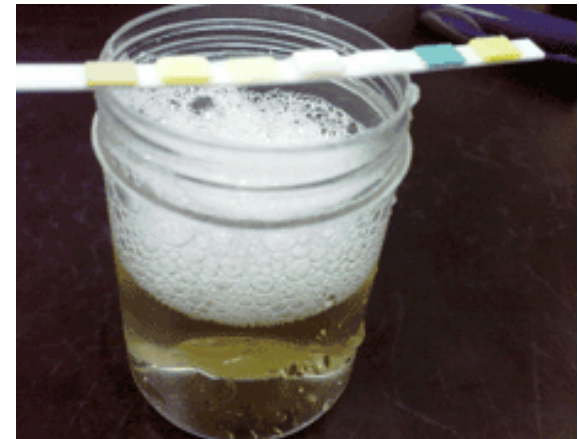


“When bubbles settle on surface of the urine, they indicate disease of the kidneys and that the complaint will be protracted...”

aforisma VII 34



Hippocrates of Kos (460-377 a.C)

Ghiggeri of Genoa

# Therapy

**Nephrotic Syndrome  
In children**

**Steroid treatment**  
(PDN 60 mg m<sup>2</sup>  
for 6 weeks)

**Steroid  
Resistance**

Calcineurin  
inhibitors

**Resistance**  
Add ACEi and  
withdraw other  
therapy

???

**Response**  
Taper steroids

**RTX / OFA  
(1 pulse)**

**Steroid  
Dependence**

< 0.3 mg/kg/die

**Cyclophosphamide  
MMF  
RTX**

From 0.3 to 0.7  
mg/kg/die

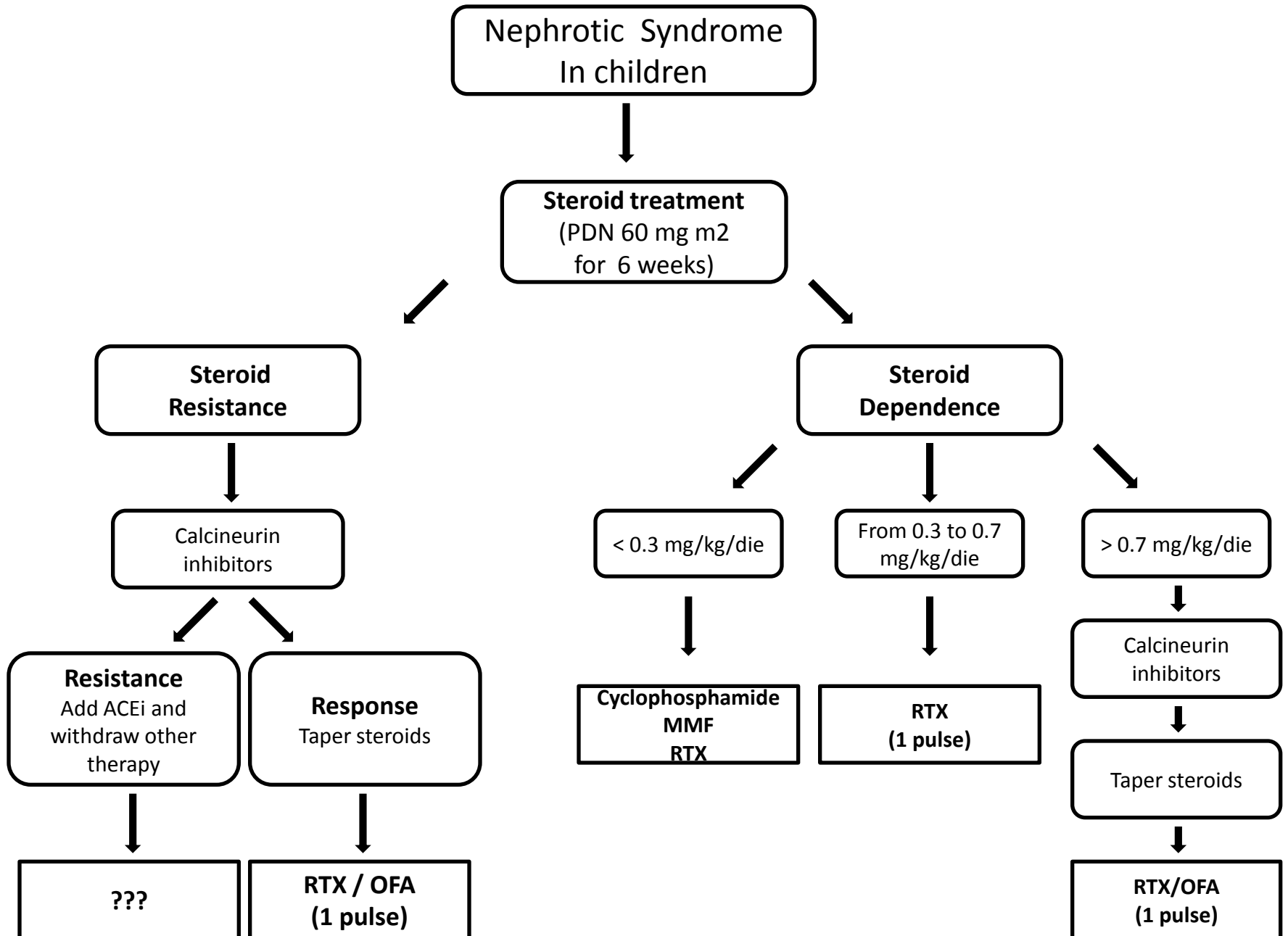
**RTX  
(1 pulse)**

> 0.7 mg/kg/die

Calcineurin  
inhibitors

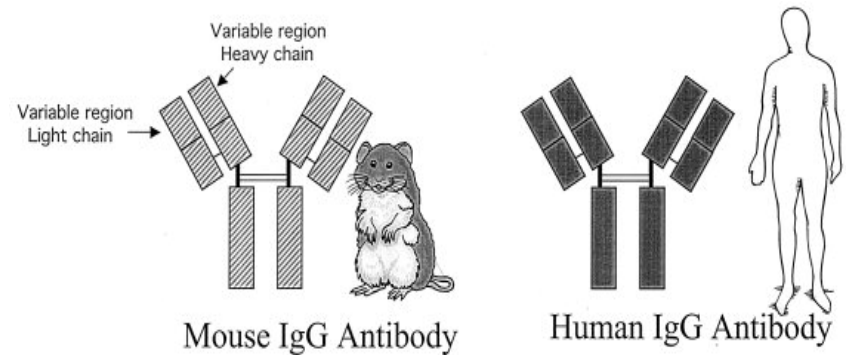
Taper steroids

**RTX/OFA  
(1 pulse)**



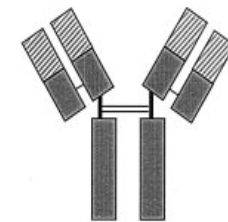
## Steroid-sparing agents in SDNS and FRNS

Drug	Efficacy	Indication	Comments
Calcineurin inhibitors	+++++	SDNS	Renal toxicity / hypertension
Mofetil mycophenolate	++++	FRNS/SDNS	Often need to use high doses (> 600 mg/m <sup>2</sup> )
Levamisole	+++	FRNS	Only 1 RCT
Cyclophosphamide	+++	?	Severe cases often relapse rapidly
Rituximab/Ofatumumab	Good	?	May compromise immunological memory



## Rituximab

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



Chimeric Antibody

# 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)*

# **Steroid Dependent Nephrotic Syndrome**

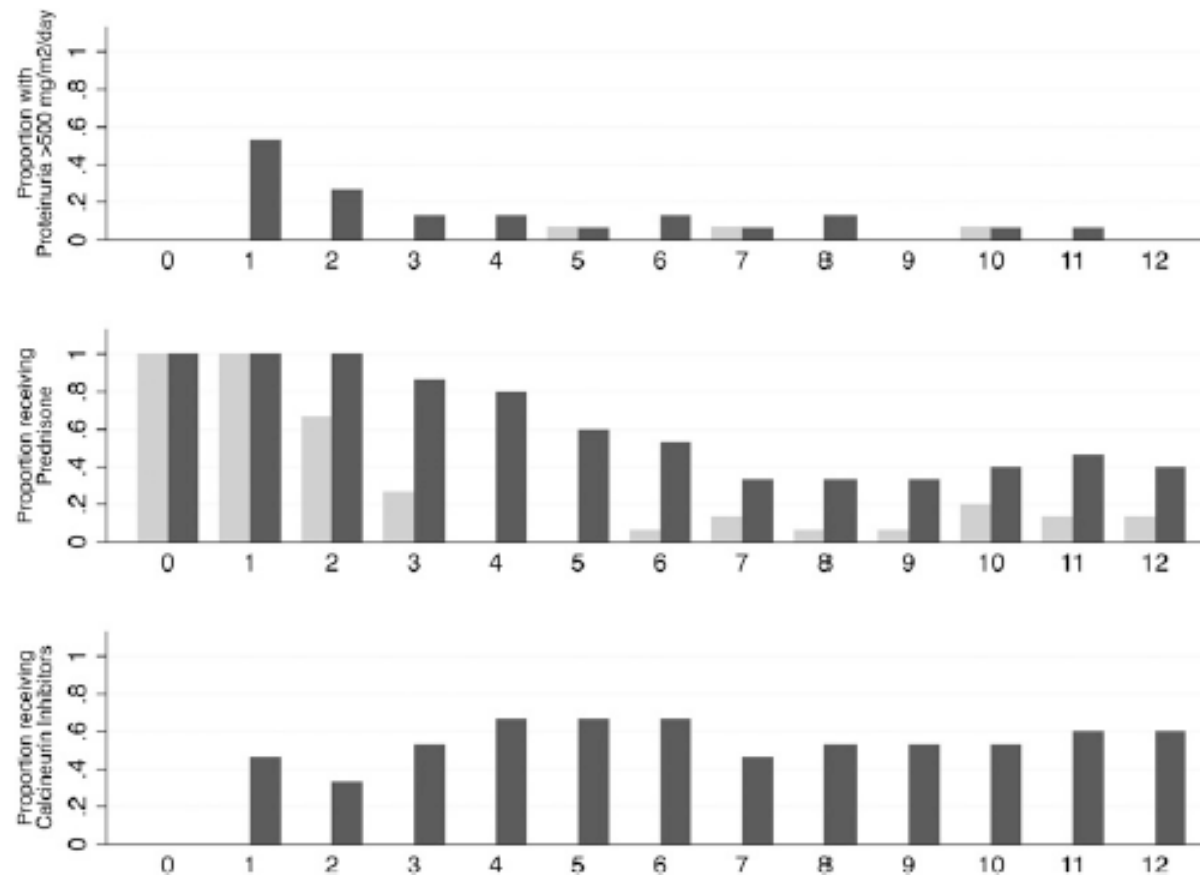
JASN 2015

CLINICAL RESEARCH

[www.jasn.org](http://www.jasn.org)

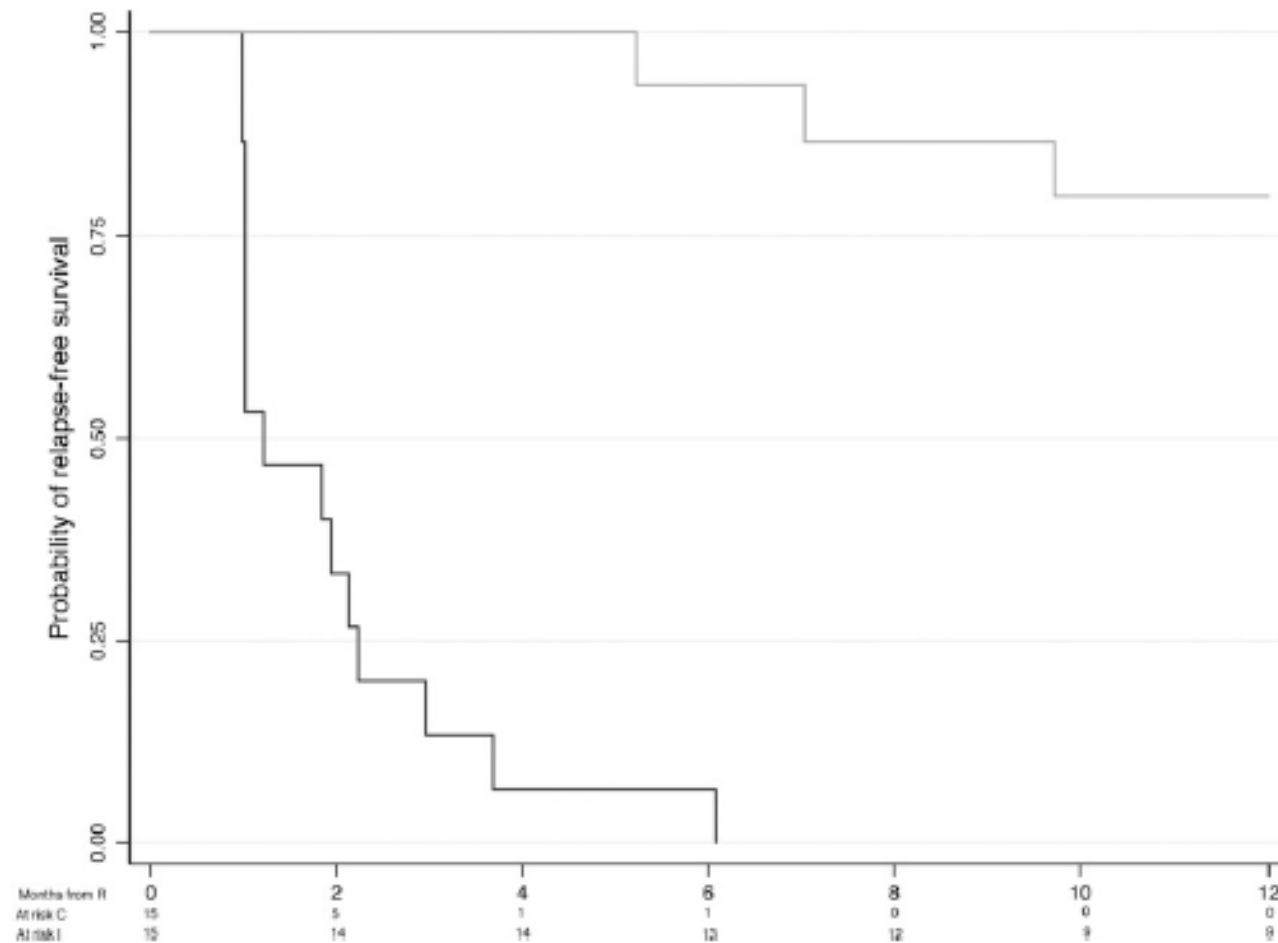
## **Rituximab in Children with Steroid-Dependent Nephrotic Syndrome: A Multicenter, Open-Label, Noninferiority, Randomized Controlled Trial**

Pietro Ravani,<sup>\*</sup> Roberta Rossi,<sup>†</sup> Alice Bonanni,<sup>†</sup> Robert R. Quinn,<sup>\*</sup> Felice Sica,<sup>‡</sup> Monica Bodria,<sup>†</sup> Andrea Pasini,<sup>§</sup> Giovanni Montini,<sup>§</sup> Alberto Edefonti,<sup>||</sup> Mirco Belingheri,<sup>||</sup> Donatella De Giovanni,<sup>‡</sup> Giancarlo Barbano,<sup>†</sup> Ludovica Degl'Innocenti,<sup>†</sup> Francesco Scolari,<sup>¶</sup> Luisa Murer,<sup>\*\*</sup> Jochen Reiser,<sup>††</sup> Alessia Fornoni,<sup>‡‡</sup> and Gian Marco Ghiggeri<sup>†</sup>



**Figure 4.** Proportion of children with proteinuria >500 mg/m<sup>2</sup> per day (top), receiving prednisone (middle) or steroid-sparing agents (bottom) by treatment group. Light gray indicates rituximab group; dark gray indicates control group.





**Figure 5.** One-year relapse-free survival by treatment group in the prednisone (control; dark gray) and rituximab (intervention; light gray) groups. The risk of relapse was reduced by 98% in children treated with rituximab (hazard ratio, 0.02; 95% CI, 0.01 to 0.15). C, comparator group; I, intervention group; R, randomization.

# **Multidrug Dependent Nephrotic syndrome**

# Short-Term Effects of Rituximab in Children with Steroid- and Calcineurin-Dependent Nephrotic Syndrome: A Randomized Controlled Trial

Pietro Ravani,\* Alberto Magnasco,<sup>†</sup> Alberto Edefonti,<sup>‡</sup> Luisa Murer,<sup>§</sup> Rossella Rossi,\* Luciana Ghio,<sup>‡</sup> Elisa Benetti,<sup>§</sup> Floriana Scozzola,<sup>§</sup> Andrea Pasini,<sup>¶</sup> Nadia Dallera,<sup>¶</sup> Felice Sica,\*\* Mirco Belingheri,<sup>‡</sup> Francesco Scolari,<sup>¶</sup> and Gian Marco Ghiggeri<sup>‡</sup>

## Summary

**Background and objectives** Prednisone and calcineurin inhibitors are the mainstay therapy of idiopathic nephrotic syndrome (INS) in children. However, drug dependence and toxicity associated with protracted use are common. Case series suggest that the anti-CD20 monoclonal antibody rituximab (RTX) may maintain disease remission.

**Design, setting, participants, & measurements** This open-label randomized controlled trial was powered to show that a strategy based on RTX and lower doses of prednisone and calcineurin inhibitors was noninferior to standard doses of these agents in maintaining 3-month proteinuria as low as baseline or up to 1 g/d greater (noninferiority margin). Participants were stratified by the presence of toxicity to prednisone/calcineurin inhibitors and centrally assigned to add RTX (Mabthera, 375 mg/m<sup>2</sup> intravenously) to lower doses of standard agents or to continue with current therapy alone. The risk of relapse was a secondary outcome.

**Results** Fifty-four children (mean age 11 ± 4 years) with INS dependent on prednisone and calcineurin inhibitors for >12 months were randomized. Three-month proteinuria was 70% lower in the RTX arm (95% confidence interval 35% to 86%) as compared with standard therapy arm (intention-to-treat); relapse rates were 18.5% (intervention) and 48.1% (standard arm) ( $P = 0.029$ ). Probabilities of being drug-free at 3 months were 62.9% and 3.7%, respectively ( $P < 0.001$ ); 50% of RTX cases were in stable remission without drugs after 9 months.

**Conclusions** Rituximab and lower doses of prednisone and calcineurin inhibitors are noninferior to standard therapy in maintaining short-term remission in children with INS dependent on both drugs and allow their temporary withdrawal.

*Clin J Am Soc Nephrol* 6: 1308–1315, 2011. doi: 10.2215/CJN.09421010

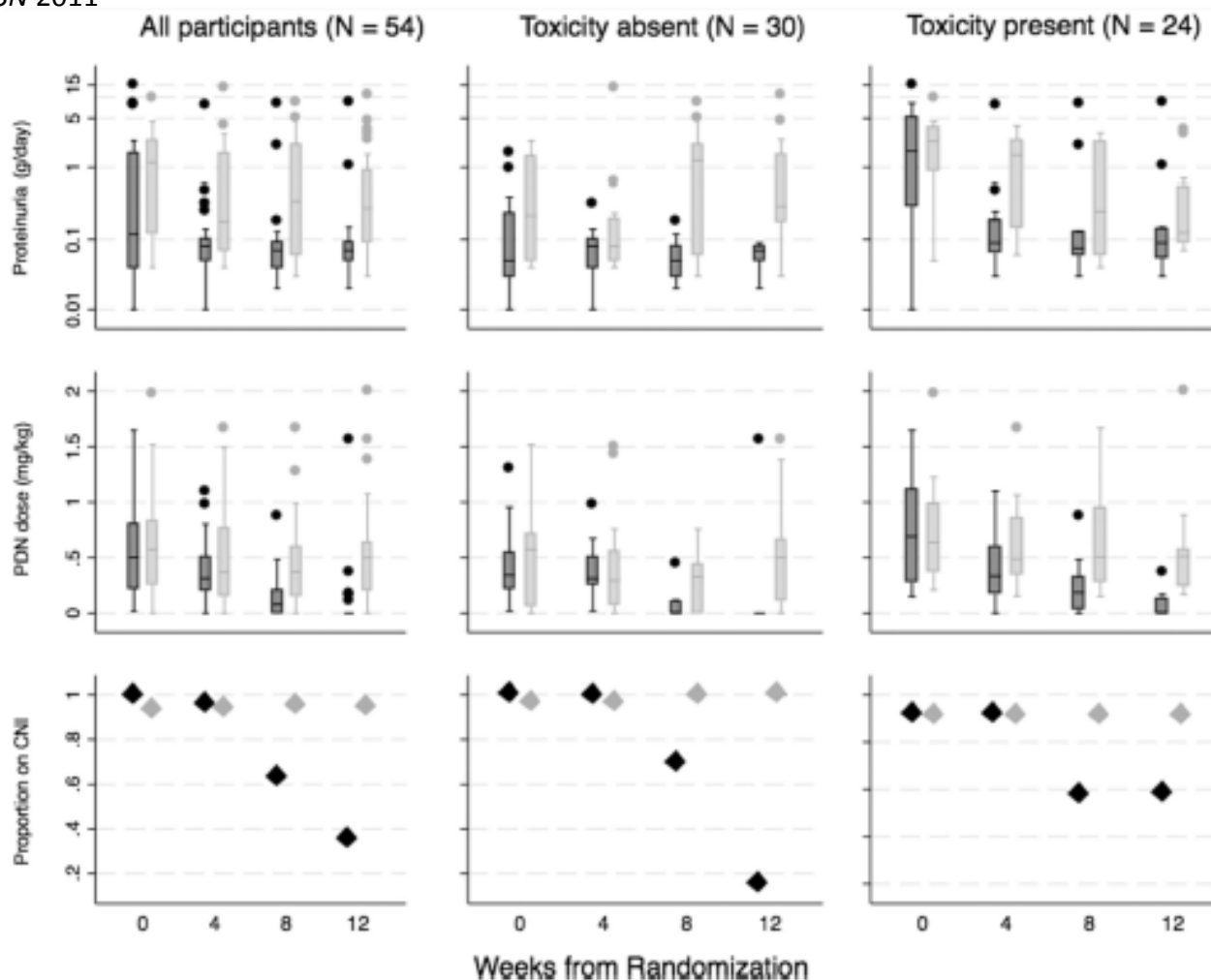
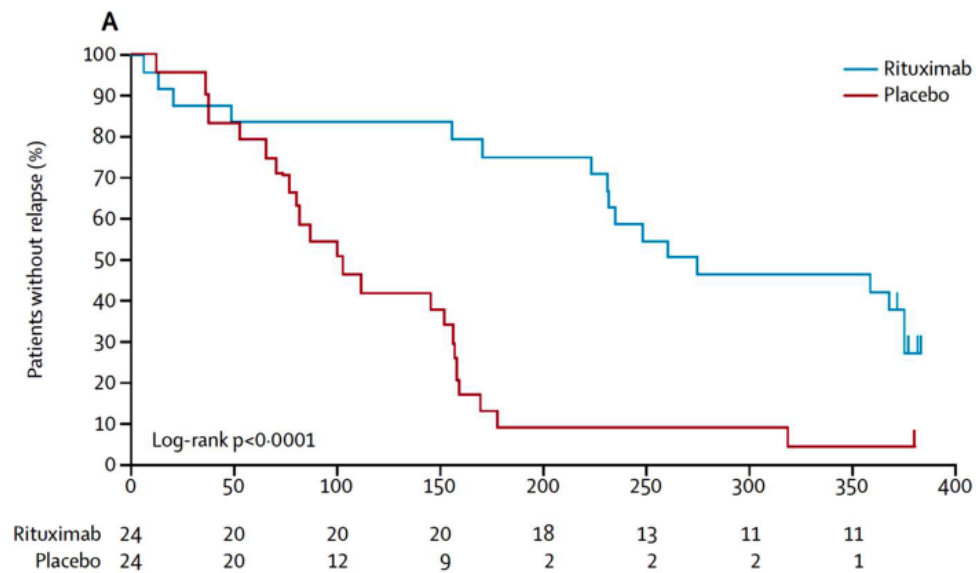


Figure 2. | The plots summarize the distribution of proteinuria (g/d [log-scale]), prednisone [PDN] in mg/kg per day, and proportion of children on full dose of calcineurin inhibitors [CNI, *i.e.*, cyclosporine or tacrolimus] over time in weeks from randomization (time zero). Dark gray bars refer to patients assigned to RTX-based strategy; light gray bars refer to standard therapy strategy. The plot on the left (all patients) refers to model 1 in Table 3; the middle and right plots refer to model 2 in Table 3. The line across the box plots (proteinuria and prednisone plots) is the median, the box hinges are the 25th and 75th percentiles, and the outliers are represented as dots lying beyond 1.5 times the interquartile range.

## **Length of remission after the first or subsequent cycles**

	<b>First cycle</b>		<b>Second and subsequent cycles</b>	
	<b>Median</b>	<b>Interquartile range</b>	<b>Median</b>	<b>Interquartile range</b>
<b>Both P and C</b>	<b>5.6</b>	<b>4.3 to 8.1</b>	<b>8.5</b>	<b>6.5 to 11.7</b>
<b>Prednisone</b>	<b>6.5</b>	<b>4.8 to 9.6</b>	<b>10.9</b>	<b>8.1 to 15.6</b>
<b>CyA/FK506</b>	<b>7.3</b>	<b>5.6 to 10.2</b>	<b>11.6</b>	<b>8.9 to 15.8</b>

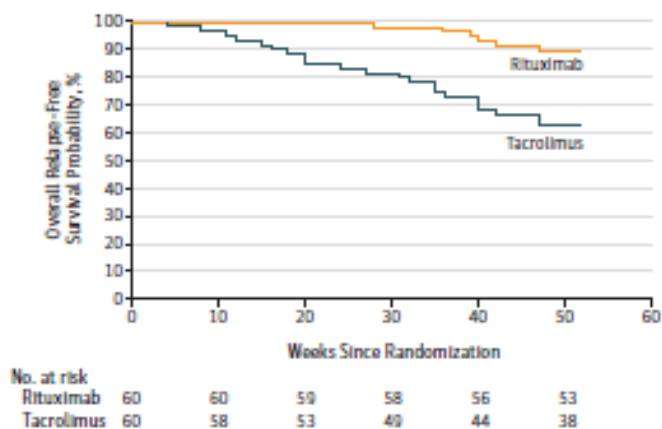
## Rituximab for INS RCT vs placebo



# Efficacy of Rituximab vs Tacrolimus in Pediatric Corticosteroid-Dependent Nephrotic Syndrome A Randomized Clinical Trial

Blswanath Basu, MD; Anja Sander, PhD; Birendranath Roy, MD; Stella Preussler, MSc; Shilpita Barua, MD; T. K. S. Mahapatra, MD; Franz Schaefer, MD

Figure 2. Probability of Relapse-Free Survival According to Treatment Group



The 12-month relapse-free survival rate was significantly higher with rituximab compared with tacrolimus (log rank  $P < .001$ ).

## Retrospective study: 551 pts (G Chan, K Tullus)

Investigating sites	Rituximab dose (per course)			With mIS (%)
	375 mg/m <sup>2</sup> (%)	750 mg/m <sup>2</sup> (%)	1500 mg/m <sup>2</sup> (%)	
London	0	13	87	42
Genoa	71	29	0	45
Hamburg	20	10	70	0
Hong Kong	0	90	10	90
Kobe	25	0	75	83
New Delhi	4	83	13	38
Paris	42	34	24	36
Rome	97	3	0	100
Singapore	46	31	23	92
Tokyo	82	0	18	80
Toronto	31	65	4	96

Unpublished, courtesy G Chan, K Tullus



## Median relapse-free period (will include in-text)

Dose	All	Concomitant immunosuppression (IS) $\geq 6$ months <sup>†</sup>		
		No	Yes	p value <sup>‡</sup>
All	12.5 (11.3 - 14.0)	11.9 (10.4 - 14.3)	13.2 (11.0 - 15.8)	0.704
Low	11.8 (10.1 - 15.8)	7.7 (6.5 - 10.3)	14.8 (11.6 - 19.4)	0.001
Medium	11.9 (10.4 - 14.3)	12.6 (10.4 - 16.8)	10.9 (10.0 - 14.2)	0.088
High	13.0 (11.8 - 17.4)	13.1 (11.8 - 17.6)	12.1 (9 - 22)	0.952
p value <sup>‡</sup>	0.391	0.002	0.103	–

\* Values are expressed as median relapse-free survival (95% CI; month)

† Missing information regarding use of concomitant immunosuppression in six patients

‡ Log-rank test

# **Multidrug Resistant Nephrotic syndrome**

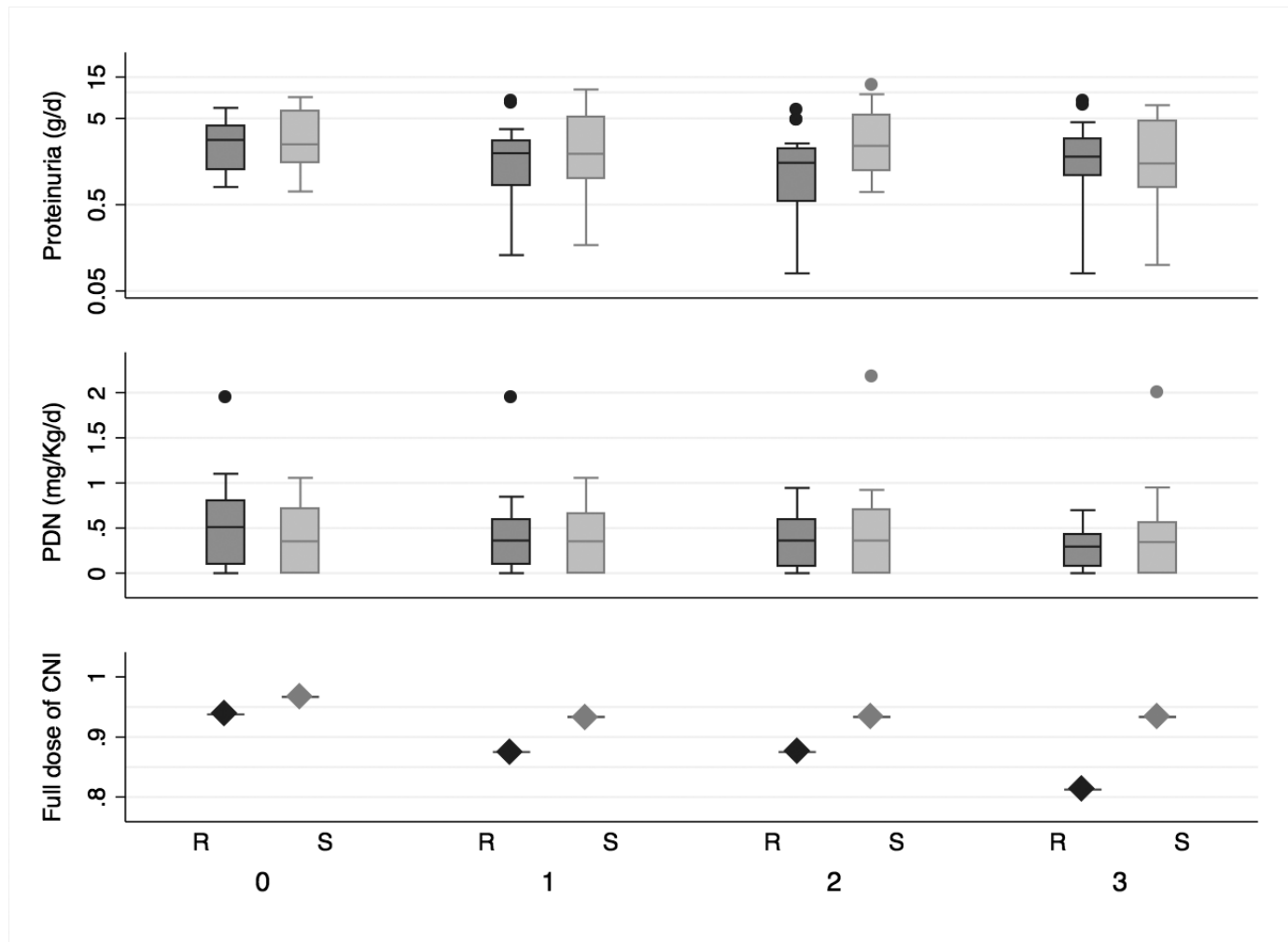
JASN 2012

CLINICAL RESEARCH

[www.jasn.org](http://www.jasn.org)

## **Rituximab in Children with Resistant Idiopathic Nephrotic Syndrome**

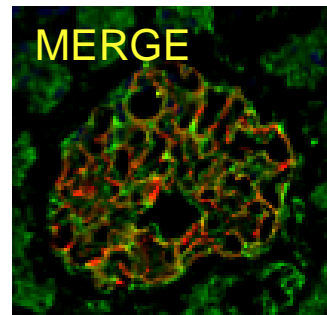
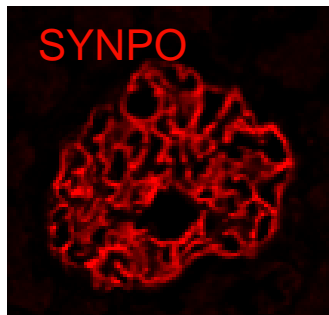
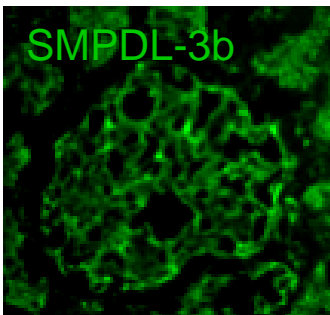
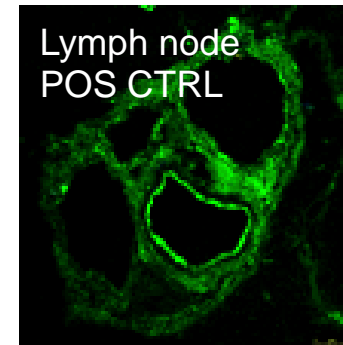
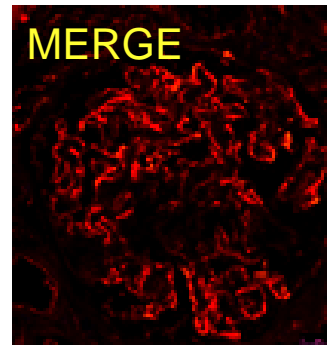
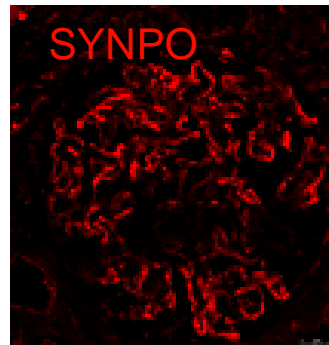
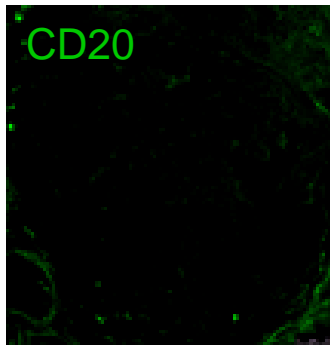
Alberto Magnasco,<sup>\*</sup> Pietro Ravani,<sup>†</sup> Alberto Edefonti,<sup>‡</sup> Luisa Murer,<sup>§</sup> Luciana Ghio,<sup>‡</sup> Mirco Belingheri,<sup>‡</sup> Elisa Benetti,<sup>§</sup> Corrado Murtas,<sup>\*</sup> Giovanni Messina,<sup>||</sup> Laura Massella,<sup>¶</sup> Maria Gabriella Porcellini,<sup>\*\*</sup> Michela Montagna,<sup>††</sup> Mario Regazzi,<sup>††</sup> Francesco Scolari,<sup>‡‡</sup> and Gian Marco Ghiggeri<sup>\*</sup>



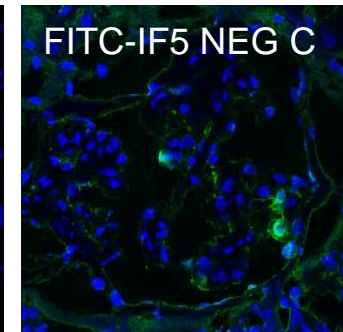
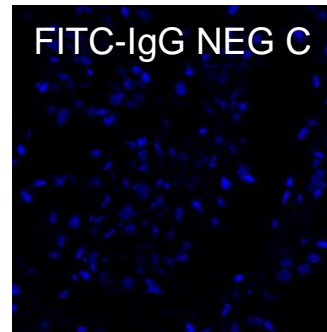
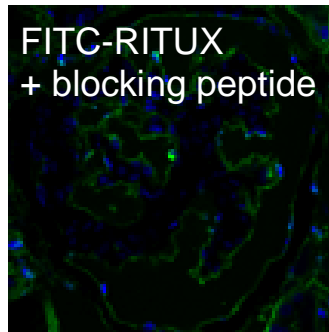
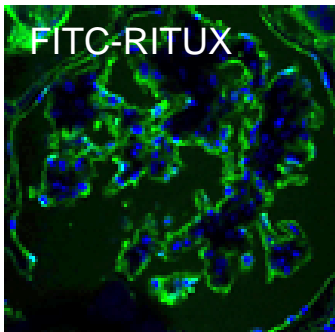
# Key points

- 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

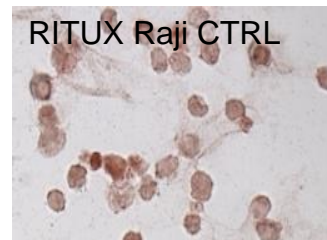
**A**



**B**



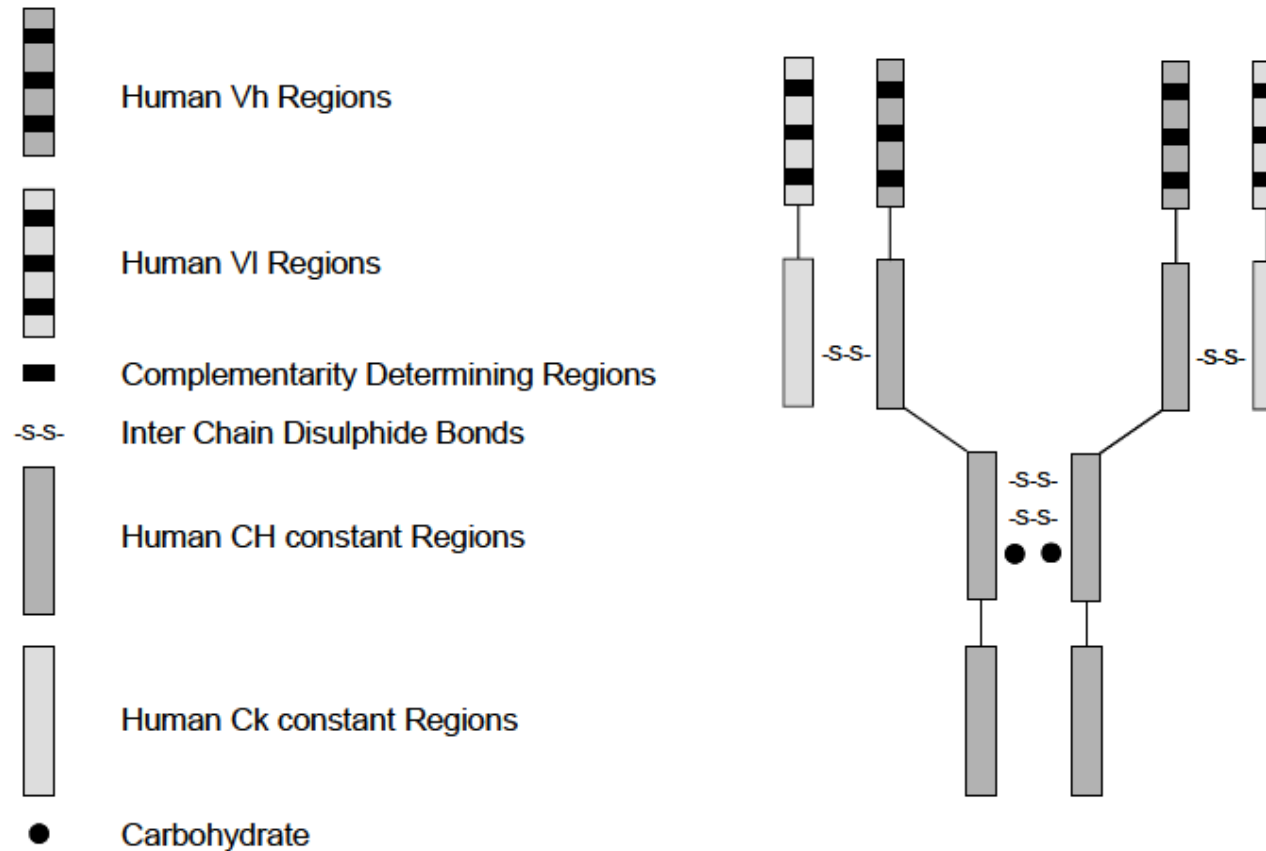
**C**



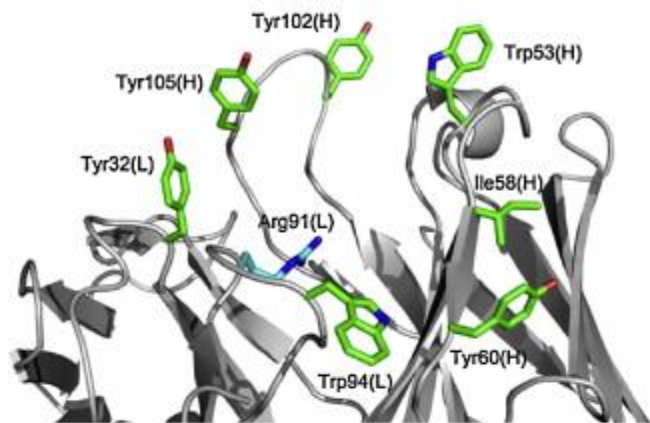
Fornoni et al, STM 2011

# HUMANIZED ANTI-CD 20 Abs

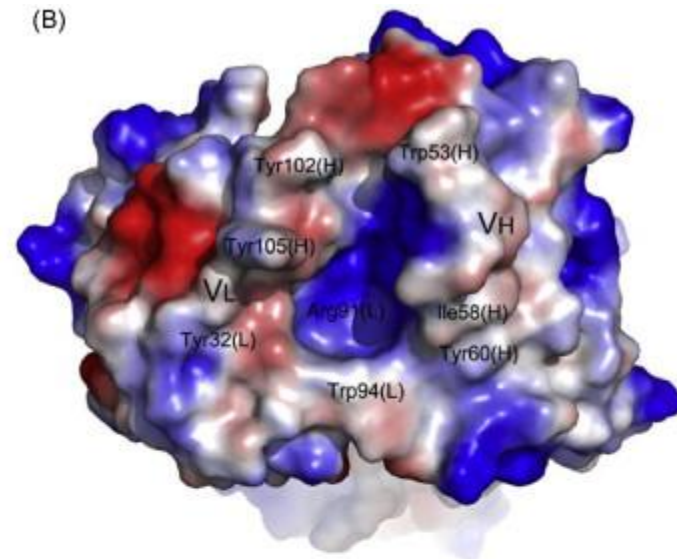
## Schematic Representation of Ofatumumab Indicating the Disulphide Bridges



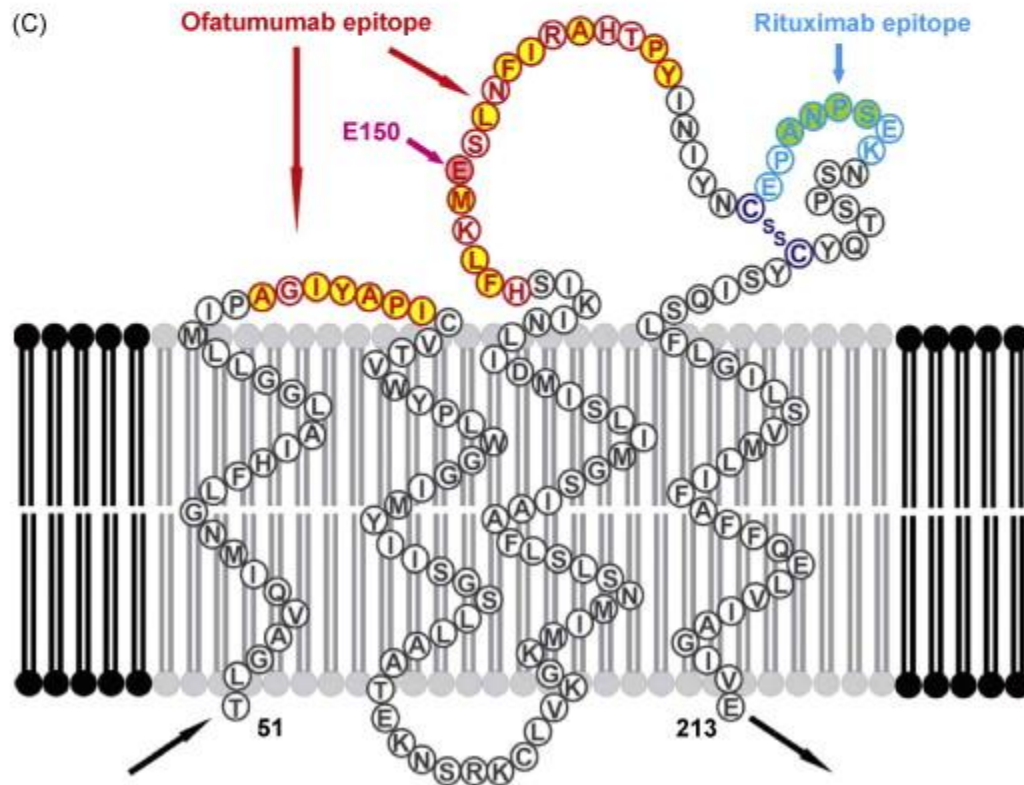
(A)



(B)



(C)





# 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  $1.73\text{m}^2$  followed by five infusion 2.000 mg  $1.73\text{ m}^2$
- 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 1.73m<sup>2</sup> T1+15

Patient No	Age (yrs)	Previous Steroid-Sparing Therapy	Proteinuria (mg/24 hours)			Serum albumin (g/dl)		eGFR* (ml/min/1,73m <sup>2</sup> )		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:

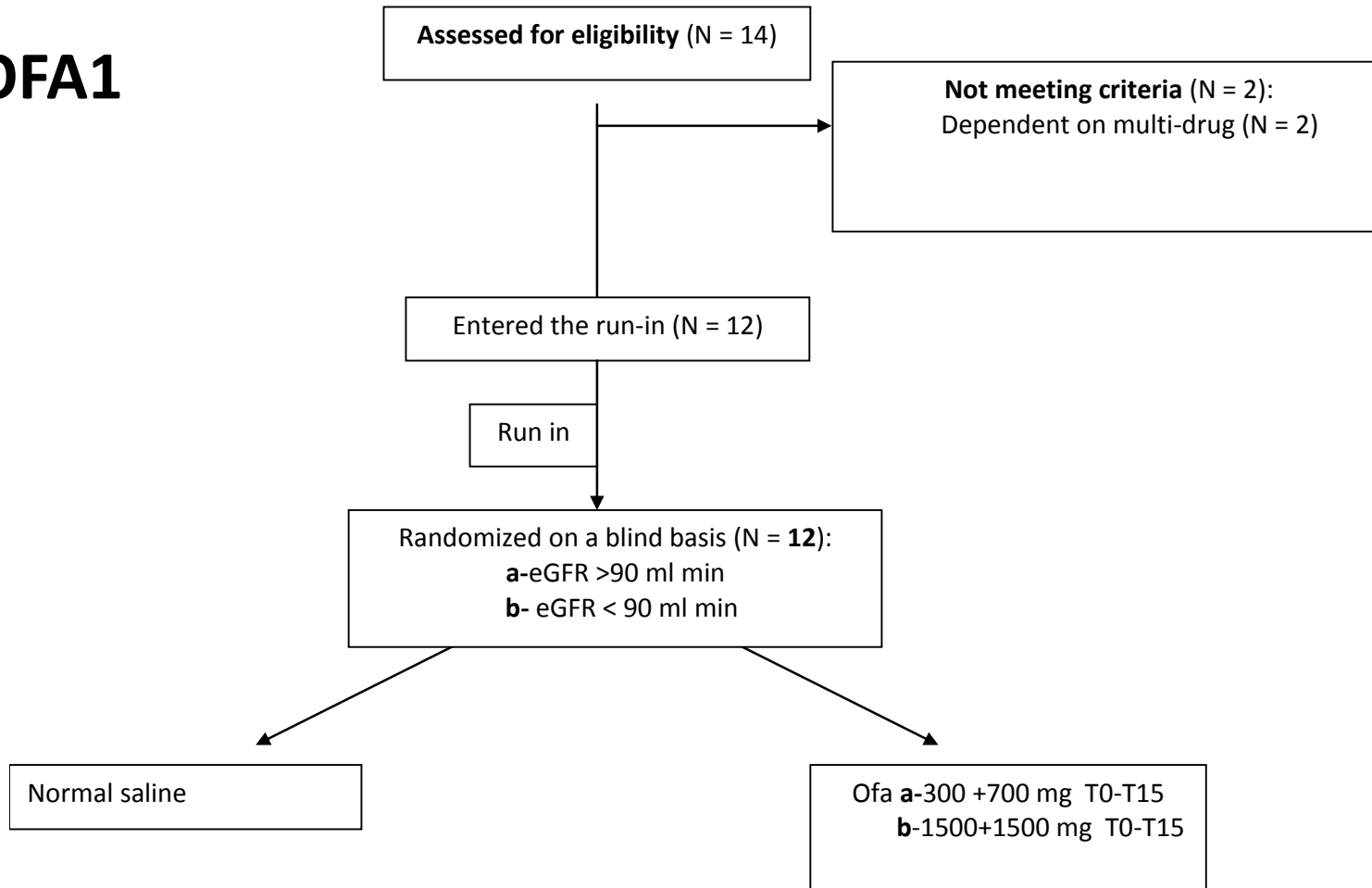
### Randomized Clinical Trials at G. Gaslini

**OFA1-** Ofatumumab in children with steroid- and CNI-resistant NS a double-blind randomized vs placebo, controlled, superiority trial (closed).

**OFA2-** Ofatumumab versus Rituximab in children with steroid and CNI- dependent INS: a double-blind randomized, controlled, non inferiority trial (closed).

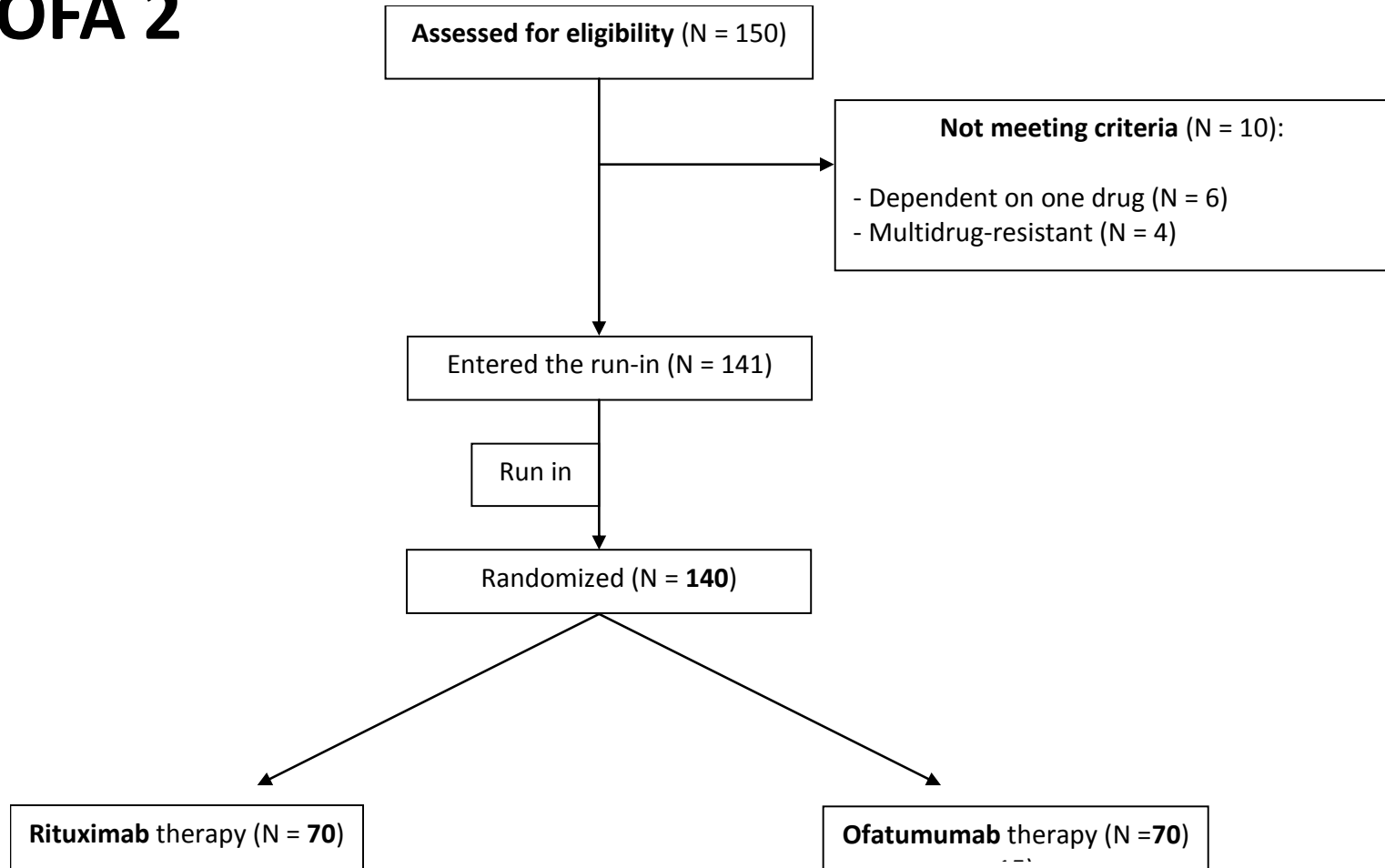
**RTX4-** Rituximab versus low-dose MMF in children with steroid dependent INS: a double-blind randomized, controlled, non inferiority trial (recruiting)

# OFA1



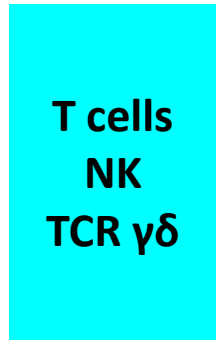
closed

# OFA 2



**closed**

# Cell phenotyping



**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**

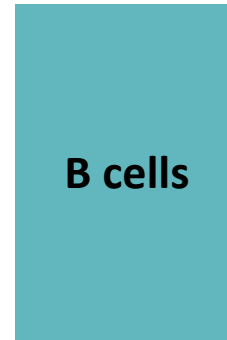


**CD4 BV421**

**CD25 PE**

**CD127 FITC**

**CD39  
APC**



**CD19 PE Cy7**

**CD20 FITC**

**CD80 PE**

**CD86 APC**

**CD39 BV510**



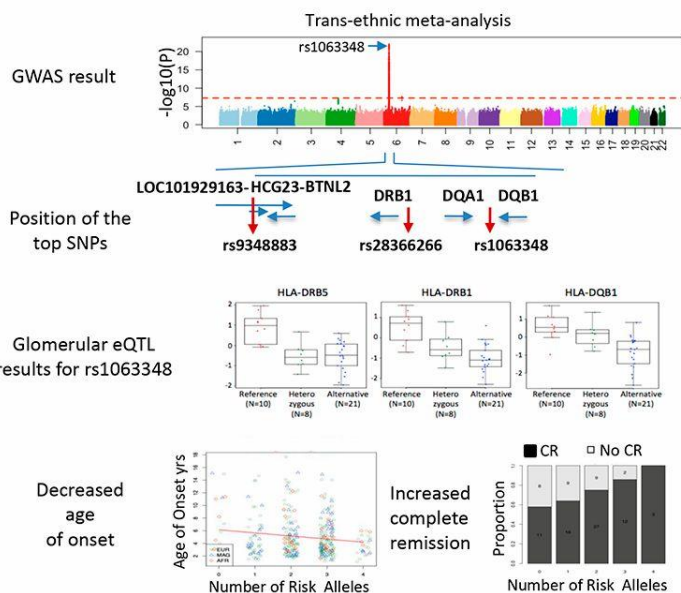
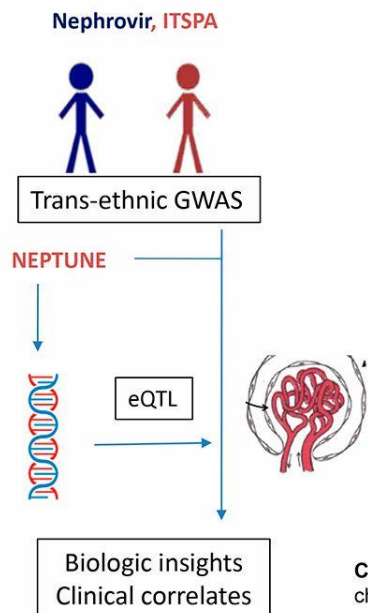
**CD20 FITC**

**CD40 PE-Cy7**

# Trans-ethnic, genome-wide analysis reveals immune-related risk alleles and their phenotypic correlates in pediatric SSNS

**RESULTS.** Three independent SNPs: 2 in *HLA-DR/DQ* region, 1 in *BTNL2* region; genes previously implicated in immune (dys)regulation

## METHODS



**CONCLUSION.** Risk alleles at these three loci may define a clinically distinct subtype characterized by an early disease onset and a relatively benign disease course.

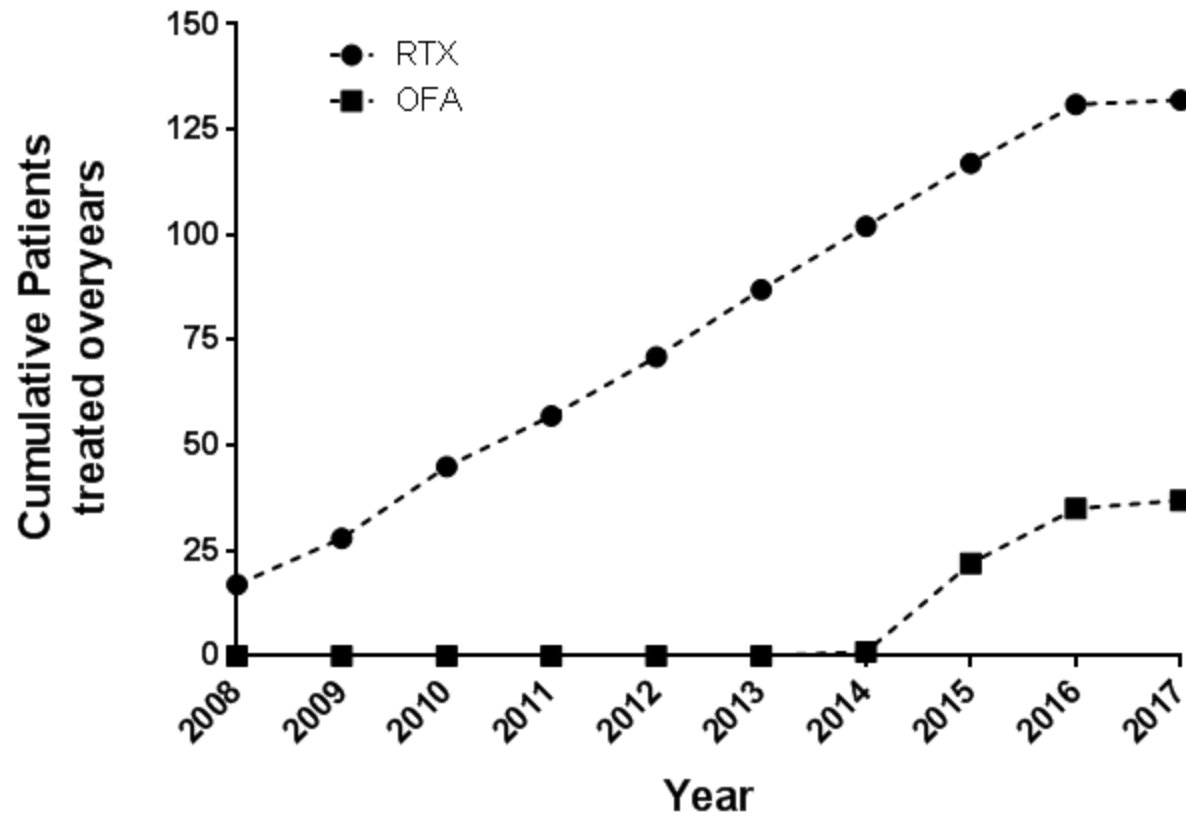
**JASN**  
JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY

# Safety

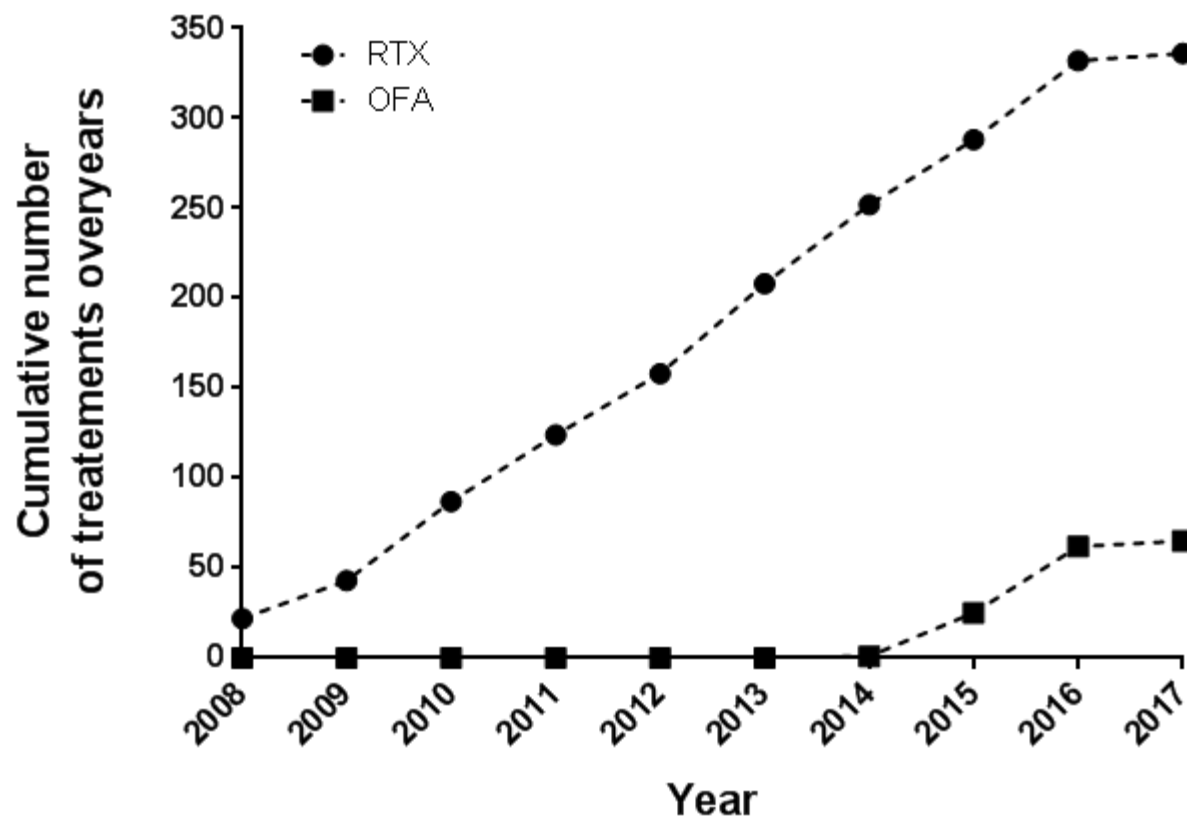
- Used in one million of patients with hematologic malignancies (first line / maintenance)
- First infusion adverse reactions (bronchospasm, cough, chills, rash, fever, headache); mild / absent thereafter
- Recent systematic review in RCTs of RA treated with biologic therapy (N = 29,423): no increased 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>		<b>Rituximab</b>		<b>Ofatumumab</b>
<i>Category</i>	<b>Low Steroid Dependence</b>	<b>High Steroid Dependence</b>	<b>Multi Drug Dependence</b>	<b>Multi Drug Dependence</b>
<i>Number of patients</i>	18	12	99	38
<b><i>Infusion reactions</i></b>				
Fever	0	0	2	1
Rash	0	0	7	7
Dyspnea	0	1	3	3
Hypotension/Hypertension	1	0	1	0
Cough/Itchy throat	0	0	5	<b>14</b>
Itch	0	0	0	2
GI symptoms	0	0	0	2
Need for treatment discontinuation	0	0	1	1
<b><i>Early adverse events (≤ 3 months)</i></b>				
Serum reaction/Arthritis	0	1	<b>5</b>	0
RALI/OALI	0	0	3	1
Pneumonia			1	1
Cutaneous manifestations	0	0	2	0
Severe Neutropenia	0	0	2	0
Infections	0	0	5	1
<b><i>Late adverse events</i></b>				
Neutropenia	0	1	1	1
Infections	0	0	2	4
Benign Neoplasia	0	0	1	0
Malignant Neoplasia	0	0	0	0
Neurological manifestation	0	0	1	1

## Delayed Side Effects linked with anti-CD20 antibodies

### *-Infections*

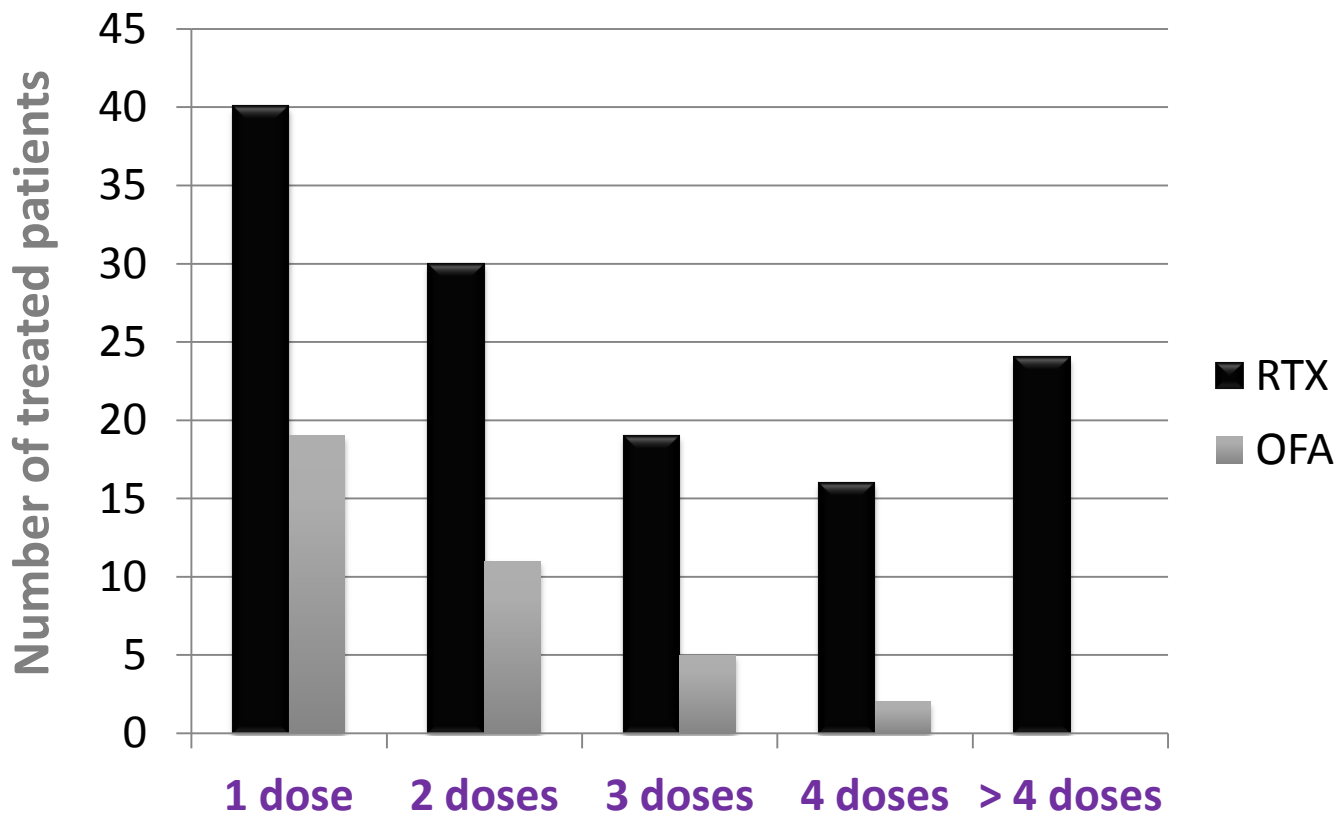
- n 1 purulent folliculitis
- n 1 pyelonephritis (complicated by renal abscess)
- n 1 dental abscess
- n 2 tonsillitis (1 complicated by tonsils abscess)
- n 2 Herpes simplex infections (Herpetic stomatitis)
- n 2 Urinary Tract Infections

### *-Rituximab/Ofatumumab Associated Lung Injury*

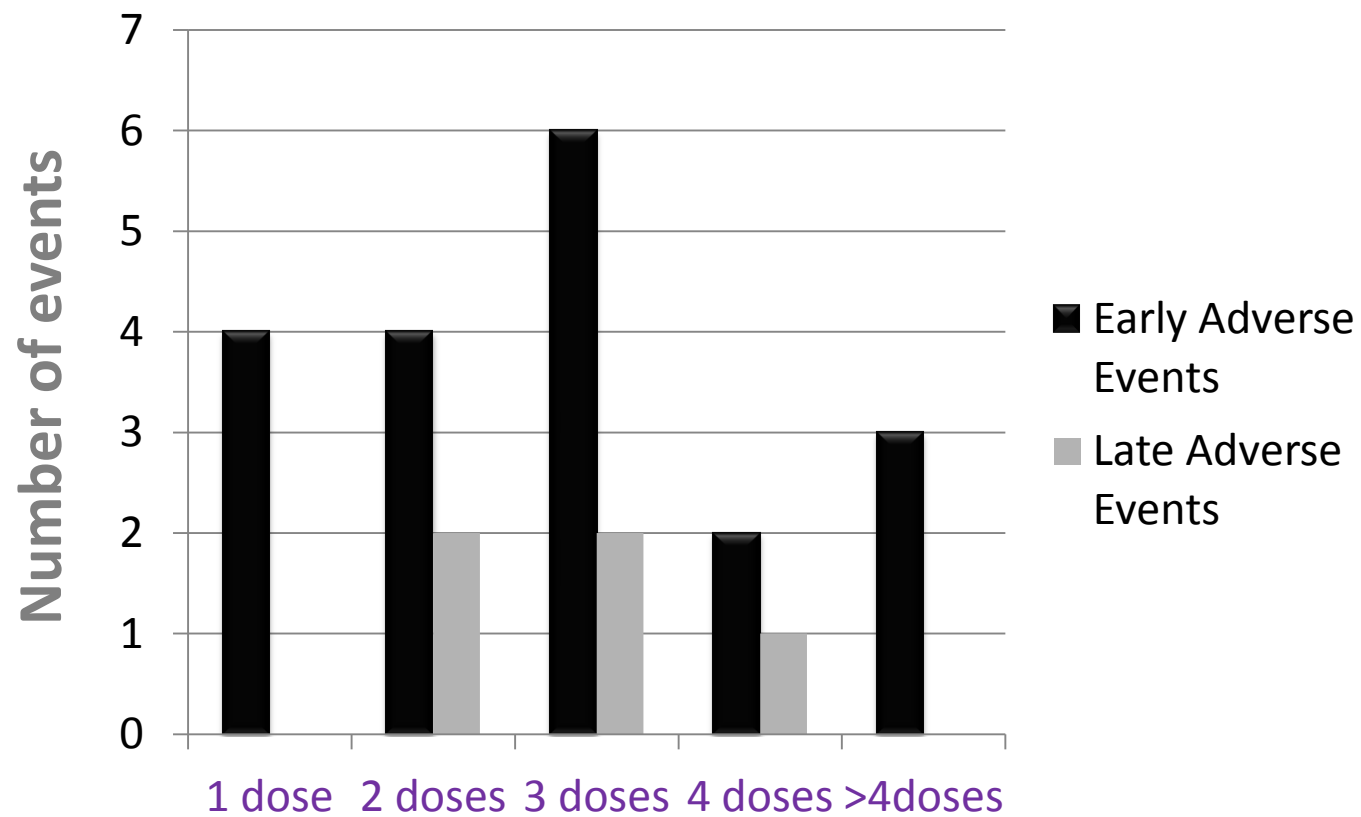
- n 2 RALI, n 1 OALI

### *-Benign Neoplasia*

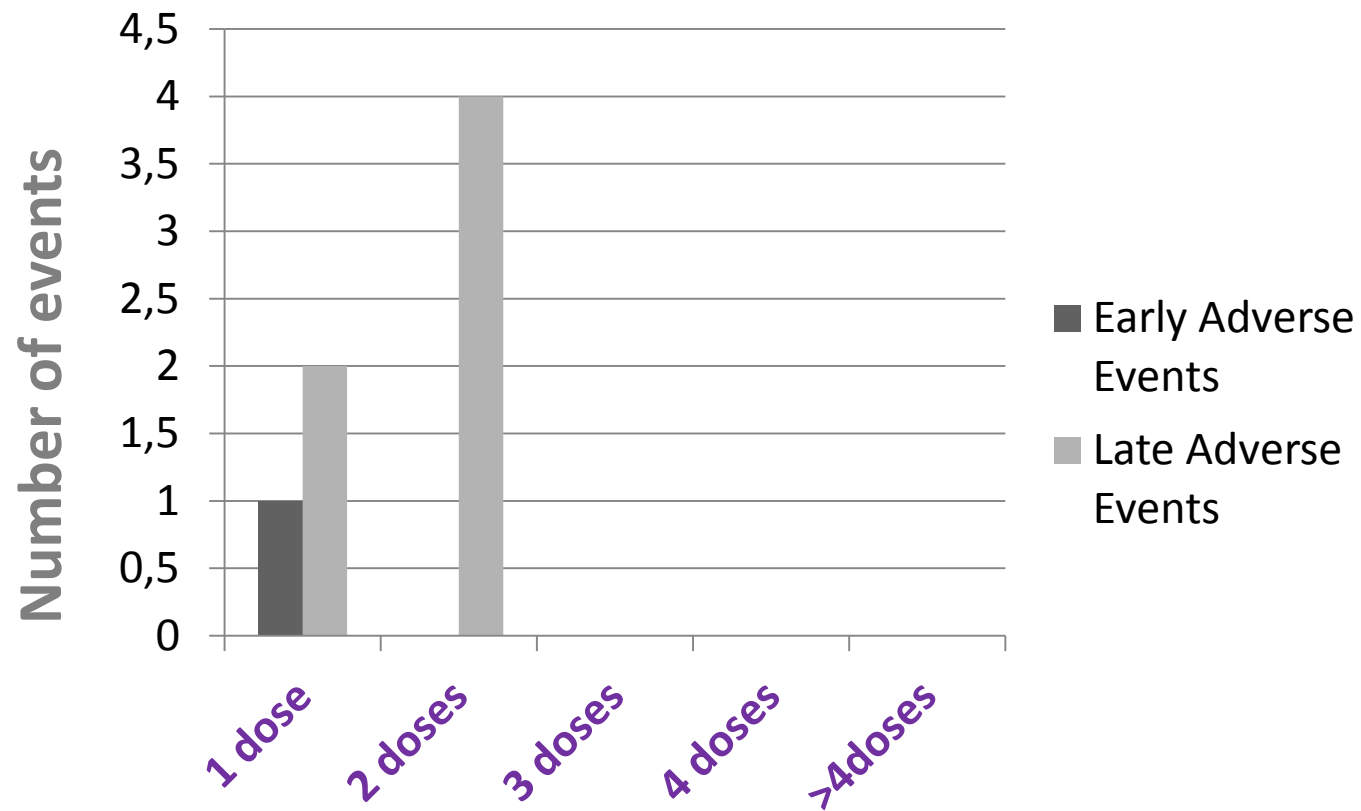
- n 1 desmoid fibromatosis



## Rituximab



## Ofatumumab



## Literature

**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

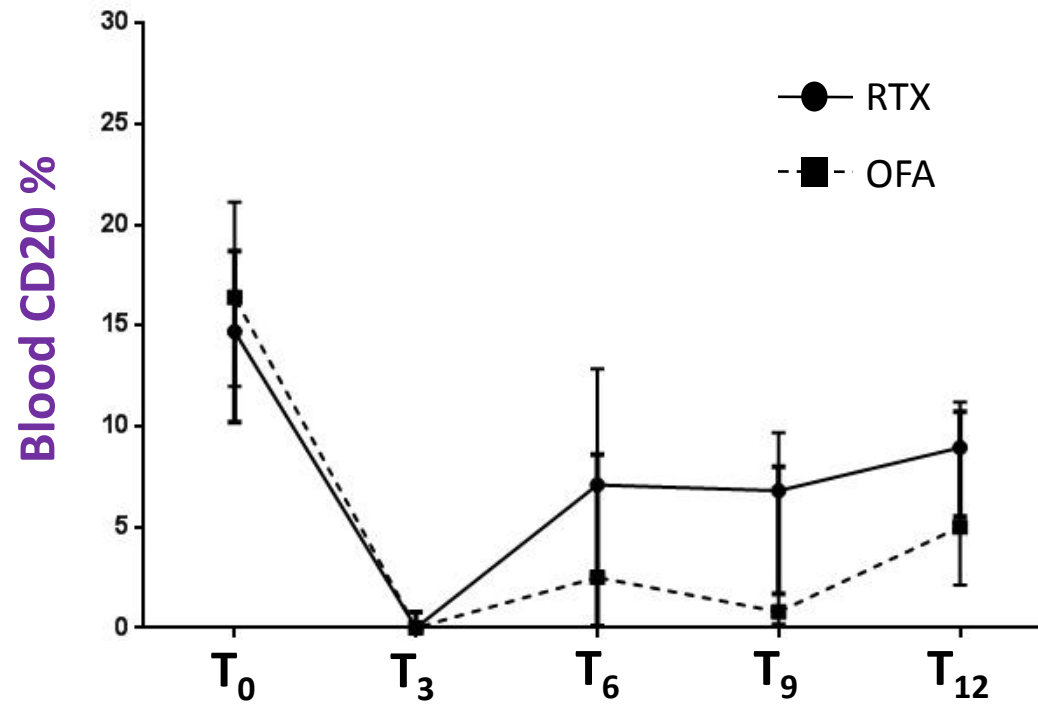
**A mild form of rituximab-associated lung injury in two adolescents treated for nephrotic syndrome.**

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

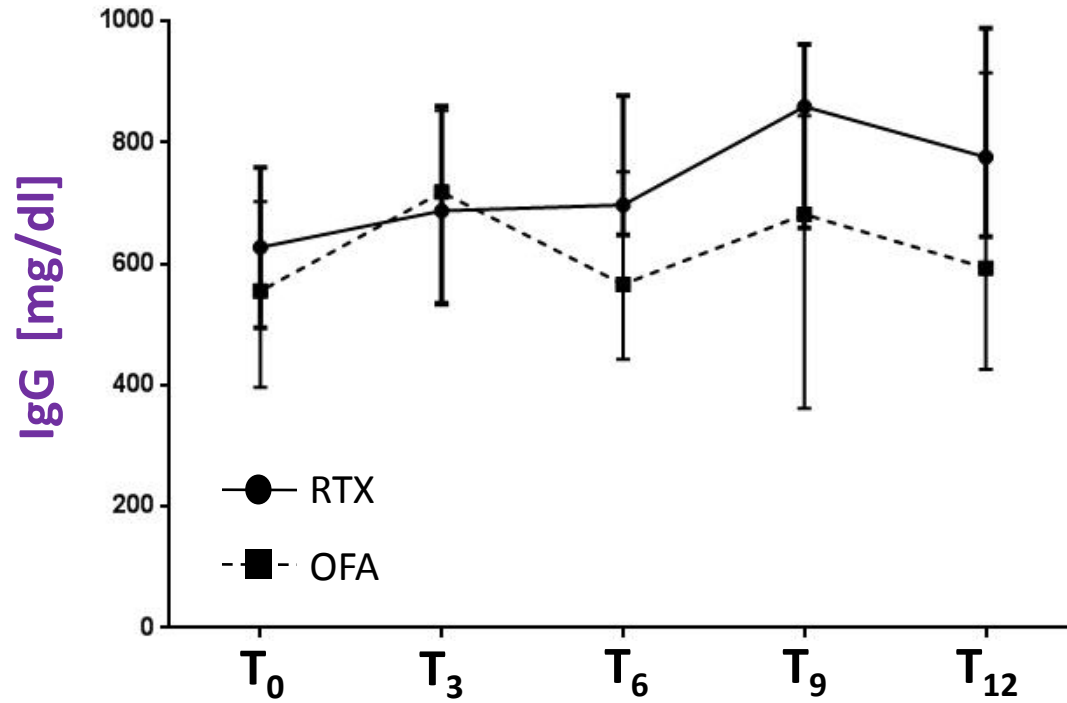
**Br Med J Case Rep.** 2015 Dec 9;2015. pii: bcr2015212694. doi



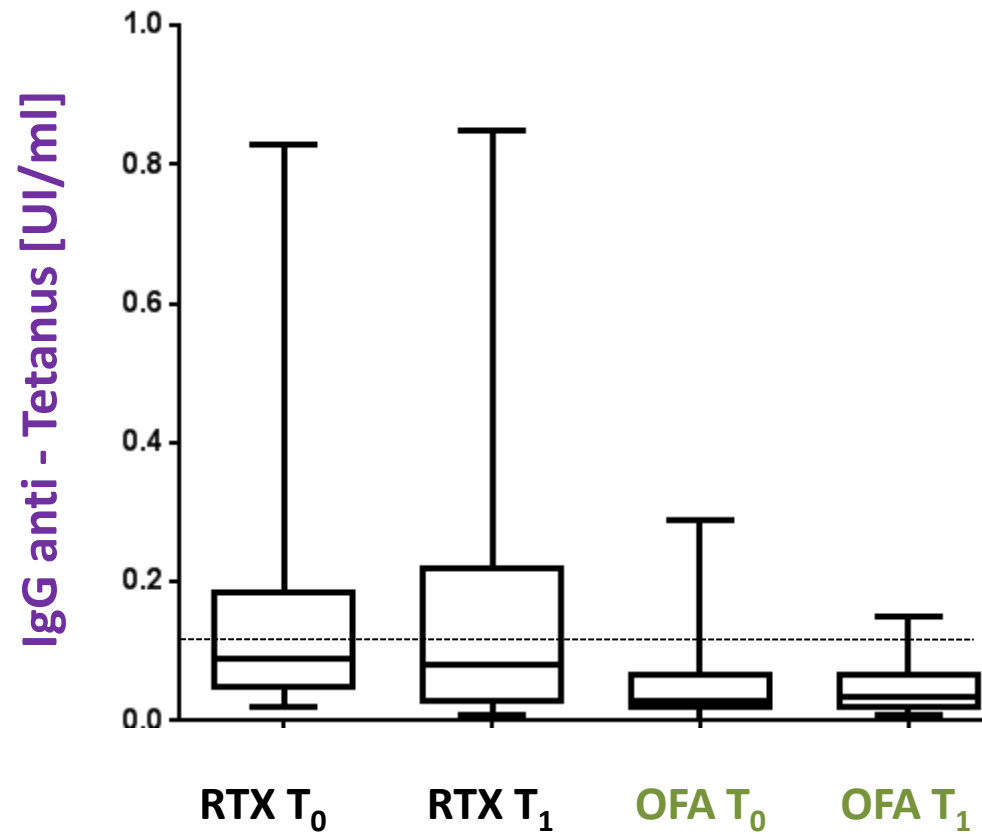
# CD20 count



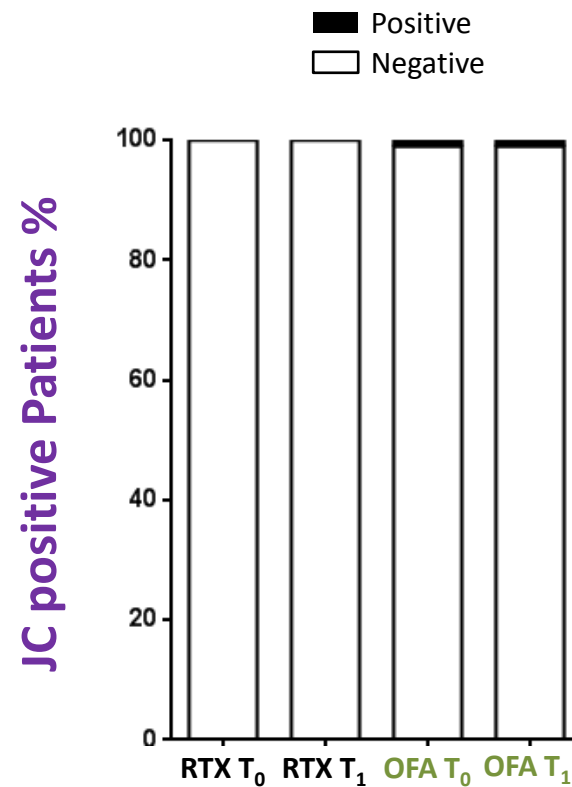
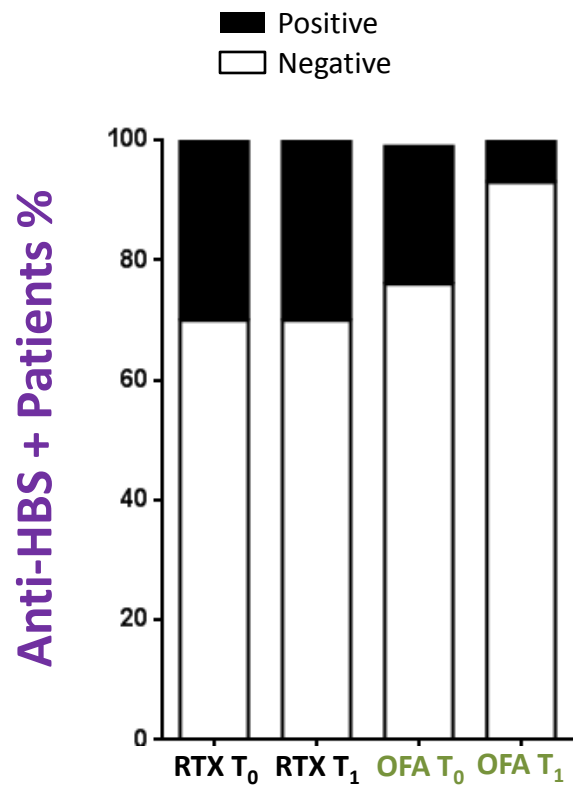
# IgG serum levels



# Serum anti -tetanus IgG



# anti-HBS



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