Il ruolo di IL-6 nella malattia di Castleman e nei processi infiammatori cronici

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The War and Peace of IL6

1. The biology of IL-6
2. IL-6 in other diseases and its contribution to chronic inflammation
3. The role of IL-6 in Castleman disease
4. Targeting IL-6

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The biology of IL-6

- Interleukin 6 is a pleiotropic cytokine with activity in several biologic systems and organs
- Initially described in 1973 by Kishimoto et al. as a soluble protein produced by T cells and inducing the differentiation of B cells into antibody-producing cells
- Following this, the same protein was independently identified by different groups and given different names according to the different contexts it was described in
- Subsequent cloning showed that these proteins were all the same, and they were grouped under the name of IL-6

> J Immunol. 1973 Oct;111(4):1194-205.

Regulation of antibody response in vitro. VII. Enhancing soluble factors for IgG and IgE antibody response

T Kishimoto, K Ishizaka

PMID: 4728681

Kang et al. Historical overview of the interleukin-6 family cytokine, J Exp Med. 2020

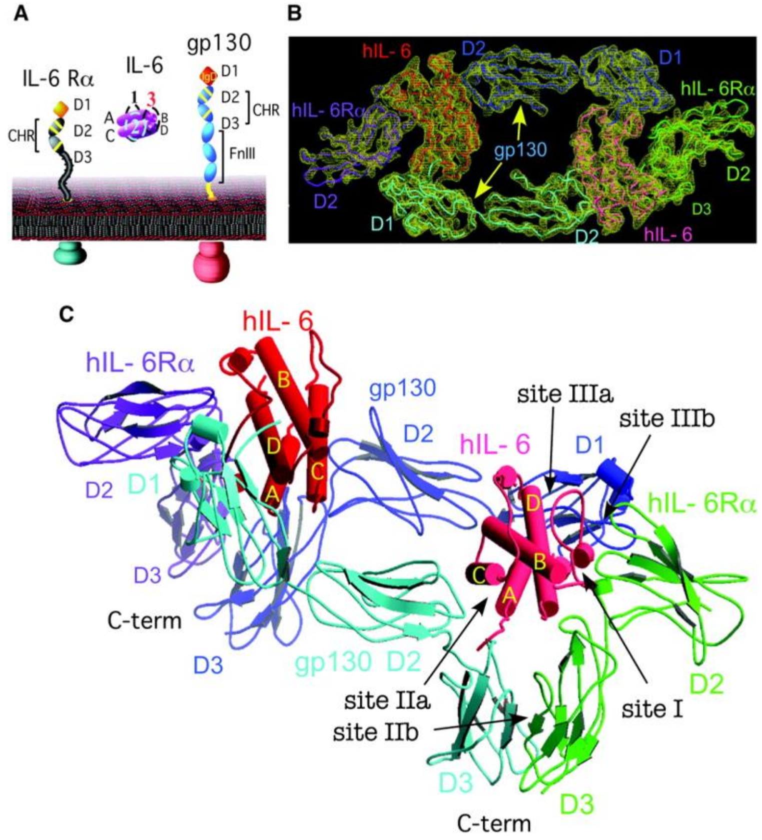
The biology of IL-6

- Human IL-6 is a variably glycosylated glycoprotein with a molecular weight from 22 to 27 kDa, which folds in a four-α helix bundle structure

- Soluble IL-6 binds to IL-6 receptor through its site I → the IL-6/IL-6R complex, through IL-6 site II, binds to the transmembrane glycoprotein gp130 → homodimerization of gp130, through the binding of IL-6 site III to the second gp130 → formation of a **hexamer structure IL-6(2)/IL6-R(2)/gp130(2)**

- Activation of JAK/STAT pathway promotes transcription of IL-6 responsive genes

- Additionally, gp130 homodimerization can activate intracellular signaling via RAS/MAPK, PI3K/AKT, YES/YAP/Notch pathways



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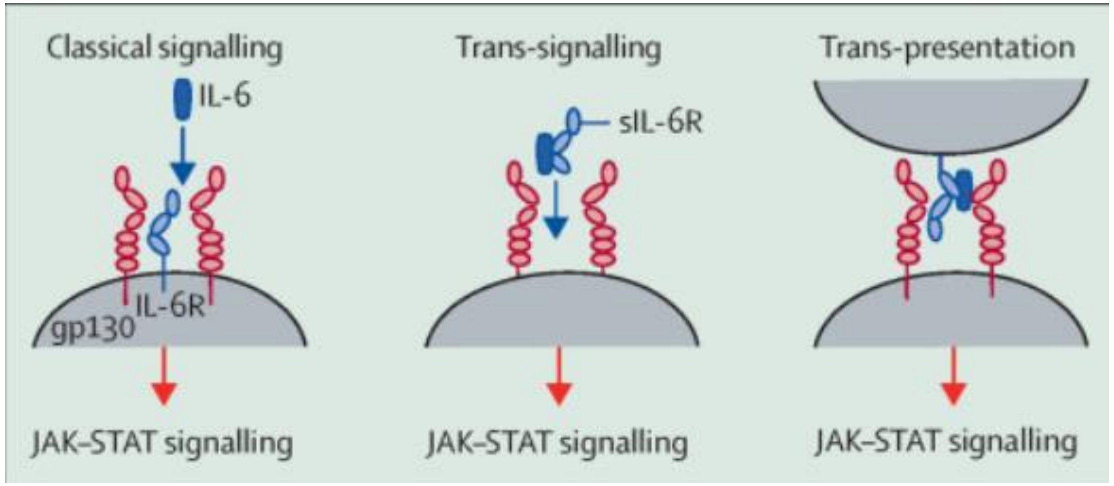
Kang et al. Targeting Interleukin-6 Signaling in Clinic. Immunity 2019
Taniguchi et al. A gp130-Src-YAP module links inflammation to epithelial regeneration. Nature 2015

The biology of IL-6 signaling

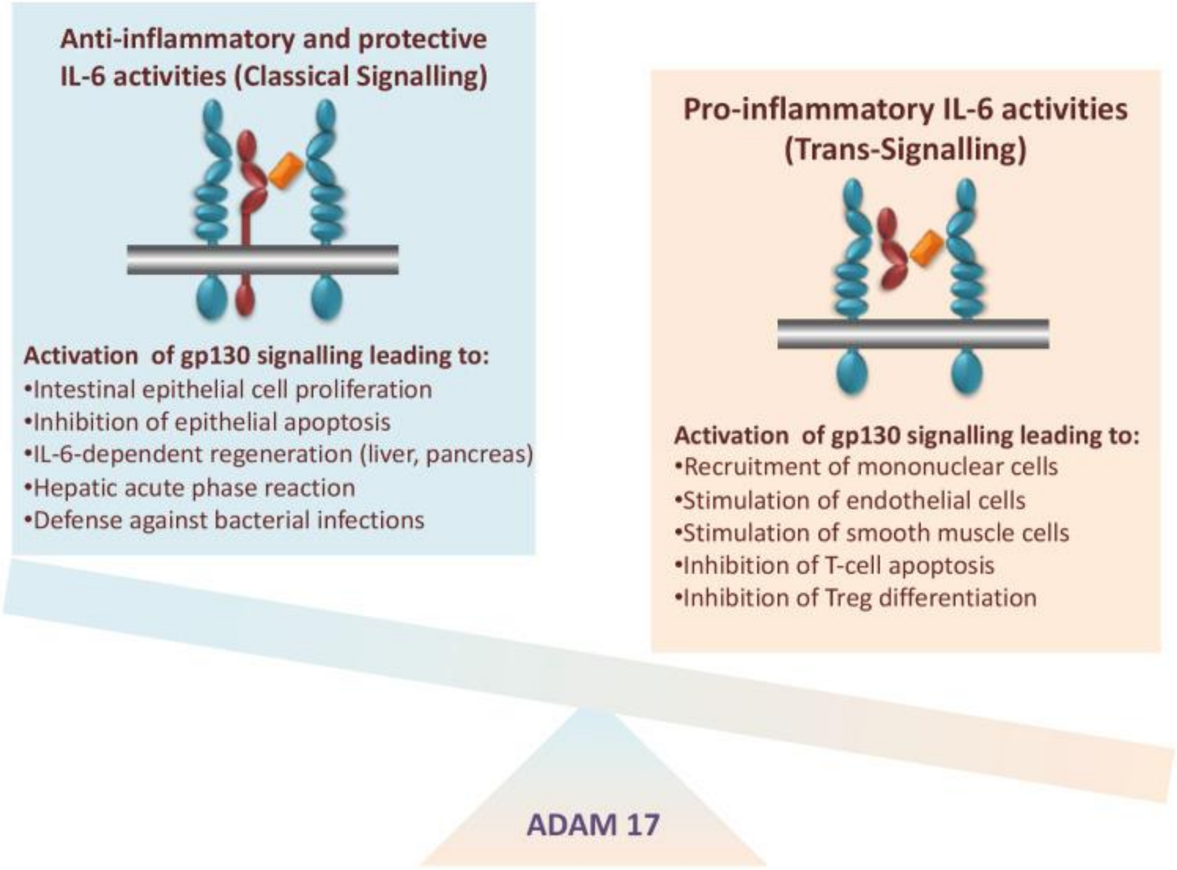
- IL-6 exerts its biological function by binding to membrane-bound IL6R and subsequent binding to gp130 → **Classical signaling**
- Alternatively, IL-6 can bind to sIL-6R and the complex can subsequently bind to membrane-bound gp130 → **Trans-signaling**
- A third mechanism is called **trans-presentation**, when a dendritic cell presents the membrane-bound IL-6/IL-6R complex from cell to cell to cognately interacting T cells, which respond through their own gp130



Rose-John. Local and systemic effects of interleukin-6 (IL-6) in inflammation and cancer. FEBS letters 2021

The biology of IL-6 signaling

- Membrane-bound IL-6R expressed by few cell types, such as hepatocytes, neutrophils, monocytes, macrophages, and some types of lymphocytes
- gp130 is ubiquitously expressed, potentially explaining the pleiotropic functions of IL-6
- ADAM17 generates sIL-6R by cleaving membrane-bound IL-6R, and is activated in response to inflammation and infection, thus acting as an important regulator of this balance
- **In healthy conditions, classical signaling prevails and only cells expressing IL-6R will respond to IL-6**
- **In pro-inflammatory conditions, sIL-6R concentrations increase and the balance shifts towards trans-signaling**



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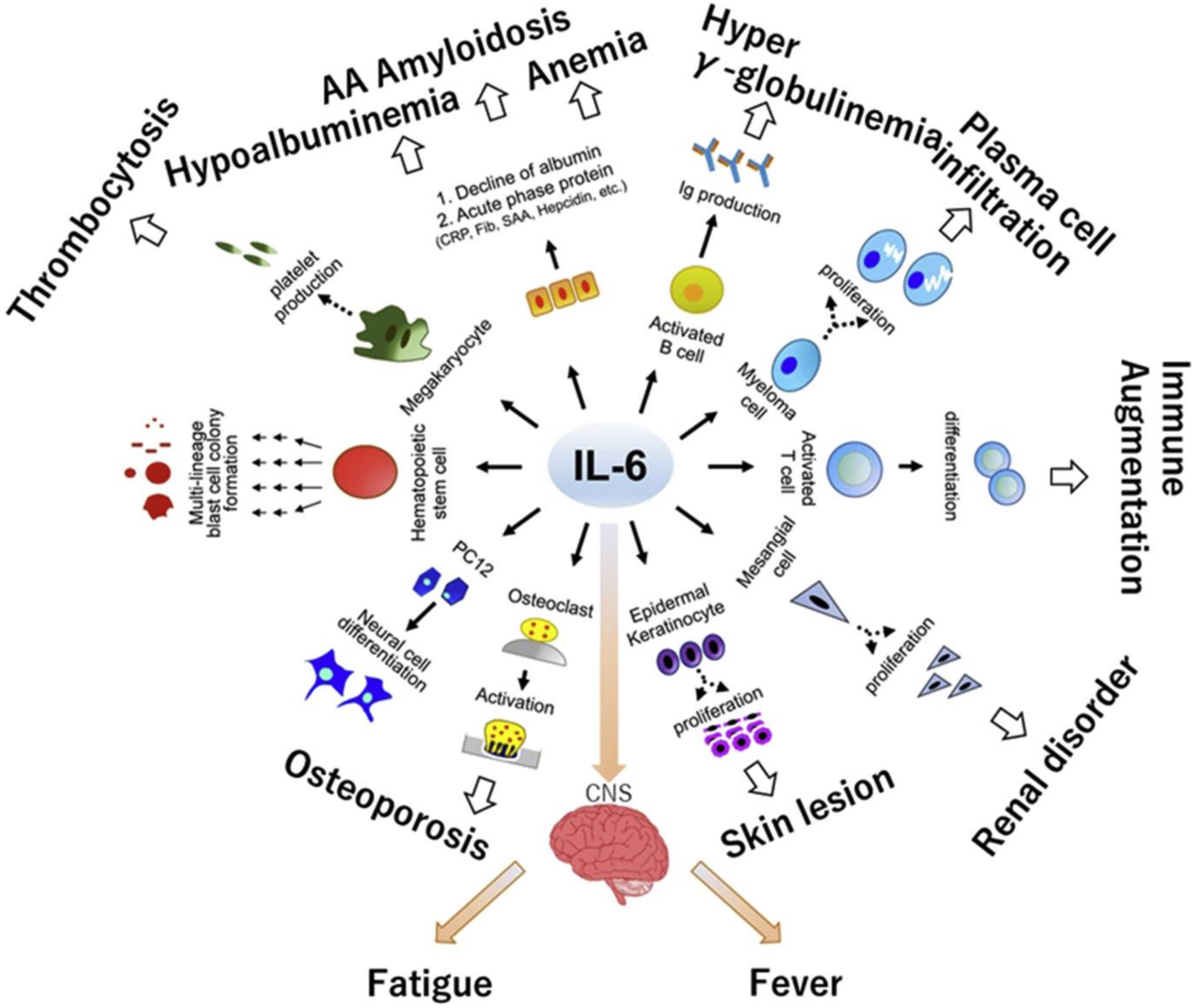
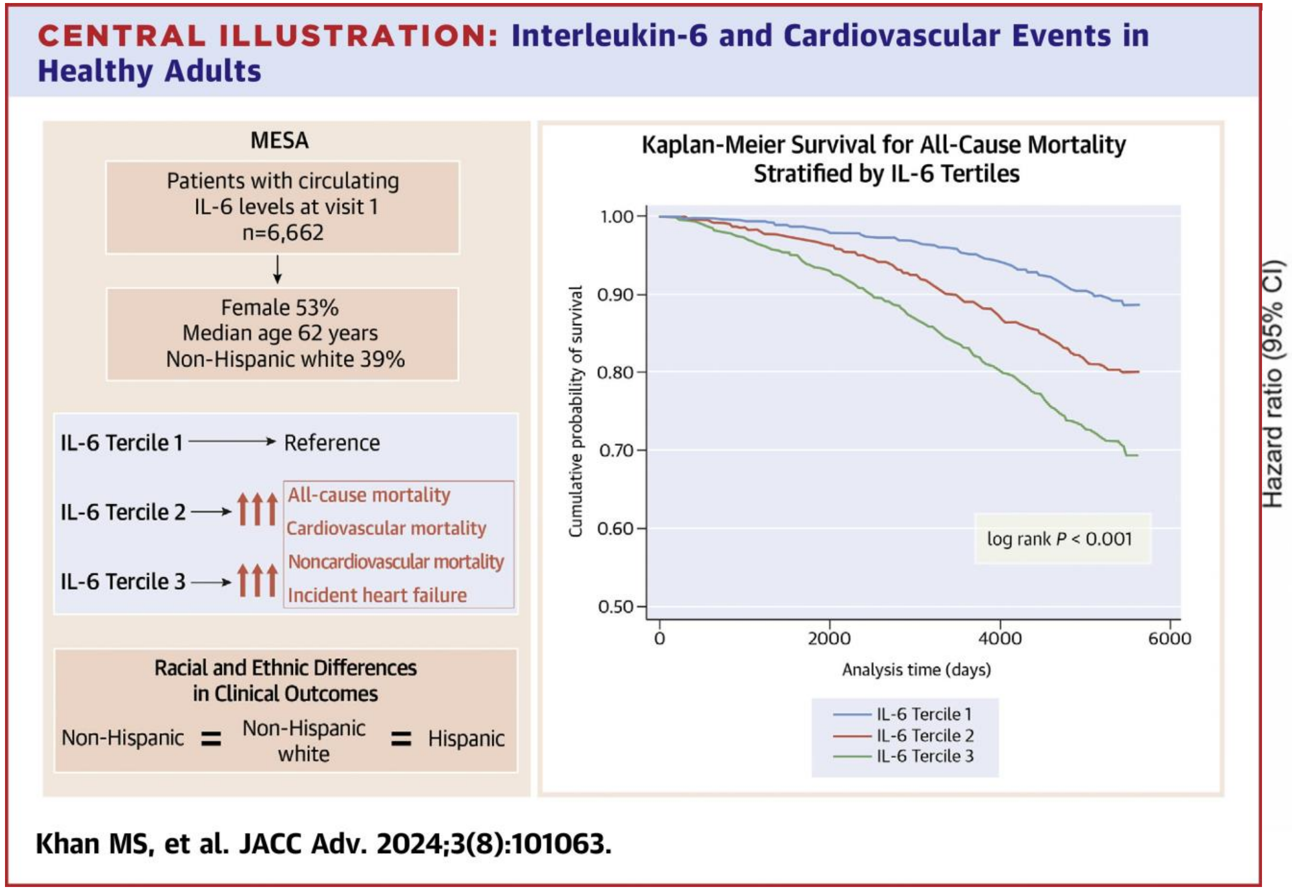
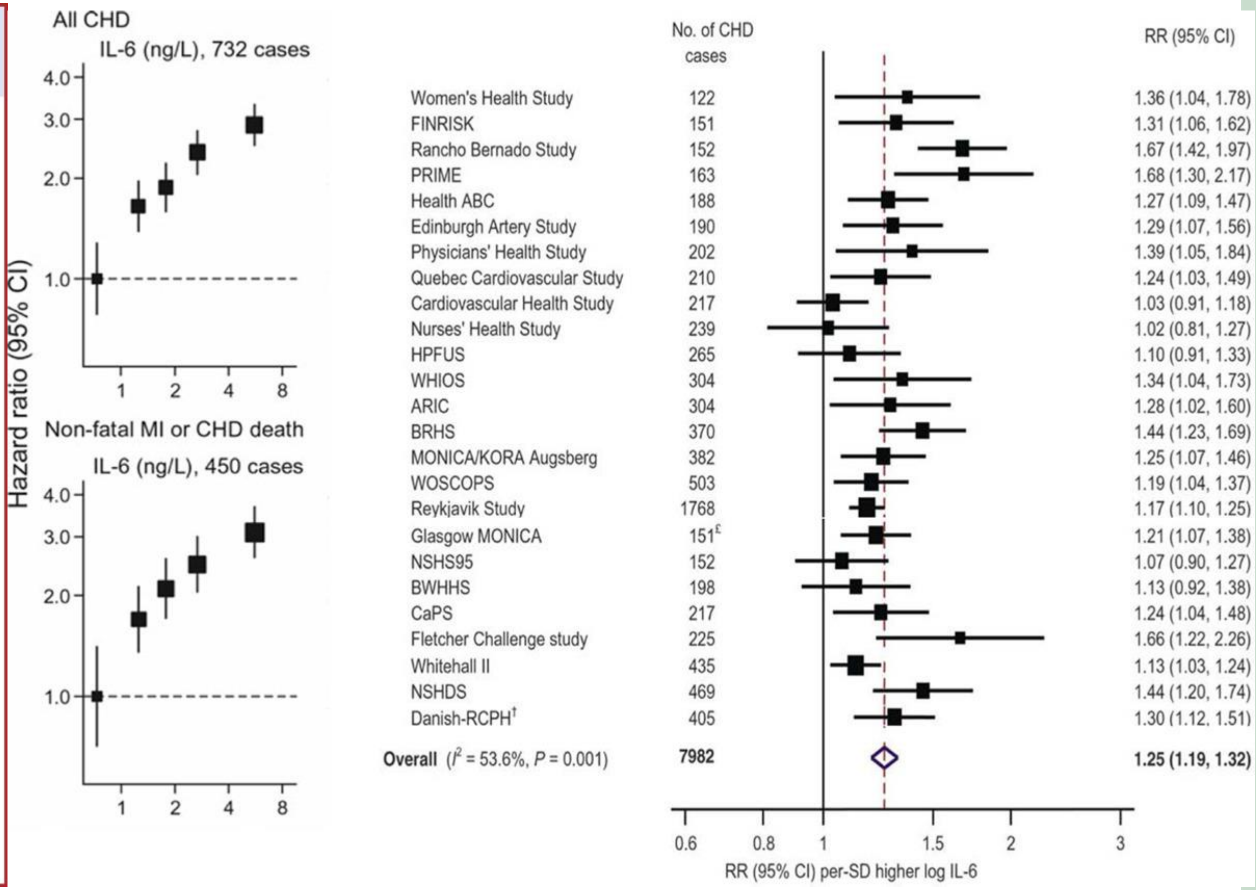
The role of IL-6 in human disease



Yoshizaki K et al. Hematol Oncol Clin North Am. 2018

IL-6 and cardiovascular disease





Khan et al. JACC Adv. 2024
Ridker et al. Circ Res. 2021

IL-6 and coagulation

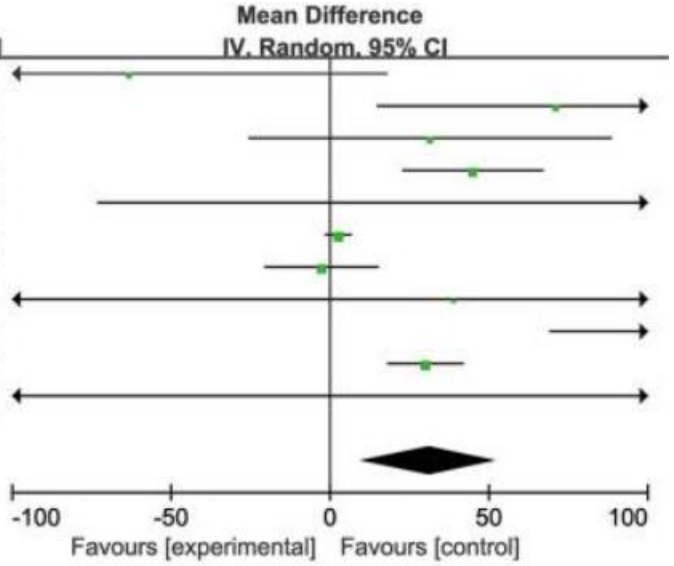
- Increase in fibrinogen levels (acute phase protein)
- Enhanced platelet synthesis, activation and aggregation
- Endothelial activation
- Upregulation of pro-coagulant factors such as tissue factors
- Inhibition of natural anticoagulants

Pro-thrombotic state

Study or Subgroup	vte Mean	vte SD	vte Total	no vte Mean	no vte SD	no vte Total	Weight	Mean Difference IV, Random, 95% CI
Alonso-Fernánde 2020	49.83	56.19	15	113.15	150.82	15	5.0%	-63.32 [-144.77, 18.13]
Cobelli 2021	121.17	132.67	23	50.15	30.26	15	8.0%	71.02 [14.68, 127.36]
Demelo-Rodríguez 2020	89.81	135.67	23	58.36	81.92	133	7.9%	31.45 [-25.72, 88.62]
Erben 2021	65.31	102.4	82	20.42	19.31	833	15.1%	44.89 [22.69, 67.09]
Galien 2021	388.86	394.93	8	185.56	139.86	48	0.6%	203.30 [-73.21, 479.81]
Li 2021	15.59	16.42	81	12.99	14.52	164	17.9%	2.60 [-1.61, 6.81]
Loffi 2021	64.48	82.64	109	67.12	70.14	224	16.0%	-2.64 [-20.67, 15.39]
Meiler 2020	258.37	410.22	14	219.48	300.61	35	0.8%	38.89 [-197.95, 275.73]
Melazzini 2020	174.51	92.2	25	68.98	37.61	234	11.8%	105.53 [69.07, 141.99]
Riyahi 2021	50.02	60.31	102	20.01	26.07	311	17.0%	30.01 [17.95, 42.07]
Salem 2020	1,014.39	1,089.81	14	735.57	865.95	38	0.1%	278.82 [-354.97, 912.61]
Total (95% CI)			**496**			**2050**	**100.0%**	**31.15 [9.82, 52.49]**

Heterogeneity: Tau² = 658.13; Chi² = 69.61, df = 10 (P < 0.00001); I² = 86%

Test for overall effect: Z = 2.86 (P = 0.004)



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IL-6 and cancer

IL-6 is a **poor prognostic marker in cancer**, due to its role in the prevention of apoptosis and elevation of proliferation, angiogenesis, invasiveness, and metastasis in cancer cells

- IL-6 has been found to be overexpressed in different cancer types
- Several studies showed that IL-6 serum concentrations are associated with clinical disease stages in patients suffering from breast cancer, hepatocellular carcinoma, lung and colorectal cancer, and soft tissue sarcomas
- Its overexpression has been reported to confer resistance to traditional cancer treatments, especially in the advanced stages of the disorder

Cancer type	Mechanism	Impact on tumor
Colorectal cancer	–	Invasion and migration of tumor cells
Colorectal cancer	Regulating HIF-1α expression	Promotion of tumor progression
Colorectal cancer	Activating the STAT3 pathway	Promotion of tumor initiation and growth
Colorectal cancer	Activating JAK2/STAT3 pathway	Promotion of tumor progression
Colorectal cancer	Enhancing white adipose tissue lipolysis and browning	Improvement of cancer cachexia
Colorectal cancer	Reducing expression of HLA-DR and CD86 on DCs and reducing in the T-cell-stimulating capacity of DCs	Having effect on tumor-infiltrating immune cells in the tumor immune microenvironment
Colorectal cancer	Activating Src-FAK-ERK/p38MAPK signaling	EMT of cancer cells
Colorectal cancer	Activating STAT3/miR-34a pathway	Enhancement the EMT and metastasis
Breast cancer	–	Promotion of cancer metastasis and the suppression of the anti-tumor immune response
Breast cancer	Elevating VEGF	Improvement of angiogenesis and metastasis
Breast cancer	Suppressing monoamine oxidases	Improvement of angiogenesis and metastasis
Breast cancer	–	Tumor relapse and growth and a poor response to neoadjuvant chemotherapy
Breast cancer	–	Facilitating communication between cancer cells and CAFs within TME
Breast cancer	Activating STAT3/Akt-PD-L1 pathway	Promotion of resistance to both chemotherapy and T cell therapy
Triple-negative breast cancer	Promoting M2 macrophage polarization	Invasion of tumor
Lung cancer	Involving in the transformation of MSCs into αSMA-positive cells	Promotion of tumor growth
Lung cancer	–	Induction of EMT and improvement of the metastasis
Lung cancer	–	Facilitating the invasiveness and metastasis
Gastric cancer	Enhancing white adipose tissue lipolysis and browning	Improvement of cancer cachexia
Gastric cancer	Activating JAK2/STAT3 pathway	Enhancement the EMT and metastasis
Ovarian cancer	Elevating VEGF and producing matrix metalloproteinase 9	Invasion and metastasis of tumor
Ovarian cancer	STAT3/HIF-1α loop	Enhancement the EMT and invasion
Ovarian cancer	Activating STAT3/NF-kB	Promotion of tumor angiogenesis
Ovarian cancer	Elevating PD-L1 level in neutrophils	Impairment of T cell function and promotion of immune evasion
Head and neck squamous cell carcinoma	Activating STAT3/xCT	Development of tumor
Thyroid cancer	–	Invasion of tumor
Oral cancer	–	Enhancement the EMT and invasion
Renal carcinoma	–	Proliferation, migration, and invasion of the cancer
Pancreatic cancer	miR-455-5p/IGF-1R axis	Proliferation and metastasis of the cancer cells

Mohamed et al. Med Oncol. 2024

IL-6R inhibition and cancer

- Human in vitro and animal studies documented treatment with TCZ to reduce tumor growth, angiogenesis, and cancer-associated cachexia
- In a phase 1 clinical study on 23 patients with advanced epithelial ovarian cancer, treatment with TCZ on top of chemotherapy demonstrated a satisfactory safety profile and encouraging immunological advantages

IL-6R	Tocilizumab	Humanized mAb	In vitro	Colon cancer	Successfully blocked IL-6R and inhibited angiogenesis and tumor growth
			Clinical trial	Resistant metastatic lung cancer	Improved prognosis and ameliorated the cachexia that so devastates their quality of life
			Murine model	Cancer cachexia	
			In vitro	Breast cancer	Considerably reduced the cancer stem cells and tumor growth in women with Notch3-expressing breast cancers
			Clinical trial	Pancreatic cancer	Inhibited CAFs
			A case report	Cancer cachexia	Had favorable effects in regulating cancer cachexia
	Tocilizumab in combination with chemotherapeutics		A phase I clinical study	Advanced epithelial ovarian cancer	Showed an acceptable safety profile and a promising immunological advantage in these patients
	Sant7	A modified IL-6	In vitro	Prostate cancer	Prevented development of the cancer cells more efficiently than other anti-IL-6 antibodies
	Iplimumab, nivolumab, and tocilizumab	–	Clinical trial	Refractory pancreatic cancer	Did not meet the prespecified criteria for expansion for full accrua

Mohamed et al. Med Oncol. 2024
Dijkgraaf et al. *Ann Oncol*. 2015

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Direct IL-6 inhibition and cancer

- Treatment with siltuximab has been associated with antitumoral effects in animal and cellular studies of lung, ovarian, prostate cancer, as well as in multiple myeloma (MM)

- In a phase 1 study on patients with metastatic and progressive castration-resistant prostate combination of docetaxel and siltuximab was associated with decline of ≥ 50% in PSA

- However, 2 phase II trials, on prostate cancer and MM, was not associated with any improvements in outcomes

IL-6	Siltuximab	Chimeric mAb	Mouse xenograft model	lung cancer	Reduced progression
			In vitro	Ovarian cancer	Effectively blocked the IL-6 signaling pathways
			In vitro	Late stage prostate cancer	Rendered the cancer cell lines more sensitive to apoptosis
			Phase I/II study	Metastatic renal cell cancer	The overall outcomes proposed more evaluation t higher doses or plus other drugs
			A case report	Relapsed refractory multiple myeloma	A complete remission achieved
	Siltuximab in combination with bortezomib	Chimeric mAb	A phase 2, randomized, double-blind, placebo-controlled	Relapsed refractory multiple myeloma	Did not contribute to enhanced effects
	Siltuximab in combination with melphalan	Chimeric mAb	In vitro	Multiple myeloma	Enhanced activation of drug-specific apoptosis in multiple myeloma cell lines and improved melphalan cytotoxicity
	Siltuximab in combination with mitoxantrone/prednisone	Chimeric mAb	Randomized open-label phase II clinical trial	Metastatic castration-resistant prostate cancer	Did not make any improvements in outcomes
	mAb 1339	Fully human mAb	In vitro	Multiple myeloma	Considerably inhibited the cncer cell growth
	mAb 1339	Fully human mAb	SCID-hu murine model	Melanoma	Improved the function of dexamethasone
	Clazakizumab	Fully human mAb	A phase I trial	Colorectal, cholangiocarginoma, mesothelioma and NSCLC	No adverse effect was found related to the treatment
	ALD518	Humanized mAb	Phase I and II trials	NSCLC	Appeared well tolerated and ameliorates NSCLC-related anemia and cachexia

Mohamed et al. Med Oncol. 2024
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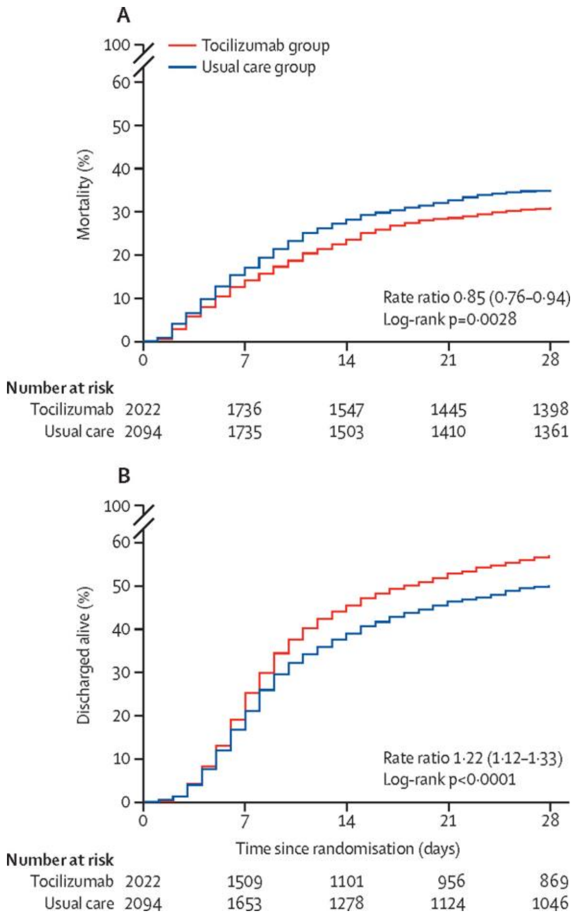
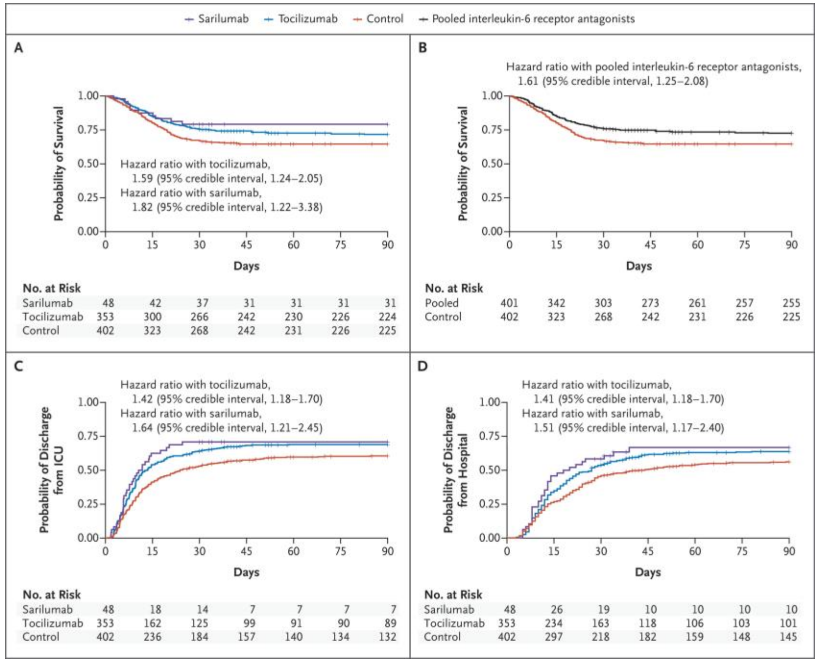
IL-6 and the paradigm of COVID-19

- Severe COVID-19 is characterized by an hyperinflammatory state, associated with severe respiratory failure, pro-thrombotic state and multiorgan dysfunction
- Elevated levels of IL-6 were observed in patients with COVID-19, and correlated with the clinical severity
- Selective IL-6 inhibition has provided robust evidence of clinical improvement when added to corticosteroid treatment (efficacy of IL-6 inhibition alone is yet to be determined)
- No difference between TCZ and sarilumab





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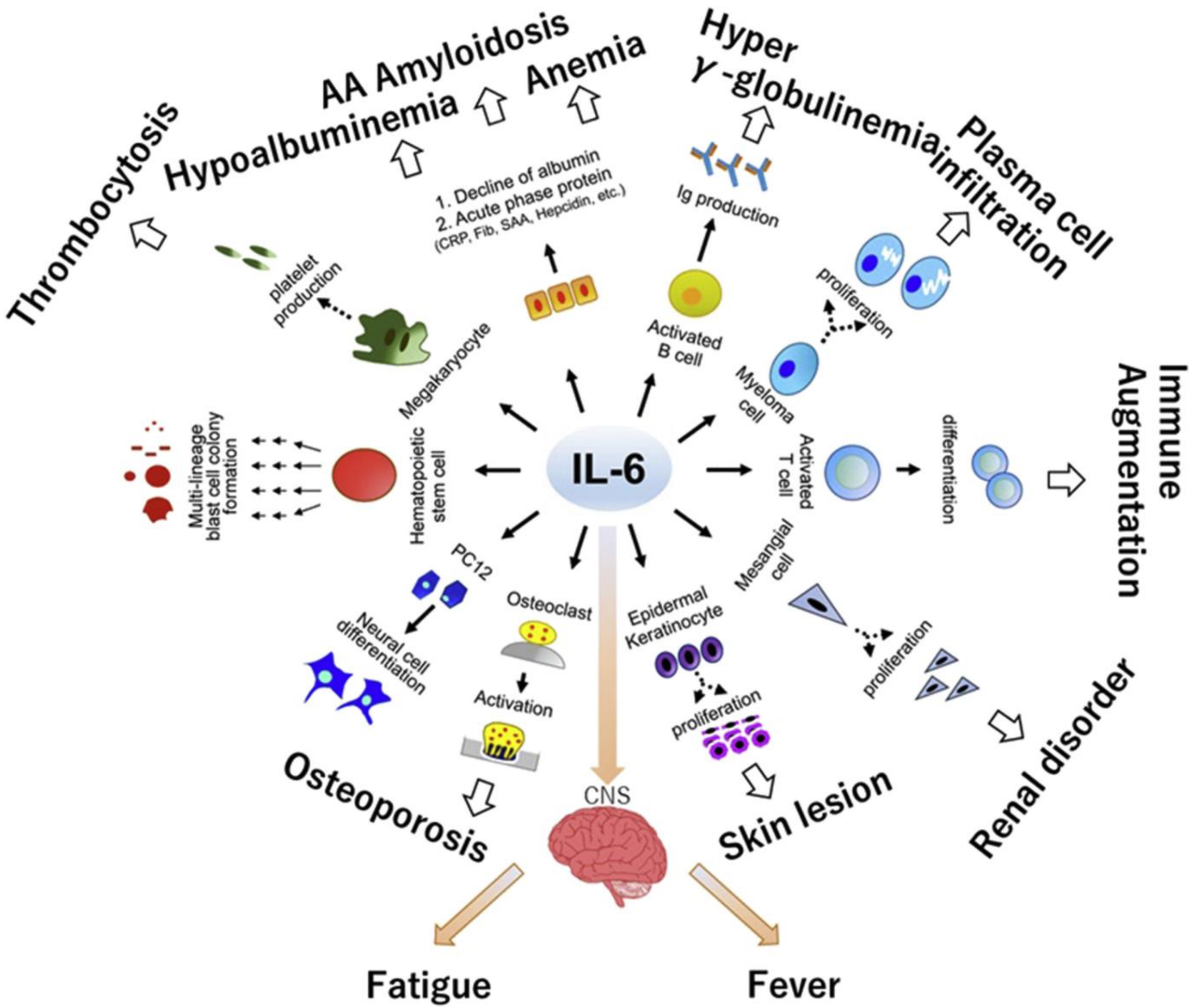

Yoshizaki K et al. Hematol Oncol Clin North Am. 2018

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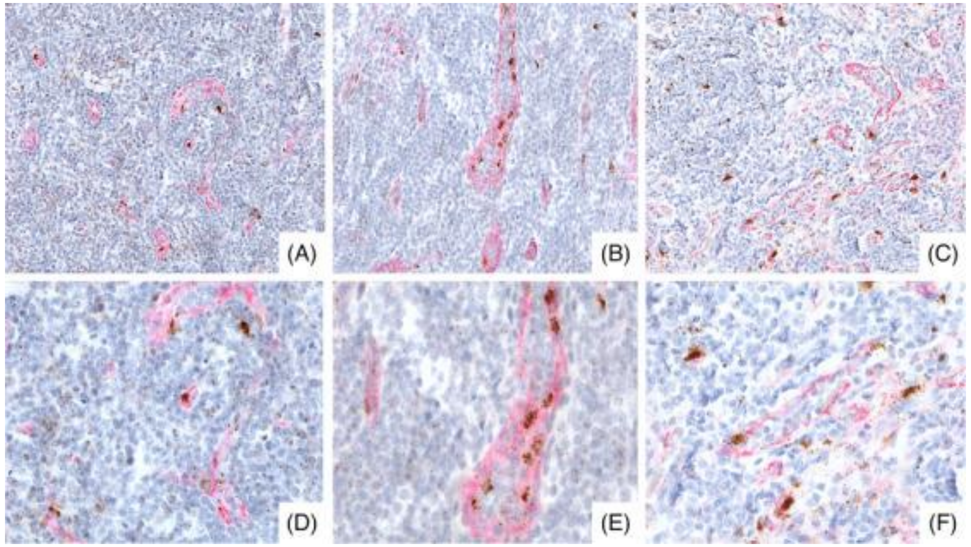
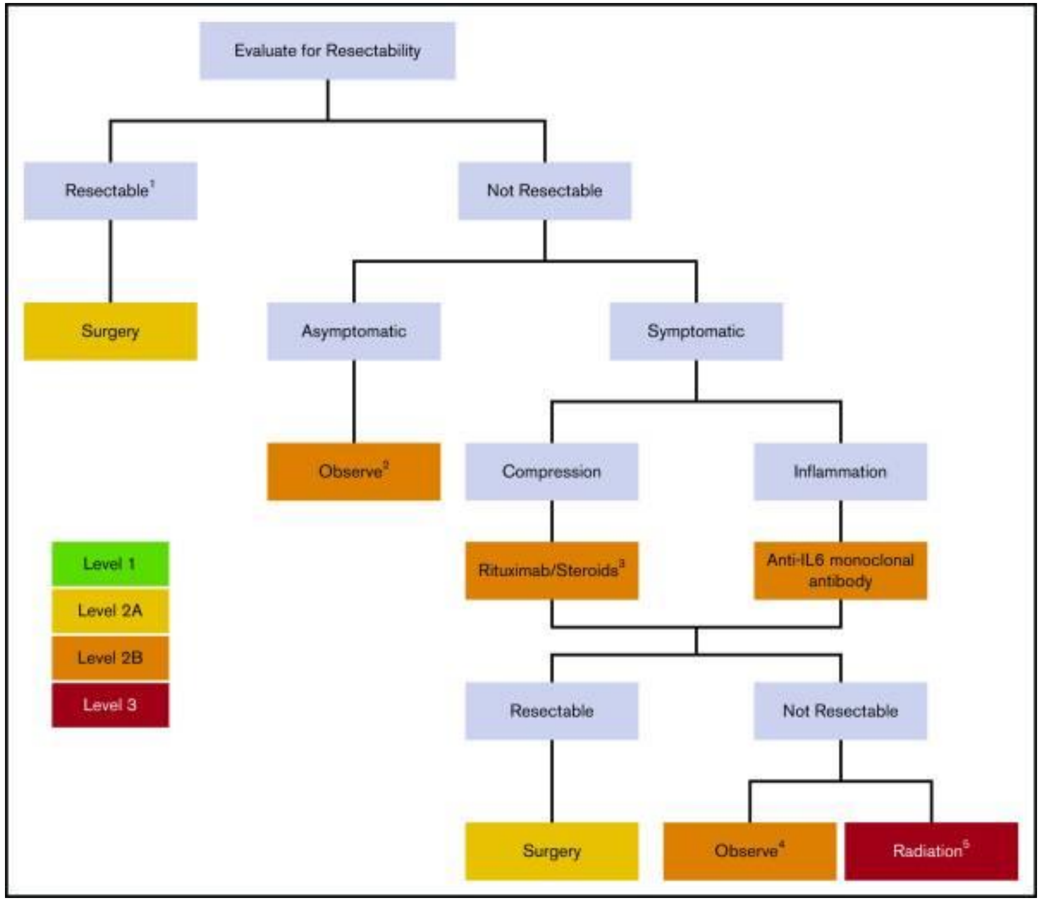
The role of IL-6 in UCD

- In the small portion of UCD cases with systemic symptoms, IL-6 is likely to be the effector cytokine driving systemic symptoms
- A recent studies showed nodal IL-6 expression in various CD subtypes, including UCD
- Anti-IL-6 mAb are included in UCD treatment guidelines
- However, IL-6 levels have not been systematically studied in UCD and most cases do not have systemic symptoms





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Fajgenbaum DC et al. Hematol Oncol Clin North Am. 2018
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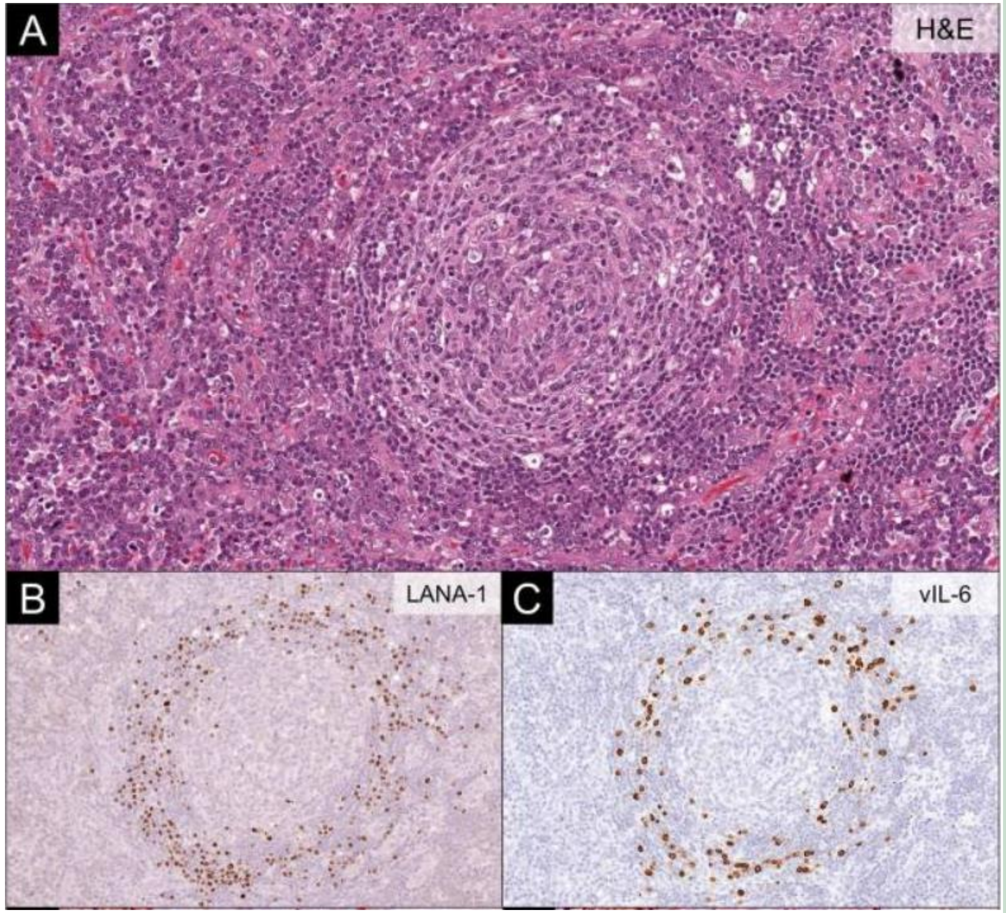
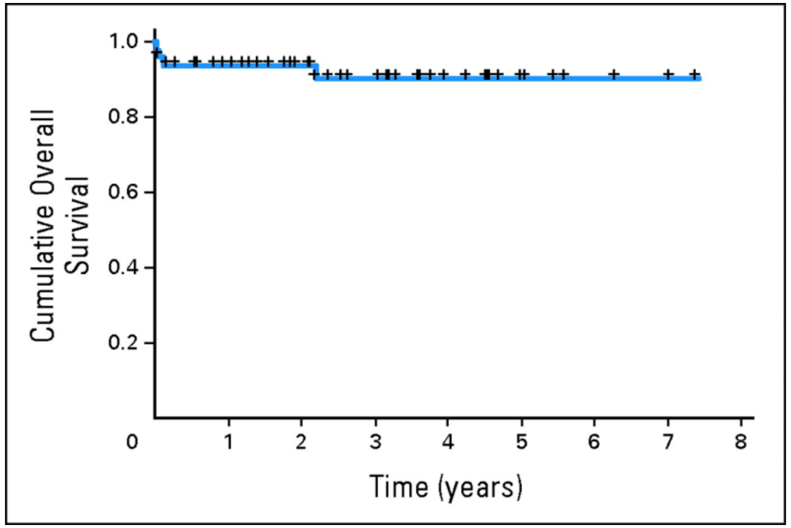
The role of IL-6 in HHV8-MCD

- In immunocompromised individuals, HHV8 can replicate in lymph node plasmablasts and transcribe the viral homolog of IL-6 (vIL-6)
- vIL-6 drives symptoms, signs, and lymph node pathology along with a cascade of other cytokines including human IL-6
- vIL-6 can bind directly to gp130, without the need of binding to IL-6R, as human IL-6 does, potentially interacting with a wider range of cells
- Even though HHV8-infected plasmablasts frequently do not express high levels of CD20, rituximab represents the mainstay of treatment (5-year OS 90%), with added etoposide for high-risk patients





Fajgenbaum DC et al. Blood. 2020
Bower et al. J Clin Oncol. 2011

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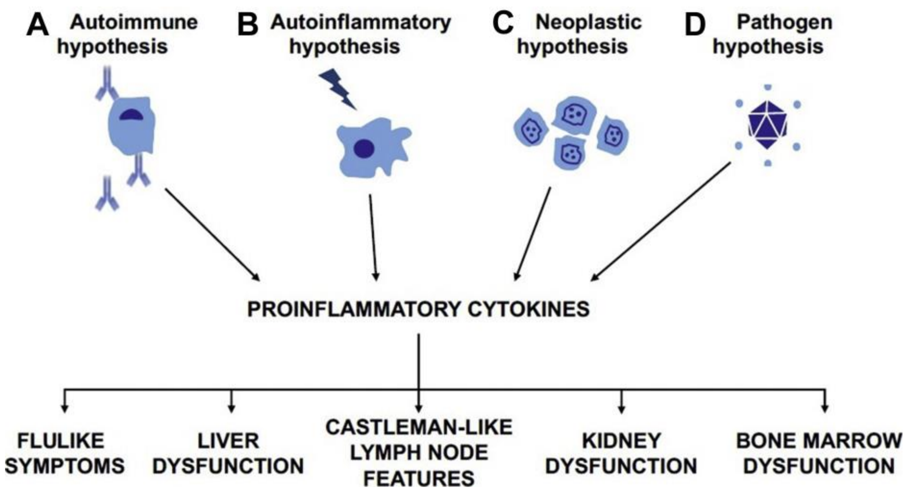
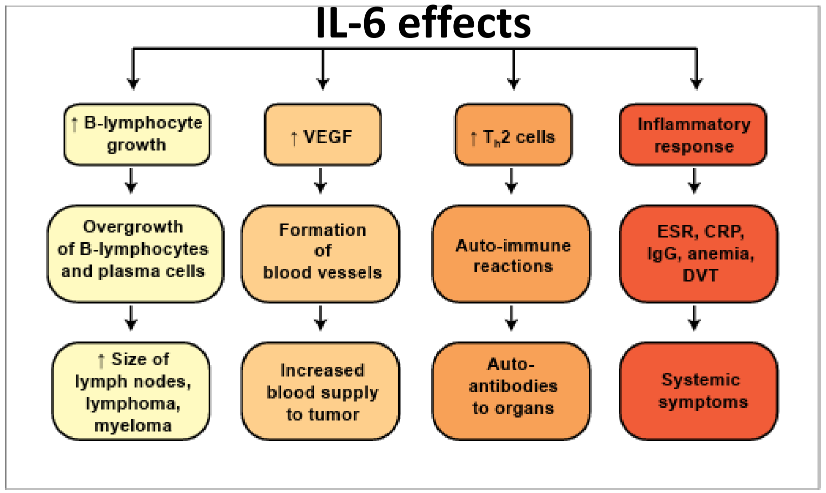
The role of IL-6 in iMCD

- The etiology and pathogenesis of iMCD is less well understood than HHV8-MCD or POEMS-MCD
- IL-6 is a critical disease driver in some patients as demonstrated by abrogation of iMCD signs and symptoms with IL-6 and IL-6 receptor antibodies and recapitulation of the iMCD phenotype with overexpression of IL-6 in mice
- Moreover, the administration of recombinant IL-6 to humans (given in studies phase I-II study on breast cancer and NSCLC) can lead to an iMCD-like syndrome
- Clinical symptoms often wax and wane with IL-6 levels, which can be highly elevated in patients with iMCD during disease flare





Fajgenbaum DC et al. Hematol Oncol Clin North Am. 2018
van Rhee F. et al. Clin Adv Hematol Oncol. 2010

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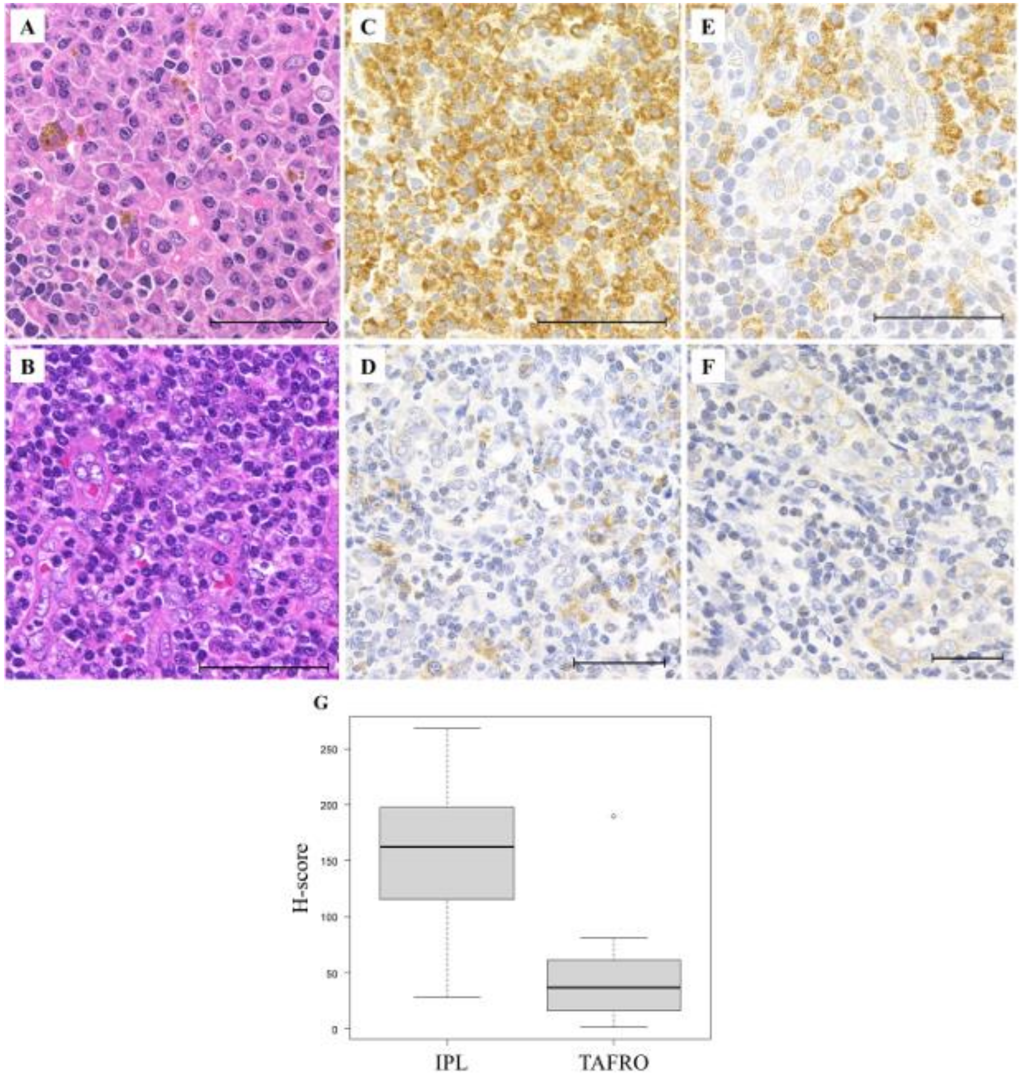
The role of IL-6 in iMCD

- iMCD patients may not have a sustained or significant response to IL-6 inhibition, and one of the reasons may be site of IL-6 production
- Plasma cells are the predominant IL-6-expressing cells in iMCD-IPL, whereas vascular endothelial cells expressed IL-6 in iMCD-TAFRO
- Autocrine IL-6 production in the ER of plasma cells may contribute to iMCD-IPL pathogenesis, potentially explaining its favorable responses to IL-6 blockade therapy
- IL-6 production in iMCD-TAFRO may be predominantly from vascular endothelial cells, suggesting that elevated serum IL-6 is a secondary phenomenon of the cytokine storm



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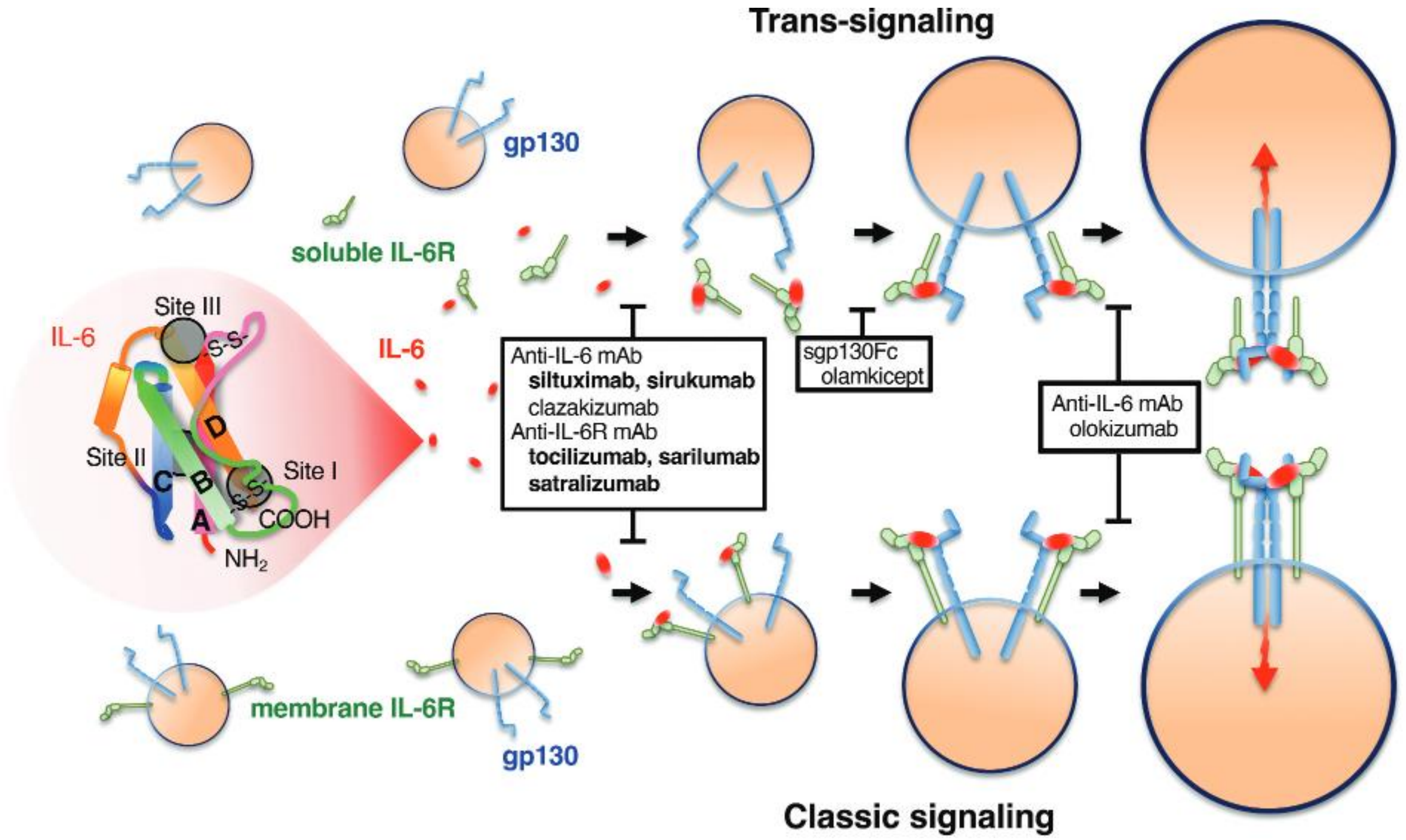
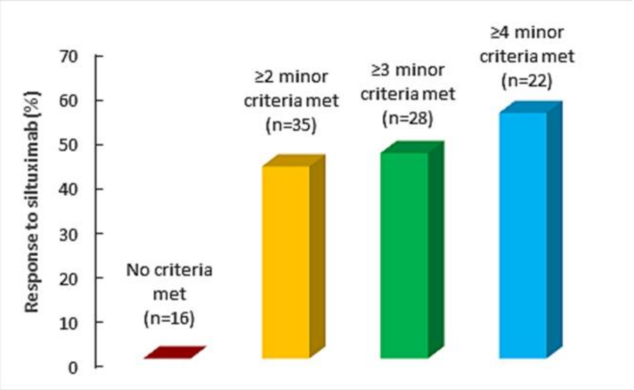
How to target IL-6?



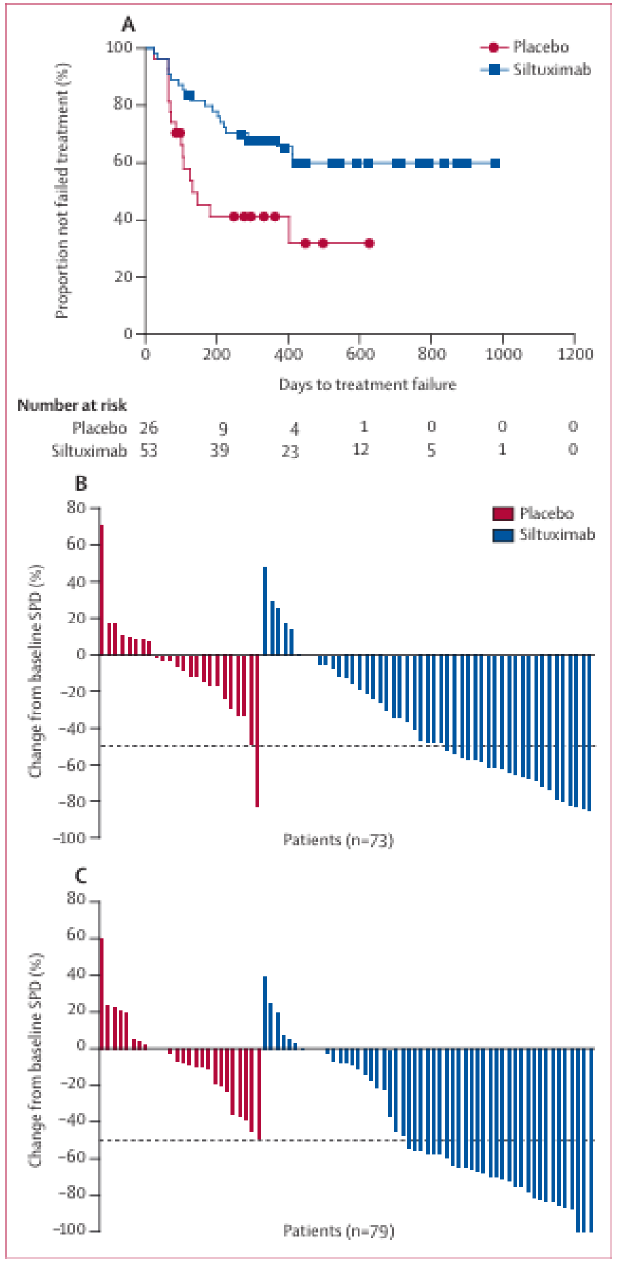
Narazaki et al. Current status and prospects of IL-6–targeting therapy. Expert Review of Clinical Pharmacology 2022

The role of IL-6 in iMCD

- Siltuximab is an anti-IL-6 chimeric antibody. In the registration study of 79 patients, 34% of patients in the siltuximab arm had durable symptomatic and tumor responses; the placebo arm had none
- Rate of response is influenced by presence of an inflammatory syndrome, as shown by the higher likelihood of response in patients that met at least 2 minor criteria for iMCD
- Real world evidence suggest disease control rates in line with data reported in clinical trial



Endpoint	Yu et al. 2017[50] (N=21)	Tonialini et al. 2018[51] (N=9)	Ostrowska et al. 2021[52] (N=11)	Min et al. 2021[42] (N=15)
Treatment duration, median (range)	NA	285 days (104 to 1113)	16 months (3 to 65)	9 months (1 to 95)
CR, n (%)	9 (42.86)	3 (33.33)[a]	2 (18.00)	3 (20.00)
PR, n (%)	7 (33.33)	0 (0)	6 (54.50)	7 (46.70)
ORR, n (%)	16 (76.19)	3 (33.33)	8 (72.50)	10 (66.67)

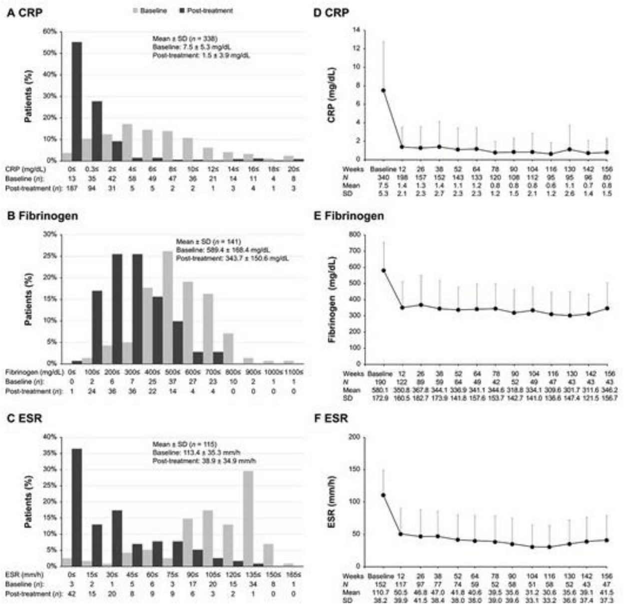


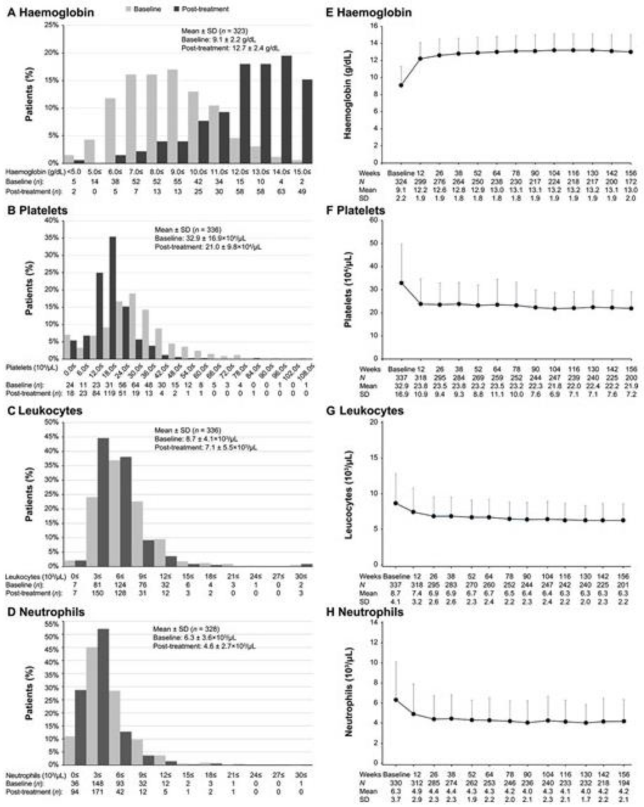
van Rhee et al. Lancet Oncol. 2014
Lang et al. Blood Rev. 2024

The role of IL-6 in iMCD

- Tocilizumab, an anti-IL6R humanized mAb is approved for the treatment for iMCD in Japan
- A recent observational study documented a response rate (partial or complete) of 58.9%
- Improvements in inflammatory markers and haematological parameters were registered as well

	Patients with evaluable response (*n*=90)
	Patients with response after treatment, *n* (%)
Complete response	14 (15.6)
Partial response	39 (43.3)
No change	32 (35.6)
Progressive disease	5 (5.6)

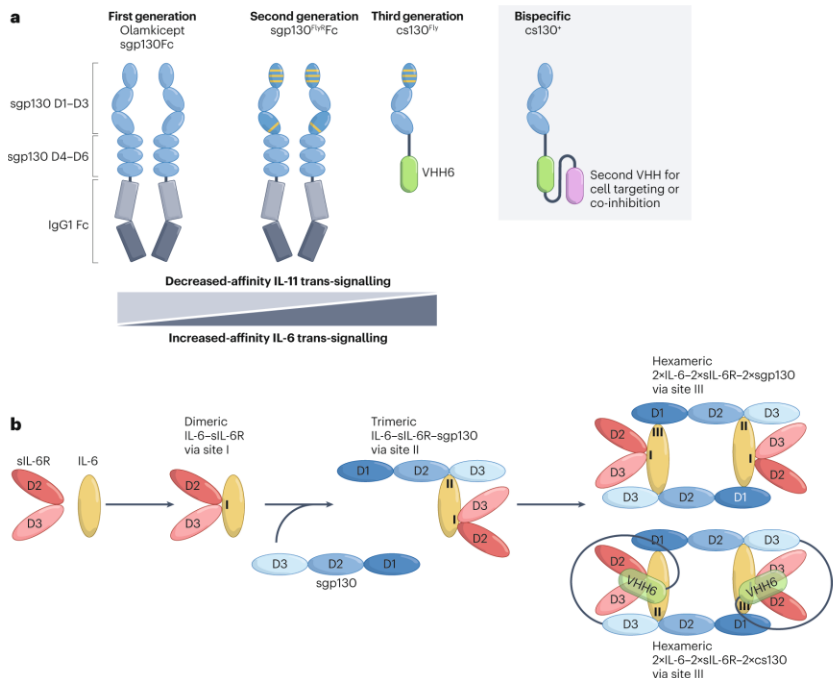


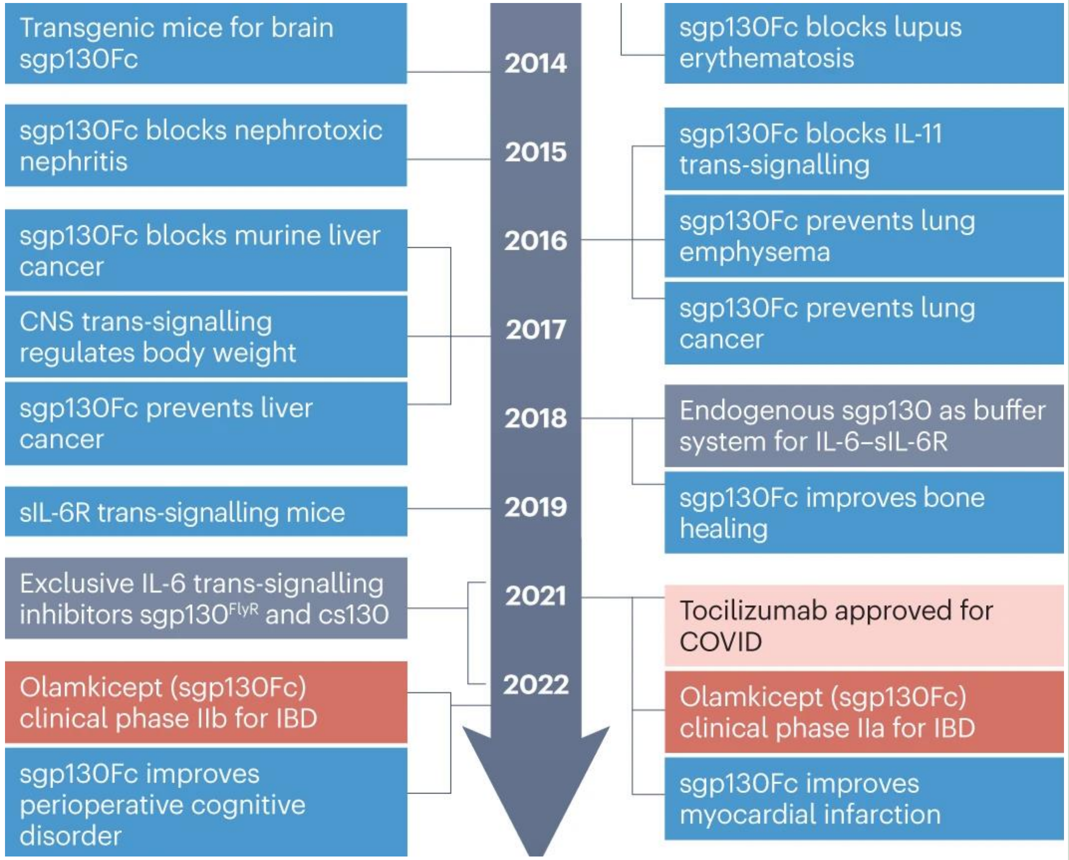


Nishimoto et al. Modern Rheumatology 2026

IL-6 signaling inhibitors

- There is growing interest on gp130 inhibition, which can selectively inhibit IL-6 trans-signaling
- However, the effect is not limited to IL-6 as gp130 is shared within IL-6 family of cytokines (issue addressed with new generations of gp130 inhibitors)





Rose-John et al. Nat Rev Immunol 2023

Final thoughts

- IL-6 is a central regulator of immunity and inflammation, with distinct effects mediated by classical and trans-signaling;
- Castleman disease is frequently driven by IL-6, especially in iMCD and HHV8-MCD;
- Cellular source and signaling context influence disease phenotype and treatment response;
- IL-6 contributes to chronic inflammation, cytokine storm, thrombosis, cardiovascular disease, and cancer;
- IL-6 blockade has significantly improved outcomes in multiple inflammatory diseases;
- Future strategies should focus on precision inhibition of pathogenic IL-6 signaling pathways.

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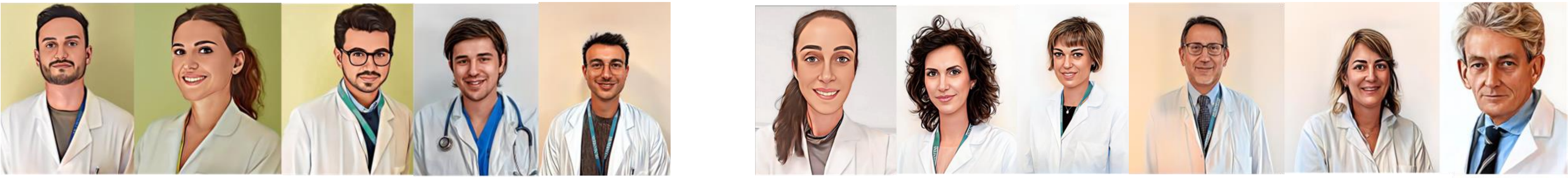

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