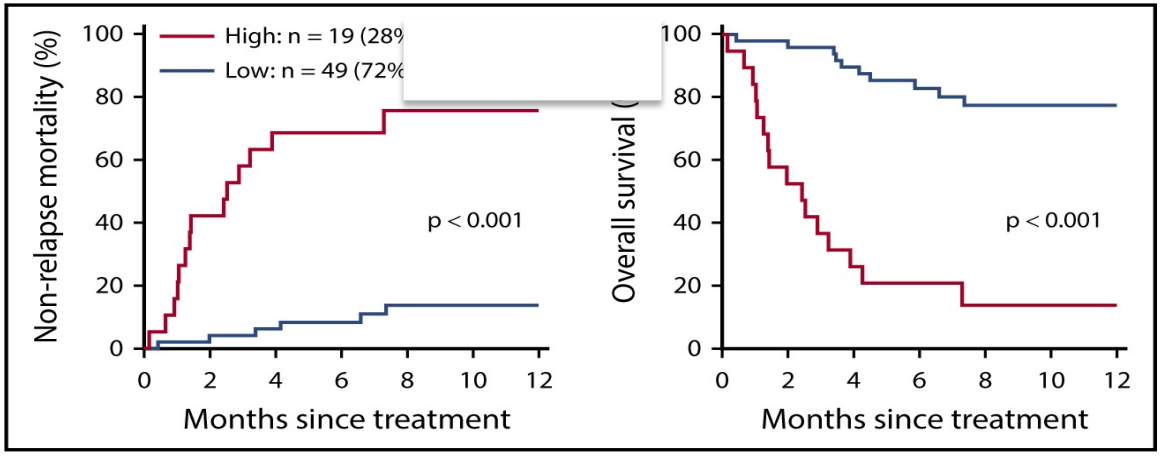
Classical approaches

New preclinical approaches



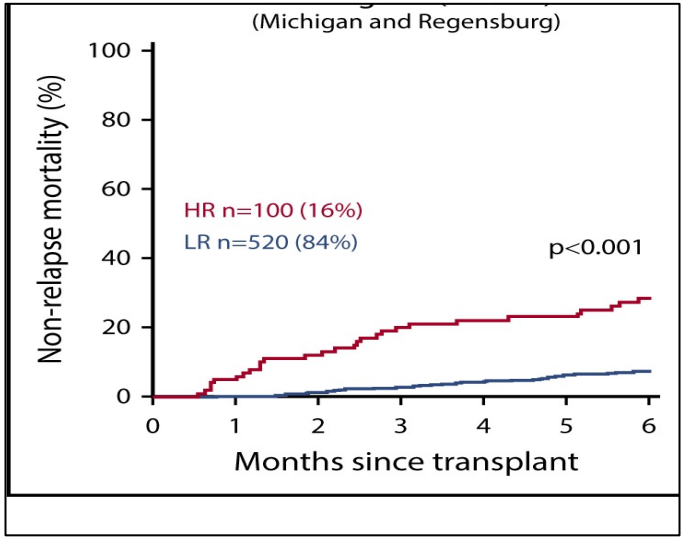

Second line

# MAGIC biomarkers predict long-term outcomes for steroid-resistant aGVHD




- REG3A (antimicrobic protein)
- ST2 (IL33 receptor)

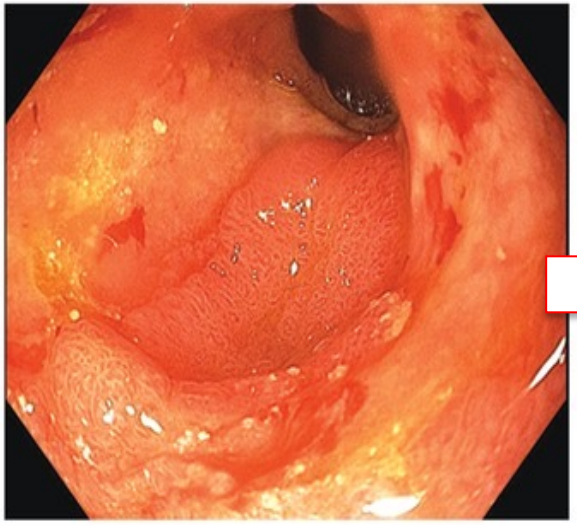
# An early biomarkers algorithm predict lethal aGVHD and survival



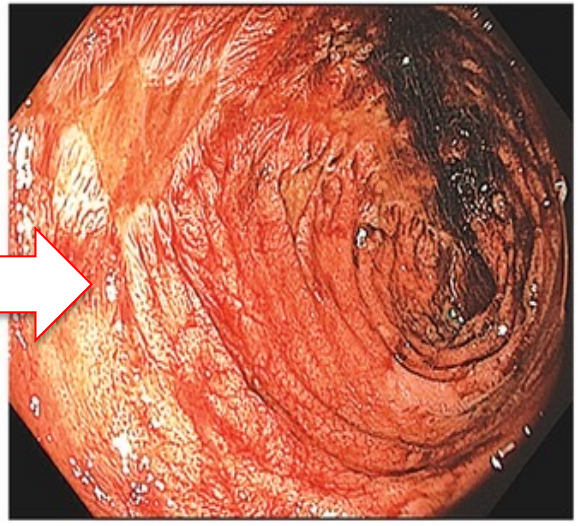
- 34aa
- LAM  trisomia 8; t(11;17) – KMT2A pos;
- RC POST FLAI5 – 3xHD ARAC
- MUD in relapse (CB 50%)
- MAC (TBF)
- CSA + MTX + ATG
- REMISSIONE MOLECOLARE (30° GIORNATA)

- COPRO POS CAMPYLOBACTER (cicli con claritromicina e meropenem)
- STOP CSA 3° ms
- 5° ms: GVHD intestino (confermata colonscopia): STEROIDE  1mg/kg
- persistenza coprocolture pos campylobacter

STEROIDE 1mg/kg



STEROIDE 1mg/kg + ruxolitinib



Dicembre ‘21

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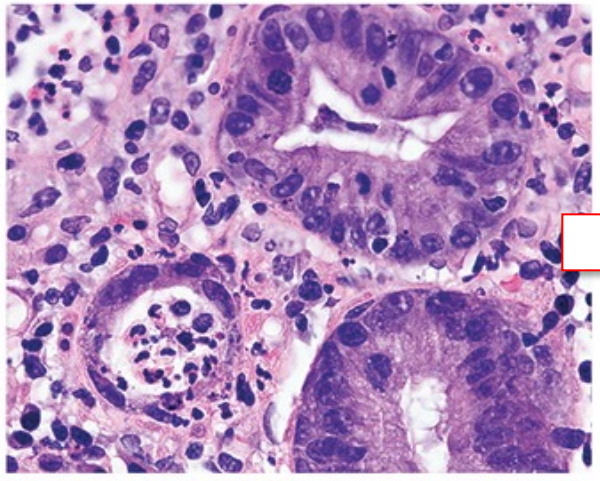
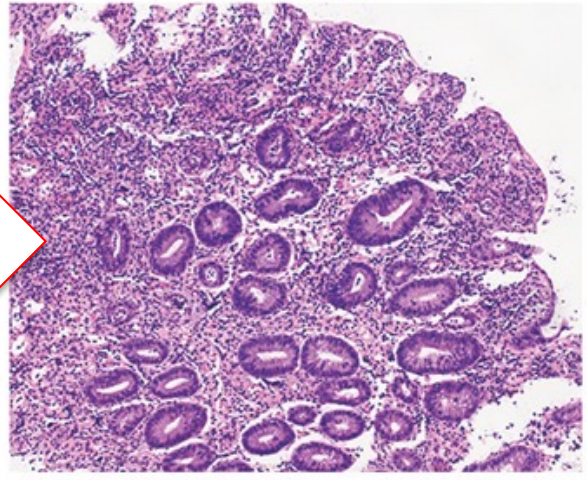
STOP ANTIBIOTICO TERAPIA

-5kg



-4kg



+7kg

Dicembre ‘21

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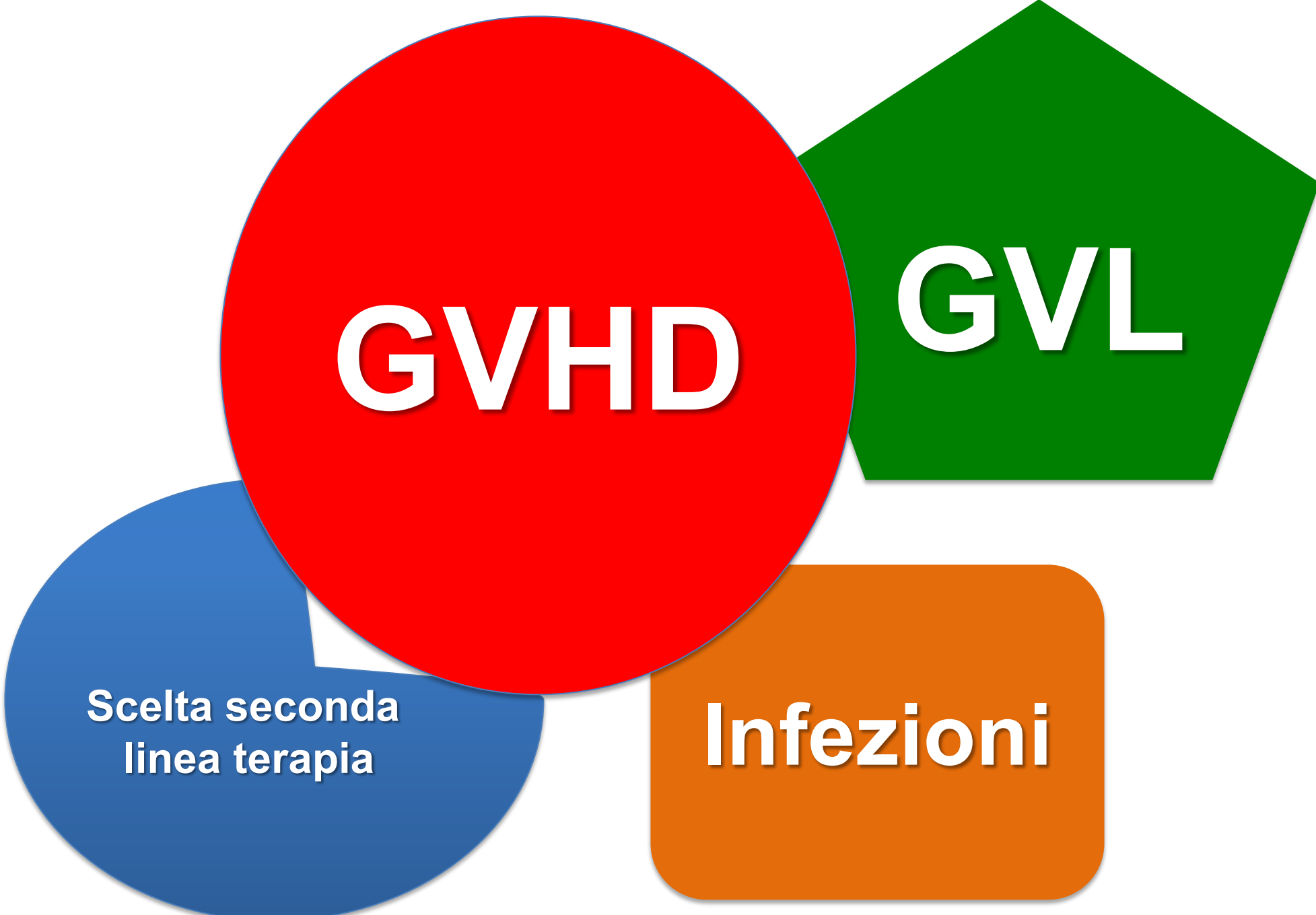
GVHD
GVL
Scelta seconda linea terapia
Infezioni