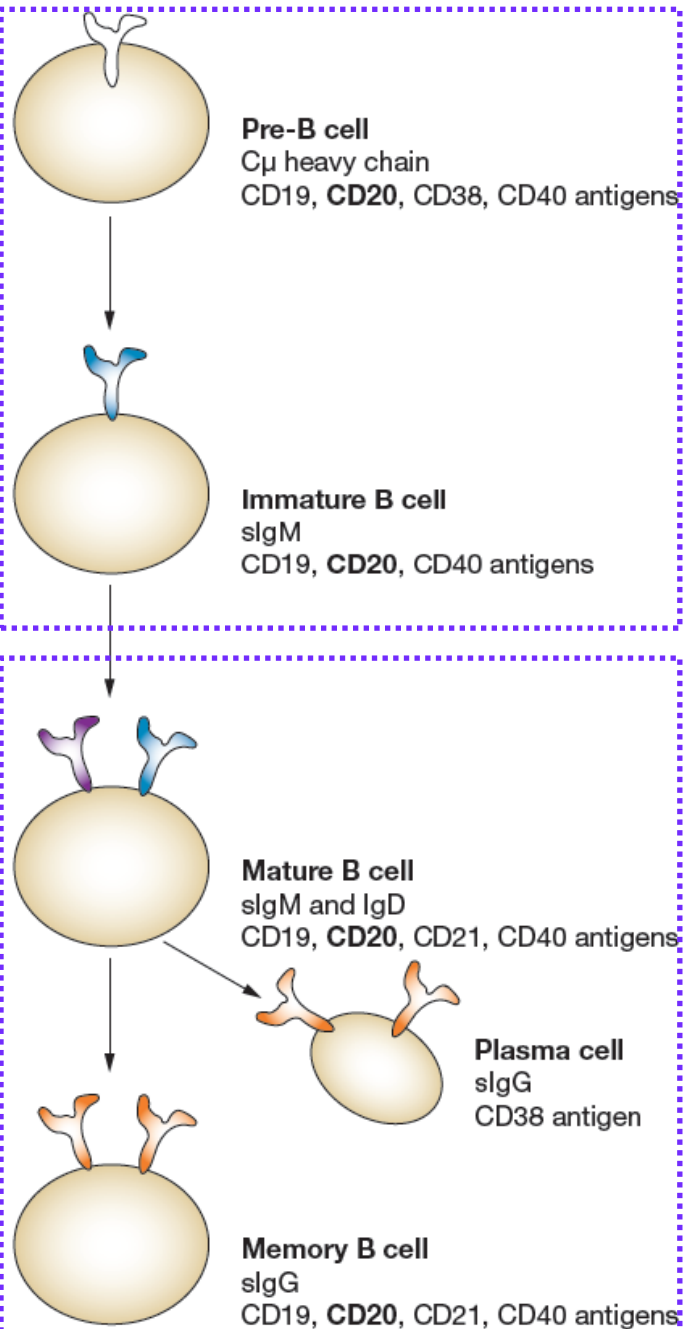


ASNIT & La Nuova Speranza Meeting 2017

Gian Marco Ghiggeri

Istituto Giannina Gaslini, Ge



CD20: 297 AA (33-37 kD)

membrane-associated, no shedding, no secretion

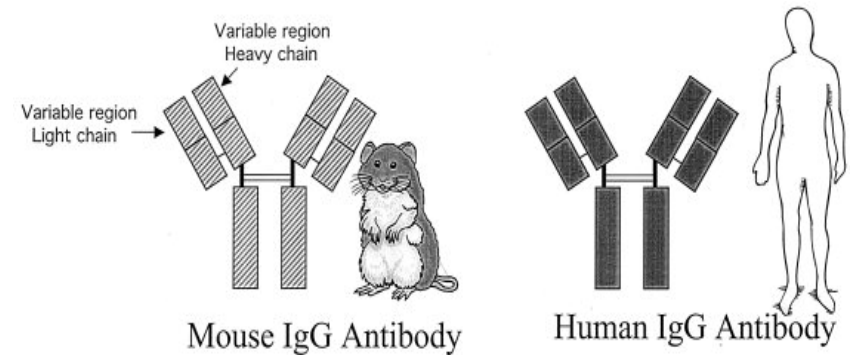
unclear function (calcium channel)
(proliferation and differentiation)

immature / mature *B cells* (>85%)

B cell lymphoma (>80%)

not on stem cells (CD34)

not on plasma cells

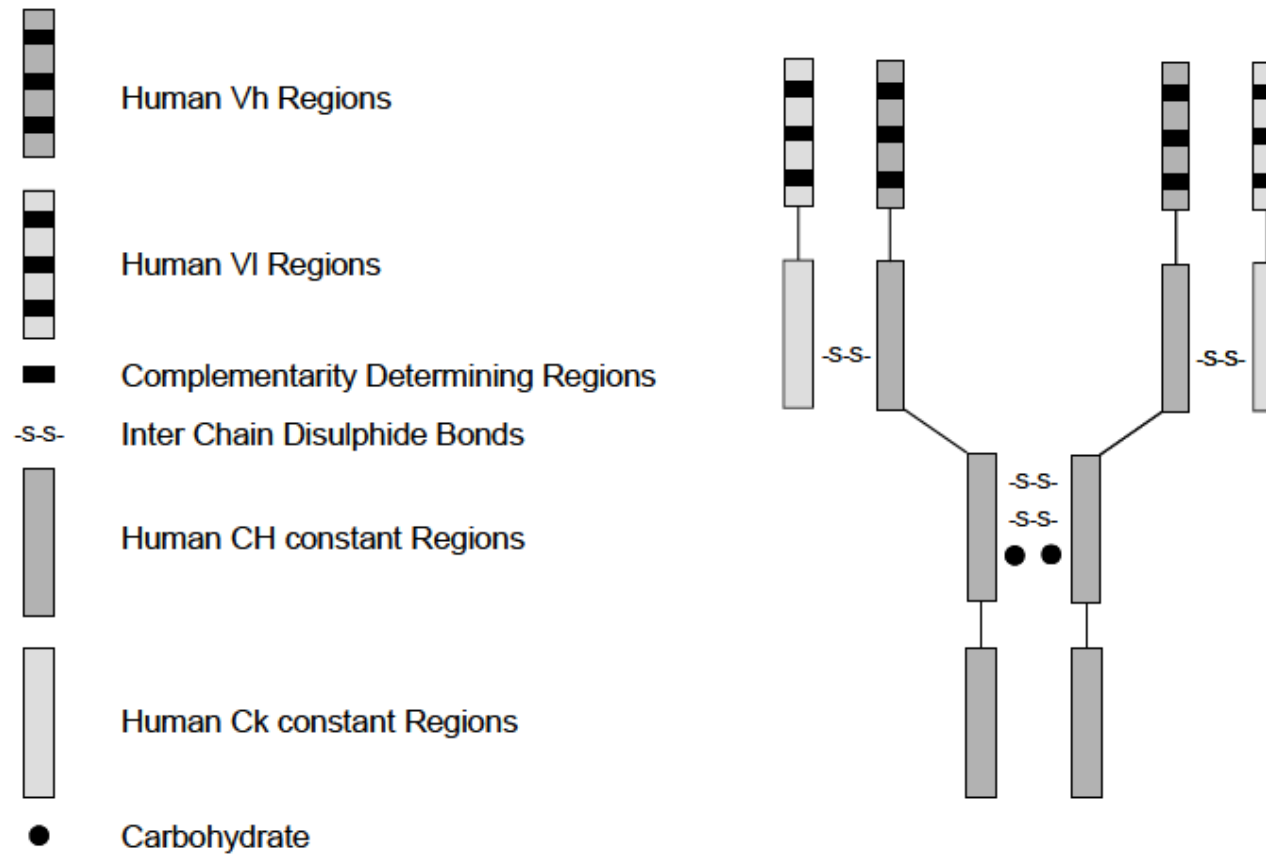


Rituximab

- *Variable regions* from mouse IgG
- *Constant regions* from human IgG1 and light kappa chain



Schematic Representation of Ofatumumab Indicating the Disulphide Bridges



(A)

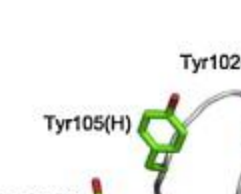


Figure 1A shows a ribbon diagram of the protein structure. The residues highlighted in green are Tyr102(H), Tyr105(H), Tyr32(L), Arg91(L), Trp94(L), Tyr60(H), and Trp53(H). The residues highlighted in blue are Ile58(H) and Arg91(L). The residues highlighted in red are Tyr102(H) and Tyr32(L).

(B)

(C)

Ofatumumab epitope

E150

Rituximab epitope

51

213

Expected effects

- ↓ Autoantibody production (IC)
- ↓ Direct cell–cell interaction (T cells, APC)
- ↓ Indirect (CK) and direct cell–cell interaction
- ↓ Direct organ infiltration

Historical use of Anti-CD20

- Introduced in the late '90s to treat NH lymphoma
- Used to treat autoimmune disease with predominant humoral component / pathogenetic auto-ab
- *Found to reduce proteinuria in children with nephrotic syndrome secondary to PTLN (Nozu, Pediatric Nephrol 2005; Pescovitz, NEJM 2006)*

Key points

- RTX may be useful in the treatment of some forms of INS in children (combined steroid/CNI dependent forms)
- RTX high superior to PDN on pure dependent forms
- SRNS not sensitive

Ofatumumab for Rituximab-Resistant Nephrotic syndrome

Basu, NEJM 2014

- 5 children with NS (2.7-13.3 yrs)
- resistance rituximab, tacrolimus/ciclosporin, cyclophosphamide
- 300 mg 1.73m^2 followed by five infusion 2.000 mg 1.73 m^2
- Proteinuria 9-21 (prot/creat) at T0
- Proteinuria 0.09-012 (prot/creat) at T6W and T6M

Bonanni and col. *BMJ Case reports* 2015 Sep 16;2015

2 doses 300-700 mg 1.73m² T1-+15

Patient No	Age (yrs)	Previous Steroid-Sparing Therapy	Proteinuria (mg/24 hours)			Serum albumin (g/dl)		eGFR* (ml/min/1,73m ²)		CD 19 (%)	
			T0	4Wk	12Wk	T0	4Wk	T0	4 Wk	T0	4Wk
1	10	Cyclosporine, tacrolimus, rituximab, IL-2	5050	3190	4200	3,27	3,37	26	23	16,7	0
2	7	Cyclosporine, tacrolimus, rituximab	2600	8760	264	2,5	2,57	71	87	9,2	0
3	16	Cyclosporine, tacrolimus, IL-2, plasmapheresis, rituximab	2900	2100	4676	3,23	3,45	27	34	NA	0
4	16	Cyclophosphamide, cyclosporine, tacrolimus, IL-2, rituximab	5580	5020	4000	3,15	3,31	30	31	17,4	0
5	14	Cyclosporine, plasmapheresis, sirolimus, tacrolimus, rituximab	2100	1180	100	3,48	3,19	123	118	NA	0
6	14	Cyclosporine, tacrolimus, rituximab, IL-2	6100	3995	NA	2,01	NA	35	NA	8,5	NA

*estimated Glomerular Filtration Rate calculated according to revised Schwartz Formula

NA = not available

NEW PERSPECTIVES:

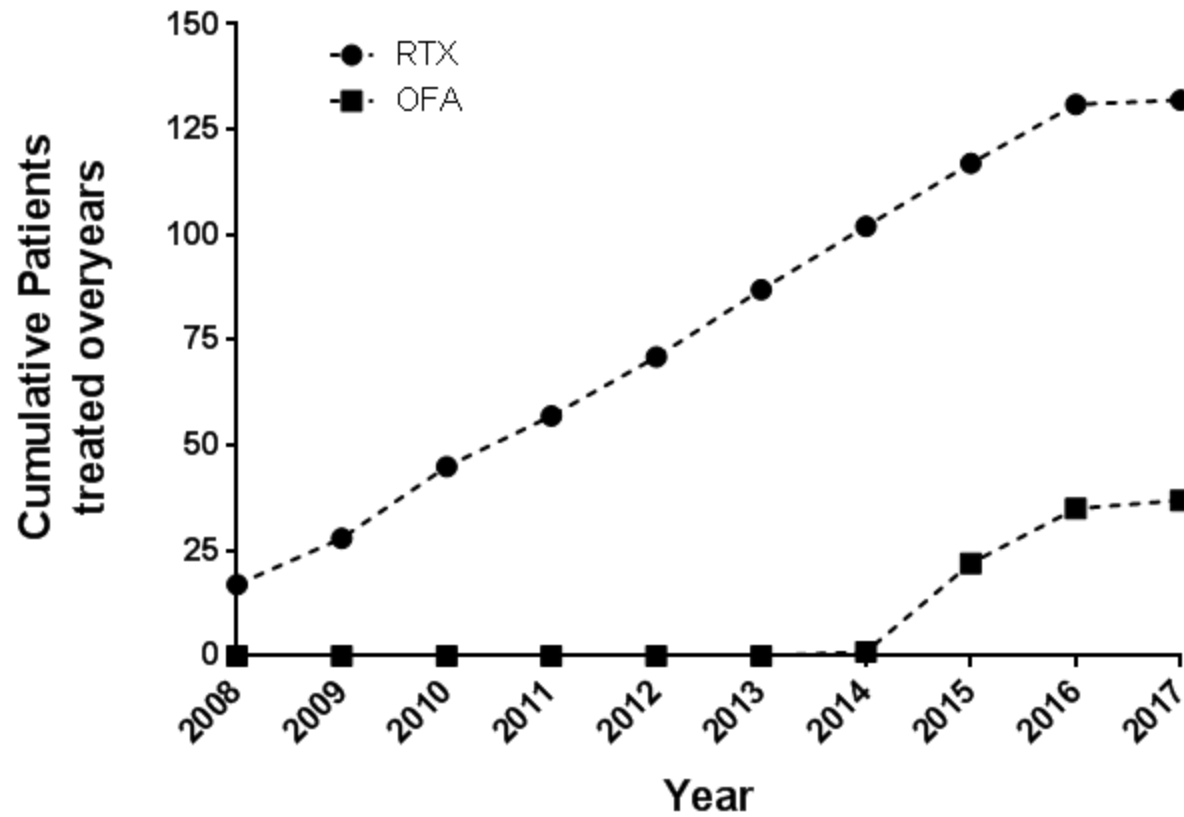
Randomized Clinical Trials at G. Gaslini

- **Ofatumumab in children with steroid- and CNI-resistant NS:
a double-blind randomized, controlled, superiority trial.**
- **Ofatumumab versus Rituximab in children
with steroid and CNI- dependent INS:
a double-blind randomized, controlled, superiority trial**

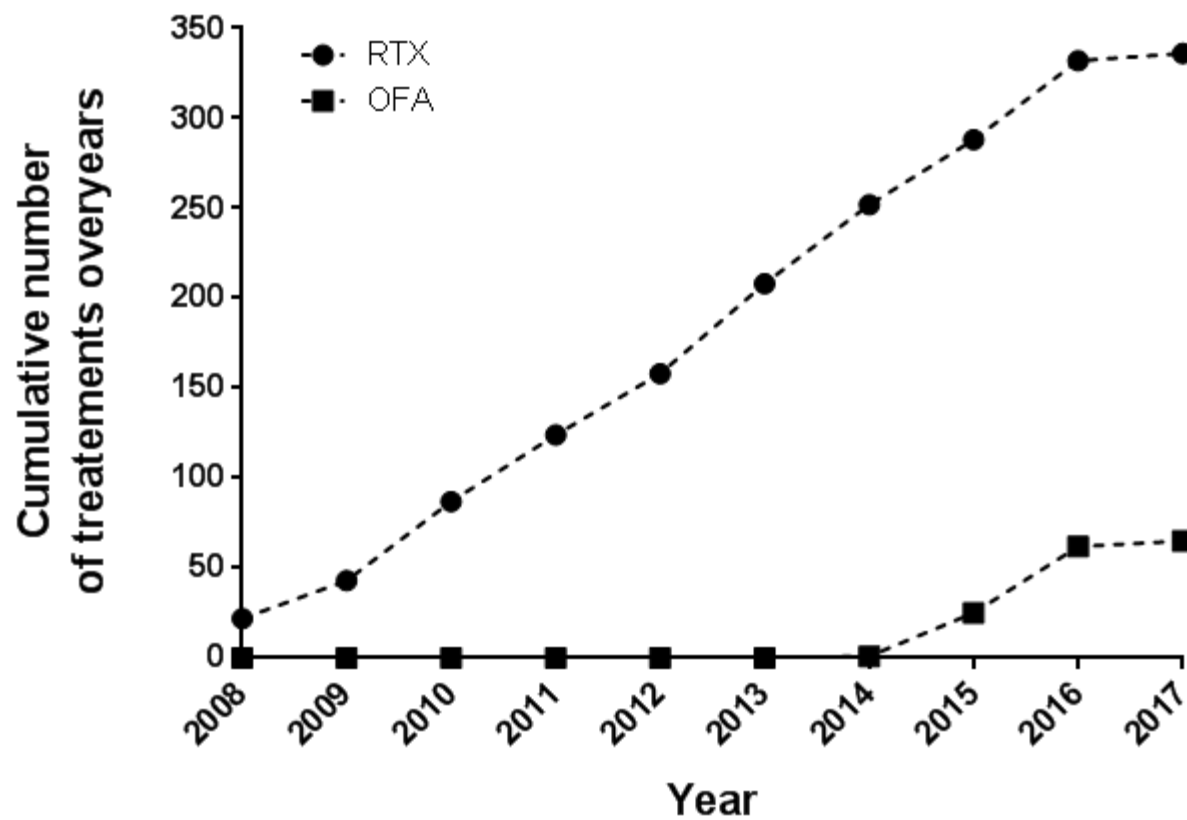
Safety

- Used in one million of patients with hematologic malignancies (first line / maintenance)
- First infusion adverse reactions (bronchospasm, cough, chills, rash, fever, headache); mild / absent thereafter
- Recent systematic review in RCTs of RA treated with biologic therapy (N = 29,423): no increased risk of malignancies (Lopez-Olivo JAMA 2012)
- 23 cases of PML in > 500,000 patients; had either B-cell cancer (20) or lupus (3); all taking chemotherapy - no cases in MS / NS
- Anti-chimeric antibodies may develop / uncertain role

Gaslini experience



Gaslini experience



<i>Drug</i>		Rituximab		Ofatumumab
<i>Category</i>		Low Steroid Dependence	High Steroid Dependence	Multi Drug Dependence
<i>Number of patients</i>		18	12	99
Infusion reactions				
Fever		0	0	2
Rash		0	0	7
Dyspnea		0	1	3
Hypotension/Hypertension		1	0	1
Cough/Itchy throat		0	0	5
Itch		0	0	0
GI symptoms		0	0	0
Need for treatment discontinuation		0	0	1
Early adverse events (≤ 3 months)				
Serum reaction/Arthritis		0	1	5
RALI/OALI		0	0	3
Pneumonia				1
Cutaneous manifestations		0	0	2
Severe Neutropenia		0	0	2
Infections		0	0	5
Late adverse events				
Neutropenia		0	0	0
Infections		0	0	2
Benign Neoplasia		0	0	1
Malignant Neoplasia		0	0	0
Neurological manifestation		0	0	1

Delayed Side Effects linked with anti-CD20 antibodies

-Infections

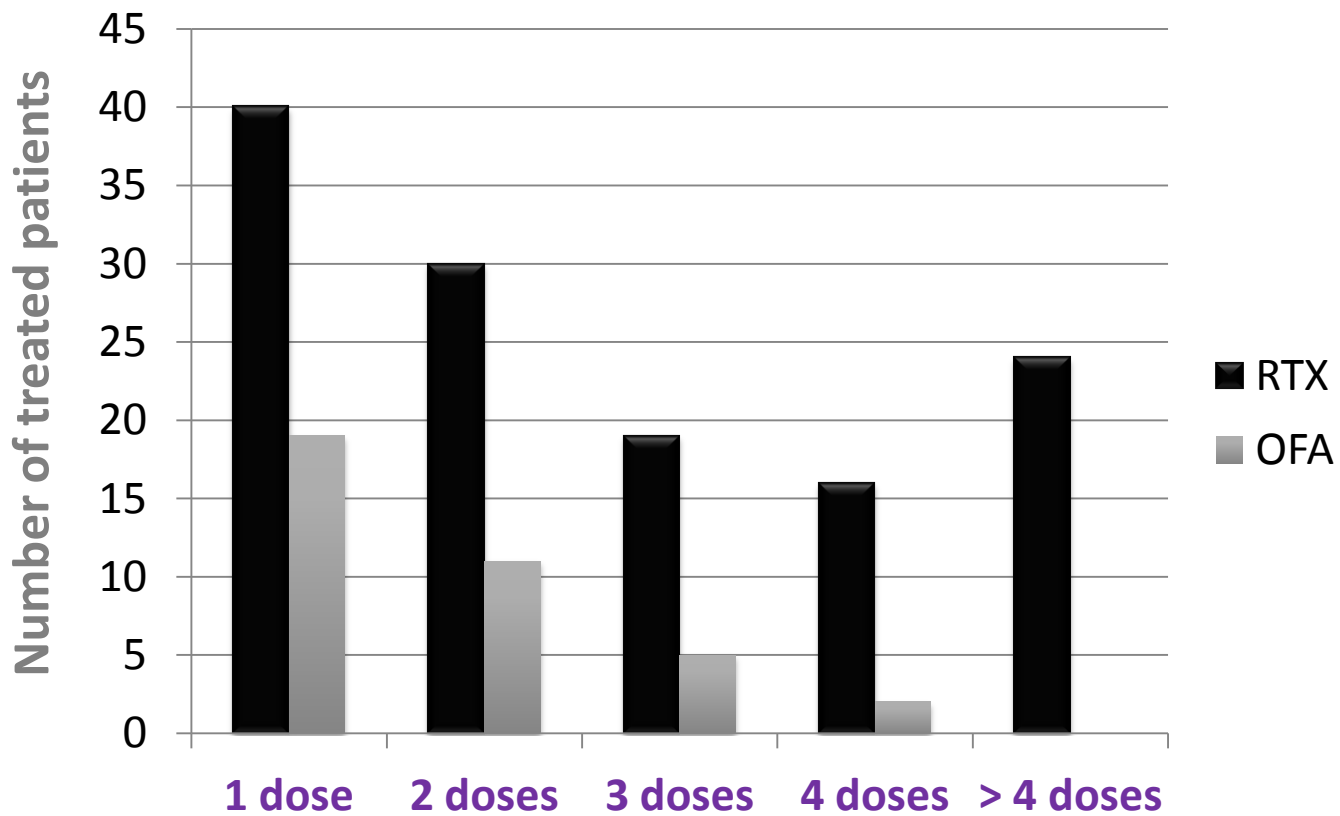
- n 1 purulent folliculitis
- n 1 pyelonephritis (complicated by renal abscess)
- n 1 dental abscess
- n 2 tonsillitis (1 complicated by tonsils abscess)
- n 2 Herpes simplex infections (Herpetic stomatitis)
- n 2 Urinary Tract Infections

-Rituximab/Ofatumumab Associated Lung Injury

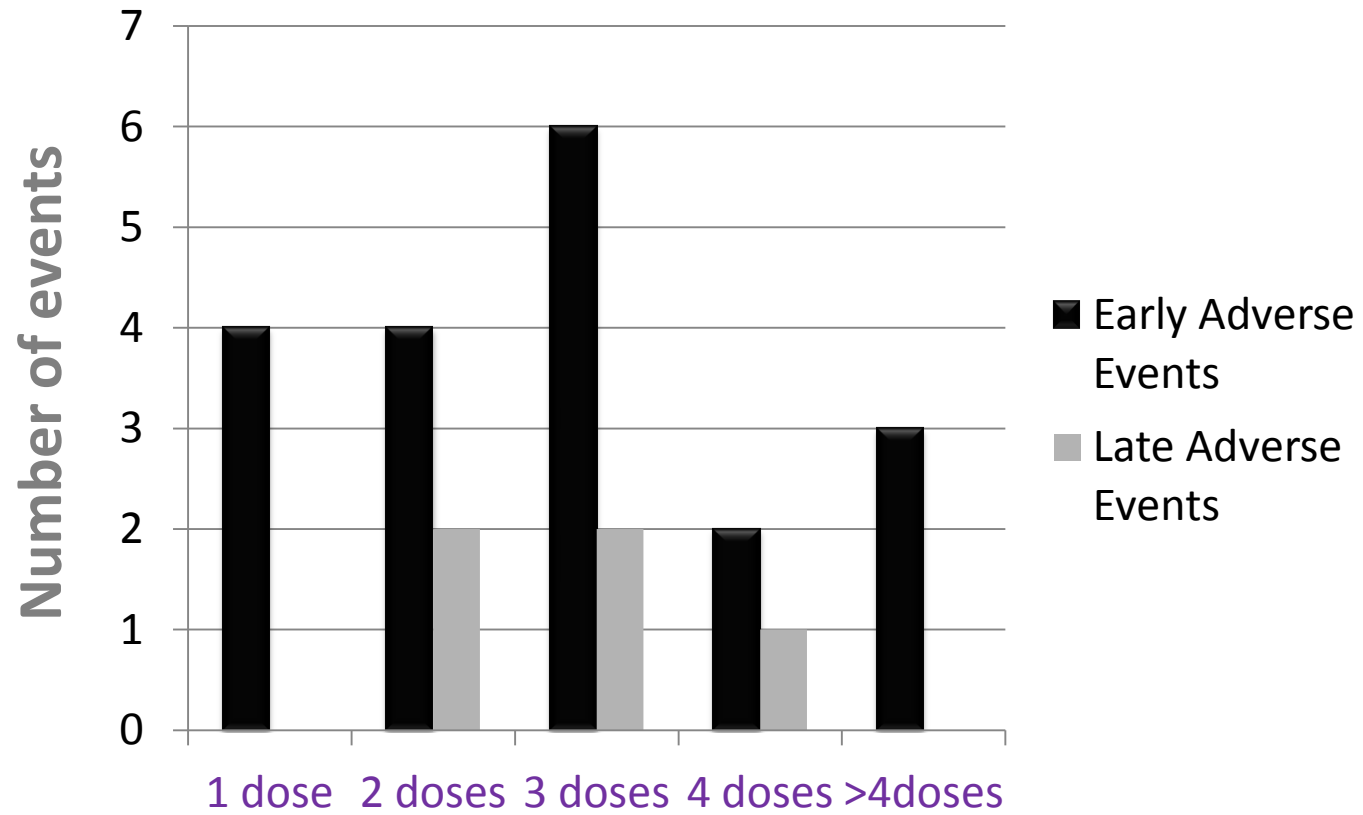
- n 2 RALI, n 1 OALI

-Benign Neoplasia

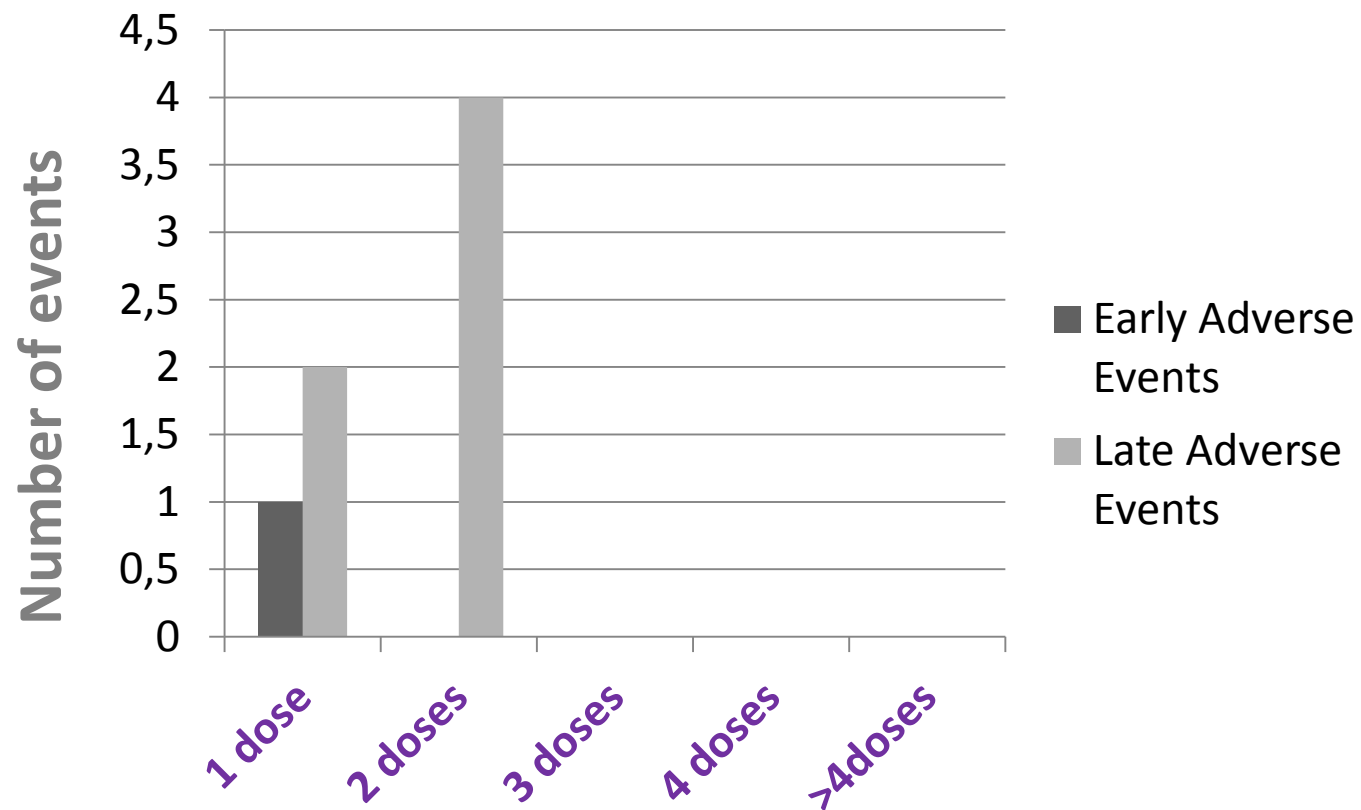
- n 1 desmoid fibromatosis



Rituximab



Ofatumumab



Letteratura

Randomised controlled trial comparing ofatumumab to rituximab in children with steroid-dependent and calcineurin inhibitor-dependent idiopathic nephrotic syndrome: study protocol

Ravani P, Bonanni A, **Ghiggeri** GM.

Br Med J Open. 2017 Mar 17;7(3).

Ofatumumab-associated acute respiratory manifestations: clinical characteristics and treatment

Bonanni A, Bertelli E, Moscatelli A, Lampugnani E, Bodria M, Ravani P, **Ghiggeri** GM.

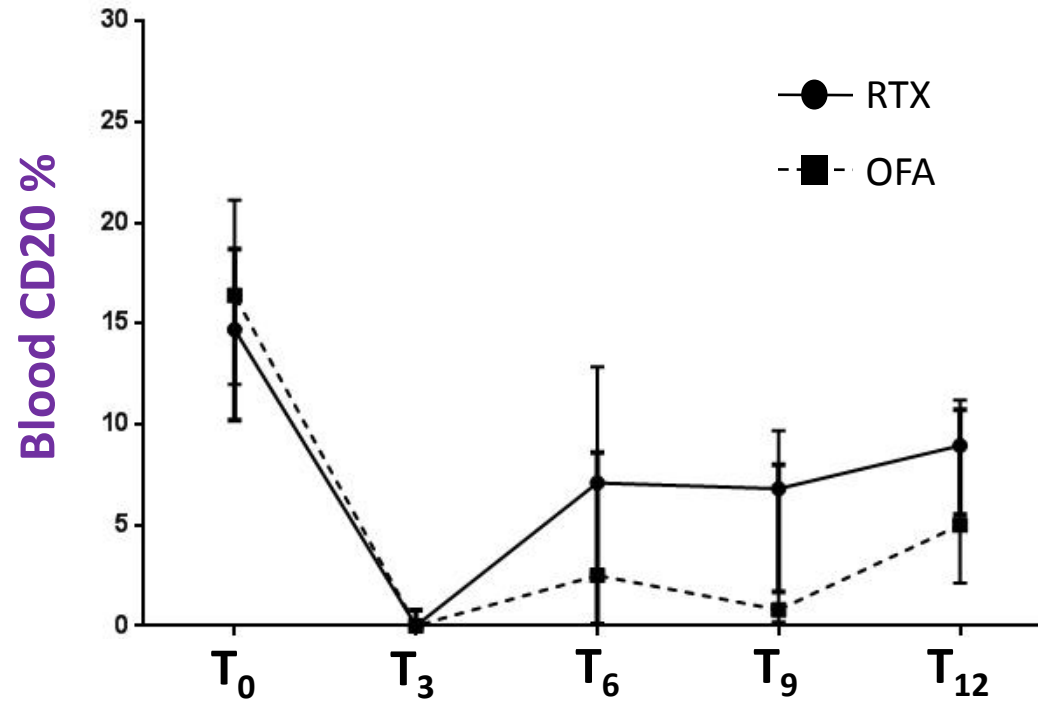
Br J Clin Pharmacol. 2016 Oct;82(4):1146-8

A mild form of rituximab-associated lung injury in two adolescents treated for nephrotic syndrome.

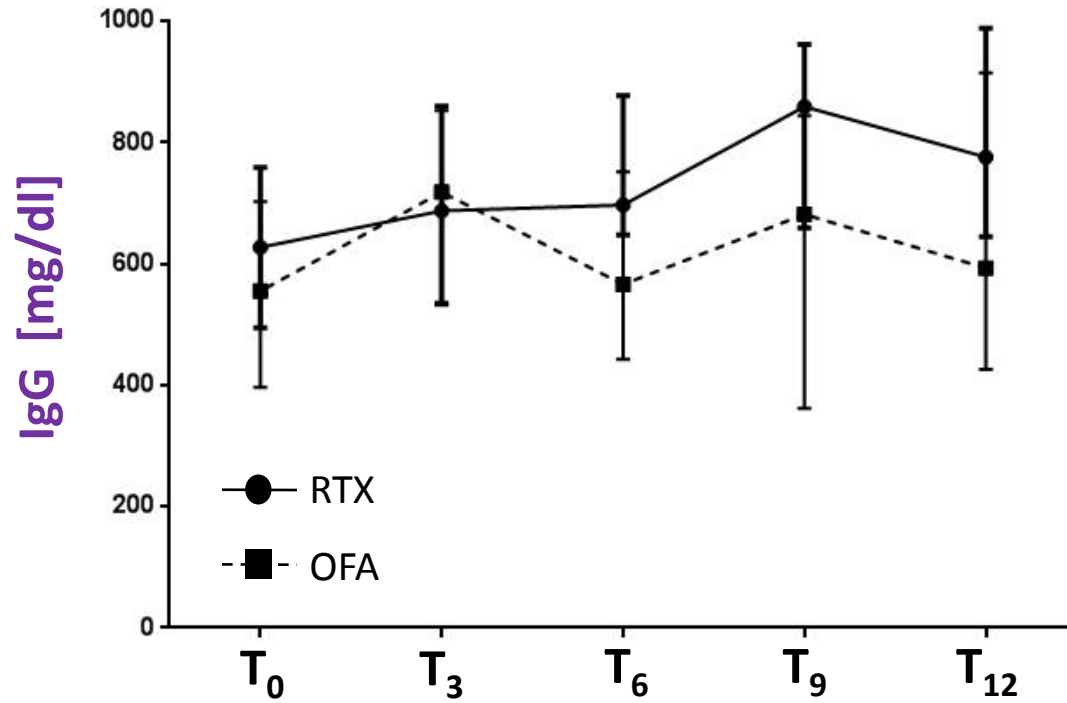
Spatafora M, Bellini T, Giordano C, **Ghiggeri** GM.

Br Med J Case Rep. 2015 Dec 9;2015. pii: bcr2015212694. doi

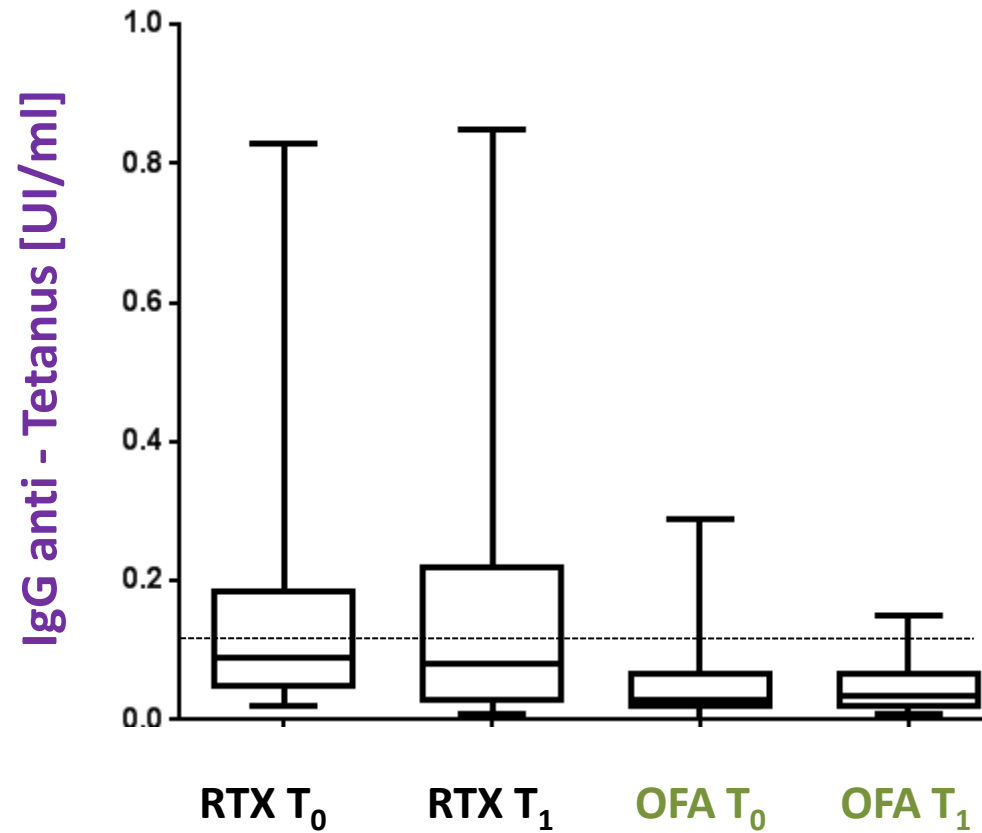
CD20 count



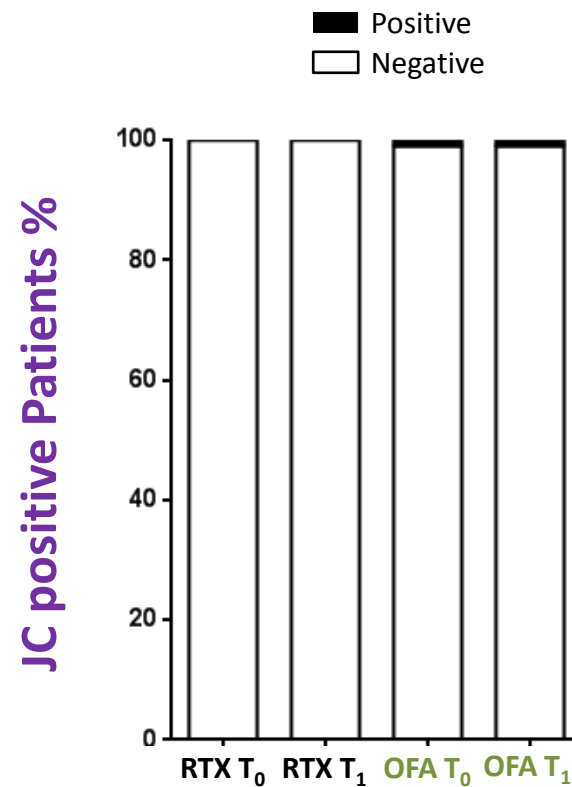
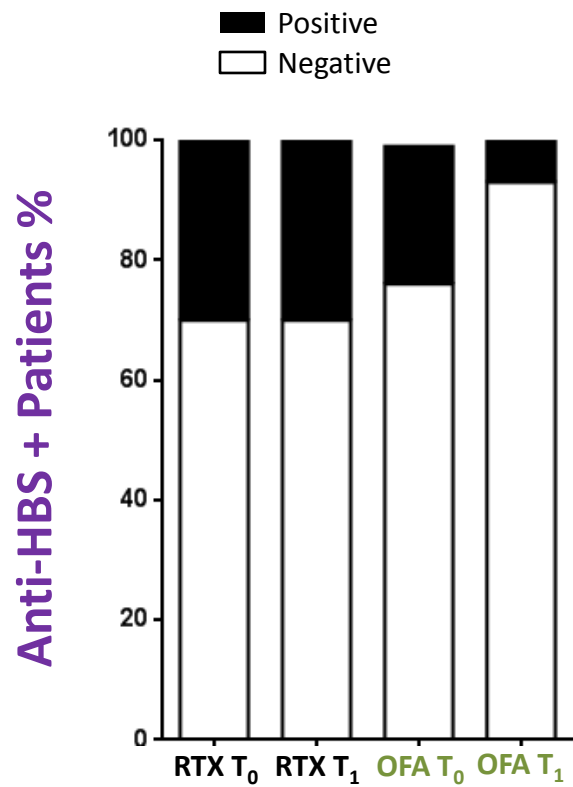
IgG serum levels



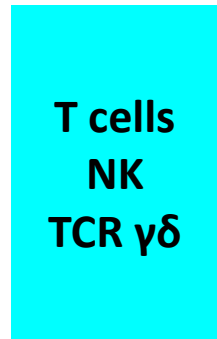
Serum anti -tetanus IgG



anti-HBS



Caratterizzazione fenotipica delle sottopopolazioni linfocitarie



CD3 FITC

CD56 PE

CD4 BV421

CD8 BV510

TCR $\gamma\delta$ PE-Cy7

CD39 APC



CD3 FITC

CD4 BV421

CD56 PE

CD161 APC

CCR6 PerCP Cy5.5

**CD39
BV510**

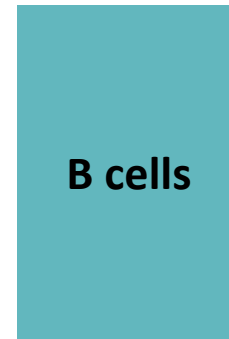


CD4 BV421

CD25 PE

CD127 FITC

**CD39
APC**



CD19 PE Cy7

CD20 FITC

CD80 PE

CD86 APC

CD39 BV510



CD20 FITC

CD40 PE-Cy7

Thanks to:

- All people contributing to RTX studies
- G. Gaslini
Medical Staff
- P. Ravani
- A Fornoni
- R. Gusmano
- The Kidney Foundation
- Wilma Querci Foundation



In memory of Prof R Gusmano