Initial anti-CD20 monoclonal antibody therapy in CLL quickly induces cytokine release syndrome with first dose infusion reaction correlating only with high IL-6, IL-8 and IP-10

Abstract
1552828

Charles C. Chu[1], Jeremiah E. Moore[1], Paige C. Bloom[1], Jennifer E. Bruno[1], Sally A. Quataert[1], Tim R. Mosmann[1], Nydia Jaimes-Delgadillo[1], Christine A. Herne[1], Clare B. Heffernan[1] Karl R. VanDerMeid[1], Andrea M. Baran[1], Derick R. Peterson[1], Ronald P. Taylor[2], Fátima Rivera-Escalera[2], Michael Rusty Elliott[2], Lauren Benoodt[1], John M. Ashton[1], Danielle S. Wallace[1], Paul M. Barr[1], and Clive S. Zent[1]

[1]University of Rochester Medical Center, [2]University of Virginia

Abstract

64% of CLL patients have a potentially life-threatening first dose infusion reaction (FDIR) within 1–2 h after initiating intravenous (IV) rituximab anti-CD20 monoclonal antibody (mAb) therapy. Once FDIR is observed, the infusion must be interrupted and patient symptoms must be managed before restarting. If FDIR is not treated, serious complications and fatalities may occur.

To better understand FDIR, we studied blood samples collected from 37 treatment-naïve CLL patients in our clinical trial NCT03788291 undergoing IV treatment with low dose (50 mg) and slow infusion (25 mg/h) anti-CD20 mAb (rituximab). All human specimen collection and usage was conducted with written informed consent after approval of the University of Rochester Research Subjects Review Board according to the ethical guidelines of the Declaration of Helsinki. We hypothesized that slow infusion of low dose mAb compared to standard infusion and dose (50 mg/h, 375 mg/m2) may produce fewer FDIRs. We analyzed four time points: baseline (0 h (pre), prior to infusion), 1 h later (during infusion), at the end of infusion (~3 h (post)) and at 48 h (Fig.1). Based on previous reports, we anticipated that CLL counts, CD20 levels and consumption of mAbs/complement would correlate with FDIRs. Furthermore, we expected cytokine release syndrome would be measurable in FDIR sera.

24 (65%) patients had a FDIR as defined by Common Terminology Criteria for Adverse Events version 5 (CTCAE) grade 2 or higher and required intervention within the first two hours. This FDIR frequency is comparable to the rate seen in standard rituximab therapy (64%), implying that dose and infusion rate of anti-CD20 mAb are not key components of FDIR, which further unexpectedly suggests that antigen/antibody levels may not correlate with FDIR.

To test this idea, flow cytometry of CLL blood samples was performed to measure CLL cell counts and CD20 levels. We further measured serum rituximab and complement levels. None of these measurements correlated with the occurrence of FDIR in CLL patients.

To explore factors outside of antigen/antibody levels, we examined CLL patient characteristics (IGHV mutation, Rai stage, cytogenetic defects/mutations) and none of these correlated with induction of FDIR.

Previous studies of rituximab FDIR noticed a cytokine release syndrome, although non-FDIR patients were not examined. In our study, we studied human sera from both FDIR and non-FDIR patients using a Luminex xMAP multiplex assay. We found cytokine release occurred in both types of patients with a ≥ 4-fold increase in one or more cytokines compared to pre-treatment levels in 95% of patients at the end of infusion (~3 hr (post)).

Because overall cytokine induction was similar between patients, we examined specific cytokines for differences in induction that might correlate with FDIR. IL-6, IL-8, and especially IP-10 induction ~3 hr (post) correlated with FDIR.

IP-10 (interferon gamma inducible protein-10), also known as CXCL10 (C-X-C motif chemokine ligand 10), is mainly secreted by monocytes and macrophages. To study the source of IP-10, we performed single-cell RNA sequencing on blood samples from two patients with FDIR at three timepoints: baseline (0 h (pre)), at the end of infusion (~3 h (post)) and at 48 h. IP-10 was detected at very low levels only in monocytes and no other cell types. This suggests that IP-10 is produced in tissue-resident cells that are monocytes or derived from monocytes, such as macrophages.

To test this, we prepared human monocyte-derived macrophages (hMDM) and added CLL cells opsonized with anti-CD20 mAb. Compared to no mAb, we found that IL-6, IL-8 and IP-10 were induced, although not necessarily at comparable levels.

To model FDIR in mice, IV anti-CD20 (5D2 mAb) treatment of C57BL/6 wildtype mice induced IL-6 and IP-10 at 2 h, which decreased at 18 h to levels similar to IgG control. This time course is similar to that seen in FDIR in human patients.

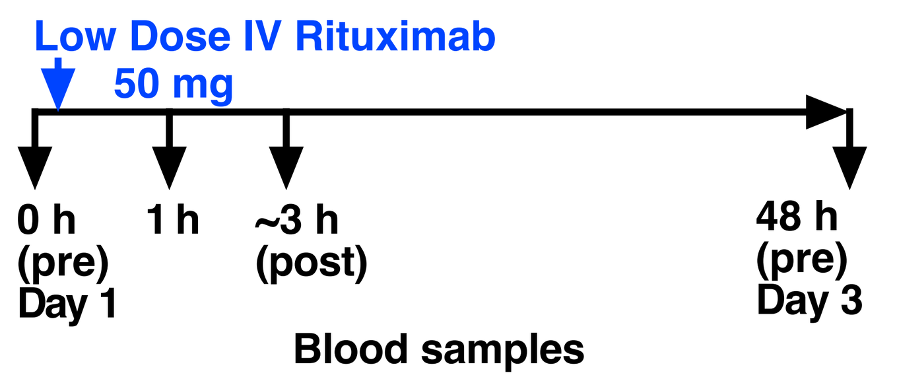
In conclusion, FDIR does NOT correlate with anticipated CLL cell characteristics or levels of antigen, antibody, or complement. Infusion of anti-CD20 mAb induces cytokines in most patients, with FDIR correlating with higher levels of IL-6, IL-8, and IP 10. Single cell RNA sequence data suggests tissue resident monocytes or macrophages, are responsible for IP-10 production. Modeling by in vitro assays and a mouse model reproduce mAb induction of cytokines, which is currently under investigation.

First Dose Infusion Reaction (FDIR)

- 64% of CLL patients have a first dose infusion reaction (FDIR) within 1–2 h after initiating IV anti-CD20 mAb therapy.
- FDIR is defined by Common Terminology Criteria for Adverse Events version 5 (CTCAE) grade 2 or higher.
- FDIR requires interruption of infusion and symptomatic management. If it is not treated, serious life-threatening complications may occur.

Salles et al. *Adv Ther* 2017 (34)2232

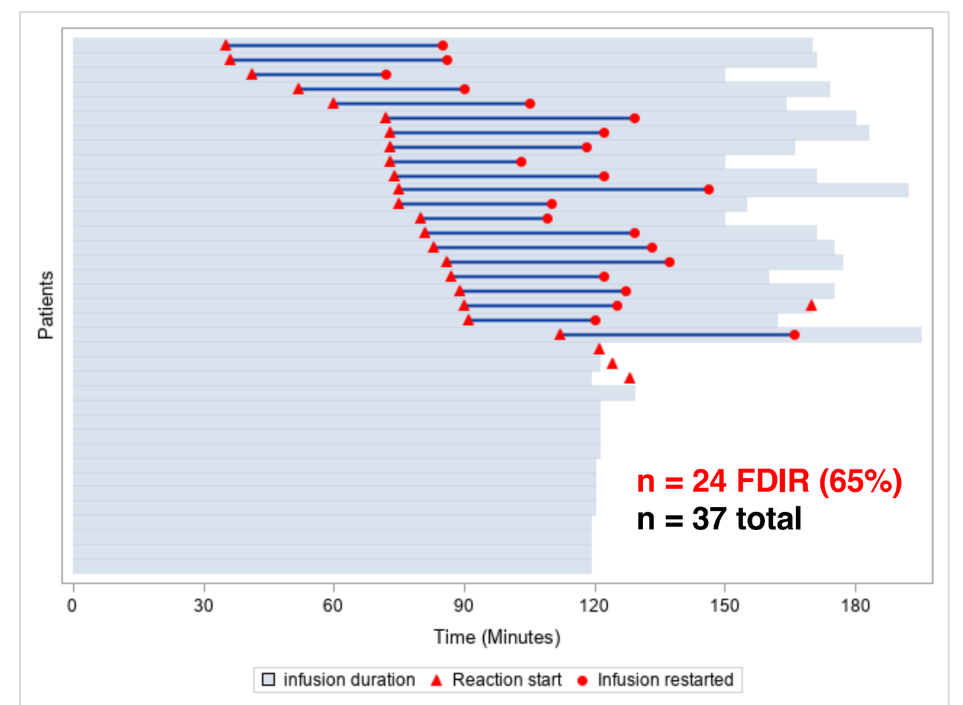
Sample Timeline



To test FDIR, blood samples were collected in our clinical trial NCT03788291 at four timepoints from treatment-naive chronic lymphocytic leukemia (CLL) patients (n = 37) undergoing IV infusion with low dose (50 mg at 25 mg/h) anti-CD20 mAb (Rituximab) on Day 1.

Wallace et al. *Blood Adv* 2023 2022009382
Moore et al. *Leuk Res* 2023 (129)107072

CLL Patients Rituximab FDIRs



Moore et al. *Leuk Res* 2023 (129)107072

Low Dose RTX Still Induces FDIR

- Standard dose IV rituximab (RTX, 375 mg/m2) induced FDIR in 77% patients.
- Low dose IV RTX (50 mg) induced FDIR in 65% of patients (with a median dose of 32 mg prior to FDIR) in our study.

What does FDIR depend on?

- Low dose FDIR induction suggests that high mAb target levels are NOT needed.
- Rituximab FDIRs are thought to be associated with higher numbers of circulating lymphocytes.
- The exact relationship between FDIRs and patient / CLL cell characteristics remains unclear.

Winkler et al. *Blood* 1999 (94)2217
Byrd et al. *J Clin Oncol* 1999 (17)791

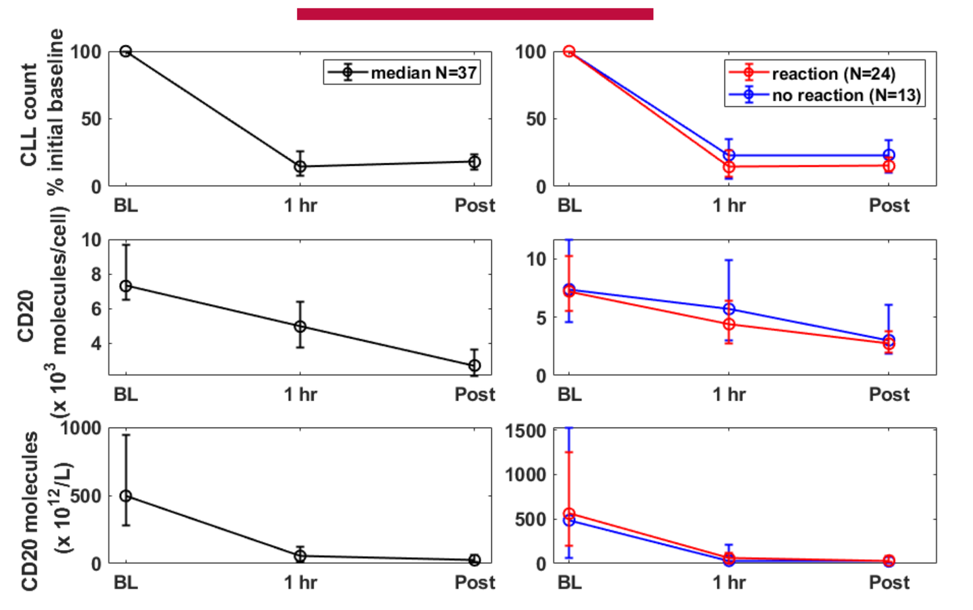
CLL Patients Description

	Patients n=37
Age at enrollment, median (min, max)	68 (42, 80)
Gender, n (%)	
Female	14 (37.8)
Male	23 (62.2)
Race, n (%)	
White	36 (97.2)
Black/African American	1 (2.8)
Performance status (ECOG), n (%)	
0	18 (48.6)
1	16 (43.2)
2	3 (8.2)
Stage (Rai) at diagnosis, n (%)	
0	6 (16.2)
I	10 (27.0)
II	11 (29.7)
III	6 (16.2)
IV	1 (2.7)
Unknown	3 (8.2)
Cytogenetic Defects[1], n (%)	
13q14 deletion	15 (40.5)
None	5 (13.5)
Trisomy 12	6 (16.2)
11q22.3 deletion	6 (16.2)
17p13 deletion	5 (13.5)
IGHV somatic hypermutation, n (%)	
Mutated (>2% vs. germline)	15 (40.5)
Unmutated	22 (59.5)
TP53 mutation, n (%)	
Positive	8 (21.6)
Negative	29 (78.4)
TP53 mutation AND 17p13 deletion, n (%)	5 (13.2)
NOTCH1 mutation, n (%)	
Positive	9 (24.3)
Negative	28 (75.7)
SF3B1 mutation, n (%)	
Positive	4 (10.8)
Negative	33 (89.2)

CLL patient characteristics do NOT correlate with FDIR (data not shown).

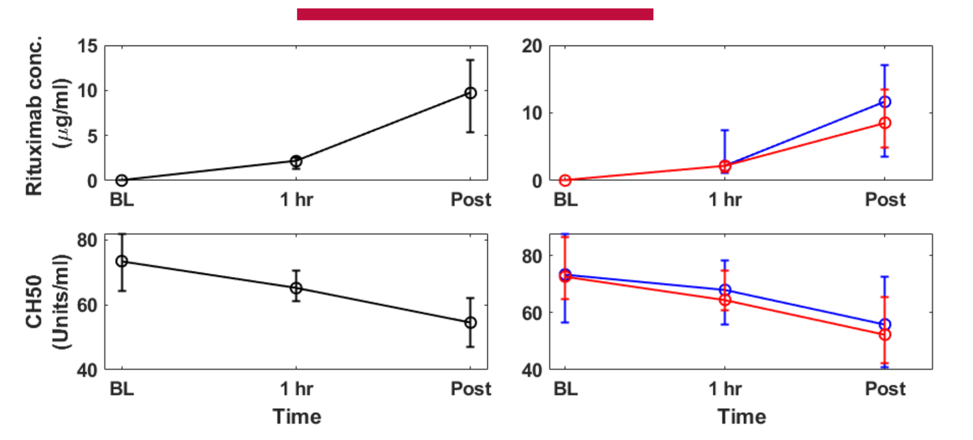
Wallace et al. *Blood Adv* 2023 2022009382
Moore et al. *Leuk Res* 2023 (129)107072

RTX FDIR is independent of CD20



Moore et al. *Leuk Res* 2023 (129)107072

FDIR is independent of mAb and Complement levels

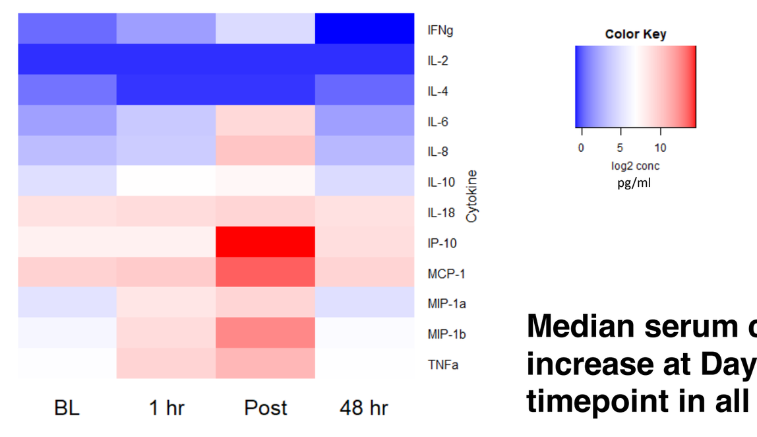


Moore et al. *Leuk Res* 2023 (129)107072

Is FDIR due to cytokine storm?

- Rituximab FDIRs correlate with increased serum TNF and IL-6 concentrations in previous studies.
- Twelve human serum cytokines were measured by Luminex xMAP multiplex assay.

Cytokine levels are increased



Median serum cytokines increase at Day 1 Post ~3 h timepoint in all patients.

Number of patients (n=37) with ≥ 4-fold increases from baseline

Cytokine	IFNγ	IL-2	IL-4	IL-6	IL-8	IL-10	IL-18	IP-10	MCP-1	MIP-1α	MIP-1β	TNFα
1 h	14	1	2	13	7	18	0	0	2	29	22	25
Post	29	0	1	35	35	23	0	27	33	34	34	33
Total	29	1	2	35	35	27	0	27	33	34	34	33

Indicates >89% patients (n = 33 or above) have ≥ 4-fold increase at Day 1 Post.

- All patients have increased serum cytokines after infusion, including non-FDIR patients.
- Therefore, cytokine storm does NOT correlate with FDIR.

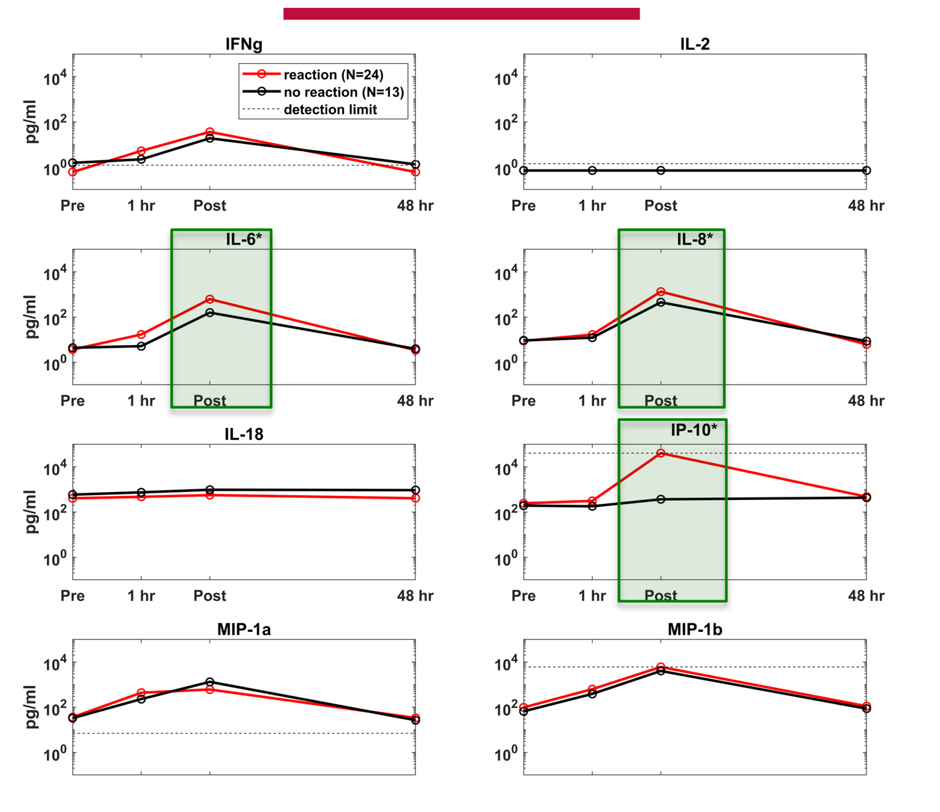
Moore et al. *Leuk Res* 2023 (129)107072

Correspondence

Email contacts for further information:
Charles_Chu@URMC.Rochester.edu or
Clive_Zent@URMC.Rochester.edu

Is FDIR due to specific cytokines?

Serum Cytokines and FDIR



- Increased IL-6, IL-8, and IP-10 correlate with infusion reaction.

Moore et al. *Leuk Res* 2023 (129)107072

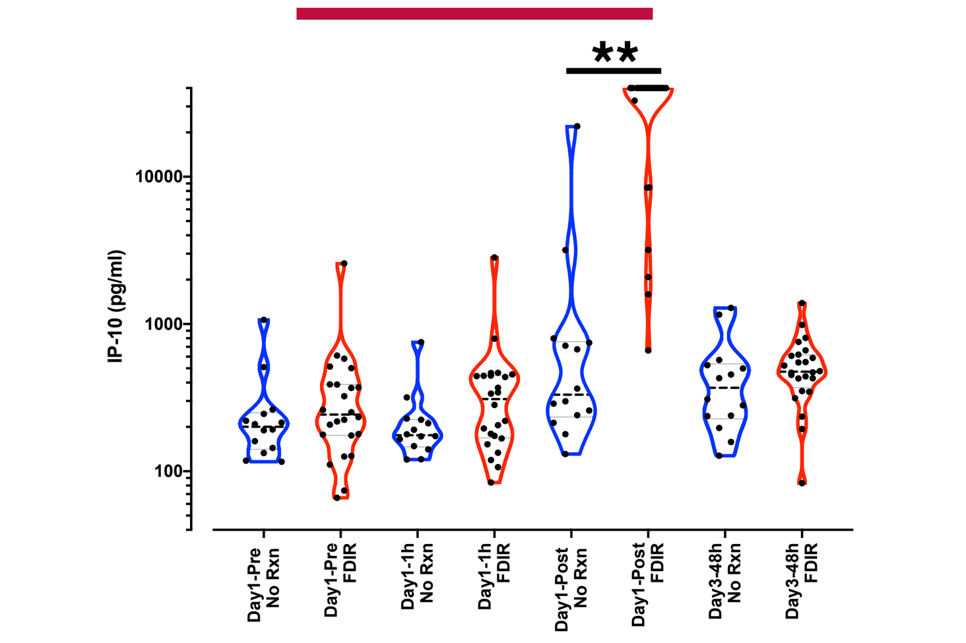
Higher IL-6, IL-8, IP10 levels increase FDIR Risk

Cytokine	Hazard Ratio (per doubling of concentration)	95% CI	p-value
IL-6	1.35	1.08, 1.69	0.008
IL-8	1.38	1.11, 1.72	0.004
IP-10	1.61	1.14, 2.29	0.007

Risk of FDIR determined by time to reaction Cox model analysis.

Moore et al. *Leuk Res* 2023 (129)107072

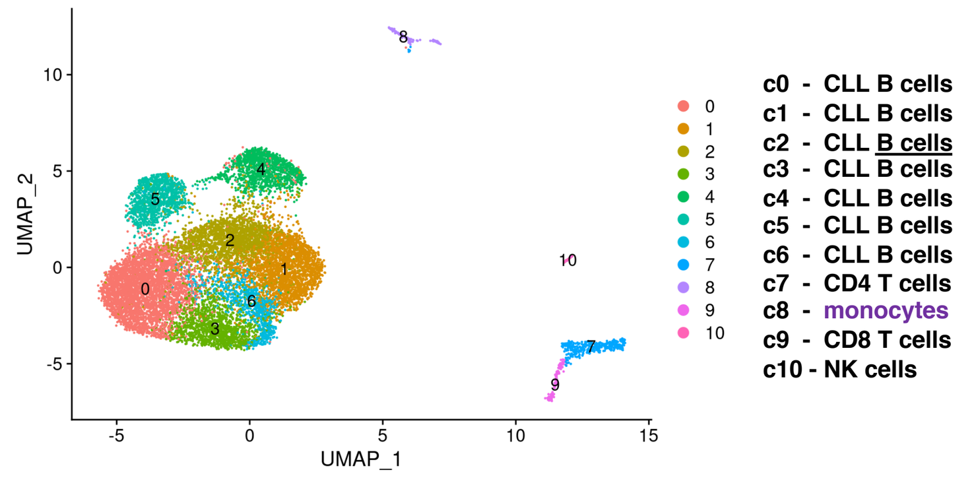
IP-10 Levels and FDIR



- FDIR patients have highest IP-10 levels immediately after FDIR (Day1-Post)

Adapted from Moore et al. *Leuk Res* 2023 (129)107072

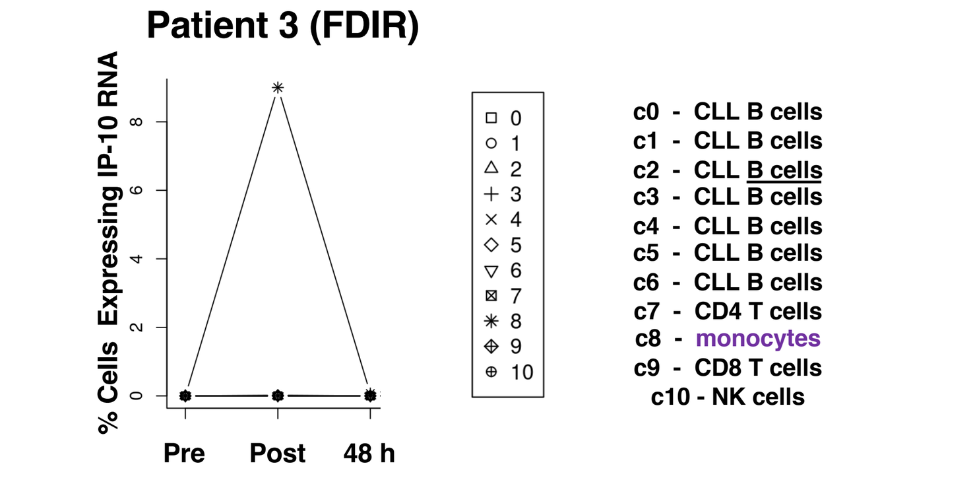
CLL PBMC cell clusters



Single cell RNA sequence obtained from peripheral blood mononuclear cells (PBMC) of CLL patient P3, who had a FDIR.

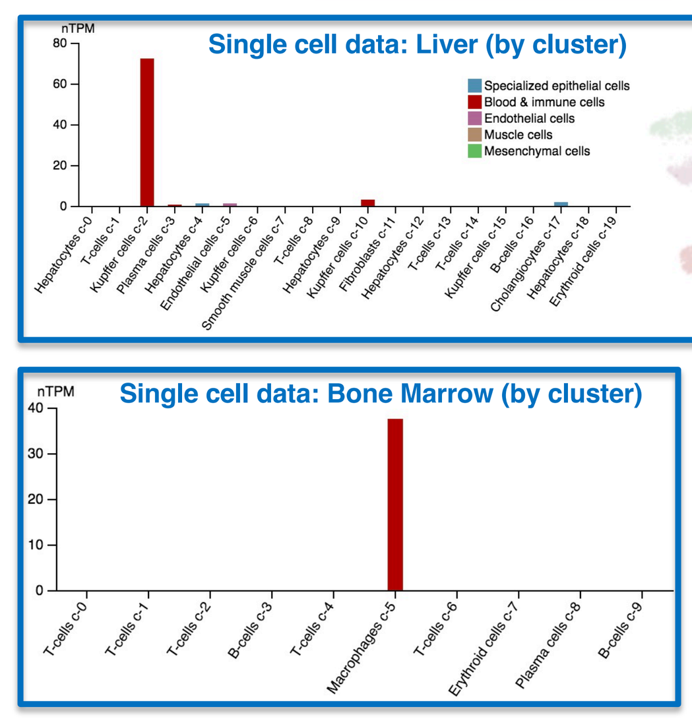
Multiple CLL clusters identified among normal cell clusters.

IP10 RNA in CLL PBMC Monocytes



- IP-10 expressed in a small percentage of CLL PBMC monocytes.
- IP-10 expressed <1% in other circulating cells (CLL B, T, NK, …)
- IP-10 is highest at Day 1 Post coinciding with FDIR in P3.
- Based on these low levels, IP-10 is suggested to be expressed in tissue resident cells of monocyte lineage.

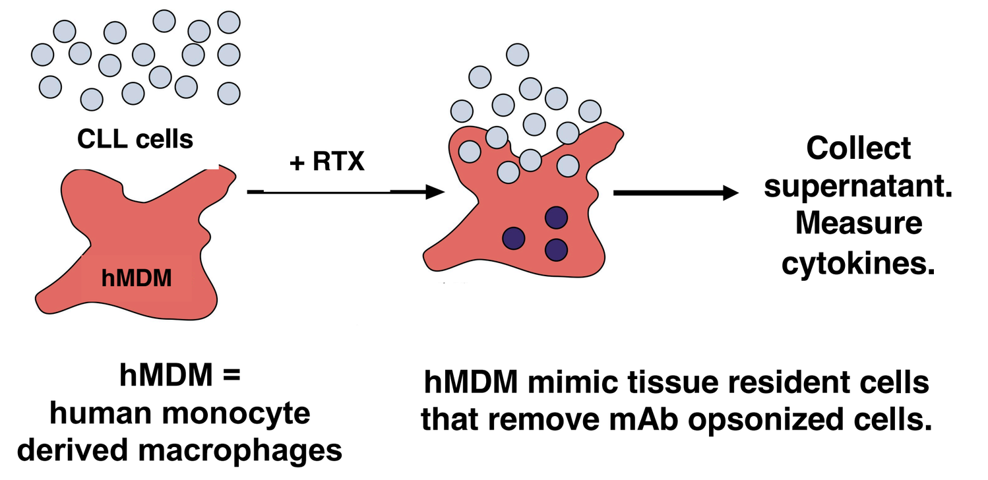
IP10 RNA in tissue macrophages



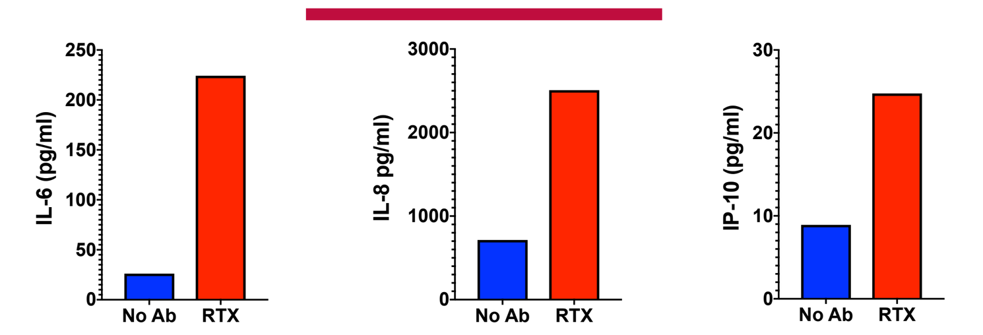
Mostly tissue macrophages express IP10.

Human Protein Atlas proteinatlas.org
Karlsson et al. Sci Adv 2021 PMID 34321199

Modeling FDIR in vitro



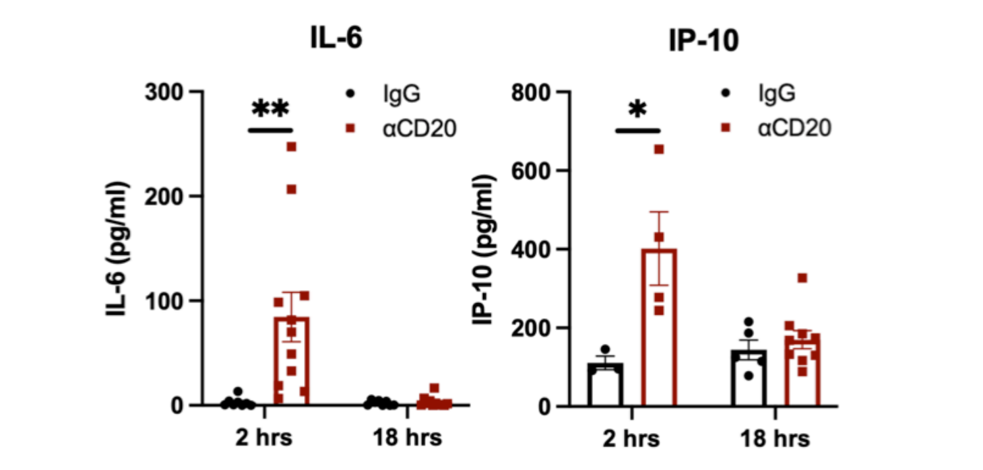
RTX induces cytokines in vitro



- Rituximab (RTX) binding to CLL cells and hMDM induces cytokines (IL-6, IL-8, and IP-10) in vitro as compared to no antibody (No Ab).
- Extent of induction is different from FDIR.

Modeling FDIR in mice

aCD20 mAb induces mouse cytokines

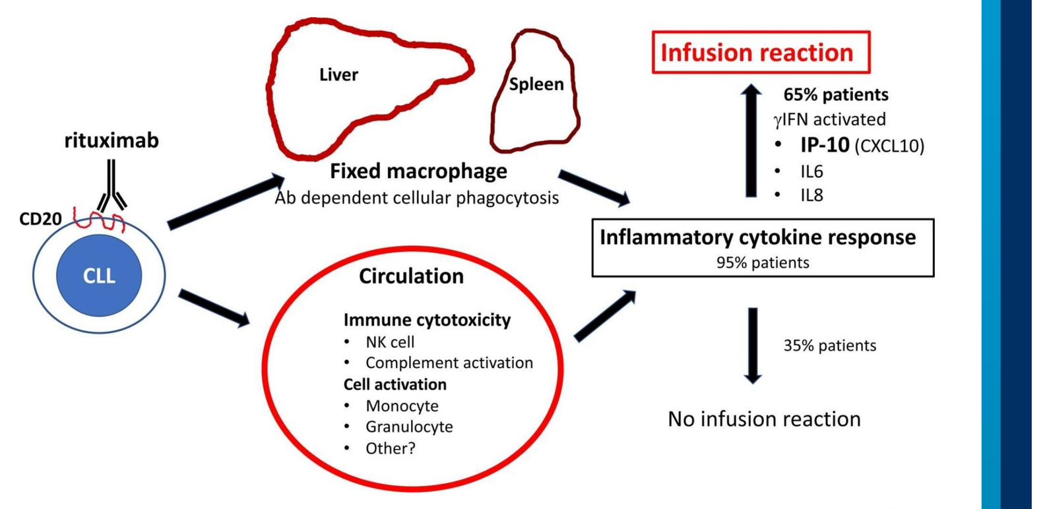


- i.v. anti-CD20 (aCD20, 5D2 mAb) treatment of mice induces IL-6 and IP-10 similarly to FDIR in human patients (2 hrs > 18 hrs post-injection).
- aCD20 mAb required (compare to IgG).

Conclusions

- FDIR occurs with low dose IV mAb treatment.
- FDIR does NOT correlate with anticipated CLL cell characteristics or levels of antigen (CLL cell counts, CLL CD20 levels, or total CD20 molecules), mAb, or complement.
- Cytokine release syndrome occurs in both FDIR and non-FDIR patients.
- FDIR correlates with elevated IL-6, IL-8, and IP-10.
- Single cell RNA sequence data suggests tissue resident cells (possibly macrophages) are responsible.
- In vitro assays that model tissue resident cells demonstrate mAb induction of cytokines.
- FDIR timing is similar in a mouse model.

mAb Infusion Reaction



Moore et al. *Leuk Res* 2023 (129)107072

Acknowledgments

We thank CLL patients at the Wilmot Cancer Institute for their participation in our research efforts and donation of specimens. Sharon Lewinski RN, Maureen Tremaine RN, Tania Orzol NP, and the nursing staff of the WCI Infusion Center provided assistance in conducting the clinical trial and collection of blood samples. This study was funded in part by a generous gift from Ms. Elizabeth Aaron, the Cadregari Endowment (CSZ), Acerta/AstraZeneca (CSZ), and the National Cancer Institute grant R21CA267040 (CSZ).

Disclosures

PBM and CSZ report funding for research through the University of Rochester from AstraZeneca and TG Therapeutics, JEM is an advisory board/consultant participant for BeiGene, CSZ reports funding for research through the University of Rochester from GenMab, and PMB reports serving as a consultant for Pharmacyclics, AbbVie, Genentech and Seattle Genetics.