Poster #310

Associations between Plasma Cytokine and Microbial Translocation Biomarkers and IRIS in AIDS Clinical Trials Group (ACTG) Study A5202

Margaret Fischl[1], Linda Harrison[2], Varghese George[1], Margaret Roach[1], Xiao-Dong Li[3], David Asmuth[3], Pablo Tebas[4], Camlin Tierney[2], Catherine Godfrey[5], Savita Pahwa[1]

[1]University of Miami School of Medicine, Miami, Florida, USA; [2]Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA; [3]University of California, Davis Medical Center, Sacramento, California, USA:

[4]New York University School of Medicine, New York, New York, USA; [5]Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA

UNIVERSITY OF MIAMI MILLER SCHOOL of MEDICINE

THE MIAMI CENTER FOR AIDS RESEARCH CFAR UNIVERSITY OF MIAMI MILLER SCHOOL OF MEDICINE

Margaret A Fischl, M.D.
Email: mfischl@med.miami.edu
Phone: 305-243-3838
Fax: 305-545-6705

Savita Pahwa, M.D.
Email: spahwa@med.miami.edu
Phone: 305-243-7732

ABSTRACT

Background: IRIS is a pathogen-specific inflammatory response associated with ART initiation. We conducted a nested case-cohort study among subjects in a large prospective randomized longitudinal clinical trial (A5202) in the USA and investigated 18 plasma biomarkers of inflammation and microbial translocation for their association with risk of IRIS.
Methods: ACTG A5202 compared ABC/3TC or TDF/FTC combined with ATV/r or EFV for first-line HIV treatment. IRIS events were defined using ACTG criteria; unmasking and paradoxical events were included. 51 of 1452 subjects with baseline CD4<350 cells/mm^3 developed IRIS (3.9 cases/100 person years). We analyzed 100 randomly selected subjects (94 non-IRIS, 6 IRIS, stratified by pre-enrollment CD4≤200 cells/mm^3) and 45 additional IRIS cases. Plasma samples at baseline (n=141), an early time-point (~week 4, n=138) and week 48 (n=136) were tested for tumor necrosis factor (TNF), sTNF receptor I, sTNF receptor II, granulocyte colony stimulating factor (G-CSF), monocyte chemoattractant protein 1 (MCP-1), interferon (IFN) gamma, IFN alpha 2, IFN gamma inducible protein 10 (IP-10), IL-1 beta, IL-2, IL-6, IL-8, IL-10, IL-12, IL-17, sCD14 (ELISA, Luminex or electrochemiluminescence), lipopolysaccharide (LPS, Limulus amebocyte lysate assay) and 16s ribosomal (r) DNA(PCR). Cox models, stratified by pre-enrollment CD4 and weighted for the case-cohort design, evaluated associations between baseline biomarkers and time to first IRIS event. Logistic models evaluated associations between biomarker levels at week 4 and 48 and IRIS.
Results: The 145 subjects included 79% male, 34% with AIDS history, median age 39 years, baseline HIV-1 RNA 4.8 (IQR 4.6-5.4) $\log_{10}$c/ml, CD4 84 (30-184) cells/mm^3. At baseline, higher IP-10, LPS, sCD14, 16s rDNA and IFN alpha 2 were associated with greater risk of IRIS (Table 2). There was a significant (p=0.012) non-linear relationship between MCP-1 and IRIS. These results were supported by adjusted models; other biomarkers had p≥0.15.
Most biomarkers (TNF, sTNFRII, G-CSF, MCP-1, IP-10, IL-8, IL-10, IFN alpha 2, LPS, sCD14) decreased after ART initiation. IP-10 and LPS at week 4 were still associated with IRIS. At week 48, this association only remained for IP-10.
Conclusions: Enhanced systemic inflammatory response presumably through persistent monocyte activation and bacterial translocation in immunosuppressed patients appear important in the pathogenesis of IRIS and may be useful to predict risk for IRIS

INTRODUCTION

- Immune Reconstitution Inflammatory Syndrome (IRIS) is widely recognized as an important pathogen-specific inflammatory response associated with the initiation of antiretroviral therapy (ART).
- To gain insight into the pathogenesis of IRIS, we conducted a nested case-cohort study to assess immune activation in ACTG A5202, a large prospective randomized equivalence clinical study conducted in the US [1,2].
- A case-cohort design was chosen for efficiency.

METHODS

A5202 Study Design
A5202 was a phase IIIB, randomized equivalence ART naive study that compared the efficacy and safety of efavirenz (EFV) and atazanavir/ritonavir (ATV/r) combined with tenofovir/emtricitabine (TDF/FTC) or abacavir/lamivudine (ABC/3TC) [1,2].

IRIS Definition and Main Outcome Measures
- IRIS events were identified using ACTG criteria prospectively by site investigators and compatible presentations retrospectively by the IRIS study team.
- All reported IRIS events and compatible presentations were independently reviewed by the A5202 IRIS review team blinded to antiretroviral regimen.
- Stored EDTA plasma specimens from A5202 baseline study visits, early/near the time of IRIS (~week 4 study visits) and week 48 study visits were evaluated for cytokines plasma-based assays and markers of gut microbial translocation.

Stratified Case-Cohort Study Design
- Subjects were selected from A5202 participants who met the following inclusion criteria:
  - Baseline CD4 count <350 cells/mm^3 (population identified as most at risk for IRIS events)
  - Initiated study treatment
  - Available stored EDTA plasma samples
- Samples were tested for 100 randomly selected participants (random sub-cohort), IRIS cases n=6; non-IRIS cases n=94, and all remaining IRIS cases (n=45)
- The random sub-cohort was stratified by the potential confounder, pre-enrollment CD4 count ≤200 cells/mm^3, to match the distribution in the IRIS cases.

Biomarkers Tested
- **Cytokine Plasma-based Assays**
  tumor necrosis factor (TNF), sTNF receptor I, sTNF receptor II, granulocyte colony stimulating factor (G-CSF), monocyte chemoattractant protein 1 (MCP-1), interferon (IFN) gamma, IFN alpha 2, IFN gamma inducible protein 10 (IP-10), IL-1 beta, IL-2, IL-6, IL-8, IL-10, IL-12, IL-17
- **Markers of Gut Microbial Translocation**
  sCD14 (ELISA, Luminex or electrochemiluminescence), lipopolysaccharide (LPS, Limulus amebocyte lysate assay), 16s ribosomal (r) DNA(PCR).

Study Objectives
1. Assess distributions of baseline biomarkers in IRIS cases and non-cases, and estimate the association of each biomarker with time to IRIS
2. Evaluate how biomarkers change over time and assess whether biomarkers differ between IRIS cases and non-cases at the early time-point and week 48

Statistical methods
- Biomarkers with >20% of values lower the laboratory detection limit were dichotomized, and effect measures compared being greater than the detection limit to less than
- Biomarkers with ≤20% of values lower than the laboratory detection limit were categorized into tertiles (i.e. 3 equally sized groups), and effect measures compared the middle and upper tertiles to the lower tertile
- If there was evidence for a non-linear effect (non-linearity p<0.05), the model using tertiles was considered primary. Otherwise, values less than the laboratory detection limit were imputed at the limit, and a linear model fitted

METHODS (continued)

- In these linear models, to improve normality, biomarkers TNF, sTNFRI, sTNFRII, G-CSF, MCP-1, IP-10, LPS and sCD14 were $\log_{10}$ transformed, and the effect per inter-tertile range increase presented.
- Univariable Cox models, stratified by pre-enrollment CD4 and weighted for the case-cohort design, evaluated associations between baseline biomarkers and time to first IRIS event.
- Univariable logistic models, conditional on pre-enrollment CD4, evaluated associations between biomarker levels at ~week 4 and IRIS.
- Normal regression models, adjusted for pre-enrollment CD4, evaluated associations between IRIS and biomarker levels at week 48
- Co-variables, baseline HIV-1 RNA, CD8 percent, prior AIDS illness and randomized treatment arm, found associated with IRIS in a previous analysis of this cohort [3] were added to multivariable models.

RESULTS

Study Sample
•Among 1452 subjects meeting inclusion criteria for this study, 51 developed IRIS within one year (3.9 cases/100 person years).
•A total of 55 IRIS related events were experienced by these 51 subjects; 11 MAC, 10 VZV, 8 HSV, 5 KS, 4 HCV, 4 mTB, 4 PCP, 2 cryptococcosis, 2 toxoplasmosis, 1 PML, 1 M. Kansasii, 1 eosinophilic folliculitis, 1 CMV colitis and 1 swollen lymph nodes (not attributed to a specific pathogen)
•37 cases were identified prospectively and 14 through retrospective review.
•First IRIS sign/symptoms occurred a median 4.4 weeks after ART initiation; with 24 (47%) occurring within 4 weeks, 20 (39%) within 16 weeks, and 4 (14%) between 16 and 43 weeks.
•Baseline characteristics of the 145 subjects selected for the nested case-cohort study are in Table 1.

Baseline Biomarkers and IRIS Status (Table 2 and Figure 1)
Cytokines
•Higher baseline IP-10 and IFN alpha 2 were significantly associated with a greater risk of IRIS with associations supported by multivariable modeling.
•There was a significant non-linear relationship (p=0.012) between baseline MCP-1 and IRIS; subjects in the middle ordered distribution had the lowest risk and those in the upper order distribution the greatest risk of IRIS. This non-linear relationship was supported by multivariable modeling.
Markers of Gut Microbial Translocation
•Higher baseline LPS, soluble CD14 and 16s rDNA were all significantly associated with a greater risk of IRIS with associations supported by multivariable modeling.

Over Time (~week 4 and 48) (Table 2 and Figures 2 and 3)
Cytokines
•Most cytokines (TNF, sTNFRII, G-CSF, MCP-1, IP-10, IL-8, IL-10, IFN alpha 2) decreased significantly after ART initiation.
•At ~week 4 and 48, only IP-10 was significantly associated with IRIS after adjustment in multivariable models.
Markers of Gut Microbial Translocation
•LPS and sCD14 decreased significantly after ART initiation.
•At ~ week 4, only LPS was significantly associated with IRIS after adjustment in multivariable models.

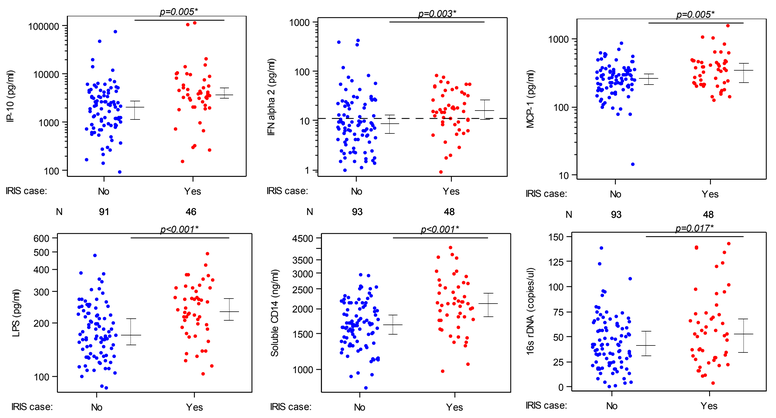
Table 1: Baseline Characteristics by Case-Cohort Group

	Random Sub-cohort (N=100)		Remaining Cases	Total
	No IRIS N=(94)	IRIS N=(6)	IRIS N=(45)	(N=145)
Men	74 (79%)	5 (83%)	36 (80%)	115 (79%)
Race/Ethnicity				
White non-Hispanic	30 (32%)	2 (33%)	14 (31%)	46 (32%)
Black non-Hispanic	32 (34%)	2 (33%)	12 (27%)	46 (32%)
Hispanic	26 (28%)	1 (17%)	17 (38%)	45 (31%)
Other/Unknown	6 (6%)	1 (17%)	2 (4%)	9 (6%)
Median age (years)	39	37	41	39
Median HIV-1 RNA ($\log_{10}$c/ml)	4.7	4.9	4.9	4.9
Median CD4 count (cells/mm^3)	108	28	56	84
Pre-enrollment CD4 count ≤200 cells/mm^3	74 (79%)	6 (100%)	36 (80%)	116 (80%)
Median CD8 count (cells/mm^3)	645	329	577	617
CD8 percent				
<50%	21 (22%)	2 (33%)	6 (13%)	29 (20%)
50-<60%	31 (33%)	1 (17%)	4 (9%)	36 (25%)
60-<70%	25 (27%)	1 (17%)	18 (40%)	44 (30%)
≥70%	17 (18%)	2 (33%)	17 (38%)	36 (25%)
Prior AIDS Illness	24 (26%)	4 (67%)	22 (49%)	50 (34%)
Study Treatment				
EFV + TDF/FTC	26 (28%)	0 (0%)	9 (20%)	35 (24%)
EFV + ABC/3TC	19 (20%)	3 (50%)	12 (27%)	34 (23%)
ATV/r + TDF/3TC	23 (24%)	0 (0%)	12 (27%)	35 (24%)
ATV/r + ABC/3TC	26 (28%)	3 (50%)	12 (27%)	41 (28%)

Table 2: Biomarkers with Significant (p-value <0.05) Associations with IRIS

Time-point	Biomarker	Inter-Tertile Range (33rd-67thcentile) or %>detection limit	Association Presented		Univariable effect, 95% CI	Univariable P-value	Multivariable effect, 95% CI	Multivariable P-value
Baseline	IP-10 (pg/ml) (n=137)	1370-3795	per inter-tertile range	Hazard ratio	1.6 (1.2, 2.2)	0.005	1.7 (1.1,2.5)	0.014
	IFN alpha 2 (pg/ml) (n=141)	46%	≤10.9 (detection limit) >10.9	Hazard ratio	1.0 (ref) 2.8 (1.4, 5.6)	0.003	1.0 (ref) 3.4 (1.3,9.0)	0.011
	MCP-1 (pg/ml) (n=141)	218-342	lower tertile middle tertile upper tertile	Hazard ratio	1.0 (ref) 0.5 (0.2, 1.3) 2.1 (1.0, 4.5)	0.005	1.0 (ref) 0.4 (0.1,1.1) 1.4 (0.5,3.8)	0.042
	LPS (pg/ml) (n=141)	162-228	per inter-tertile range	Hazard ratio	1.7 (1.3, 2.3)	<0.001	1.8 (1.2,2.6)	0.006
	sCD14 (ng/ml) (n=141)	1580-2082	per inter-tertile range	Hazard ratio	2.1 (1.5, 3.0)	<0.001	2.1 (1.2,3.7)	0.008
	16s rDNA (copies/ul) (n=141)	32-58	per inter-tertile range	Hazard ratio	1.4 (1.1, 1.9)	0.017	1.4 (1.1,2.0)	0.018
Early (~Week 4)	IP-10 (pg/ml) (n=123)	699-1745	per inter-tertile range	Risk ratio	1.6 (1.2, 2.2)	0.004	1.6 (1.1,2.2)	0.008
	LPS (pg/ml) (n=138)	174-244	per inter-tertile range	Risk ratio	2.2 (1.5, 3.3)	<0.001	2.3 (1.5,3.6)	<0.001
Week 48	IP-10 (pg/ml) (n=131)		non-IRIS IRIS	Multiplicative effect on IP-10	1.0 (ref) 2.2 (1.4, 3.4)	<0.001	1.0 (ref) 2.1 (1.3,3.2)	0.002

Figure1: Baseline Biomarkers with Significant (p-value <0.05) Associations by IRIS Status



* p-values from univariable models in Table 2. Dashed line displays laboratory detection limit. Black lines display the median, inter-tertile range (33rd-67th centile)

Figure 2: Early (~Week 4) IP-10 and LPS by IRIS Status

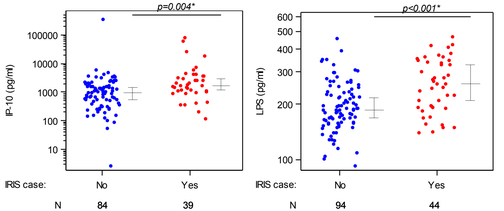
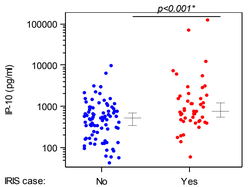


Figure 3: Week 48 IP-10 by IRIS Status



* p-values from univariable models in Table 2. Black lines display the median, inter-tertile range (33rd-67th centile)

SUMMARY

➢For plasma cytokines, higher baseline IP-10 and IFN alpha 2 were associated with a greater risk of IRIS
➢There was a significant non-linear relationship with baseline MCP-1 and IRIS; subjects in the middle tertile had the lowest risk and those in the upper tertile the greatest risk of IRIS
➢ For markers of microbial translocation, higher baseline LPS, soluble CD14 and 16s rDNA were all associated with a greater risk of IRIS
➢Many plasma cytokines (TNF, sTNFRII, G-CSF, MCP-1, IP-10, IL-8, IL-10, IFN alpha 2) and markers of microbial translocation (LPS, soluble CD14) decreased after ART initiation
➢At ~week 4, higher IP-10 and LPS were still associated with IRIS. At week 48, IRIS was only associated with higher IP-10

CONCLUSION

Enhanced systemic inflammatory response presumably through persistent monocyte activation and bacterial translocation in immunosuppressed patients appear important in IRIS pathogenesis and may be useful in understanding pathways leading to development of IRIS

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