ABIRISK

EIP
European Immunogenicity Platform

"Role of T-cells in immunogenicity of biologics in Intestinal Bowel disease"

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GA²LEN
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Network of Excellence

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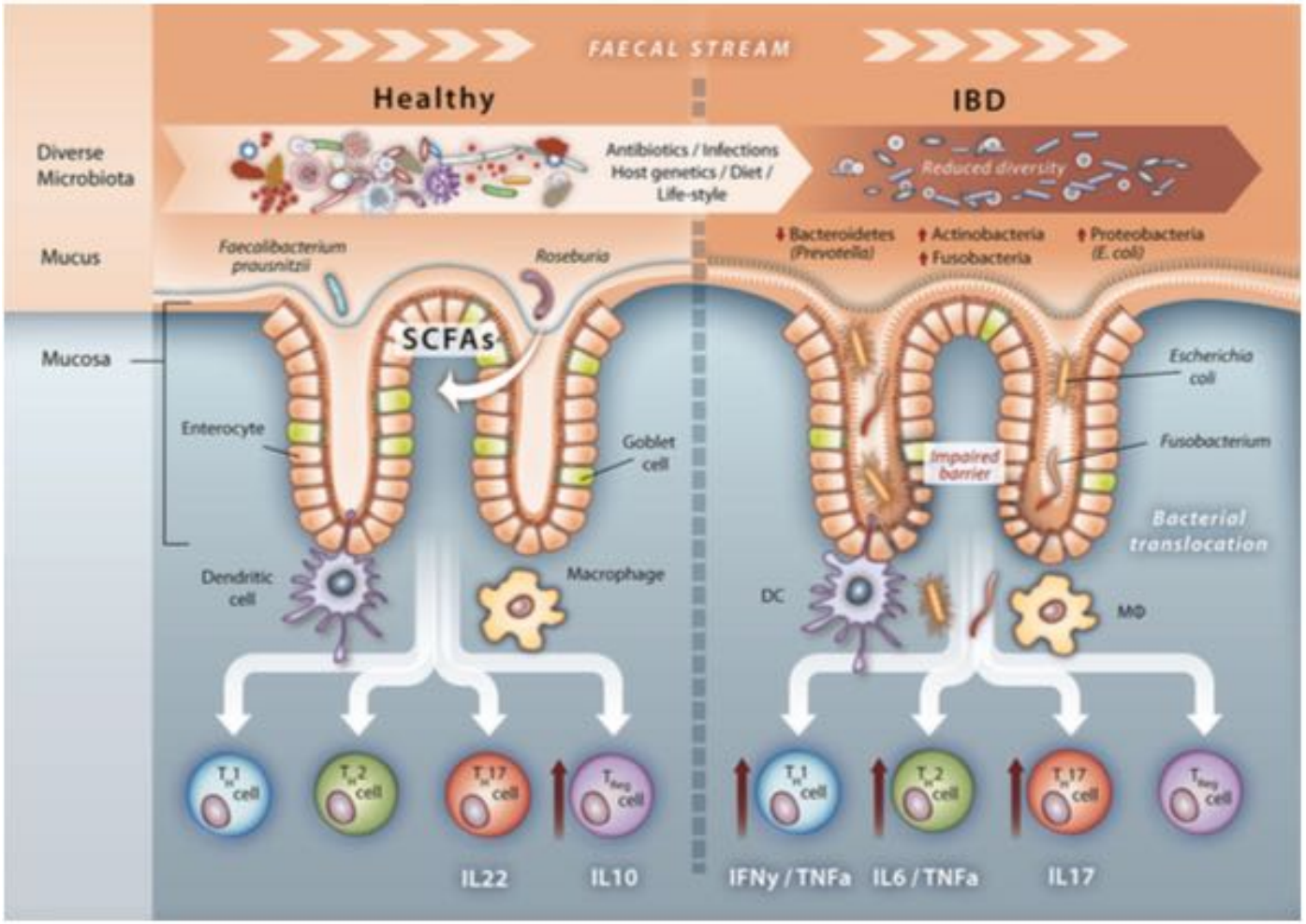
Disclosure

In relation to this presentation, I declare that there are no conflicts of interest.

A conflict of interest is any situation in which a speaker or immediate family members have interests, and those may cause a conflict with the current presentation. Conflicts of interest do not preclude the delivery of the talk, but should be explicitly declared. These may include financial interests (eg. owning stocks of a related company, having received honoraria, consultancy fees), research interests (research support by grants or otherwise), organisational interests and gifts.

Human inflammatory bowel disease (IBD) is a spontaneously relapsing, immunologically mediated disorder of the gastrointestinal tract, characterized by uncontrolled inflammation resulting from inappropriate and persistent activation of the mucosal immune system.



Biologics in IBD

TNF-a Blockers

Infliximab

Adalimumab

Certulizumab

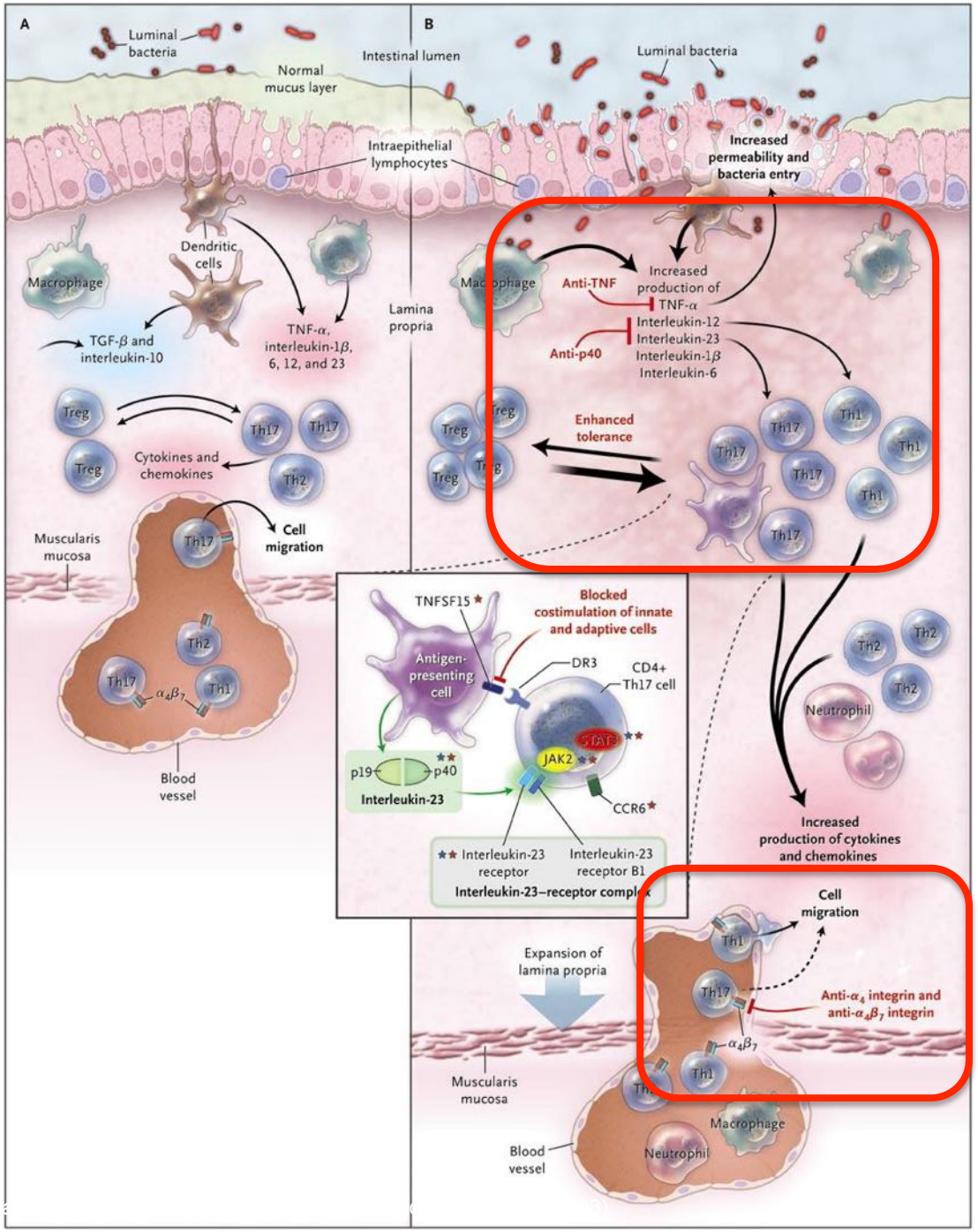
Golimumab

Anti-p40 mAbs

Ustekinumab

Anti- $\alpha 4\beta 7$ integrin

Vedolizumab



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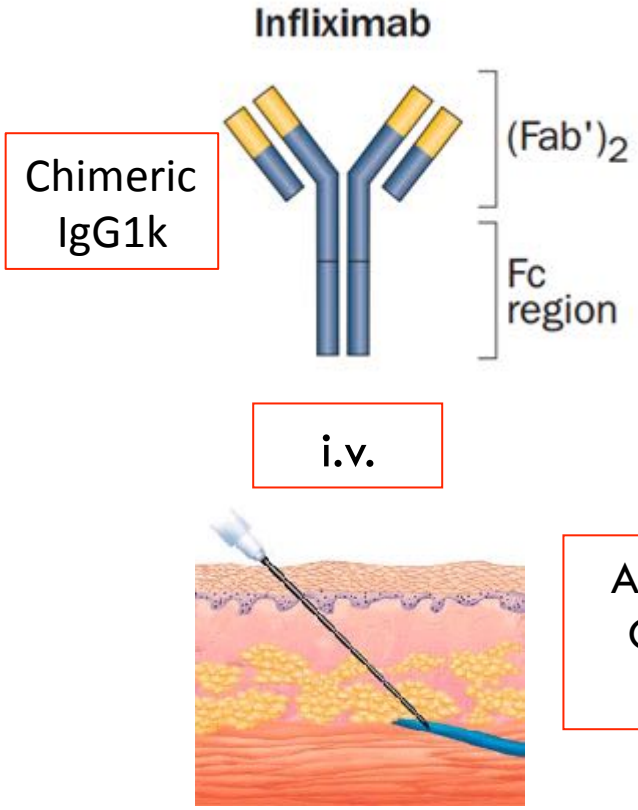


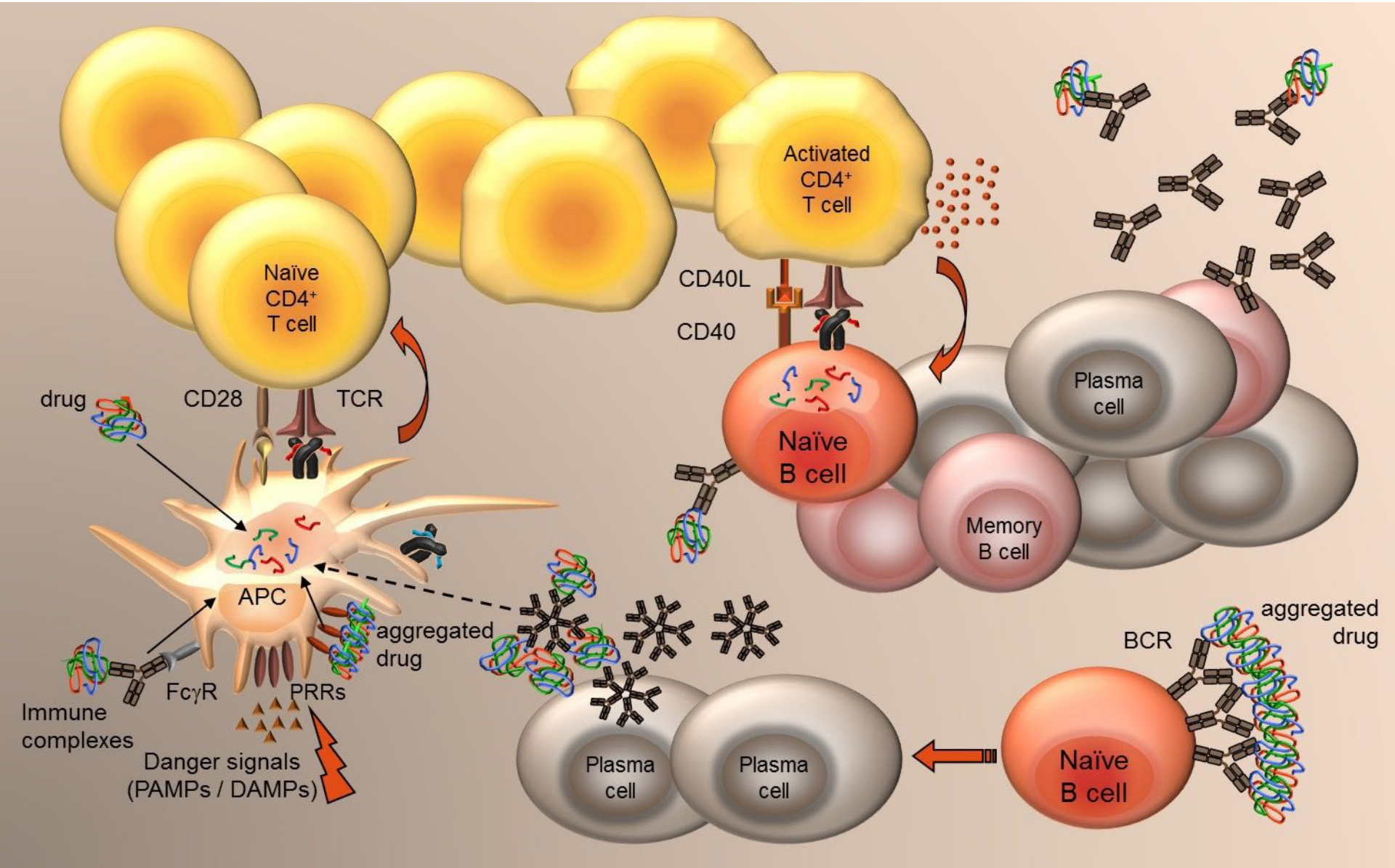
ADA in 30 % Of Treated patients

VIRTUALLY ALL BIOLOGICALS ARE IMMUNOGENIC

- **Large and complex proteins**
- **Different degree of glycosylation**
- **Presence of xenoantigens**
- **Partial or complete human sequence (allotypes)**
- **Neoantigens on junction-sites**
- **Repetitive Idiotype (mAbs)**
- **Cross-reactivity with recall epitopes**

(Schouwenburg et al. Nature Reviews, Rheumatology, 2013)

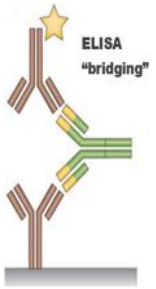
The Immune response to Biologics

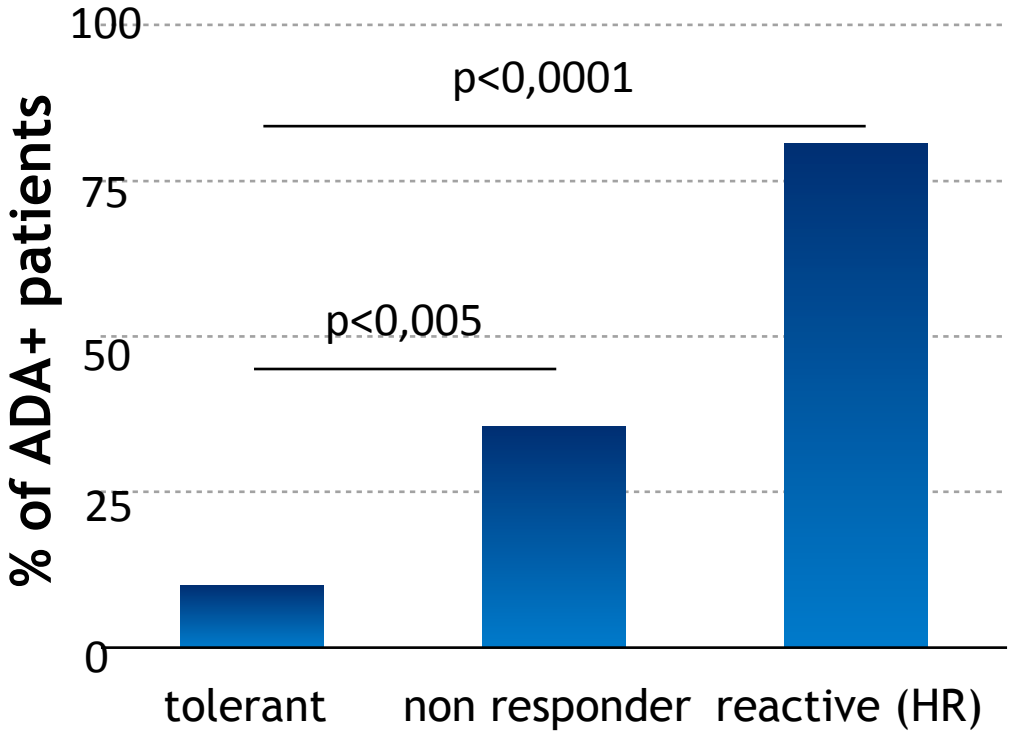


ANTI-DRUG ANTIBODIES (ADA): Cross sectional study

346 Infliximab-treated patients

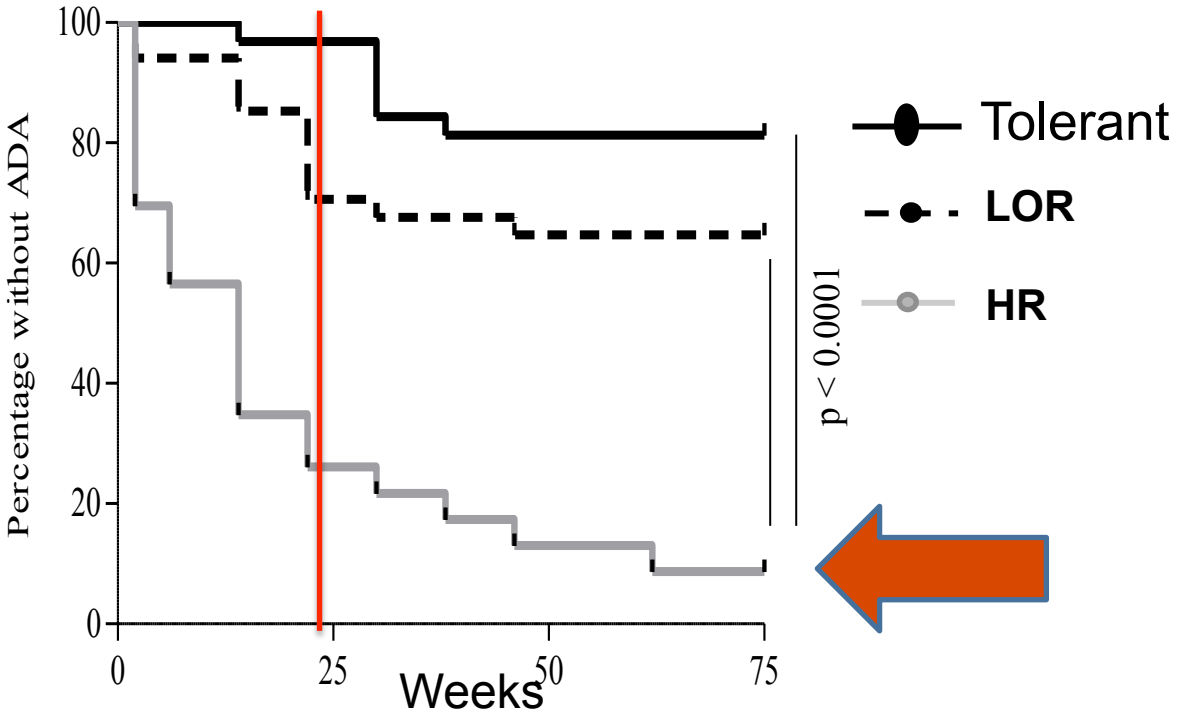
Tolerant n=200
Non responder n=93
Reactive n=53





(personal unpublished data)

ADA AND CLINICAL OUTCOMES
Longitudinal Study



*(**Nencini F et al,** submitted)*

From 2007 to 2015,
91 IFX-treated patients
Were longitudinally monitored
(up to 40 infusions)*
for ADA development and clinical outcome
[about 1200 serum samples evaluated]

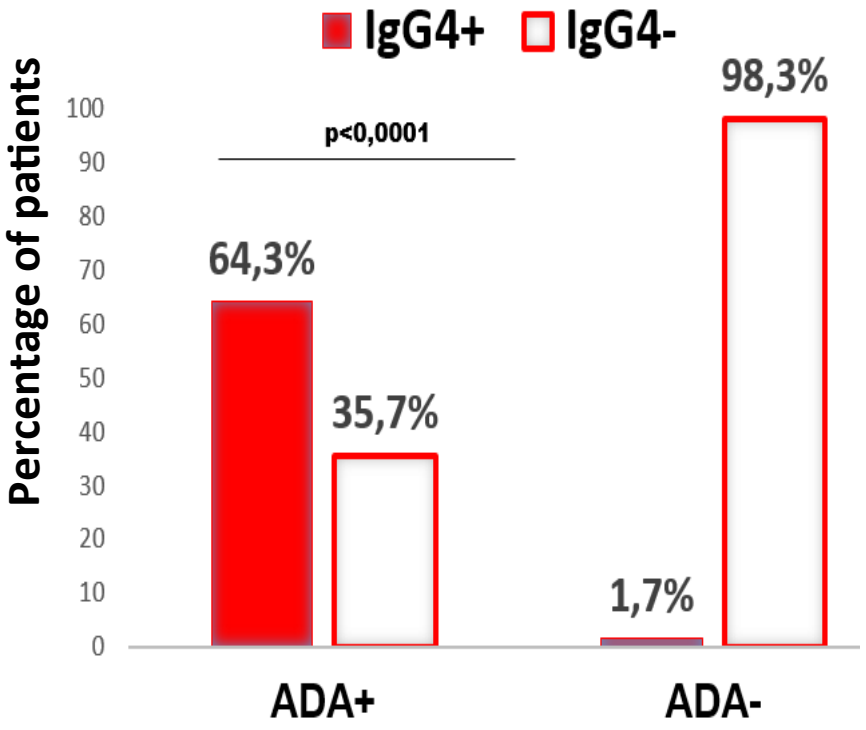
* Serum collected before each infusion

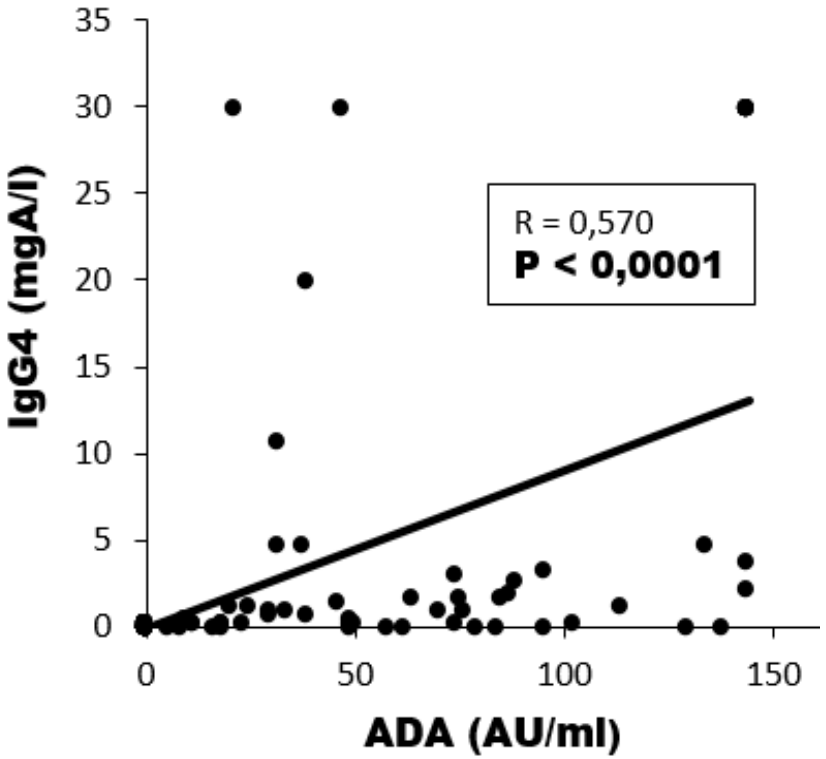
- 46.8 % of IFX-treated patients developed ADA,
- Among ADA+ patients, the large majority of them (85%) developed ADA within the first 5 infusions (about 24 weeks)

Anti-IFX IgG4 are mainly detectable in ADA+ patients

ASSAY: Modified IFX-coated ImmunoCAP provided by Thermo Fisher

PATIENTS: 172 exposed subjects with different diseases (56 ADA+ ; 116 ADA-)

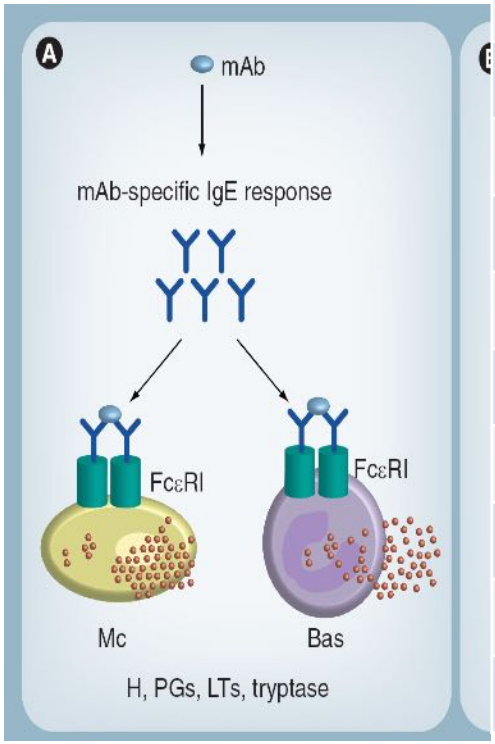




(Vultaggio A et al, in preparation)

IgE ADA ISOTYPE: A TRUE ALLERGIC REACTION

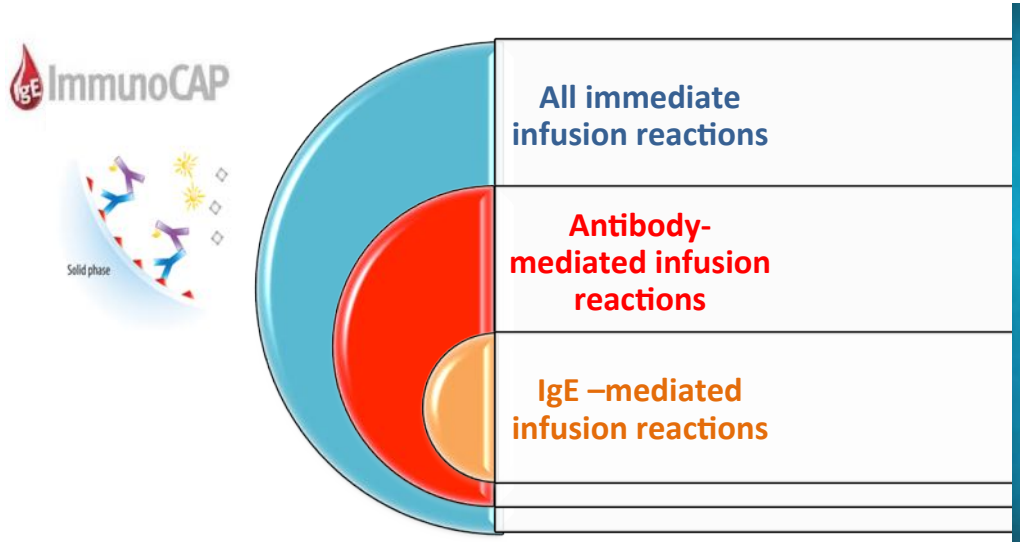
IgE-mediated reactions have been described towards several BAs

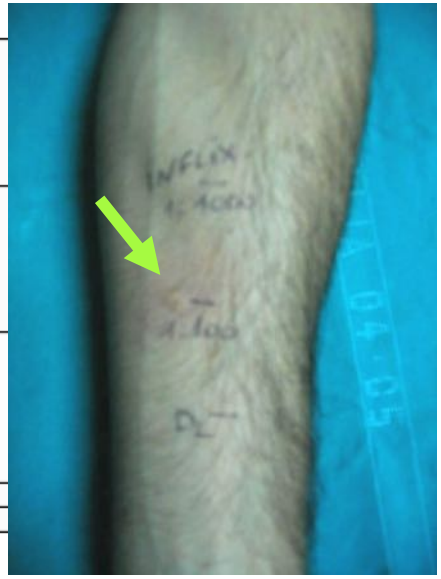


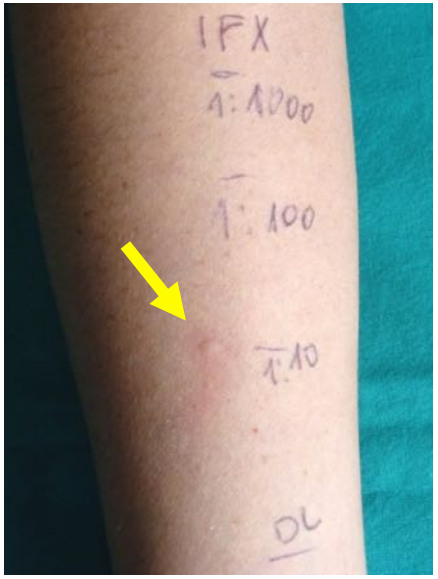
Drug	In vivo	In vitro	Ref
Muromonab	No	Yes	Georgitis, 1991
Cetuximab	No	Yes	Chung, 2008
Tocilizumab	No	Yes	Stubenrauch, 2010
Basiliximab	No	Yes	Baudouin , 2003
Omalizumab	Yes	No	Price, 2007
Etanercept	Yes	No	Bavbek, 2011
Adalimumab	Yes	No	Paltiel, 2008
Rituximab	Yes	No	Brennan , 2009
Natalizumab	Yes	Yes	MunozCano, 2010
Infliximab	Yes	Yes	Vultaggio, 2010
Rituximab	Yes	Yes	Vultaggio, 2012

Maggi E, Vultaggio A, Matucci A, *Exp Rev Clin Immunol 2011*

Vultaggio A et al, *Allergy 2010*
Matucci A et al, *Clin Exp Allergy 2013*







60 patients with immediate reactions to IFX

86.6% (n=52) with detectable non isotype-specific ADA (ADA+)

25% (n=13) with detectable IFX-specific IgE (IgE+)

(21.6% of all reactions) highly correlating with SPT

Personal unpublished data

Remarks

The presence of high affinity ADA of IgG/ IgE isotypes and of IgG1/IgG4 subclasses in HR patients indicates that the immune response towards IFX is essentially a T-cell dependent phenomenon
(Baker MP et al, Slf nonself, 2010)

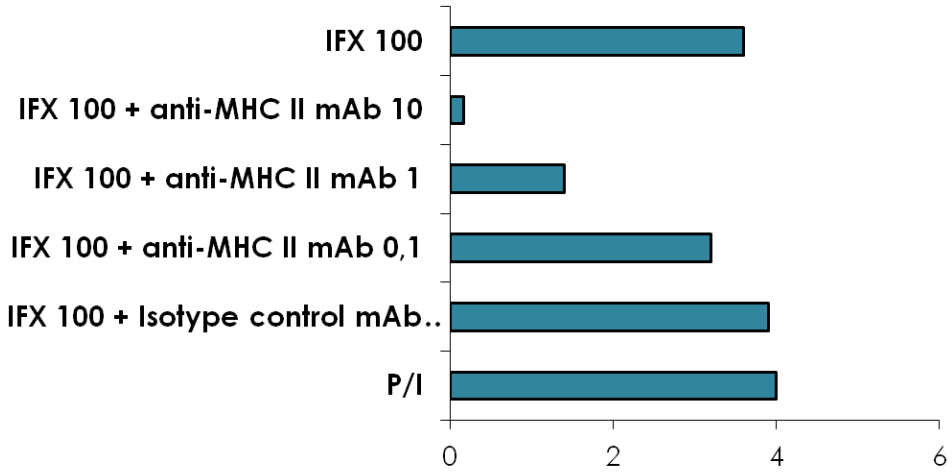
Topics

- T cell response to IFX is related to ADA positivity and HR in exposed patients
- A number of IFX-specific memory T cells produces biologically active IL-10
- Role for drug-induced IL-10 in the detection of cellular response to IFX and drug sensitization
- Potential biomarkers predicting drug sensitization, ADA induction and clinical outcomes to IFX

THE PROLIFERATIVE RESPONSE TO IFX IN EXPOSED PATIENTS

Clinical outcome:
Tolerant = 23
Non responder= 26
Reactive = 22

ADA status
N=39 ADA-
N=32 ADA+



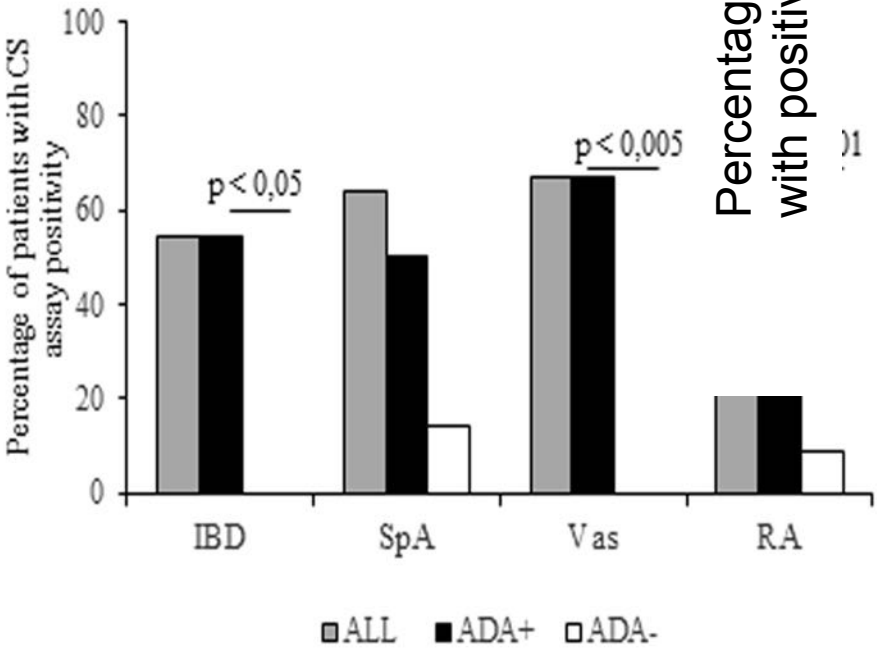
. The proliferative response was detectable even some years after drug interruption

. The proliferative response to IFX correlates significantly with ADA levels in a disease independent manner

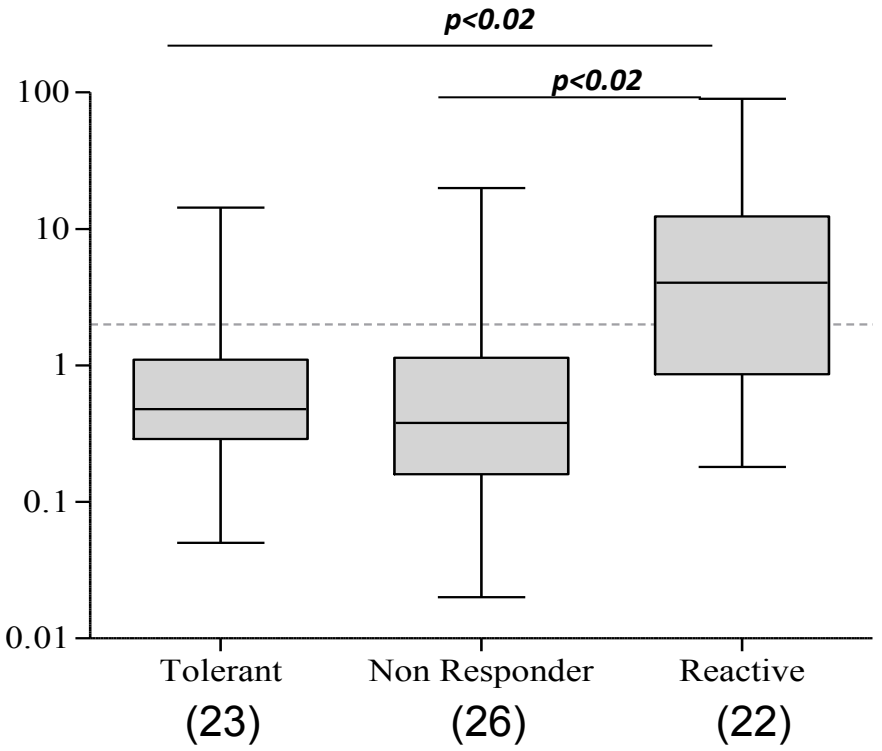
(Vultaggio A, et al, Clin Exp Immunol 2016)

The proliferative response to IFX correlates with ADA+ positivity and clinical outcomes, irrespective of diseases

IBD Inflammatory bowel diseas
SpA Spondiloarthropathies;
Vas Vasculitis;
RA Rheumatoid arthritis.

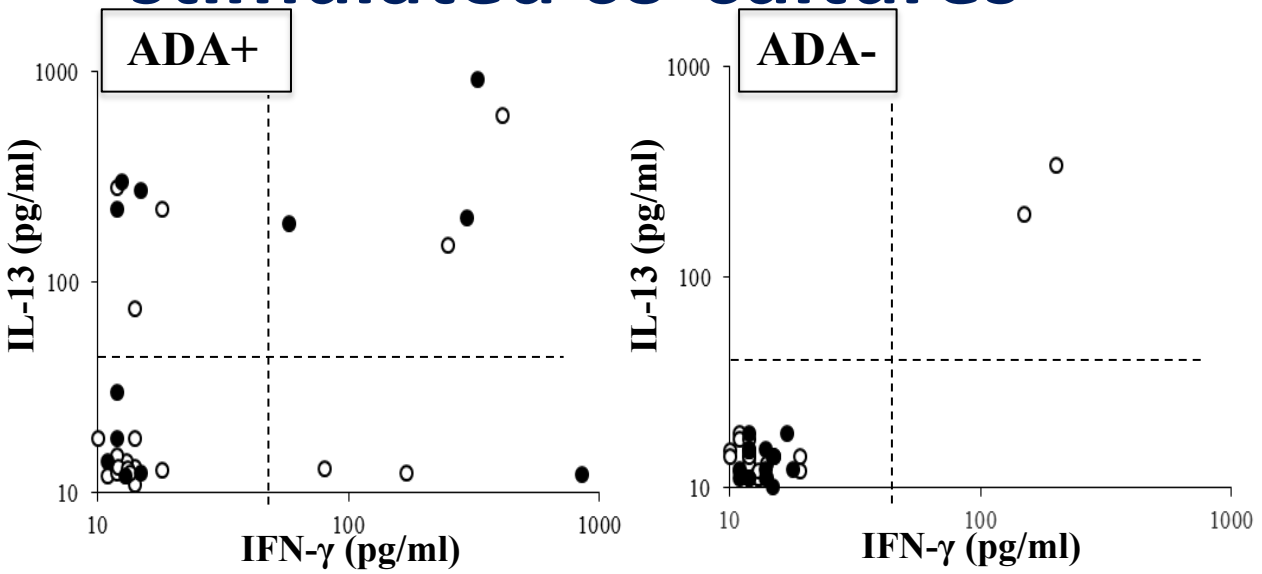


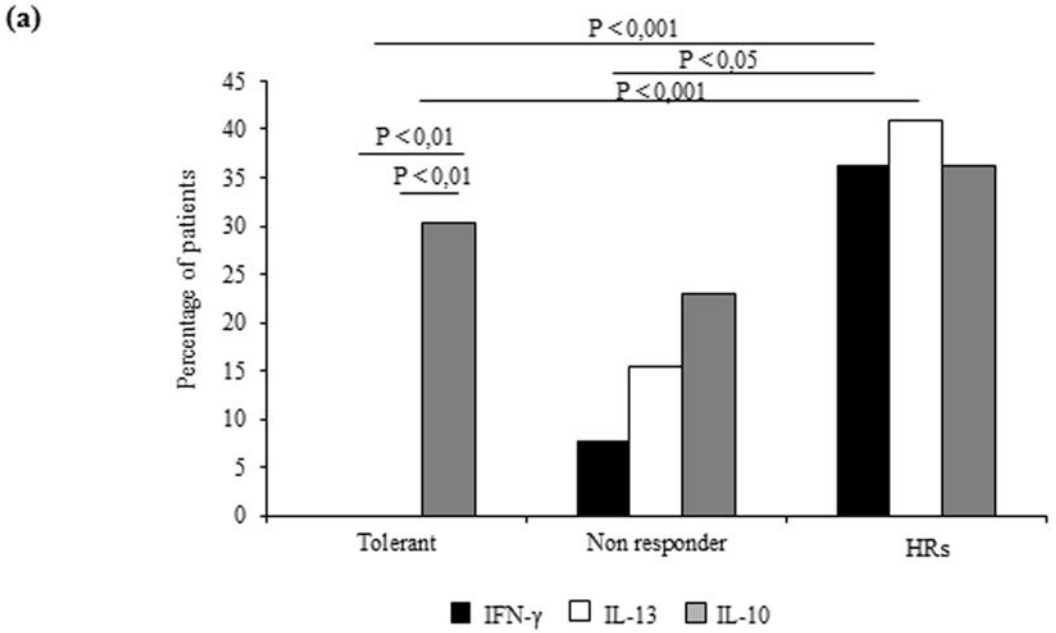
Percentage of patients with positive CS assay



IL-10 is the frequently detected in IFX-stimulated co-cultures

- IL-10 positive supernantants



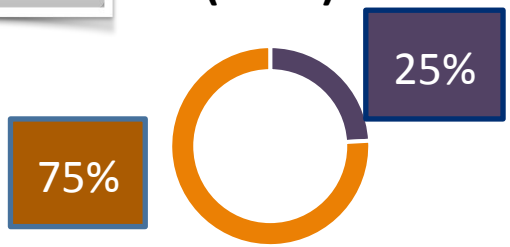


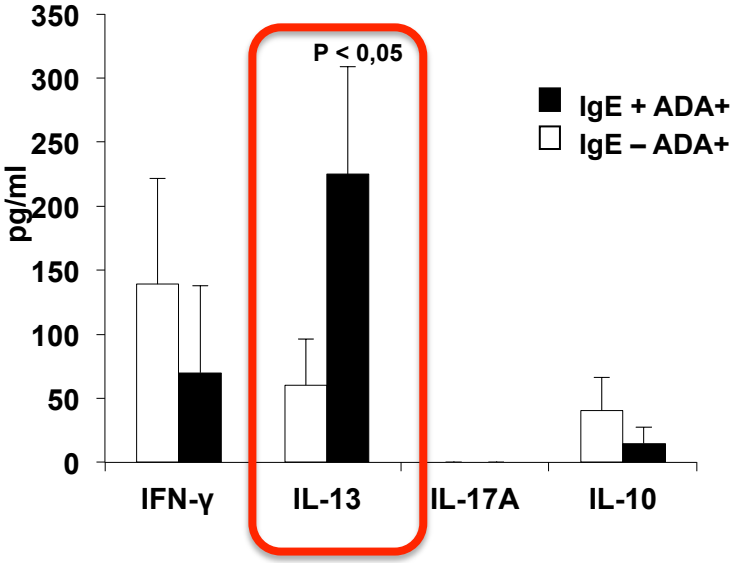
In Coculture assay (IFX-pulsed DC + CD4+ T cells) IL-10 was the most frequently detectable cytokine in supernatants

TYPE 2 CYTOKINES ARE PRODUCED IN PATIENTS WITH IgE ADA

INFLIXIMAB

HRs patients (n=22)

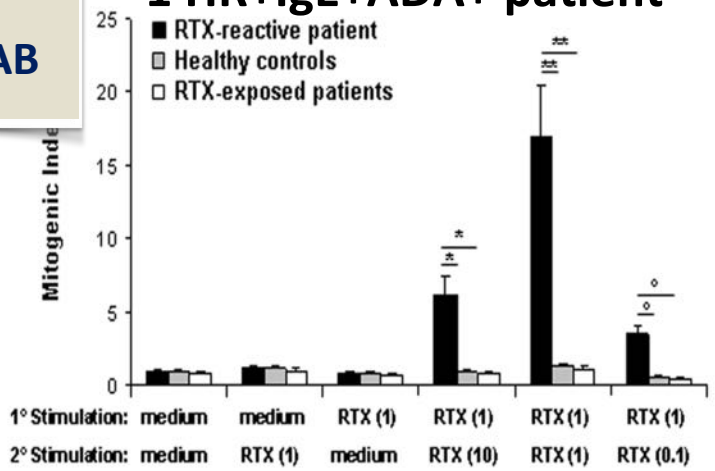




(Vultaggio A et al, Clin Exp Immunol 2016)

RITUXIMAB

1 HR+IgE+ADA+ patient



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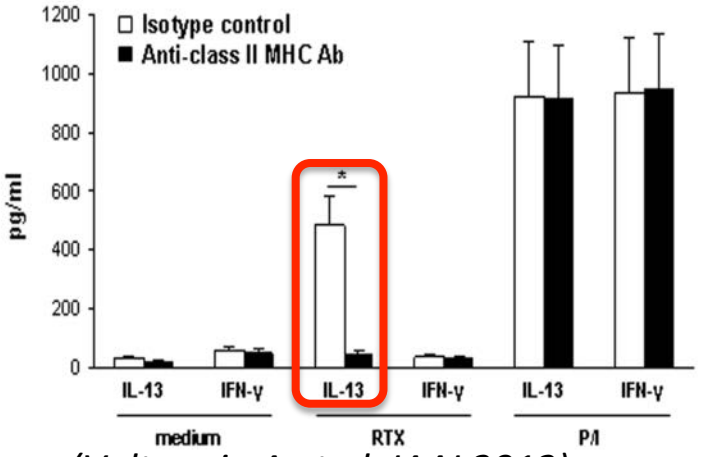

(Vultaggio A et al, IAAI 2012)

Remarks

The cellular response to IFX is related with the clinical outcomes, as LOR and HR

The in vitro response to IFX associates with the production of IL-10 in tolerant ADA- patients and of IL-10 plus adaptive cytokines in HR+ ADA+ patients

A Th2-like response to the drug is present in HR+ IgE+ patients, whereas an anti-IFX type 1 response characterizes HR+ IgE- patients. IL-10 is upregulated in both group of patients.

Topics

- T cell response to IFX is related to ADA positivity and HR in exposed patients
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TNFa-BLOCKERS (TNFb) INDUCE REGULATORY CELLS AND CYTOKINES

IN HEALTHY DONORS (in vitro)

- TNFb inhibit T cell proliferation, IFN-g production and expand Treg cells in PBMC by blocking TNFa-TNFR interactions (Aspalter RM et al, J Leukoc Biol, 2003)

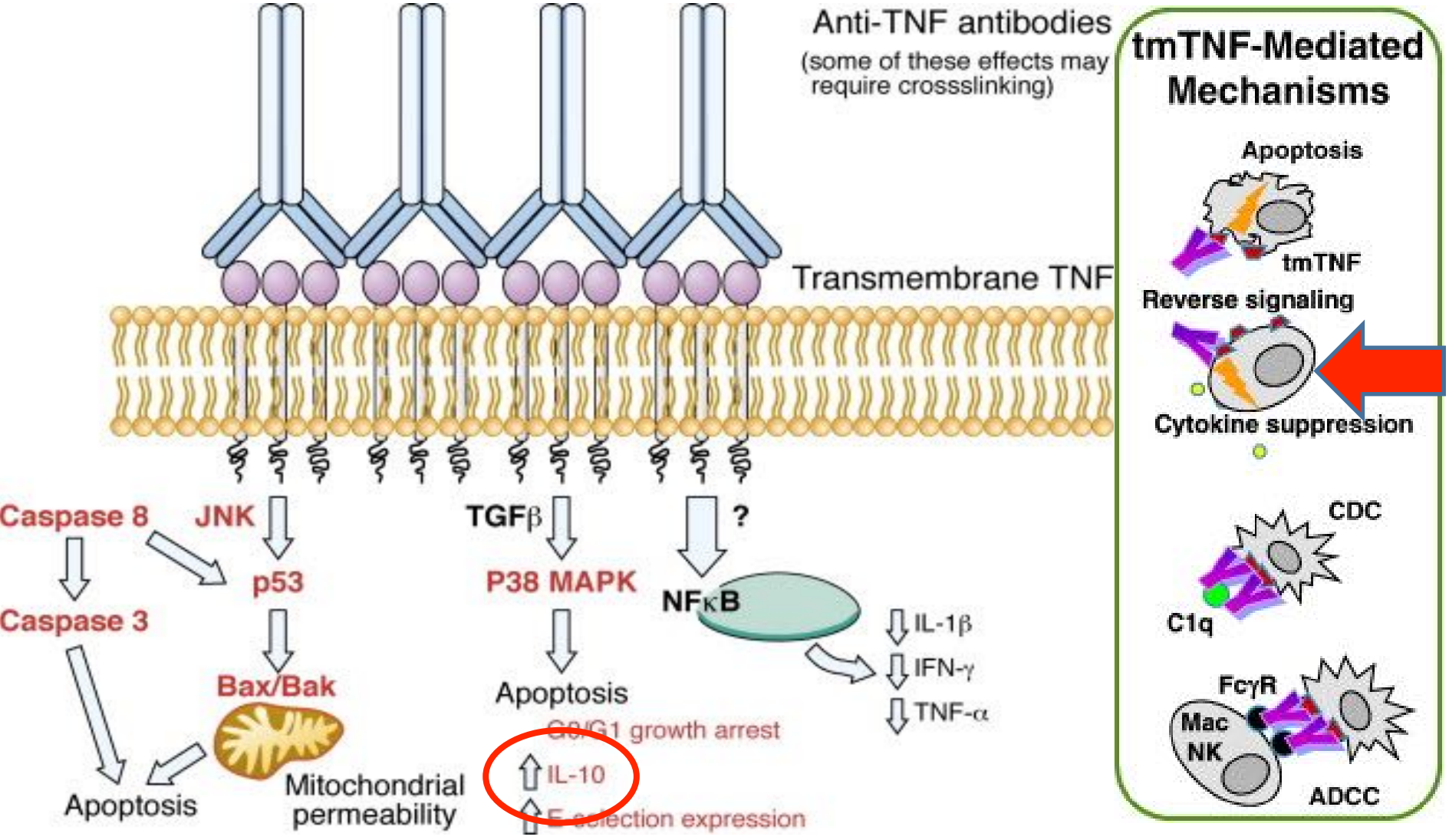
- TNFb enhance the suppressive function by favouring the development of antigen-specific IL-10+ Th cells and the regulatory gene profile in TNFb-treated T cells (Kleijwegt FS et al, J Immunol, 2010, Boks MA et al, Clin Immunol 2014)

IN PATIENTS (ex vivo)

- Contradictory results on the in vivo effects of TNFb on Treg cells from RA or Sarcoidosis patients (Valencia X et al, Blood, 2006; Nadkarni S et al, J Exp Med 2007, Nie H et al, Nat Med 2013; Byng − Maddiak R et al, Rheumatology −Oxford- 2015, Verwoerd A et al, Clin Exp Immunol, 2016)

- TNFb increase IFN-g levels from peripheral T cells and enhance IL-10 serum levels in RA patients (Aerts NE et al, Rheumatology −Oxford- 2010, Ehrenstein MR et al, J Exp Med 2004)

Pathways of TNF-α blockers



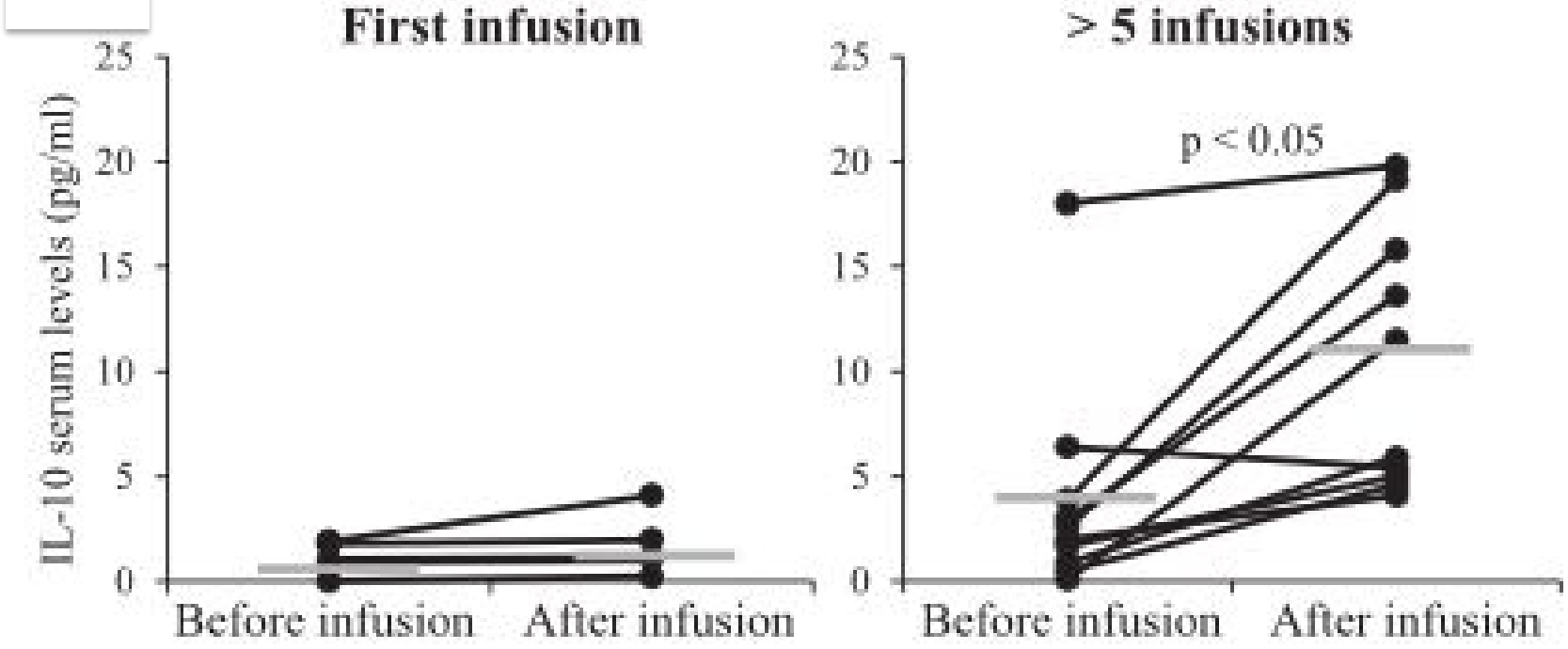
The Journal of Immunology

IL-10–Producing Infliximab-Specific T Cells Regulate the Antidrug T Cell Response in Exposed Patients

Alessandra Vultaggio, Francesca Nencini, Sara Pratesi, Daniele Cammelli, Maria Totaro, Sergio Romagnani, Enrico Maggi and Andrea Matucci

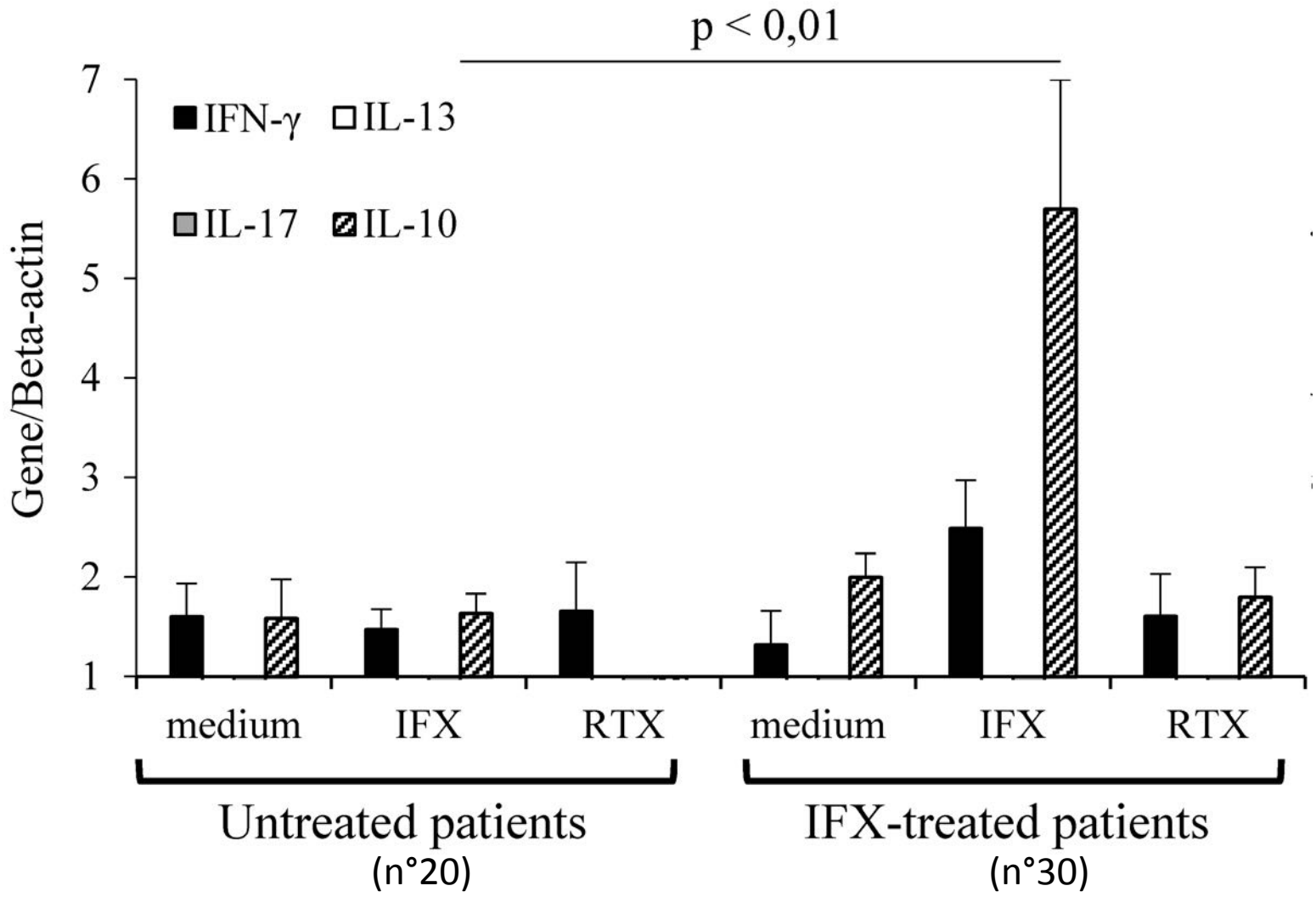
J Immunol 2017; 199:1283-1289; Prepublished online 17 July 2017;

IFX treatment induces IL-10 production in vivo



Vultaggio A et al, J Immunol, 2017

Cross sectional study: IL-10 mRNA is upregulated In drug-stimulated PBMC from exposed patients



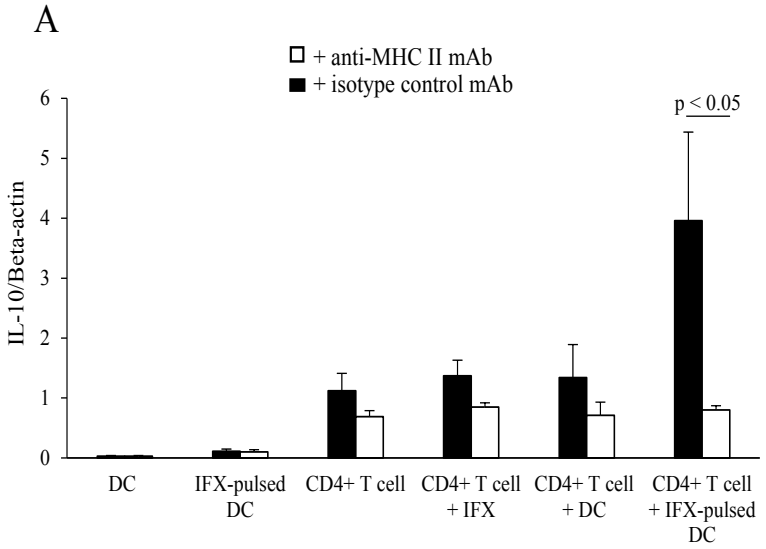
Vultaggio A et al, J Immunol, 2017

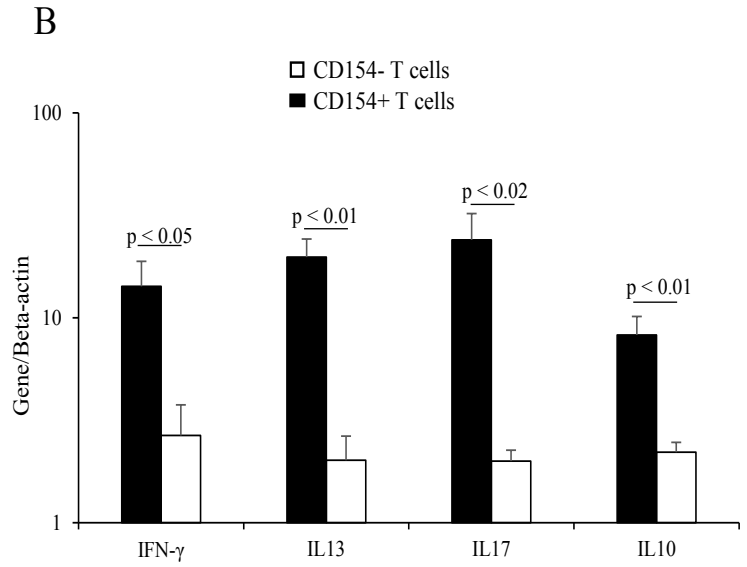
T CELL INVOLVEMENT IN IFX-INDUCED IL-10 PRODUCTION

A

Coculture assay (DC/T cells obtained from n=4 patients)

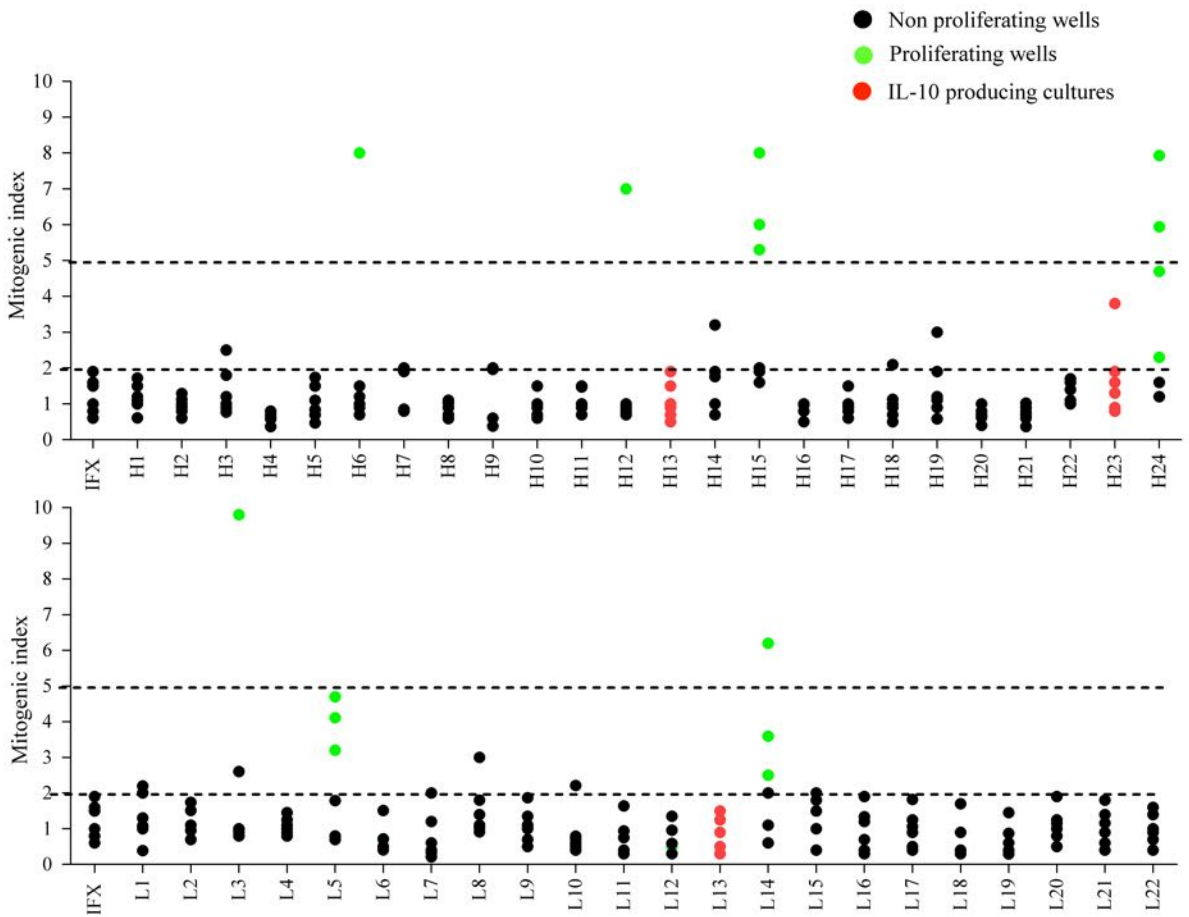
IFX-induced TCL from PBMC of treated patients (2 steps of stimulation) and then CD154(CD40L)+ T cells were obtained





T CELL INVOLVEMENT IN IFX-INDUCED IL-10 PRODUCTION

PBMC from one ADA+ patient
In vitro stimulated with the drug or a panel of 46 overlapping (10 mer) 15 mer-peptides covering the entire VH and VL domains of IFX

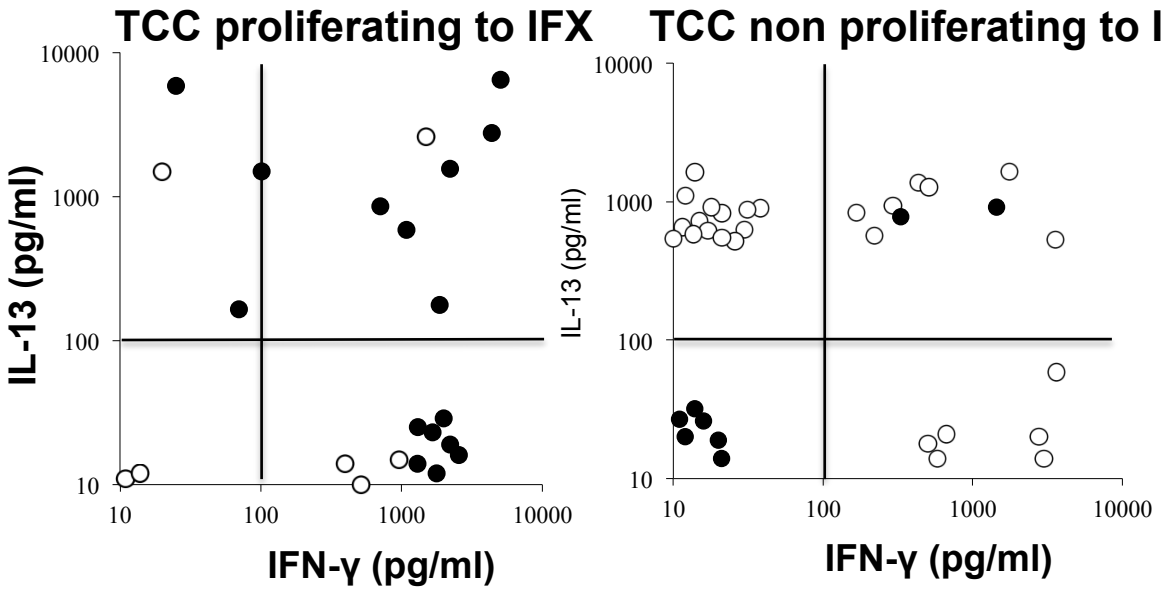


(Vultaggio A et al, J Immunol, 2017)

T CELL INVOLVEMENT IN IFX-INDUCED IL-10 PRODUCTION

Total TCC	188
Clonal Efficiency*	24.5%
TCC CD4+ CD8-	156 (83%)
TCC CD8+ CD4-	28 (14.9%)
TCC γδ CD4- CD8-	4 (2.1%)
TCC CD4+CD39+CD73+	13 (7%)
IFX-Specific TCC:	58 (29,8%)
Proliferating TCC	23 (12.2%)
Non proliferating cytokine-producing TCC	35 (18.6%)

● IL-10 producing TCC



IFX-specific T cell clones (TCCs) were generated (from 1 ADA+ patient)

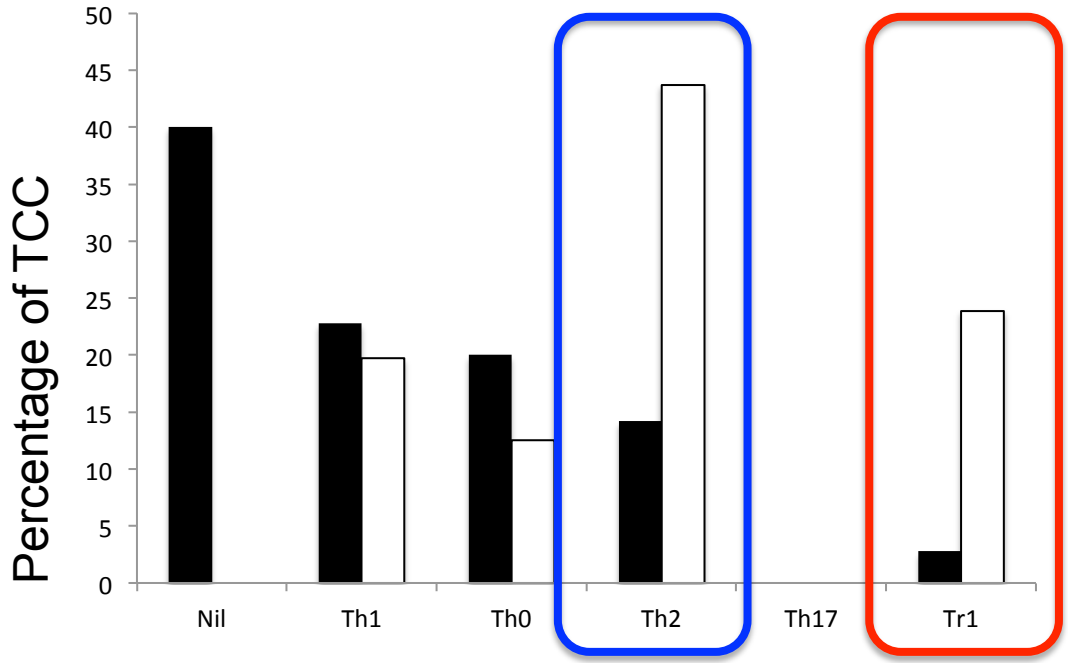
(Vultaggio A et al, J Immunol, 2017)

IL-10 and IL-13 are frequently produced by IFX-specific T cell clones

Methods: Limiting dilution procedure of cloning from blasts of T cell lines derived from IFX stimulated PBMC from 5 HR patients

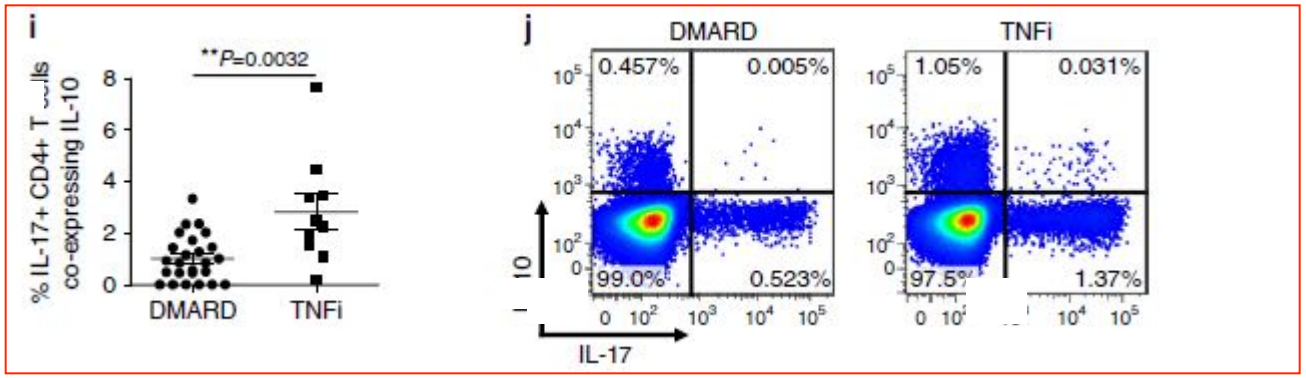
TCC: analysed: >1000

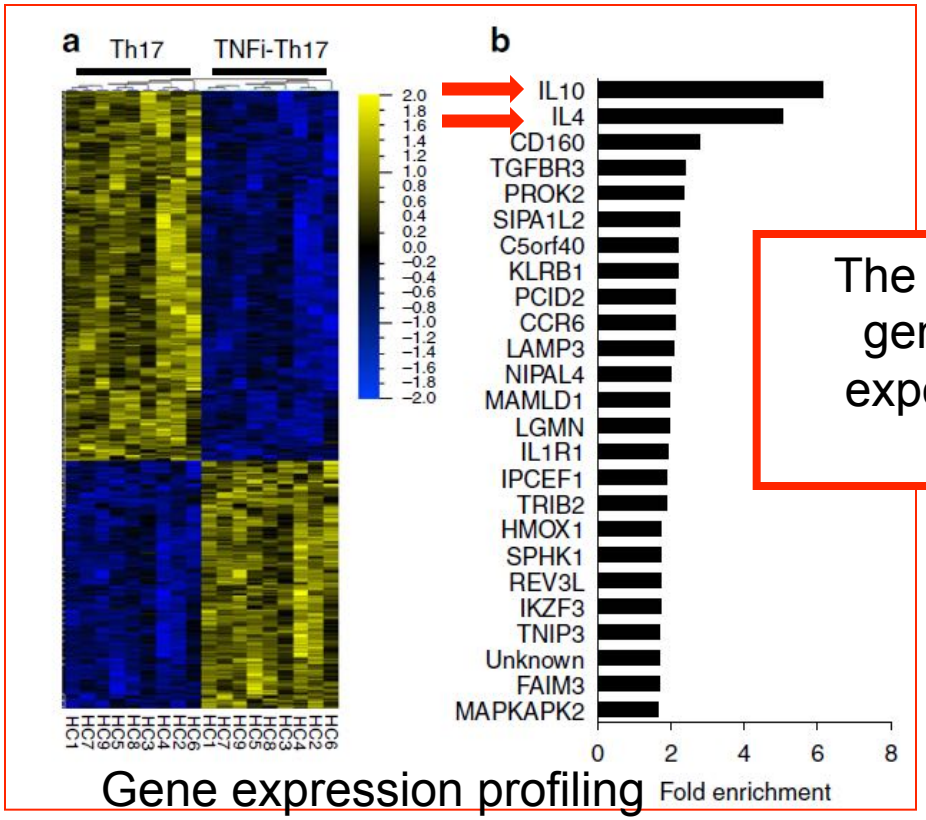
- TCC proliferating to IFX (N°50)
- TCC Producing CKs without proliferation to IFX (N° 123)



(Vultaggio A et al, unpublished)

TNF-α blockers induce Th17 to produce IL-10 via the upregulation of "Aiolos" transcription factor



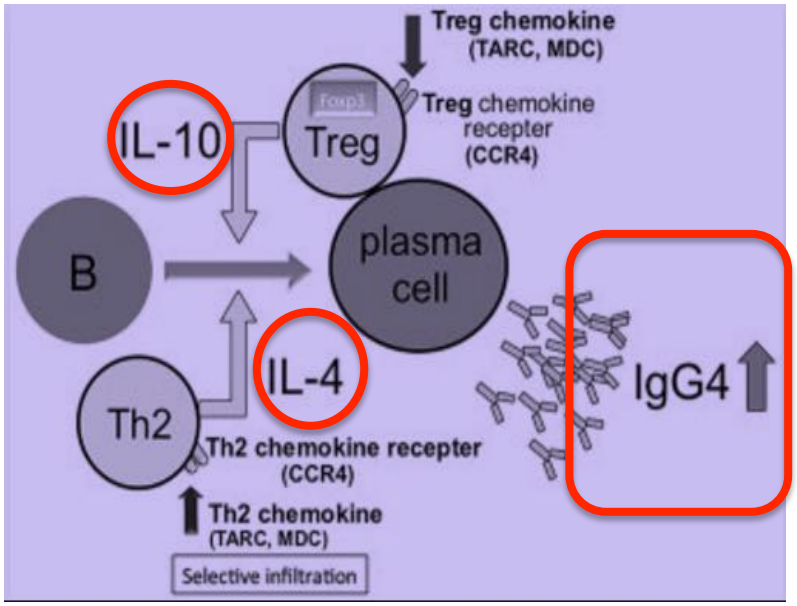


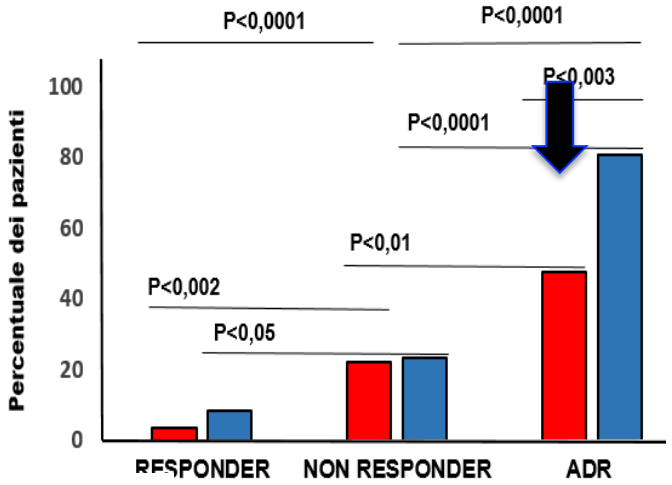
Gene expression profiling

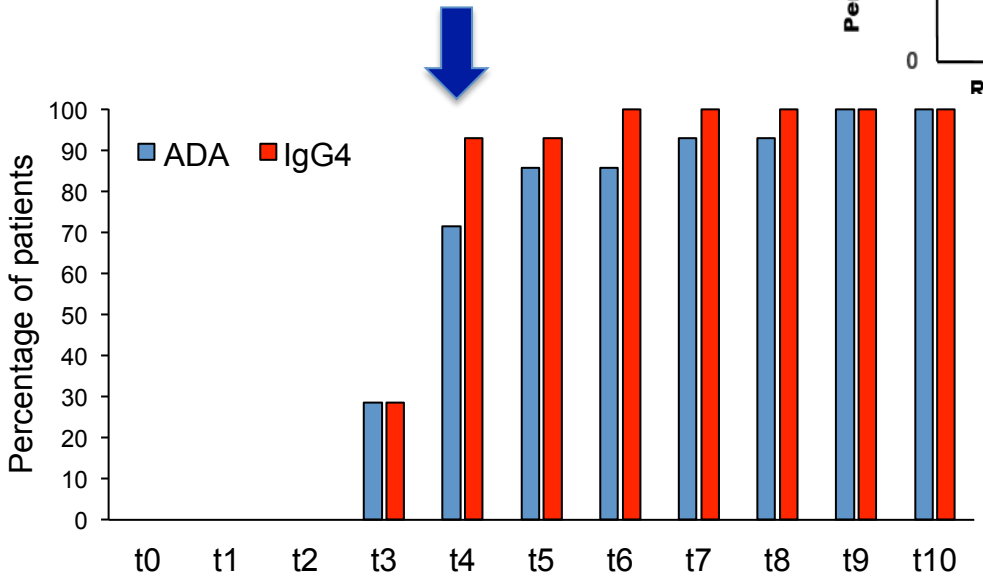
The top two upregulated genes in Adalimumab exposed Th17 cells are IL-10 and IL-4

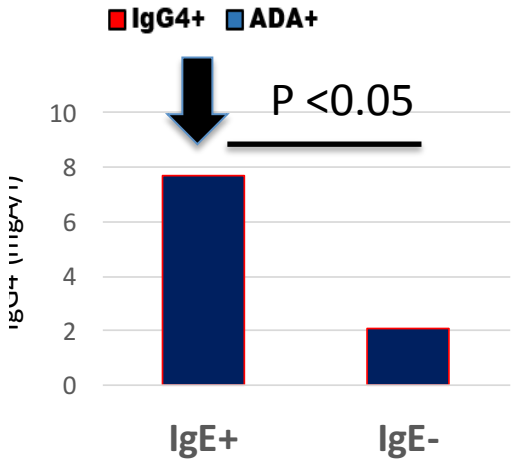
Evans HG. et al, Nature Communications, 2014

EARLY ONSET AND HIGH LEVELS OF IgG4 ADA IN IgE+ HR+ PATIENTS



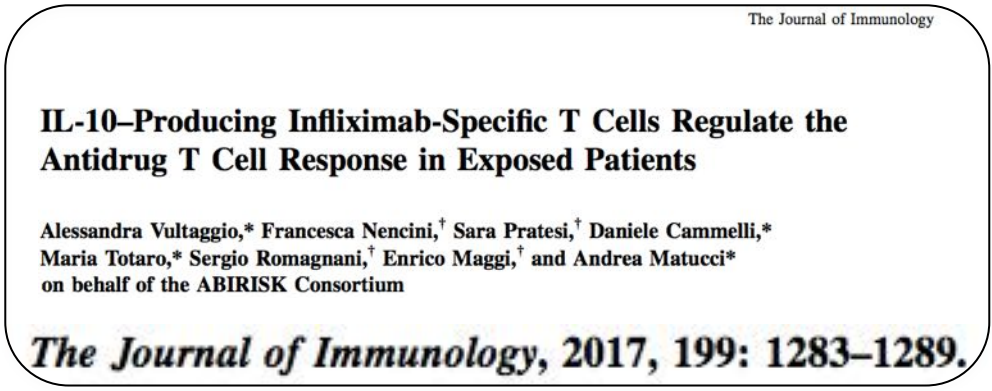






(Vultaggio A, in preparation)

QUESTIONS



The Journal of Immunology

IL-10–Producing Infliximab-Specific T Cells Regulate the Antidrug T Cell Response in Exposed Patients

Alessandra Vultaggio,* Francesca Nencini,† Sara Pratesi,† Daniele Cammelli,* Maria Totaro,* Sergio Romagnani,† Enrico Maggi,† and Andrea Matucci* on behalf of the ABIRISK Consortium

The Journal of Immunology, 2017, 199: 1283–1289.

IFX-specific memory T cells
are a source of biologically active IL-10

Does IL-10 from memory T cells interfere with detection of IFX-specific T cells ?

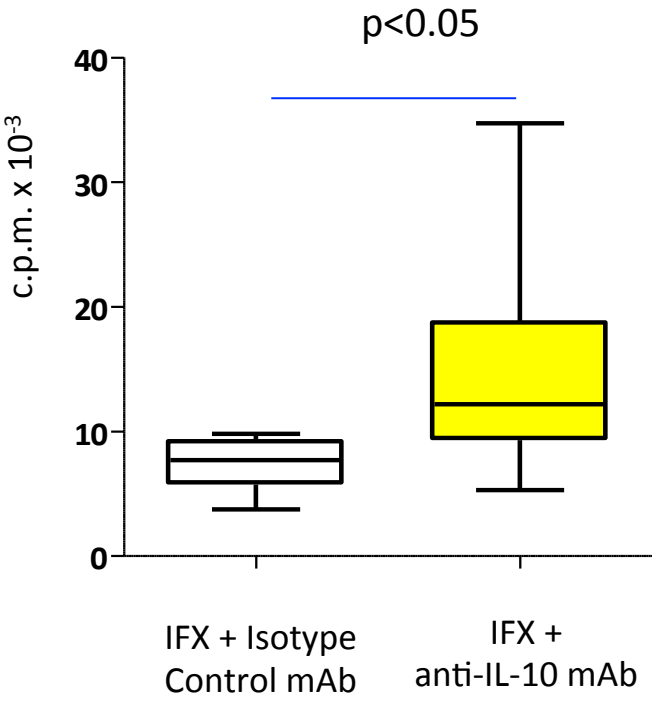
Does it prevent drug sensitization?

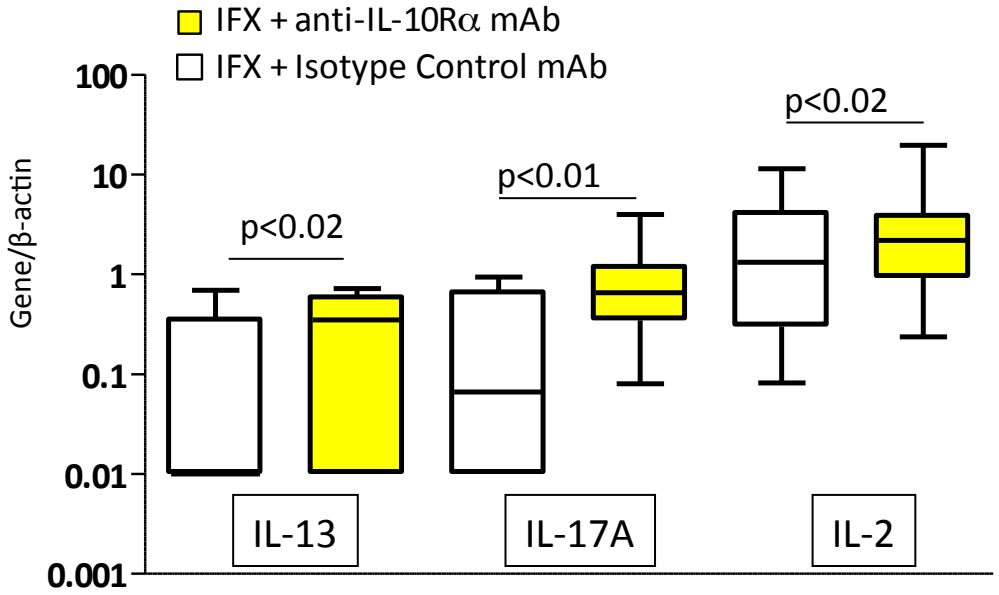
Topics

- T cell response to IFX is related to ADA positivity and HR in exposed patients
- A number of IFX-specific memory T cells produces biologically active IL-10
- Role for drug-induced IL-10 in the detection of cellular response to IFX and drug sensitization
- Potential biomarkers predicting drug sensitization, ADA induction and clinical outcomes to IFX

IFX-INDUCED IL-10 IS FUNCTIONALLY ACTIVE

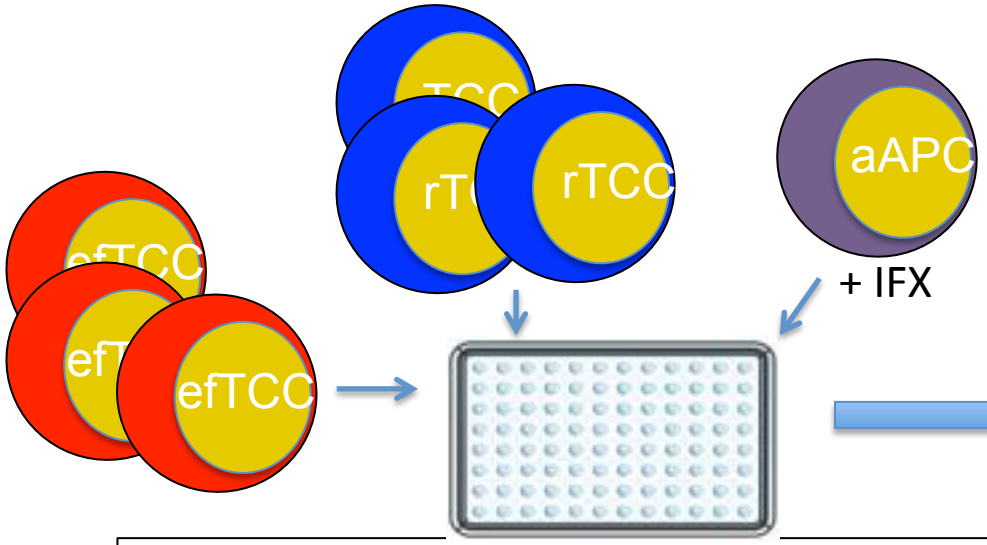
IFX displays an IL-10-dependent inhibitory activity on spontaneous, on anti-CD3/CD28-induced and on SEB-induced proliferation



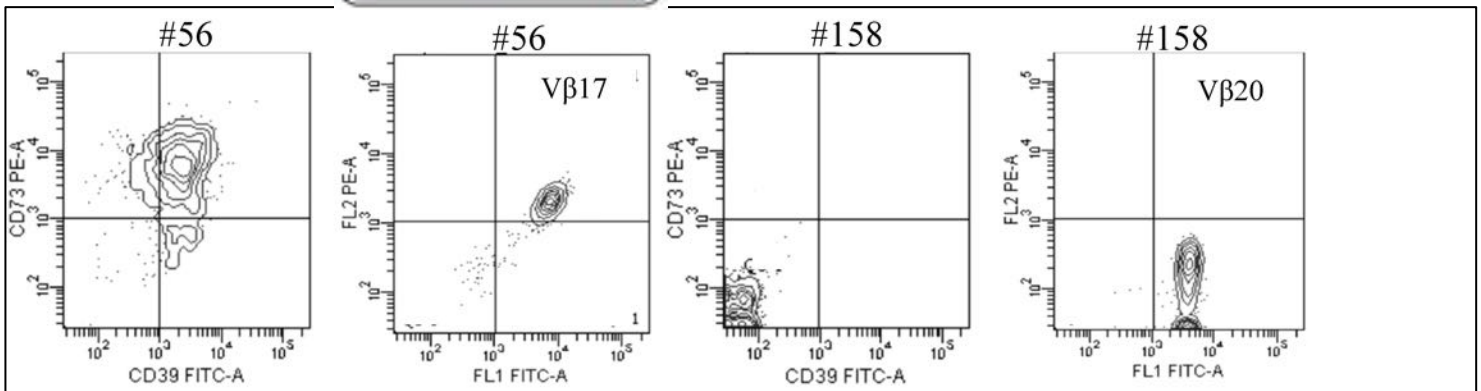


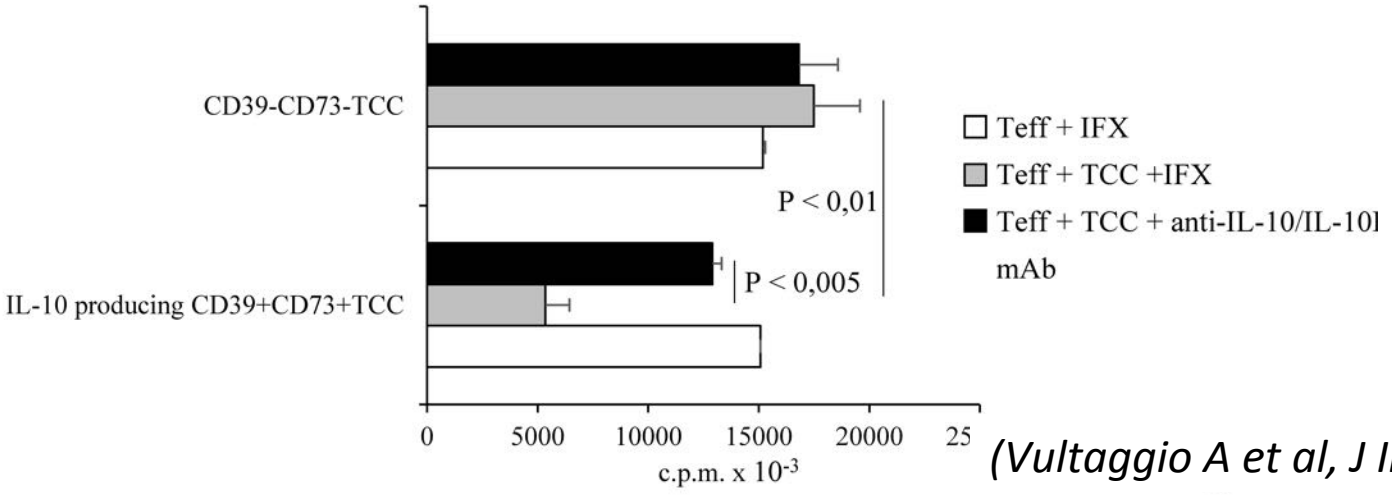
Vultaggio A et al, J Immunol, 2017

IL-10 FROM MEMORY T CELLS IS FUNCTIONALLY ACTIVE



3H-TdR uptake





(Vultaggio A et al, J Immunol, 2017)

Temporal evolution of cellular response to IFX

Patients

- **17 naïve patients** enrolled before the beginning of treatment (IFX)
- they have been monitoring during the first 8 infusions

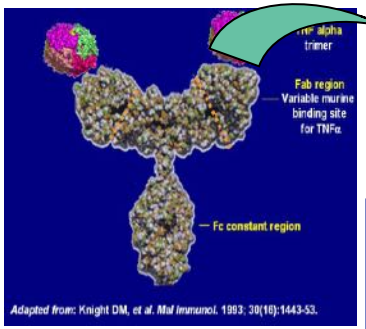
Methods

- MHC Class II (DR and DQ) assessment
- Cytokine mRNA expression of PBMCs upon re-stimulation with IFX (24h)
- Proliferative response of PBMC to peptides covering VH and VL chains (n=11 pts)
- ADA assessment
- Clinical outcome monitoring

- 5 patients developed ADA (all after the 4th infusion)
- 3 patients displayed immunogenicity-related events
- 2 patient w/o events

IFX induces memory T cells in the great majority of exposed patients

(Manuscript in preparation)



IFX full molecule

IFX-peptides

IfxH1	EVKLEESGGGLVQPG	Ifxl1	DILLTQSPAILSVSP
IfxH2	ESGGGLVQPGGSMKL	Ifxl2	QSPAILSVSPGERVS
IfxH3	LVQPGGSMKLSCVAS	Ifxl3	LSVSPGERVSFSCRA
IfxH4	GSMKLSCVASGFIFS	Ifxl4	GERVSFSCRASQFVG
IfxH5	SCVASGFIFSNHWMN	Ifxl5	FSCRASQFVGSSIHW
IfxH6	GFIFSNHWMNWVRQS	Ifxl6	SQFVGSSIHWYQQRT
IfxH7	NHWMNWVRQSPEKGL	Ifxl7	SSIHWYQQRTNGSPR
IfxH8	WVRQSPEKGLEWVAE	Ifxl8	YQQRTNGSPRLLIKY
IfxH9	PEKGLEWVAEIRSKS	Ifxl9	NGSPRLLIKYASESM
IfxH10	EWVAEIRSKSINSAT	Ifxl10	LLIKYASESMSGIPS
IfxH11	IRSKSINSATHYAES	Ifxl11	ASESMSGIPSRFSGS
IfxH12	INSATHYAESVKGRF	Ifxl12	SGIPSRFSGSGSGTD
IfxH13	HYAESVKGRFTISRD	Ifxl13	RFSGSGSGTDFTLSI
IfxH14	VKGRFTISRDDSKSA	Ifxl14	GSGTDFTLSINTVES
IfxH15	TISRDDSKSAVYLQM	Ifxl15	FTLSINTVESEDIAD
IfxH16	DSKSAVYLQMTDLRT	Ifxl16	NTVESEDIADYYCQQ
IfxH17	VYLQMTDLRTEDTGV	Ifxl17	EDIADYYCQQSHSWP
IfxH18	TDLRTEDTGVYYCSR	Ifxl18	YYCQQSHSWPFTFGS
IfxH19	EDTGVYYCSRNYYGS	Ifxl19	SHSWPFTFGSGTNLE
IfxH20	YYCSRNYYGSTYDYW	Ifxl20	FTFGSGTNLEVKRTV
IfxH21	NYYGSTYDYWGQGTT	Ifxl21	GTNLEVKRTVAAPSV
IfxH22	TYDYWGQGTTLTVSS	Ifxl22	VKRTVAAPSVFIFPP
IfxH23	GQGTTLTVSSASTKG		
IfxH24	LTVSSASTKGPSVFP		

ADA-

ADA+

Mitogenic Index

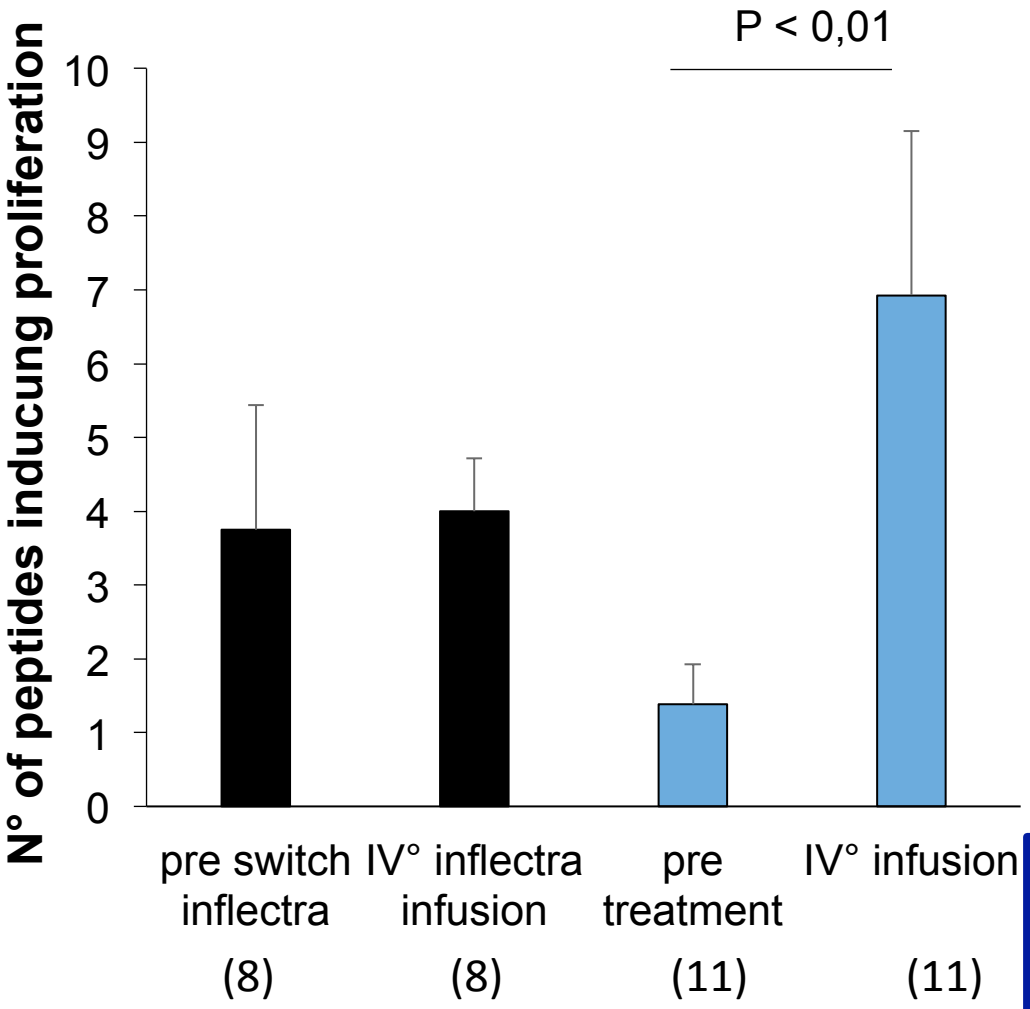
MI

pre treatment

After four Infusions (6 months of treatment)

EARLY ONSET OF CELL SENSITIZATION TO IFX

	DRB1	DRB1	DQA1	DQA1	DQB1	DQB2
pz#1	01:01	07:01	01:01	02:01	02:02	05:01
pz#2	01:01	07:01	01:01	02:01	02:02	05:01
pz#3	11:02	16:01	01:02	05:05	03:01	05:02
pz#4	04:04	13:05	03:02	05:05	03:01	04:02
pz#5	13:01	14:01	01:01	01:03	05:03	06:03
pz#6	11:01	15:01	01:02	05:05	03:01	06:02
Pz#7	04:04	13:05	03:02	05:05	03:01	04:02
Pz#8	13:01	14:01	01:01	01:03	05:03	06:03
Pz#9	11:01	15:01	01:02	05:05	03:01	06:02
Pz#10	01:01	14:01	01:01	01:04	05:01	05:03
Pz#11	01:01	16:01	01:01	01:02	05:01	05:02

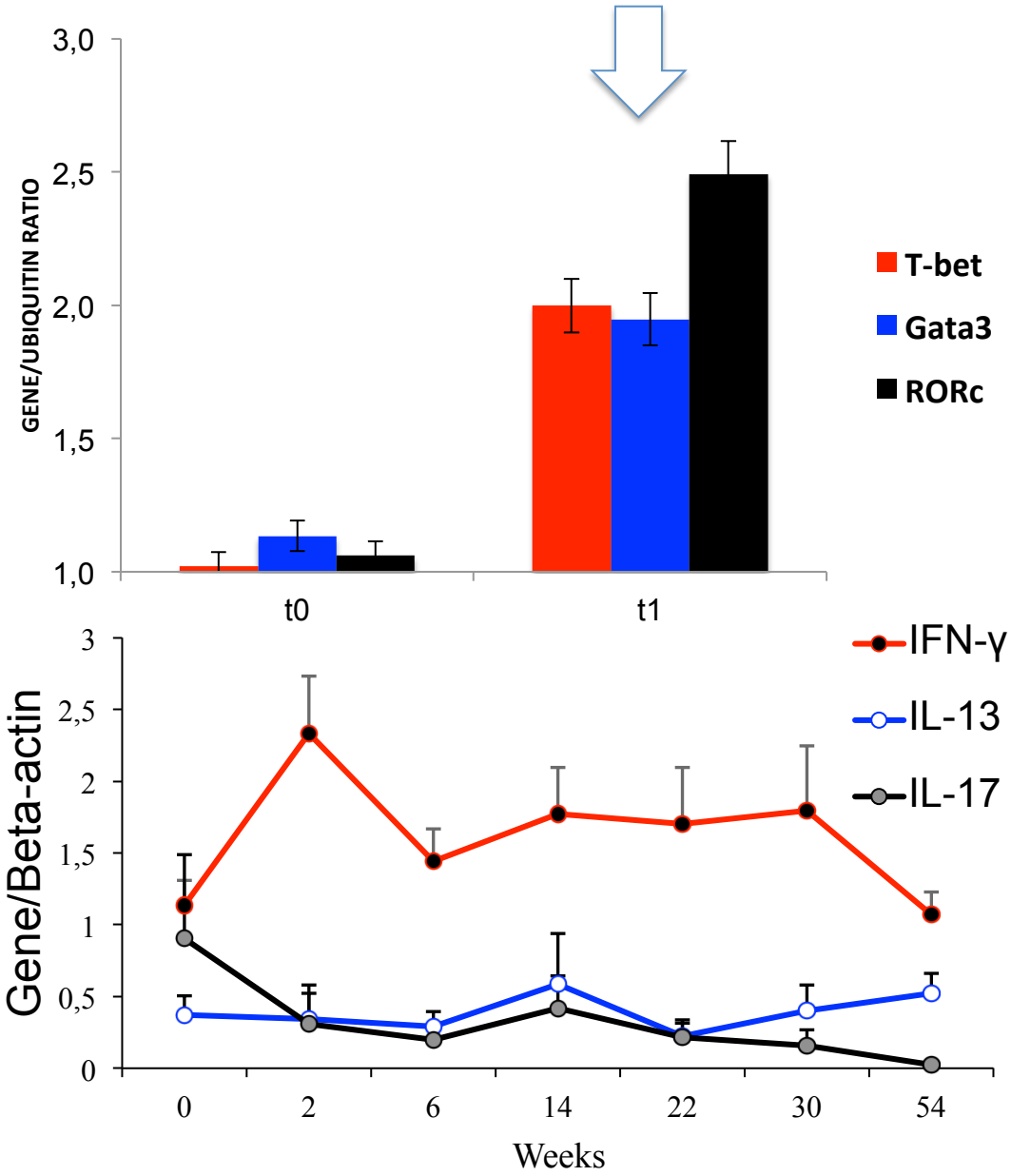


IFX biosimilar-switched pts

IFX originator-treated pts

Nine out of 11 patients (81,8%) increased the number of peptides recognised after IV inf.

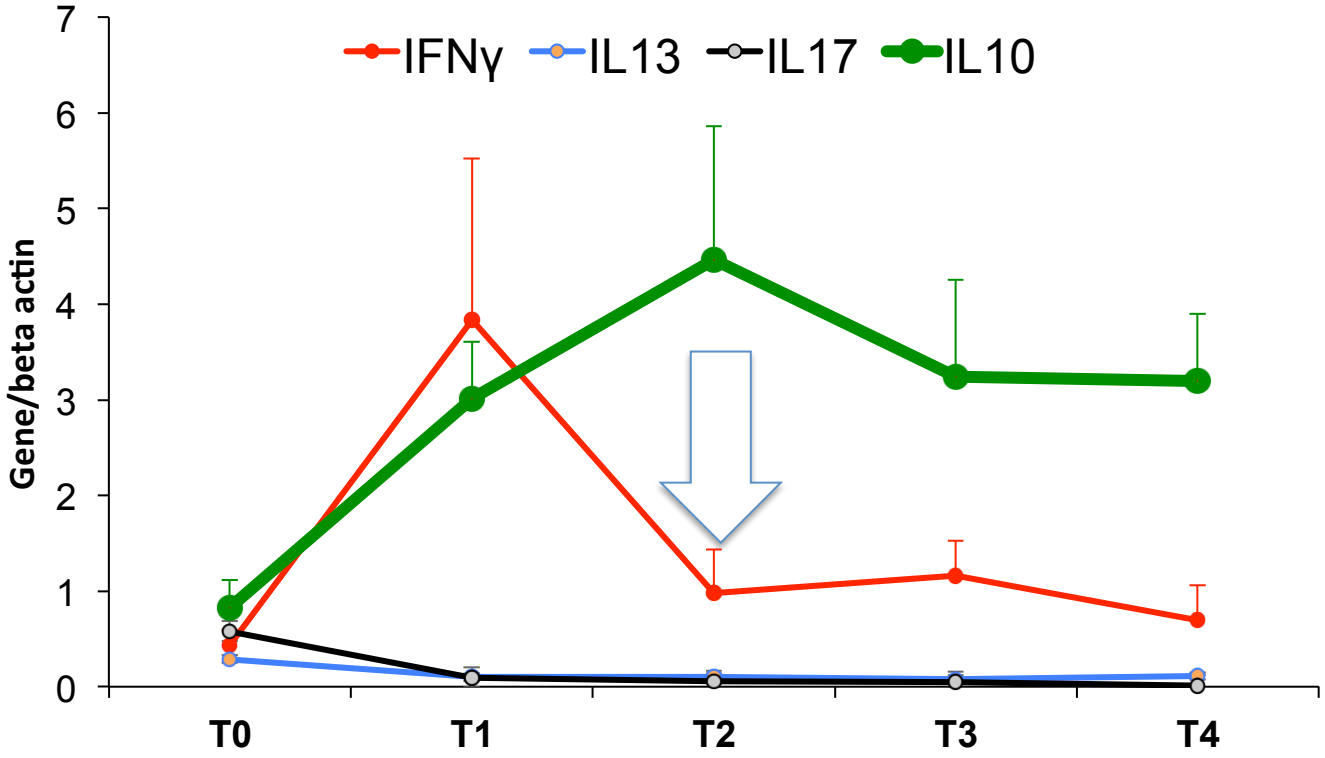
EARLY ONSET OF SENSITIZATION TO IFX IN EXPOSED PATIENTS

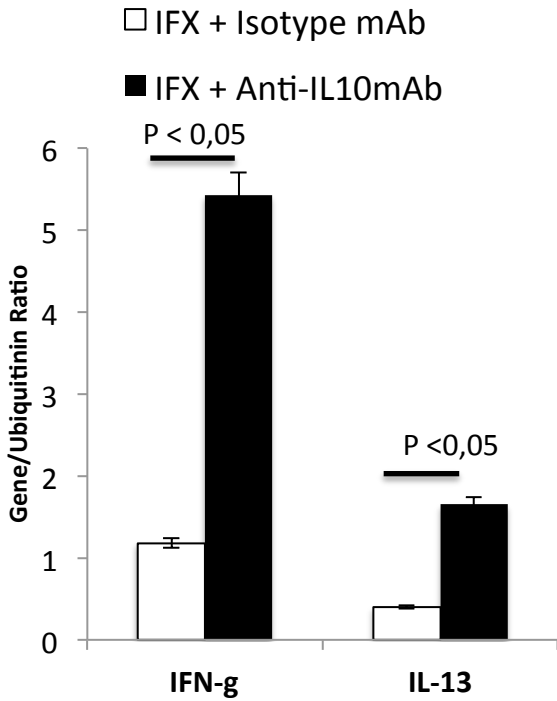


The increased mRNA Expression of main regulators and/or of adaptive cytokines was found in 16 out of 17 patients (94.1%) after the first drug infusions

(Manuscript in preparation)

IL-10 mRNA is early and stably expressed In exposed patients and dowregulates adaptive cytokines





(manuscript in preparation)

REMARK AND QUESTION

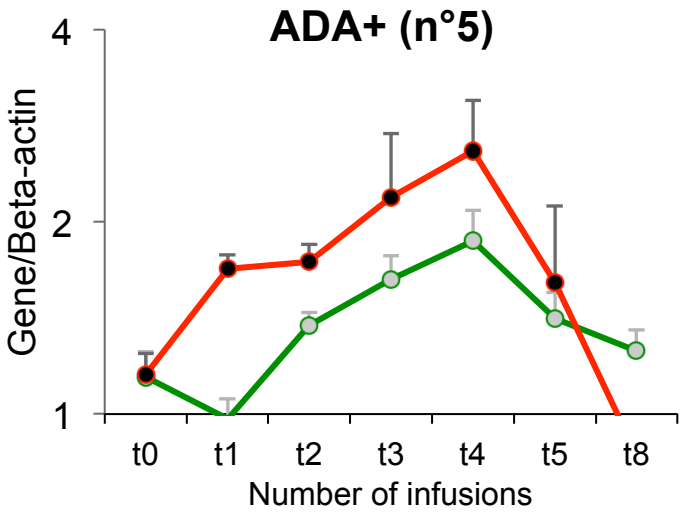
IFX-induced IL-10 does not prevent the drug sensitization even though it can interfere with the detection/activity of memory T cells

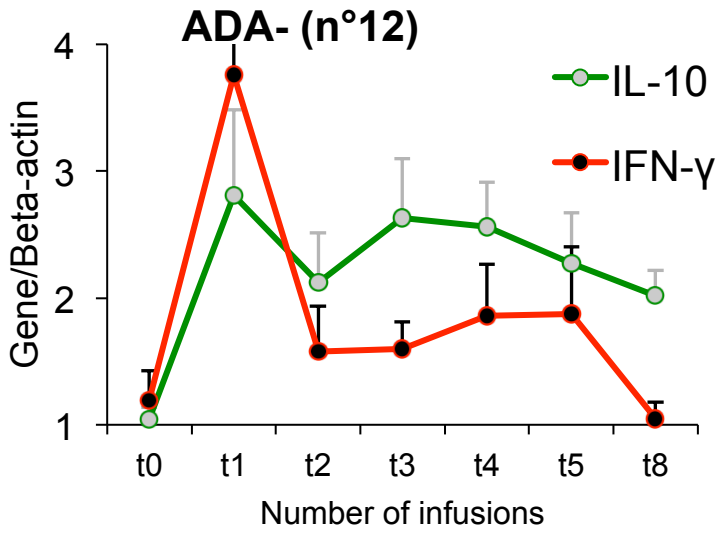
Does cytokines increase predict clinical outcomes?

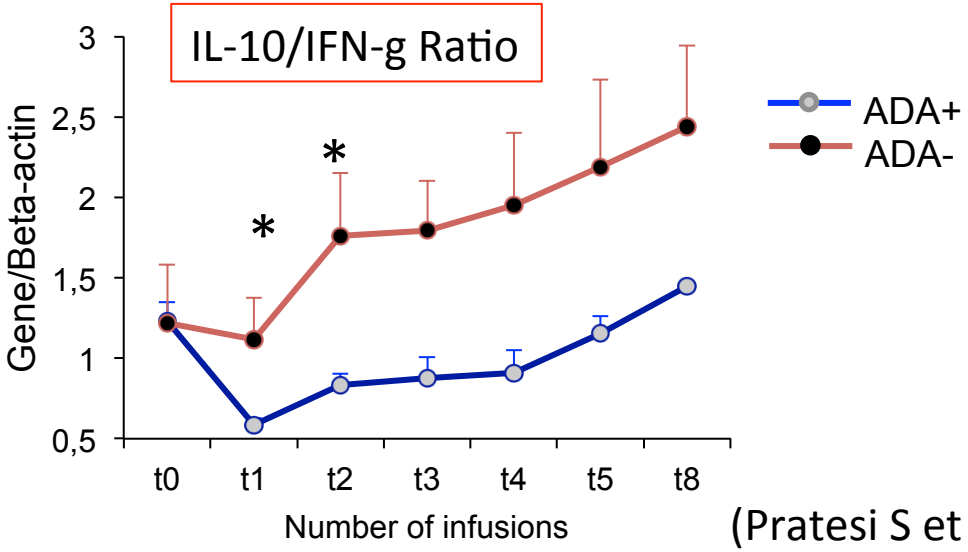
Topics

- T cell response to IFX is related to ADA positivity and HR in exposed patients
- A number of IFX-specific memory T cells produces biologically active IL-10
- Role for drug-induced IL-10 in the detection of cellular response to IFX and drug sensitization
- Potential biomarkers predicting drug sensitization, ADA induction and clinical outcomes to IFX

Opposite IL-10 and IFNg RNA expression in ADA+ and ADA- patients

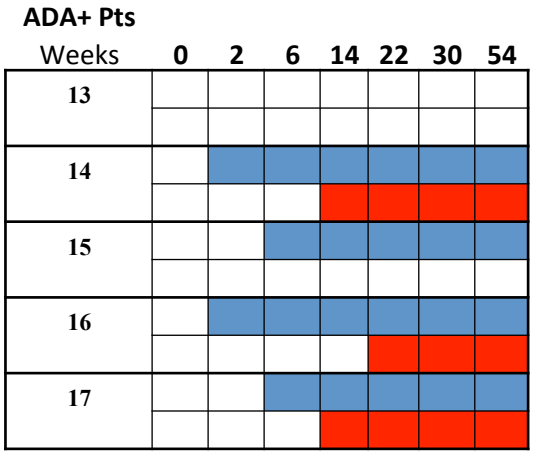


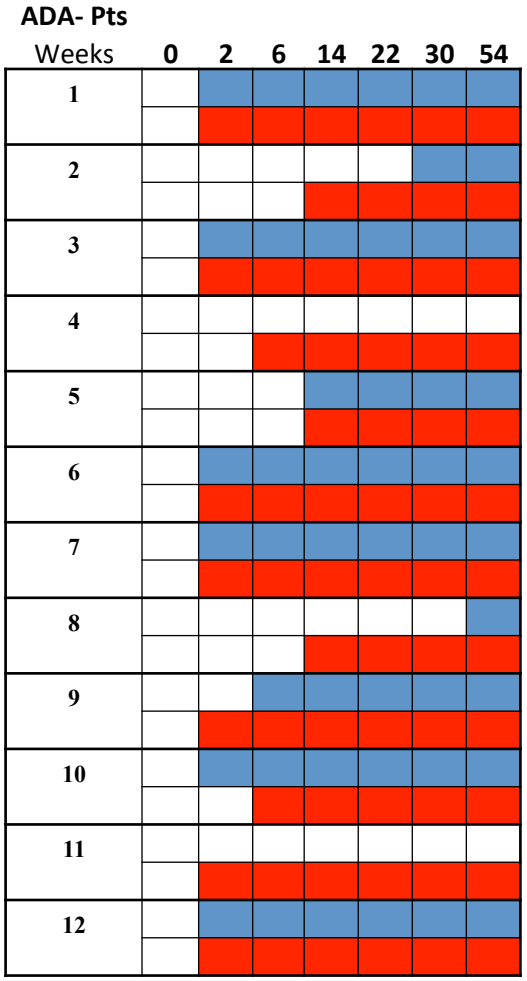


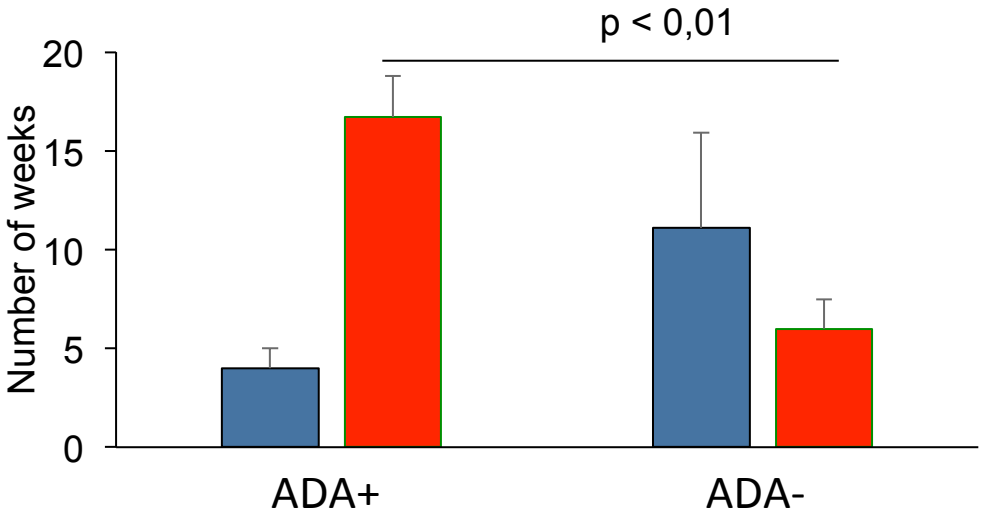


(Pratesi S et al, in preparation)

The IFN-γ mRNA usually preceeds IL-10 mRNA expression in ADA+ patients







(Pratesi S et al, in preparation)

Adaptive cytokines highly preceed ADA onset and clinical outcomes

ADA+ PATIENTS	IFNγ mRNA expression (weeks)	IL-10 mRNA Expression (weeks)	ADA Onset (weeks)	Outcome onset (weeks)
Pt # 13	2	-	2	30
Pt # 14	2	14	22	30
Pt # 15	6	-	22	30
PT # 16	2	22	22	–
Pt # 17	6	14	30	–
Mean (weeks)	**3,6**	**16,6**	**19,6**	**30**

(Manuscript in preparation)

IFX-specific T cells from patients during drug reaction Don't express IL-10 mRNA in IFX-stimulated PBMC

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CONCISE COMMUNICATION

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Anti-adalimumab antibodies in rheumatoid arthritis patients are associated with interleukin-10 gene polymorphisms

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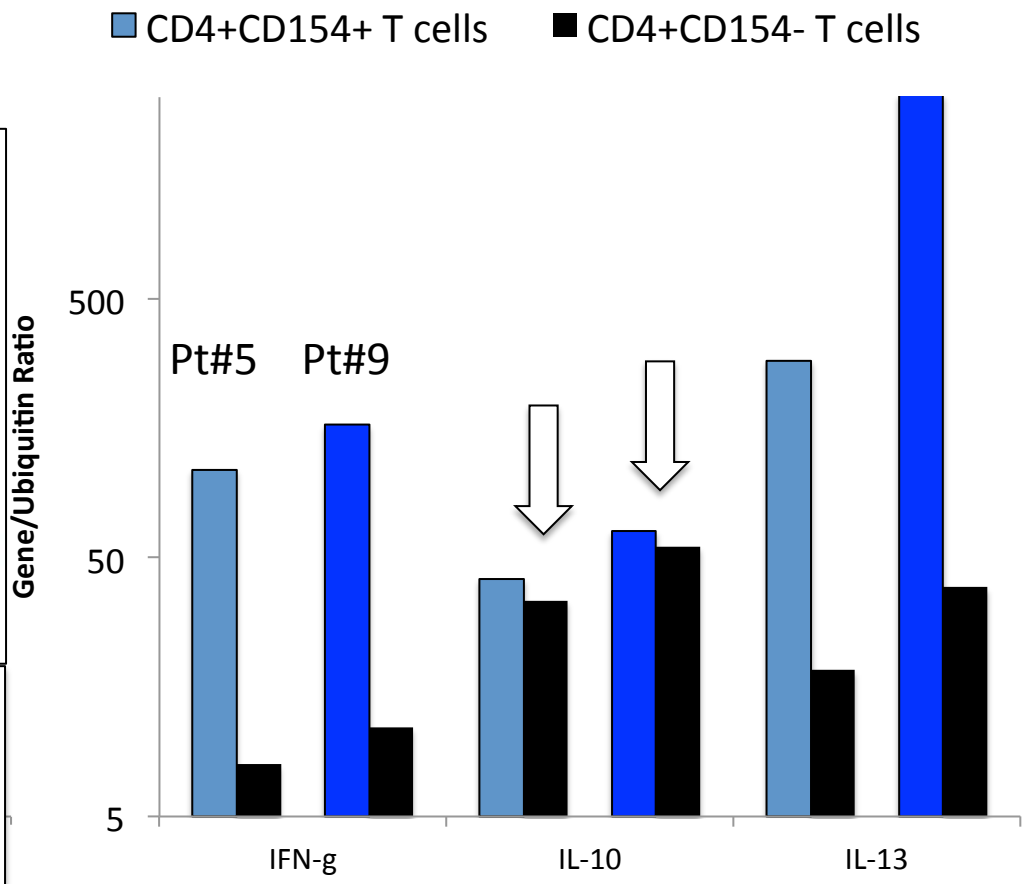

(Vultaggio A et al, submitted)

Conclusions

- Humoral and cellular response to IFX is related to ADA positivity and clinical outcomes in exposed patients
- IgE ADAs are detected in a proportion of patients with severe HR, showing also positive skin tests and drug-specific type2 response.
- Drug sensitization is induced in the majority of exposed patients (including tolerant) during the very first infusions,
- A number of IFX-specific memory T cells produces biologically active IL-10 which prevents the detection of drug-specific cellular response.
- The IFX-induced IL-10 (or IFNg) may be considered a potential biomarker to predict drug sensitization, ADA induction and clinical outcomes.

"Role of T-cells in immunogenicity of biologics in Intestinal Bowel disease"

Center of Excellence DENOTHE University of Florence
(E. Maggi, chair)

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