GITMO

GITMO Symposium

HOT TOPICS IN CHRONIC GVHD

Non immune effector targets in GVHD

Attilio Olivieri ,Head of Hematology Unit

Dpt. of clinical and molecular sciences Ancona, Italy

OCTOBER 20-21, 2024

ANCONA, Ego Hotel

Monday

08:45-09:45 **cGVHD part II: burning topics in lung cGVHD**

Chairmen: A. Bacigalupo (Genova), G. Moroncini (Ancona)

08:45 Round table: common and atypical lung manifestations in cGVHD in idiopatic pulmonary fibrosis and in CLAD
D. Wolff (Regensburg, DE), M. Bonifazi (Ancona), F. Meloni (Padova)

Sunday

15:20-18:00 **cGVHD part I**

Chairmen: F. Ciceri (Milano), M. Martino (Reggio Calabria)

15:20 cGVHD prophylaxis: are new strategies better than conventional tools? Yes
A. Nagler (Tel Aviv, IL)

15:40 cGVHD prophylaxis: are new strategies better than conventional tools? No
F. Bonifazi (Bologna)

16:00 ECP in the era of new drugs for cGVHD: which role and which schedule?
H. Greinix (Graz, AT)

16:20 Non immune effector targets in cGVHD
A. Olivieri (Ancona)

16:40 Ruxolitinib-refractory GVHD: it is still a worth definition?
M. Mohty (Paris, FR)

17:00 What to do in ruxolitinib-refractory cGVHD?
D. Wolff (Regensburg, DE)

17:20 Overlap cGVHD: assessment in the real life and proposal of a prospective GITMO study
D. Pulanic (Zagreb, HR), J. Mariotti (Milano)

17:40 Discussion

18:00 Closing part I

18.,15-19.45
MUSIC FROM THE WORLD
Israel, Balkans, Austria, Germany, France, Italy,

Azienda Ospedaliero Universitaria delle Marche

Attilio Olivieri

Head of Hematology Unit

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Università Politecnica delle Marche

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
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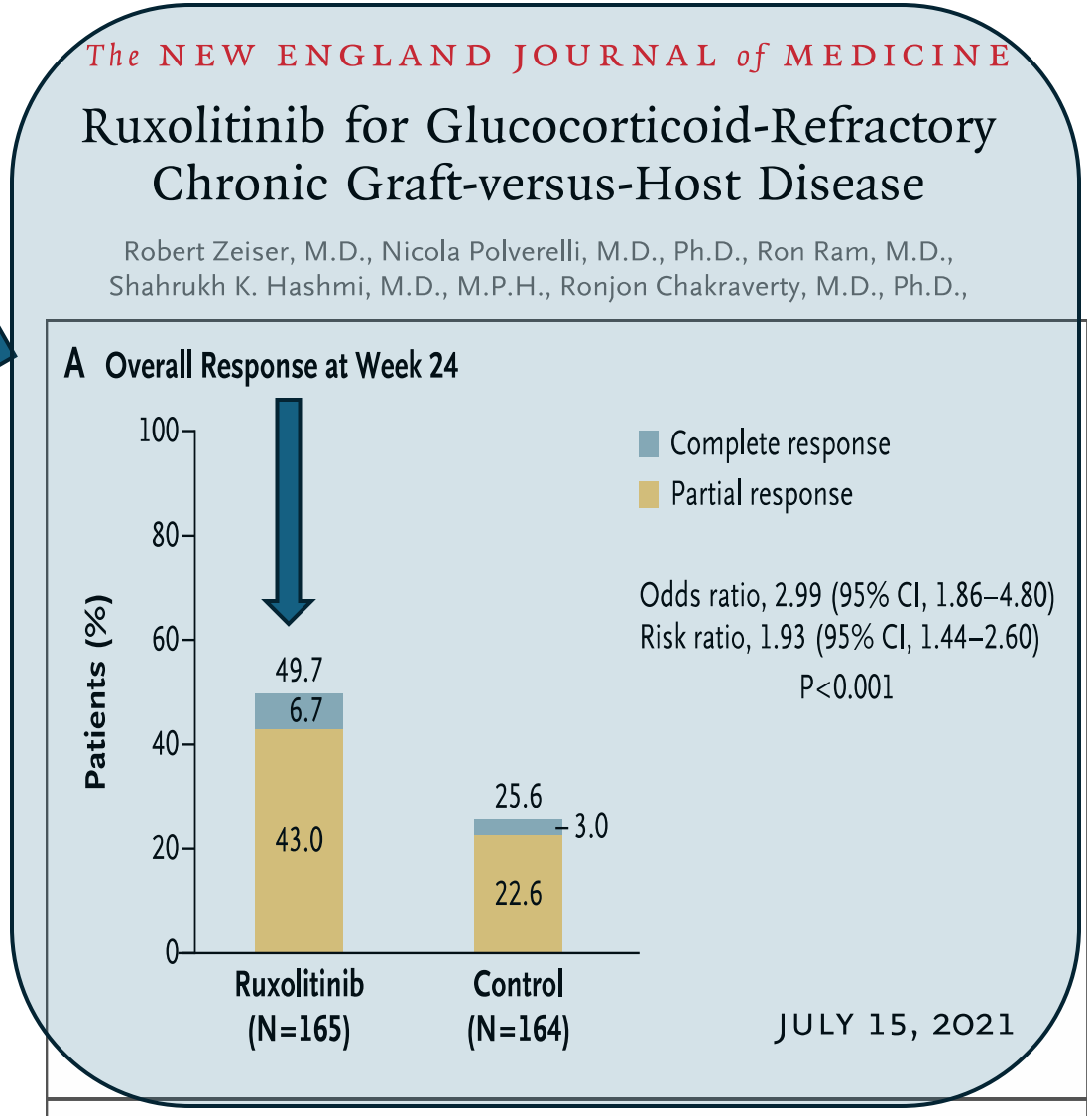
JAZZ	yes	x	x	x	x	yes	
Novartis	yes	x	x	x	x	yes	
Incyte	yes	x	x	x	x	yes	
Sanofi	yes	x	x	x	x	no	

Background-1

SR-GVHD....still unmeet medical need...

- Treatment resistance in both acute and in cGVHD is common and emerges quickly during disease evolution.
- JAK1/2 inhibitors fail to rescue 50% of patients with SR-cGVHD
- *During aGVHD irreversible organ damage may occur (e.g. Thymus, intestinal crypt; biliary ductules..other..); these sequelae deeply influence the course of subsequent cGVHD and the response to treatment.*

.....often the cause of a present failure lies in the past.....



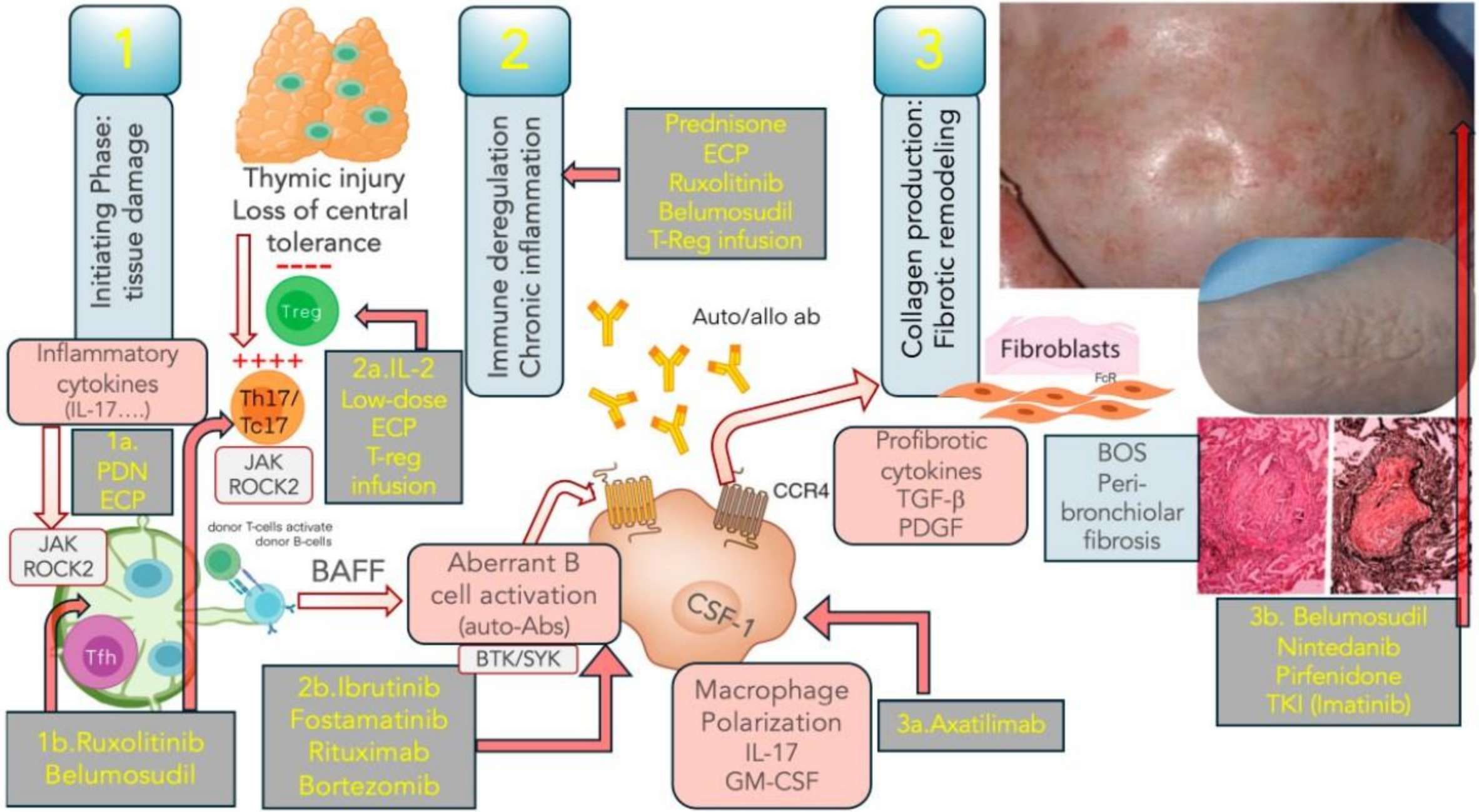
Background-2

……often the cause of a present failure lies in the past……

- In aGVHD the derangement of intestinal Stem Cell–supportive niches drastically reduces enterocyte regeneration….shortened enterocyte telomeres have been found in biopsies from patients with severe forms of intestinal aGVHD…*

- …Similarly, the thymic damage, the exhaustion of T reg pool or the irreversible peribronchiolar fibrotic damage in lung cGVHD, suggest that *some manifestations cannot be cured simply with immunosuppressive/anti-inflammatory drugs…. Representing some examples of the limitations of treatments targeting the traditional immune effectors* (e.g. alloreactive T-cells; aberrant B-lymphocytes)….

**Voermans C, Hazenberg MD. Cellular therapies for graft-versus-host disease: a tale of tissue repair and tolerance. Blood. 2020; 136(4):410-417.*


1
Initiating Phase: tissue damage
Thymic injury
Loss of central tolerance
Treg
++++
Th17/ Tc17
JAK ROCK2
2a.IL-2 Low-dose ECP T-reg infusion
2
Immune deregulation Chronic inflammation
Prednisone ECP Ruxolitinib Belumosudil T-Reg infusion
3
Collagen production: Fibrotic remodeling
Auto/allo ab
Fibroblasts
FcR
Inflammatory cytokines (IL-17….)
1a. PDN ECP
JAK ROCK2
donor T-cells activate donor B-cells
BAFF
Aberrant B cell activation (auto-Abs)
BTK/SYK
CCR4
CSF-1
Profibrotic cytokines TGF-β PDGF
BOS Peri-bronchiolar fibrosis
Tfh
1b.Ruxolitinib Belumosudil
2b.Ibrutinib Fostamatinib Rituximab Bortezomib
Macrophage Polarization IL-17 GM-CSF
3a.Axatilimab
3b. Belumosudil Nintedanib Pirfenidone TKI (Imatinib)

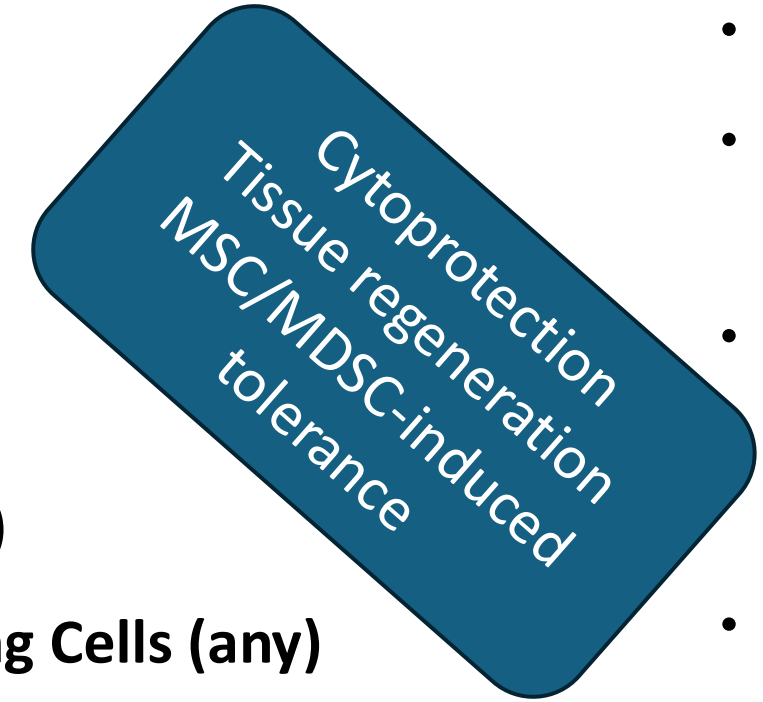
AGENDA

- Non Immune Effector Cells **(NIEC)** involved in cGVHD
- Antifibrotic drugs (for Scleroderma; lung fibrosis, *joints, fasciae…..*)
- Pathways associated with NIEC
- Strategies potentially usefull with NIEC and future scenarios

Non-immune cell targets in cGVHD

…and pathways not directly involving immunological effectors

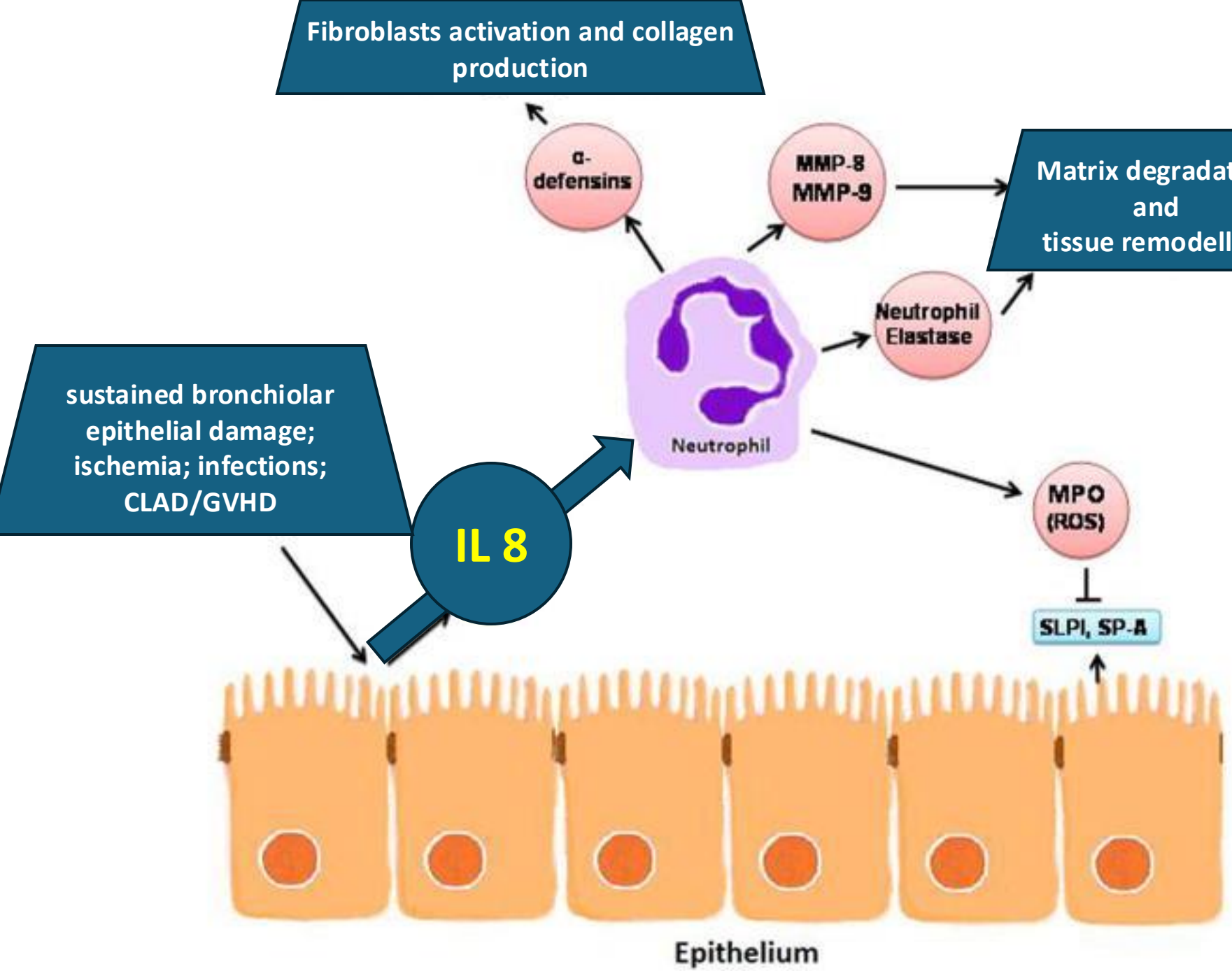
- **PMN**
- **Fibroblasts**
- **Monocytes**
- **Machrophages**
- **MDSC***
- **MSC° (secretome)**
- **Antigen Presenting Cells (any)**
- **NK…**



Involved pathways

- *Elastase production: tissue damage (IL8-TNF)*
- *Collagen production: TGF-β; fibroblast activation; myofibroblast generation*
- *Monocyte polarization (M1 or M2):*
  CSF1 induced M2 shift (profibrotic; proinflammatory)
- *Secretome and Induction of tolerogenic APC*

MDSC*: myelod derived suppressor cells; °MSC: mesenchymal stem cells



PMN in cGVHD

PMN play

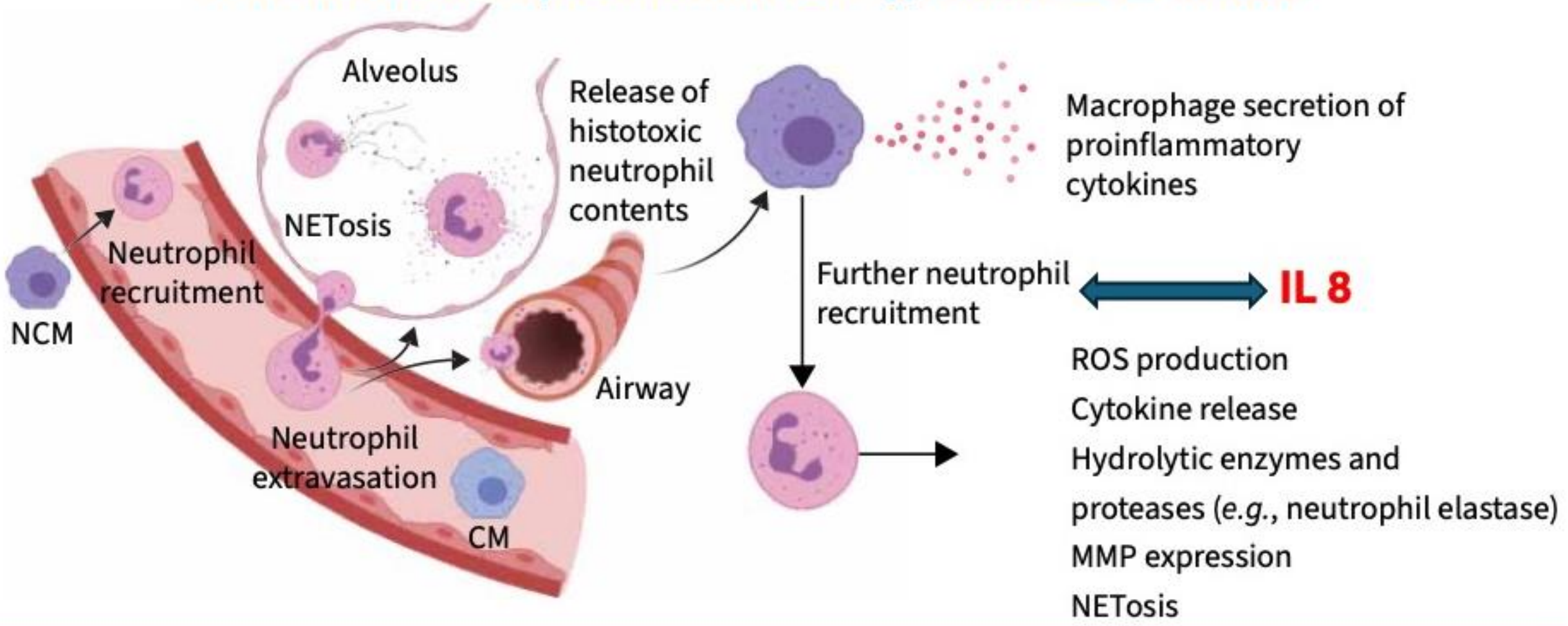
a central role

in the development

or progression

of BOS

PMN are the most predominant cell type in the BALF in BOS*



*Devouassoux G, et al. Alveolar neutrophilia is a predictor for the bronchiolitis obliterans syndrome, and increases with degree of severity. Transpl Immunol. 2002; 10(4):303–10.

*Bronchoalveolar Lavage as a Tool to Predict, Diagnose, and Understand BOS Vanessa E., Am J Transplant. 2013 March ; 13(3): 552–561.

Hubner RH, et al. Matrix metalloproteinase-9 in BOS after lung transplantation. Eur Respir J. 2005.

Elssner A, et al. Elevated levels of interleukin-8 and TGF-beta in bronchoalveolar lavage fluid from patients with BOS syndrome: proinflammatory role of bronchial epithelial cells. Transplantation. 2000.

- NE: serine protease stored in azurophilic granules of PMN in its inactive form; When PMN are exposed to inflammatory stimuli*, active NE is released.

- Release of NE degrades extracellular matrix components: elastin, laminins, and collagens, resulting in tissue damage *(e.g. elastic fibers of the bronchiolar wall)*

Inhibitors of neutrophil elastase (NE): alvelestat, sivelestat and α1-antitrypsin are promising in aGVHD and in BOS

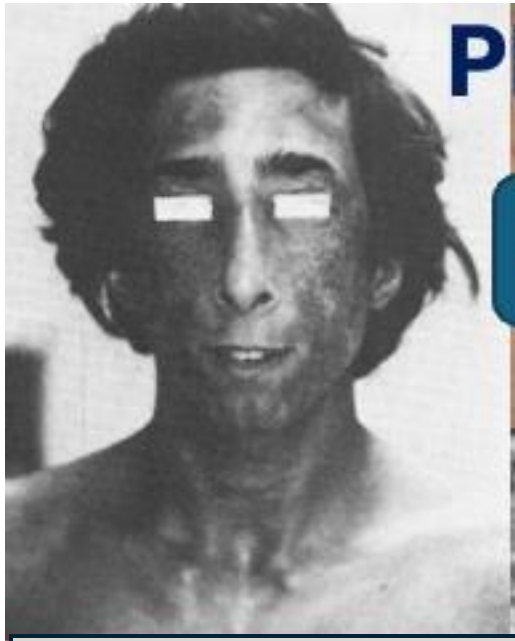
Stockley, R. et al Phase II study of a NE inhibitor (AZD9668) in patients with bronchiectasis. *Respir. Med.* **2013**, *107*

*Henriksen P.A. The potential of NE inhibitors as anti-inflammatory therapies. *Curr. Opin. Hematol.* **2014**

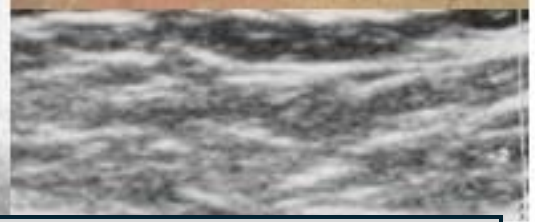
Altered Serum Alpha1-Antitrypsin Protease Inhibition before and after Clinical Hematopoietic Stem Cell Transplantation: Association with Risk for Non-Relapse Mortality

Ido Brami [1,*], Tsila Zuckerman [2], Ron Ram [3], Batia Avni [4], Galit Peretz [5], Daniel Ostrovsky [6], Yotam Lior [7], Caroline Faour [8], Oisin McElvaney [9], Noel G. McElvaney [9] and Eli C. Lewis [1]

Phase 3 of cGVHD



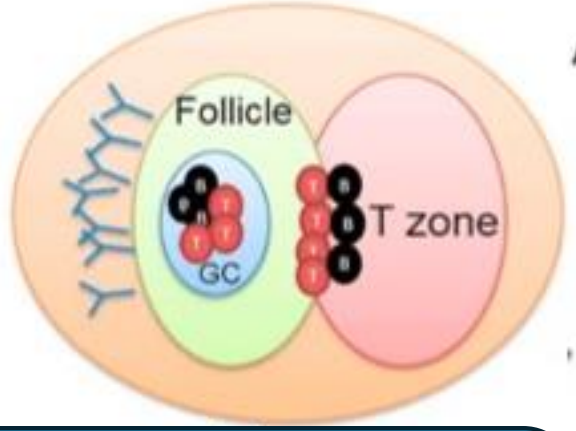
Skin&derma; lung, esophagous, fascia,



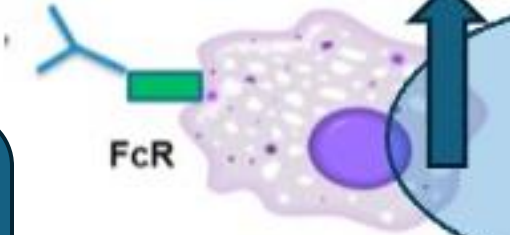
Allo/auto reactive B cells

Ab production

Biologically-driven Tx for fibrotic manifestations (scleroderma, lung, joints, genitalia,esofagous)



IL-17/GM-CSF



monocyte / Macrophage Activation

TGFβ

RESEARCH ARTICLE

The Journal of Clinical Investigation

CSF-1–dependant donor-derived macrophages mediate chronic graft-versus-host disease

Kylie A. Alexander,[1] Ryan Flynn,[2] Katie E. Lineburg,[1] Rachel D. Kuns,[1] Bianca E. Teal,[1] Stuart D. Olver,[1] Mary Lor,[1] Neil C. Raffelt,[1] Motoko Koyama,[1] Lucie Leveque,[1] Laetitia Le Texier,[1] Michelle Melino,[1] Kate A. Markey,[1] Antiopi Varelias,[1] Christian Engwerda,[1] Jonathan S. Serody,[3] Baptiste Janela,[4] Florent Ginhoux,[4] Andrew D. Clouston,[5] Bruce R. Blazar,[3] Geoffrey R. Hill,[1,6] and Kelli P.A. MacDonald[1]

Th17 cells secrete CSF-1 driving monocyte recruitment

In SS-like GVHD and in BOS, inflammatory milieu induces macrophage-2 polarization with TGF-beta production inducing myofibroblast differentiation from fibroblasts, pericytes or endothelial cells

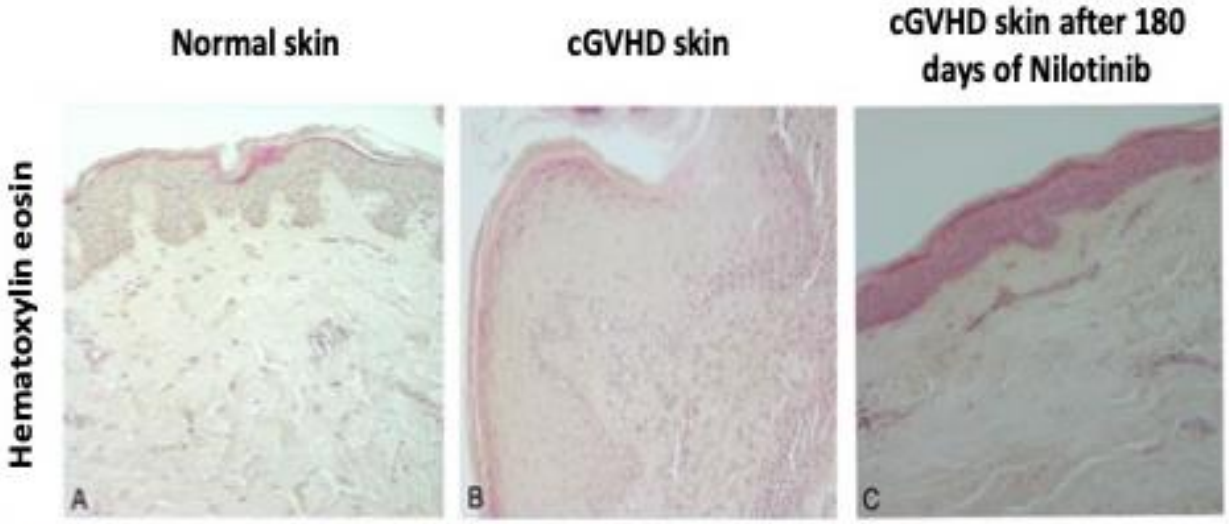
Fibroblast proliferation and Collagen Production

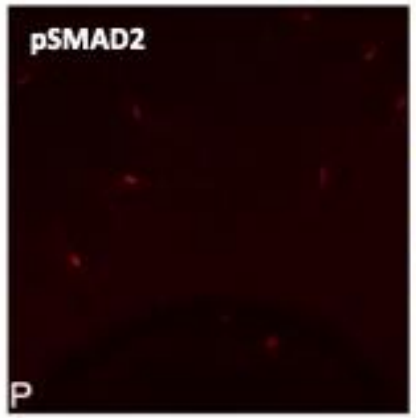
Aberrant tissue repair

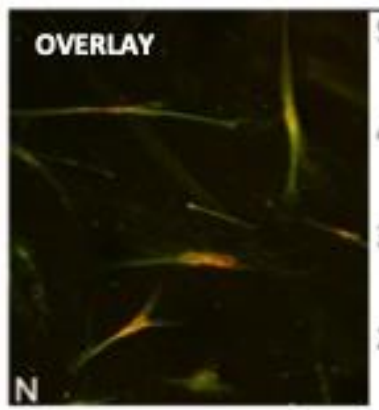
Collagen deposition & tissue fibrosis

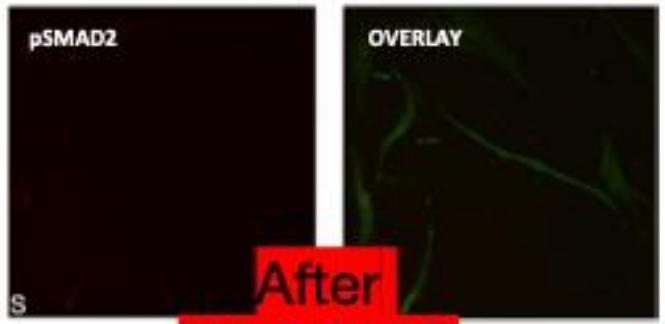
Treatment area/drug	Mechanism of action	Current trials	Comments
Fibrosis			
Anti-CSF1R	Monoclonal anti-CSF1R blocks macrophage infiltration of lung and skin.[119]	NCT03604692, NCT04710576	Timing of intervention may be important because early administration of CSF1 ameliorates experimental aGVHD.[120]
Pirfenidone Nintedanib Ibrutinib Imatinib	Broad effects include reduced lung infiltration by macrophages, reduced frequency of T follicular helper cells, inhibition of TGF-β signaling and STAT3 activation in cultured fibroblasts, and attenuation of hedgehog signaling.[105]	NCT03315741,	In preclinical models of cGVHD, pirfenidone was effective in bronchiolitis obliterans but less effective in skin disease.[94] It has been approved for treatment of idiopathic pulmonary fibrosis.[105]
Nilotinib	Tyrosine kinase inhibitor that blocks activation via C-ABL, PDGFR-α/β, and TGF-β.[105]	NCT01810718, NCT01155817	The safety profile was shown to be adequate in a phase 1 trial; efficacy testing is in progress.
Belumosudil (KD025)	Rho-associated kinase 2 inhibitor inhibits IL-17, IL-21, and		Phase 1/2 dose escalation study and a phase 2 randomized
Mesenchymal stromal cells (MSCs)	Reduces apoptosis and promotes re-epithelialization after radiation-induced gut injury[112] and promotes ILC3 function.[38]	NCT0060330, NCT01045382	Efficacy and mechanism of action in GVHD remains unclear (reviewed in Voermans and Hazenberg[113]). Different sources of MSCs and different methods of manipulation are potential confounders. MSCs derived from iPSCs show potential efficacy in a phase 1 study.[114]

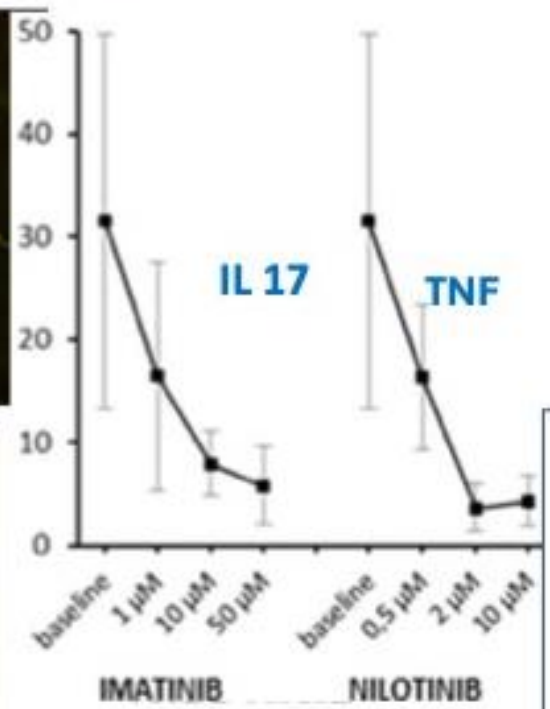
Compounds potentially useful for cGVHD fibrotic manifestations







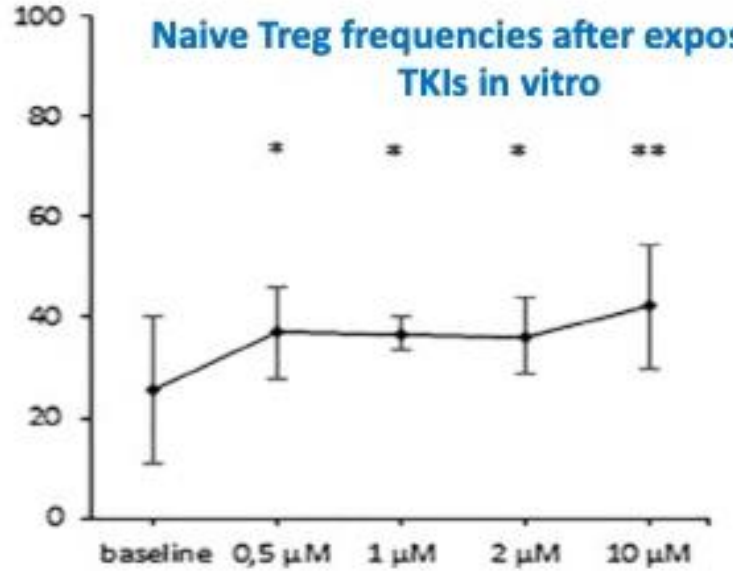




Biol Blood Marrow Transplant 24 (2018)

Immunomodulatory Effects of Tyrosine Kinase Inhibitor In Vitro and In Vivo Study

Elena Marinelli Busilacchi [1,2,†], Andrea Costantini [1,3,†], Nadia Viola [3], Benedetta Costantini [4], Jacopo Olivieri [5], Luca Butini [3], Giorgia Mancini [2], Ilaria Scortechini [2], Martina Chiarucci [2], Monica Poiani [1,2], Antonella Poloni [1,2], Pietro Leoni [1,2], Attilio Olivieri [1,2,*]



Nilotinib Treatment of Patients Affected by Chronic Graft-versus-Host Disease Reduces Collagen Production and Skin Fibrosis by Downmodulating the TGF-β and p-SMAD Pathway

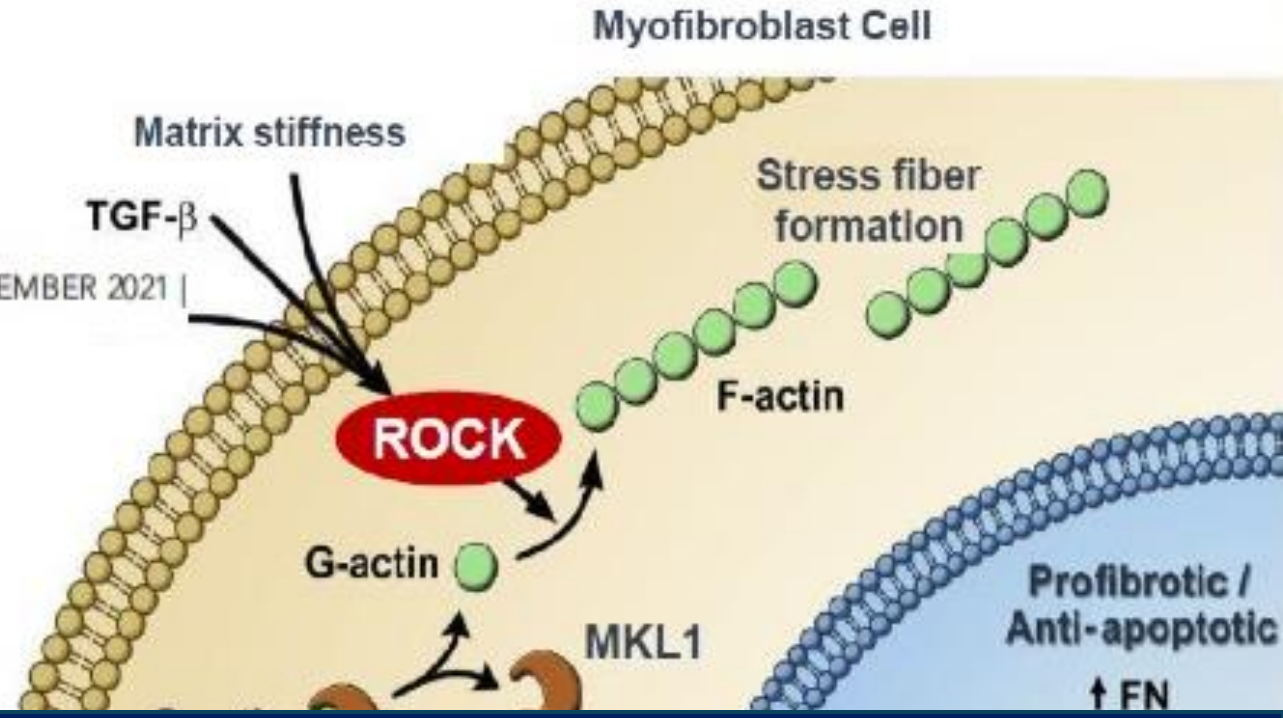
Elena Marinelli Busilacchi[1,2,†], Andrea Costantini[1,3,†], Giorgia Mancini[2], Giovanni Tossetta[4], Jacopo Olivieri[5], Antonella Poloni[1,2], Nadia Viola[3], Luca Butini[3], Anna Campanati[6], Gaia Goteri Daniela Marzioni[4], Attilio Olivieri[1,2,*]

Biol Blood Marrow Transplant 26 (2020) 823–834

ROCK2 signal regulates proinflammatory cytokines (IL-21&IL-17) both in AID and incGVHD

ROCK Regulates Multiple Profibrotic Processes, Including Myofibroblast Activation

- ROCK is downstream of major pro-fibrotic mediators
- ROCK regulates fibroblast differentiation to myofibroblasts,



blood® 2 DECEMBER 2021 |

TRANSPLANTATION

Belumosudil for chronic graft-versus-host disease after 2 or more prior lines of therapy: the ROCKstar Study

Corey Cutler,[1] Stephanie J. Lee,[2] Sally Arai,[3] Marcello Rotta,[4] Behyar Zoghi,[5] Aleksandr Lazaryan,[6] Aravind Ramakrishnan,[7]

Best ORR: 72-77%; DOR (median) 54 Weeks;
44% of patients on Tx at >1 year

Clinical response to belumosudil in bronchiolitis obliterans syndrome: a combined analysis from 2 prospective trials

Blood Advances Dec 2022 **best ORR for lung: 32%**

Zachariah DeFilipp,[1] Haesook T. Kim,[2] Zhongming Yang,[3] John Noonan,[3] Bruce R. Blazar,[4] Stephanie J. Lee,[5,6] Corey Cutler[3]

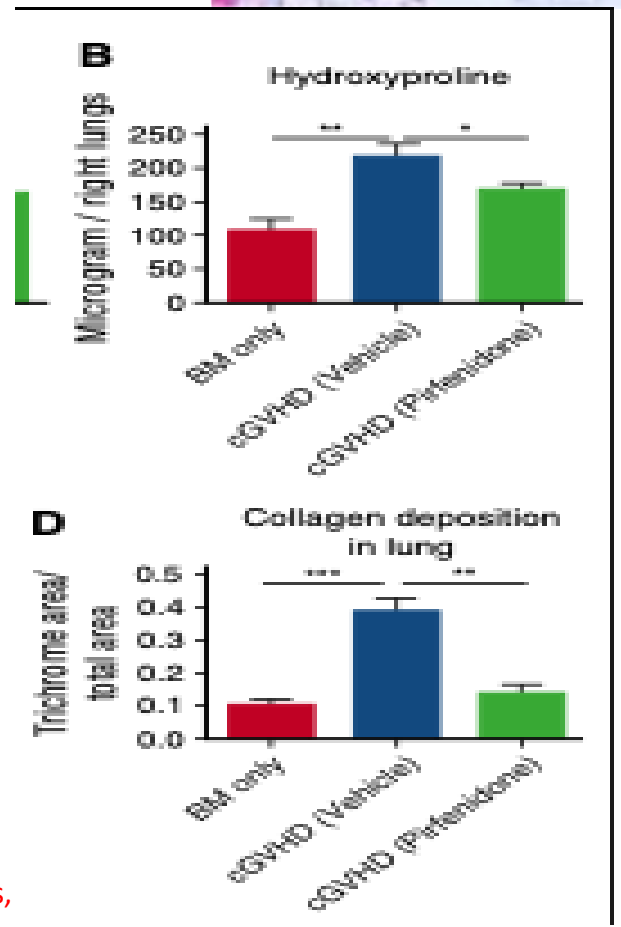
Belumosudil showed reduction of fibrosis in animal models of BOS and sclerodermatous cGVHD, inhibiting multiple fibrotic pathways

Pirfenidone ameliorates murine chronic GVHD through inhibition of macrophage infiltration and TGF-β production

Jing Du,[1] Katelyn Paz,[1] Ryan Flynn,[1] Ante Vulic,[2] Tara M. Robinson,[2] Katie E. Lineburg,[3] Kylie A. Alexander,[3] Jingjing Meng,[4] Sabita Roy,[4] Angela Panoskaltsis-Mortari,[1] Michael Loschi,[1] Geoffrey R. Hill,[3] Jonathan S. Serody,[5] Ivan Maillard,[6] David Miklos,[7] John Koreth,[8] Corey S. Cutler,[8] Joseph H. Antin,[8] Jerome Ritz,[8] Kelli P. MacDonald,[3] Timothy W. Schacker,[9] Leo Luznik,[2] and Bruce R. Blazar[1]

- Hydroxyproline (HP) in lung correlates with the amount of collagen in lung.
- HP in cGVHD mice is two-fold higher than the non-cGVHD mice
- Pirfenidone reduced lung HP content

Pirfenidone reduces fibrosis, F4/801 macrophage accumulation and TGF-b deposition in lung



pirfenidone, (FDA)-approved drug for idiopathic pulmonary fibrosis,

N ENGL J MED 391;11 NEJM.ORG SEPTEMBER 19, 2024

Axatilimab in Recurrent or Refractory Chronic Graft-versus-Host Disease

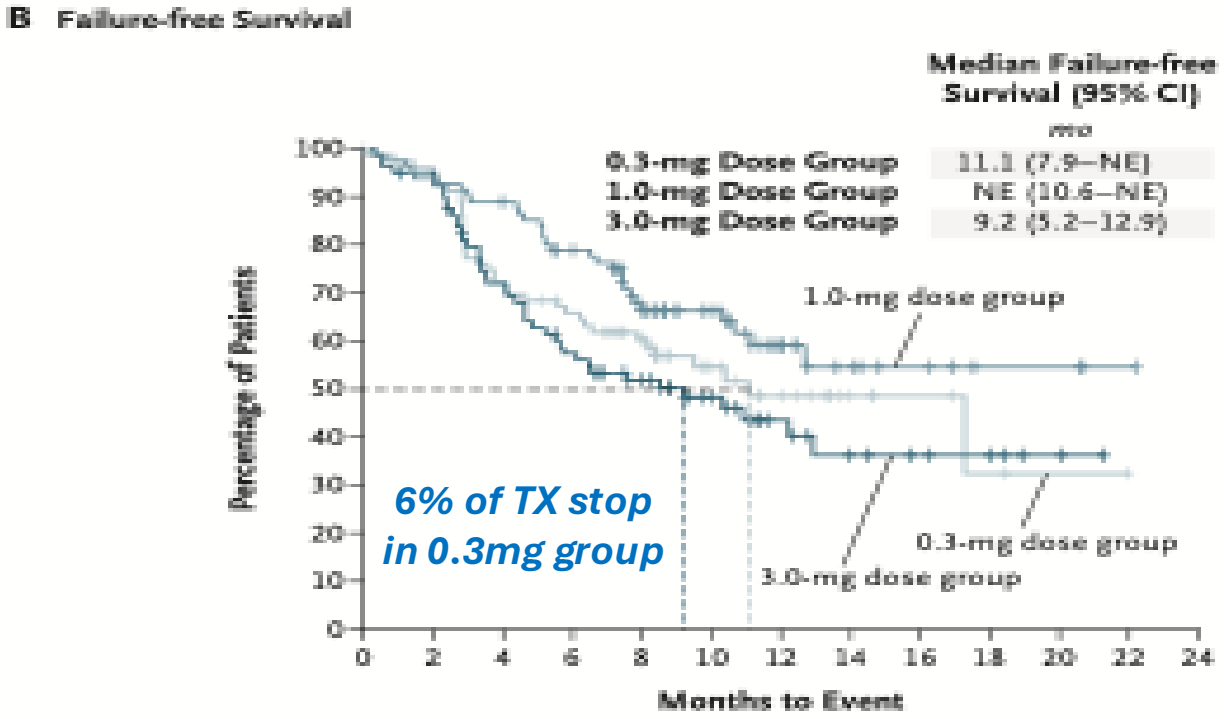
D. Wolff, C. Cutler, S.J. Lee, I. Pusic, H. Bittencourt, J. White, M. Hamadani, S. Arai, A. Salhotra, J.A. Perez-Simon, A. Alousi, H. Choe, M. Kwon, A. Bermúdez, I. Kim, G. Socié, S. Chhabra, V. Radojcic, T. O'Toole, C. Tian, P. Ordentlich, Z. DeFilipp, and C.L. Kitko, for the AGAVE-201 Investigators*

CSF1R signaling– dependent monocytes and macrophages mediate inflammation and fibrosis in cGVHD

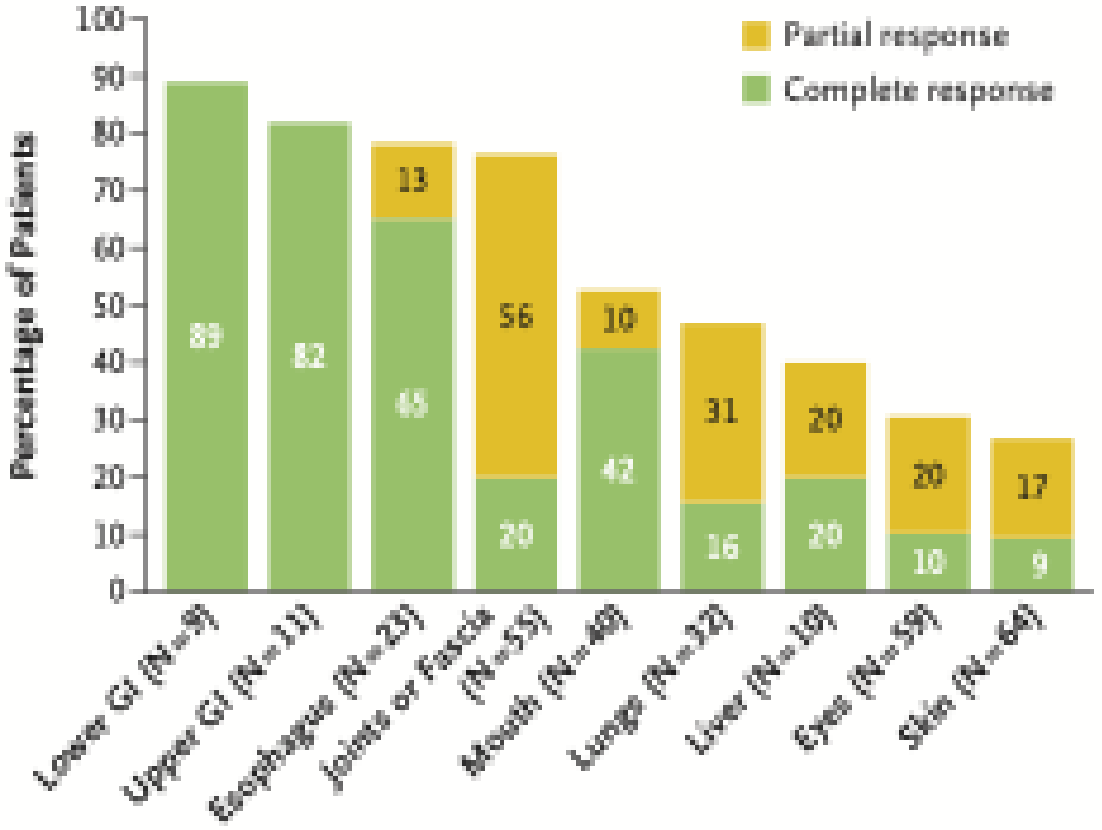
Alexander KA, et al. CSF-1-dependant donor-derived macrophages mediate chronic graft-versus- host disease. J Clin Invest 2014;124: 4266-80.

Axatilimab, humanized IgG4 moAb, inhibits CSF1R signaling in *CSF1R-expressing macrophages*

241 pts with SR-cGVHD enrolled



C Overall Response in the 0.3-mg Dose Group → **74%**



MDSC: myeloid derived suppressor cells

Messmann JJ, Blood. 2015; Wang D, Biol Blood Marrow Transplant. 2013

Heterogeneous mixture of immature myeloid cells: 2 major subsets (granulocytic MDSCs and monocytic MDSCs)

MDSC mediate suppressive activity via several mechanisms*, and recover rapidly after alloHCT with G-CSF-mobilized PBSC

Highfill SL, et al. BM MDSCs inhibit GVHD via an arginase-1-dependent mechanism up-regulated by IL-13. Blood. 2010.

Baumann T, al. Regulatory myeloid cells paralyze T cells through cell-cell transfer of metabolite methylglyoxal. Nat Immunol. 2020.

Ex Vivo Generated Human Cord Blood Myeloid-Derived Suppressor Cells Attenuate Murine Chronic Graft-versus-Host Diseases

Ji-Young Lim[1], Da-Bin Ryu[1], Mi-Young Park[2], Sung-Eun Lee[1], Gyeongsin Park[3], Tai-Gyu Kim[2], Chang-Ki Min[1,*]

Biol Blood Marrow Transplant 24 (2018) 2381–2396

Non GVHD control | GVHD | GVHD + hCB-MDSCs

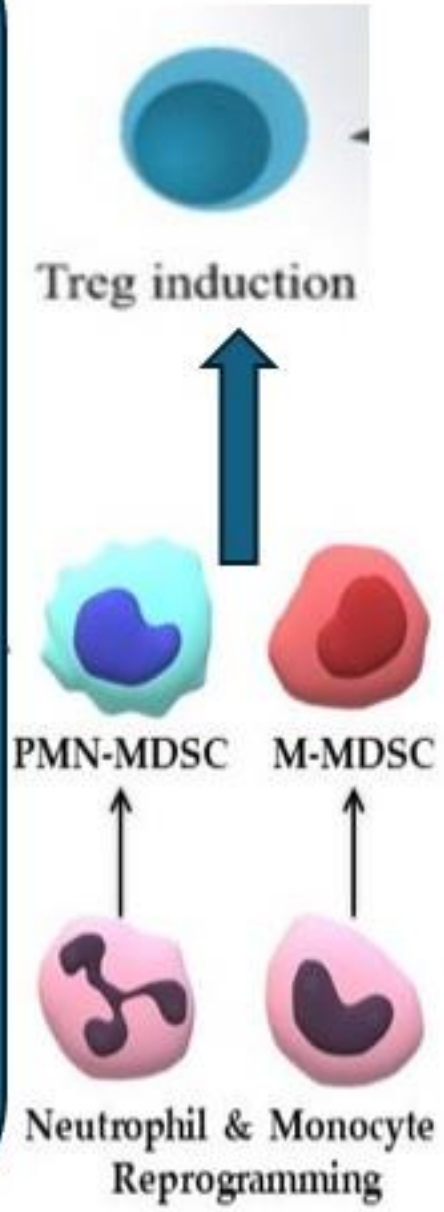
MDSC phenotype:

CD11b+CD33+HLA-DR−/low

- CD14+ (monocytic, M-MDSCs)
- CD15+ (granulocytic, PMN-MDSCs),
- CD14-/CD15- (early stage, eMDSCs

Mechanisms of Action of MDSCs

T reg induction/TH inhibition
T-cell homing blockage
Extracellular vesicle cargo
Induction of tolerogenic cells (DC/APC)
.....
Angiogenesis and metastasis
Expression of negative immune checkpoint molecules



Increased circulating MDSCs in patients treated with ECP for acute or cGVHD

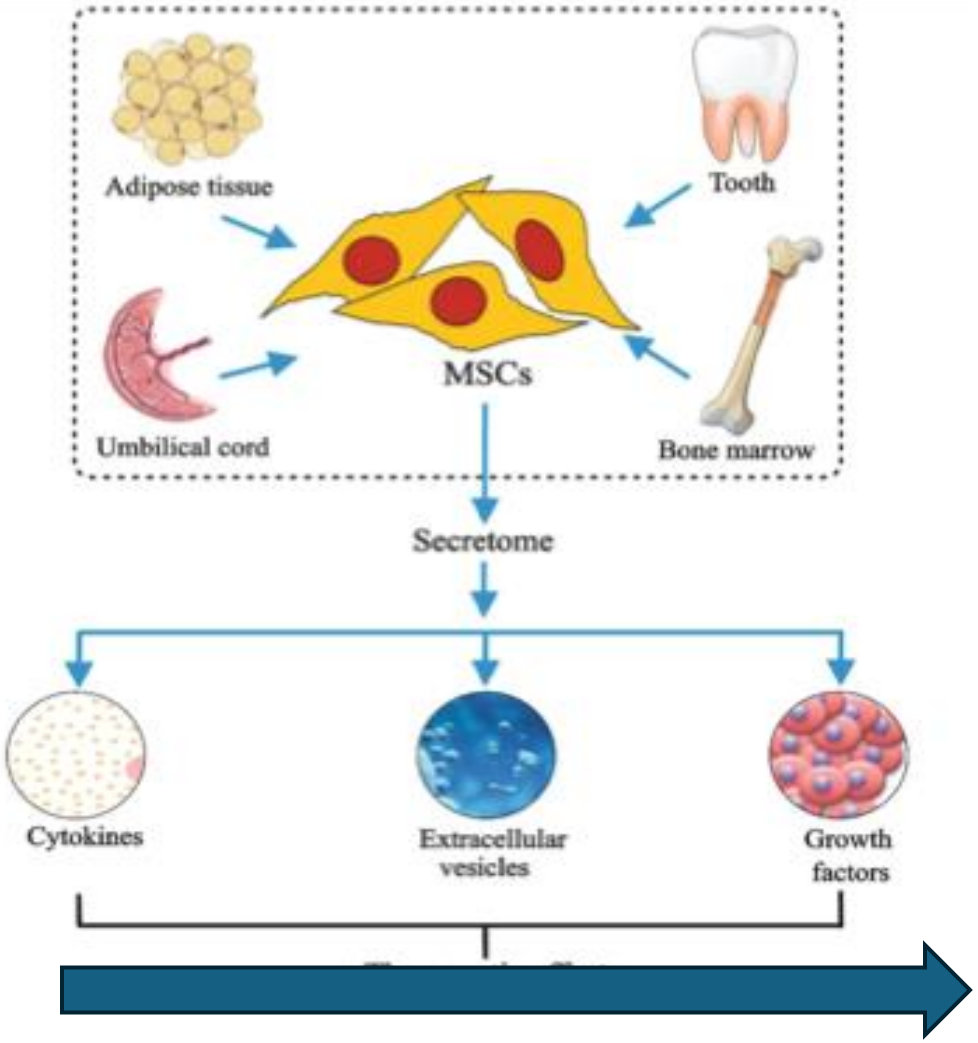
Wang L, Ni M, et al.
Front Immunol. (2018) 9:2207

MDSC increased in mice treated with PTCy

Cy prevents GVHD not by eliminating alloreactive T cells but by inducing alloreactive T-cell functional impairment

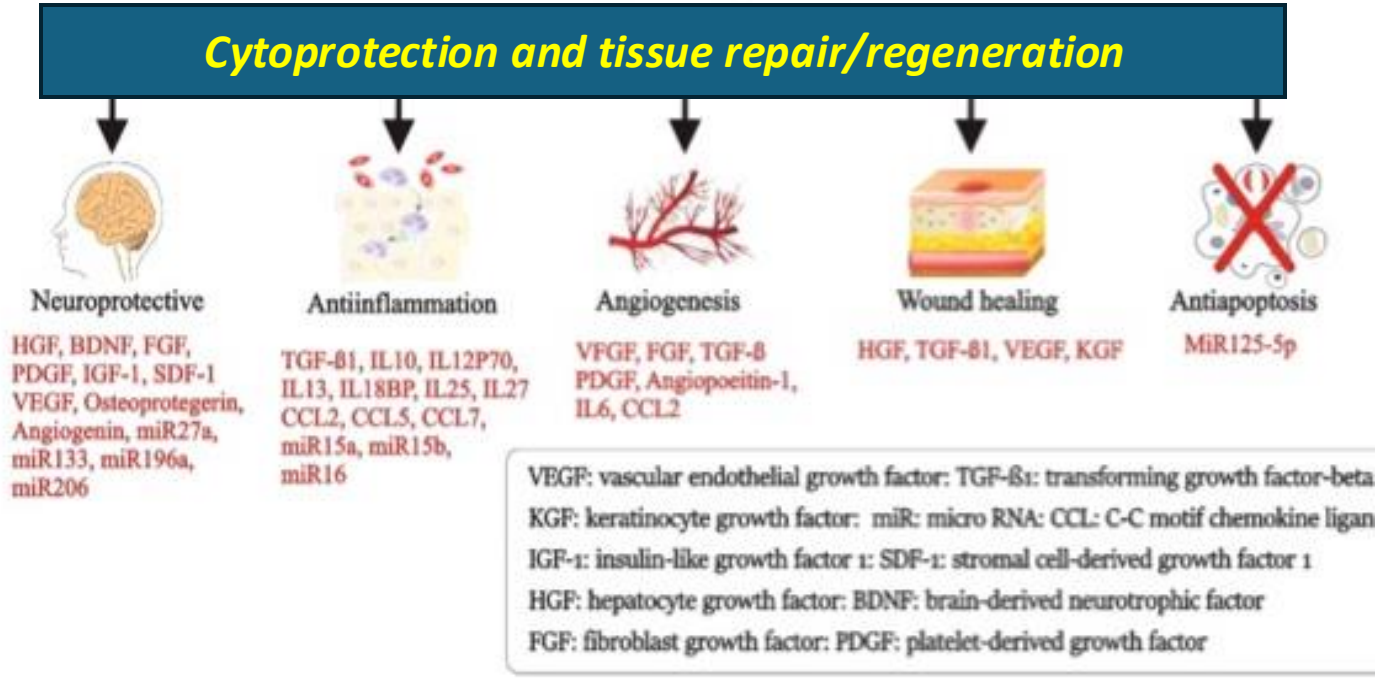
Fletcher R.E.et al,
Blood Adv april 2023

MSCs exert pleiotropic activities both in acute and cGVHD*



Paracrine effects of MSCs occur in response to their microenvironment……..

- inhibit the activation and proliferation of CD4+T cells
- inhibit the migration of macrophages;
- facilitate proliferation of Treg;↑ IL-10
- ↓BAFF
- ↓Collagen production

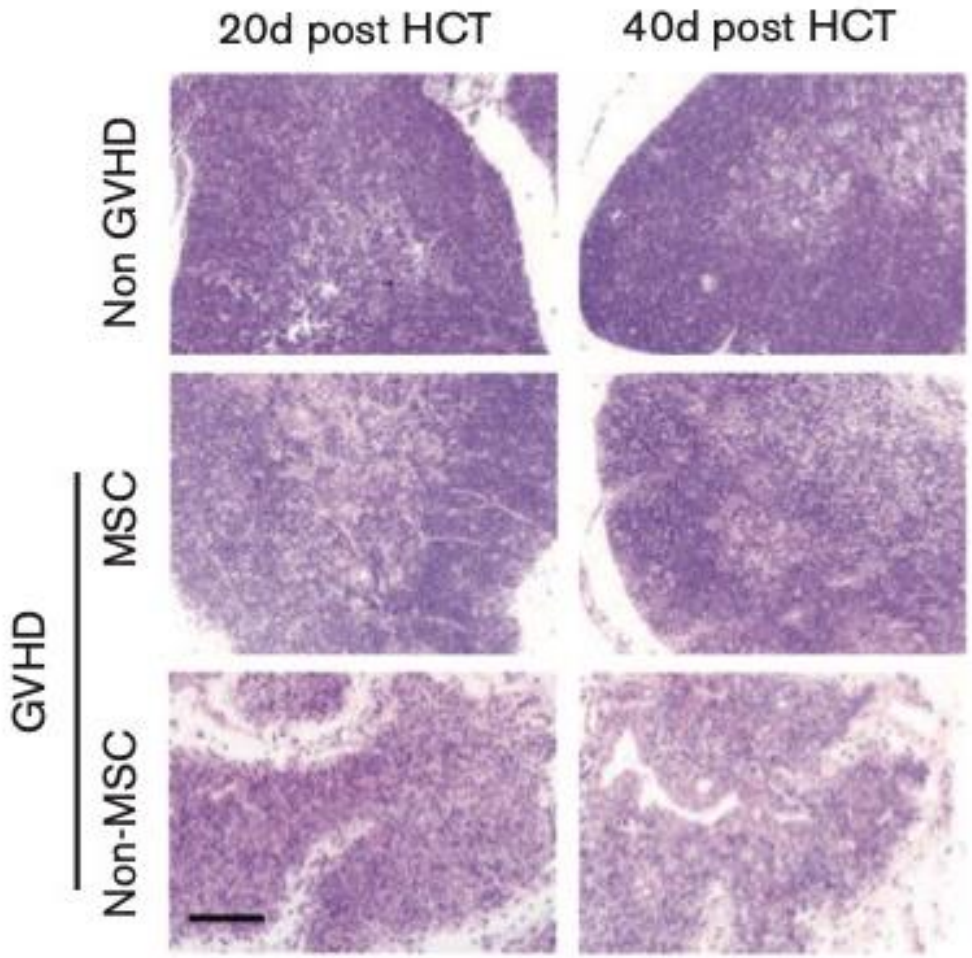


Morata-Tarifa C,. MSC for the prophylaxis and treatment of GVHD: meta-analysis. Stem Cell Res Ther. 2020

Mesenchymal stromal cells ameliorate chronic GVHD by boosting thymic regeneration in a CCR9-dependent manner in mice

Blood Adv. 6 Sept 2023 • VOL 7, N 18

Xin Zhang,[1,2,*] Jiabao He,[1,3,*] Ke Zhao,[1,3,*] Shiqi Liu,[1,3,*] Li Xuan,[1,3] Shan Chen,[1,3] Rongtao Xue,[1,3] Ren Lin,[1,3] Jun Xu,[1,3] Yan Zhang,[1,3] Andy Peng Xiang,[4] Hua Jin,[1,3] and Qifa Liu[1,3]

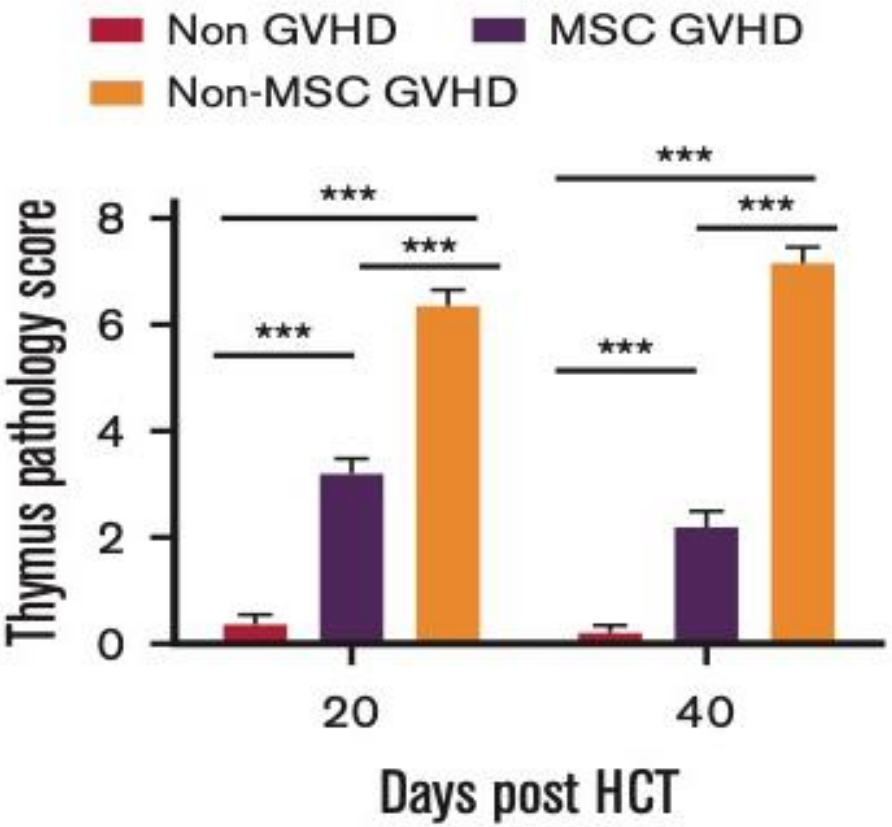


…thymic architecture recovered,

…….

…….incidence and severity of subsequent cGVHD decreased after MSCs treatment

- MSCs can home to the thymus via the CCL25-CCR9 axis and repair the damaged thymus caused by aGVHD.



JAMA Oncology | Original Investigation

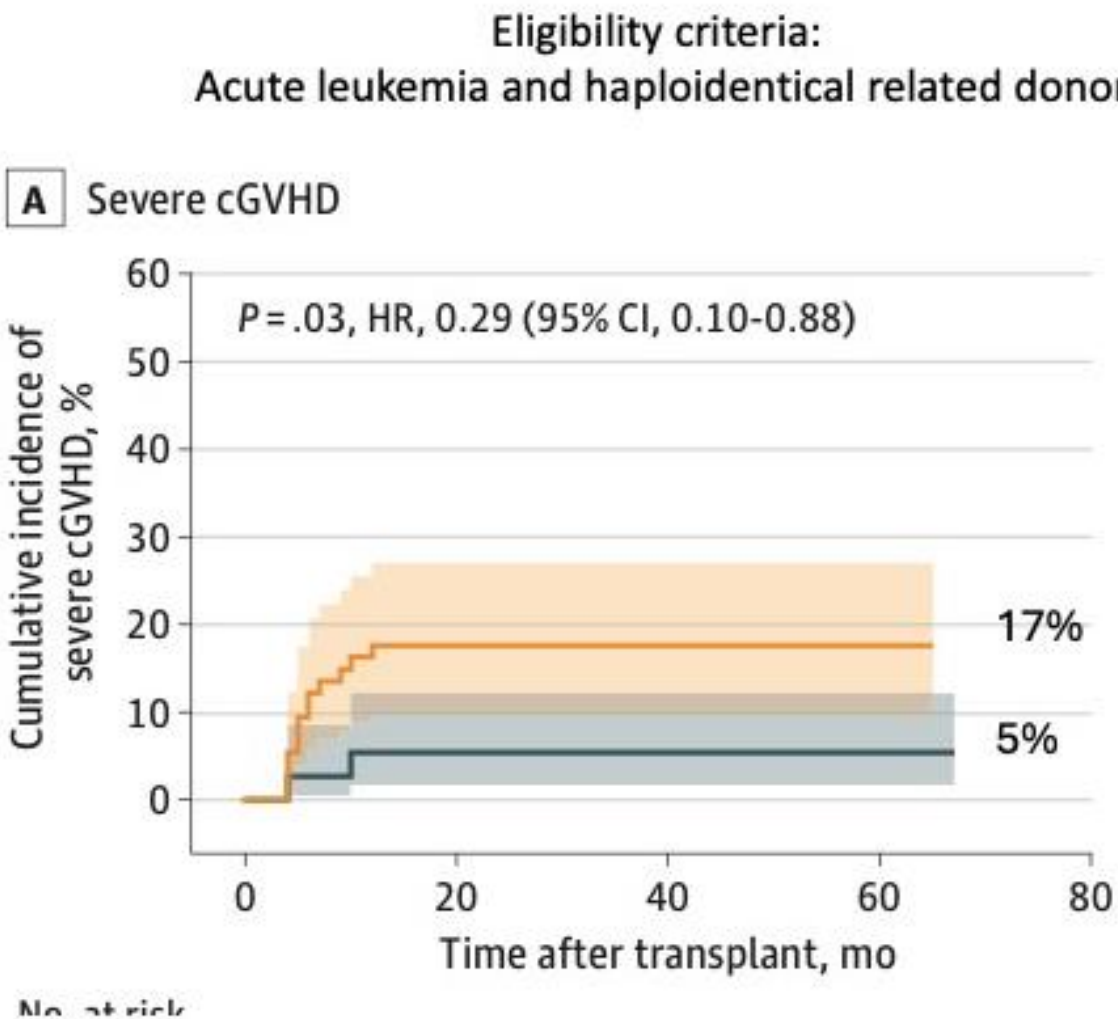
Mesenchymal Stem Cells for Prophylaxis of Chronic Graft-vs-Host Disease After Haploidentical Hematopoietic Stem Cell Transplant

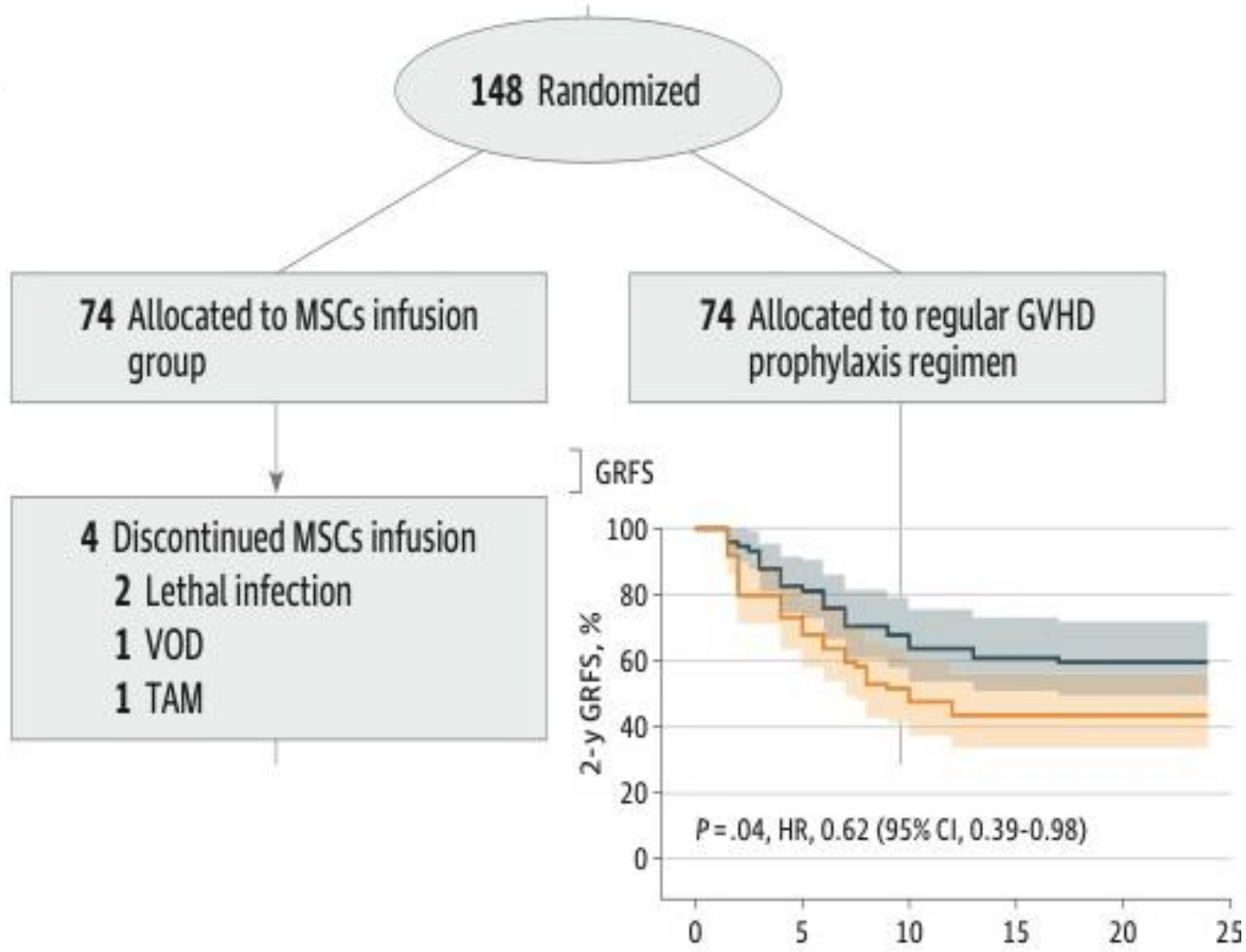
An Open-Label Randomized Clinical Trial

Ruihao Huang, MD; Ting Chen, MD; Sanbin Wang, MD; Jishi Wang, MD; Yi Su, MD; Jing Liu, MD; Yanqi Zhang, PhD; Xiangyu Ma, PhD; Qin Wen, MD; Peiyan Kong, MD; Cheng Zhang, MD; Lei Gao, MD; Jiang F. Zhong, PhD; Li Gao, MD; Xi Zhang, MD, PhD

Random to receive MSCs
($1 \times 10_6$ cells/kg,
every 2 weeks for 4 infusions,
starting from 45 days
after HSCT) or NOT

Eligibility criteria:
Acute leukemia and haploidentical related donor





Mouse models and data from humans suggest that immunomodulatory properties of MSCs are mediated by secretome (micro-vesicles and exosomes: EV)

- EVs contain microRNA and chemokines able to alter gene expression of target cells, to modulate immune and inflammatory responses in GVHD.

  Łacina P et al. Differential expression of miRNAs from EVs in cGVHD: a preliminary study. Adv Clin Exp Med (2022)

- EVs from MSC can inhibit profibrotic pathway: miRNA miR-29-3p (contained in MSC-EVs) regulates hub genes involved in TGF-beta signaling and fibrotic processes.

MSC-EVs to treat and prevent GVHD…..

MSC-EVs to treat dry eye in patients with ocular GVHD (NCT04213248)

APCs play as cGVHD drivers

Non-hematopoietic cells can develop antigen-presenting ability under specific conditions:

keratinocytes, fibroblasts alveolar epithelial cells and intestinal epithelial cells (IEC).*

**IEC were found to initiate lethal gastrointestinal GVHD*

Both MDSC and MSC can modify the APC functions and polarization

APC type	Origin	Function
$CD141^+$ /$CD123^+$ dendritic cells	Donor	Regulate Tcell Tolerance T reg expansion
$CD19^+$ B cells	Host Donor	Production of auto-Abs; disregulation of B-cell homeostasis. Expansion of autoreactive T cells
Macrophages	Host Donor	Production of auto-Abs Promote fibrosis via TGF-beta; activate Th17; shift M2 via CSF1R
Medullary thymic epithelial cells (mTECs)	Host Donor	Restore Thymus microenvironment and tolerance Defective autoreactive T-cell selection

CONCLUSIONS

- NIEC are pivotal in cGVHD: MSC, MDSC, macrophages, myofibroblasts, PMN and APC represents both potential tools and potential targets.

- MSC: predominantly used in aGVHD, but data are accumulating about their efficacy also in cGVHD prevention/treatment.

- MSC show pleiotropic immunomodulatory abilities mediated by secretome, modulating both immune effector and NIEC and by their unique ability to promote the regeneration of critical tissues (e.g. Thymus, lung, bowel).

- Multiple studies have shown an excellent safety profile of MSCs and indicated their efficacy in treating and preventing cGVHD.

Recent acquisitions on MSC secretome (EVs), promoted preclinical research with therapeutic applications of specific EVs, isolated in cell factories able to guarantee the standards for an easier and cheaper product, compared to the cell therapy.

Thanks....

Azienda Ospedaliero Universitaria delle Marche

Simone Angeletti & nurses team

All patients and their families

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GITMO
GRUPPO ITALIANO PER IL TRAPIANTO DI MIDOLLO OSSEO, CELLULE STAMINALI EMOPOIETICHE E TERAPIA CELLULARE