Marta Epeldegui, PhD
UCLA AIDS Institute

Research in the Epeldegui lab is focused on studying AIDS-malignancies

How does HIV infection lead to B-cell lymphoma?

Many AIDS-associated cancers are associated with infection with oncogenic viruses (EBV, HHV8, HPV).

HIV infection results in the **loss of specific immunity to cells infected with such viruses**, leading to uncontrolled viral infection, transformation of infected cells, and cancer.

However, not all cancers seen in HIV+ populations are infected with an oncogenic virus.

HIV infection also results in the **chronic inflammation and immune system activation** (both antigen-specific and polyclonal), leading to DNA-modifying events and **oncogenic molecular errors** that result in cancer.

AIDS-associated non-Hodgkin lymphoma

- Two major mechanisms are believed to contribute to the development of AIDS-NHL:

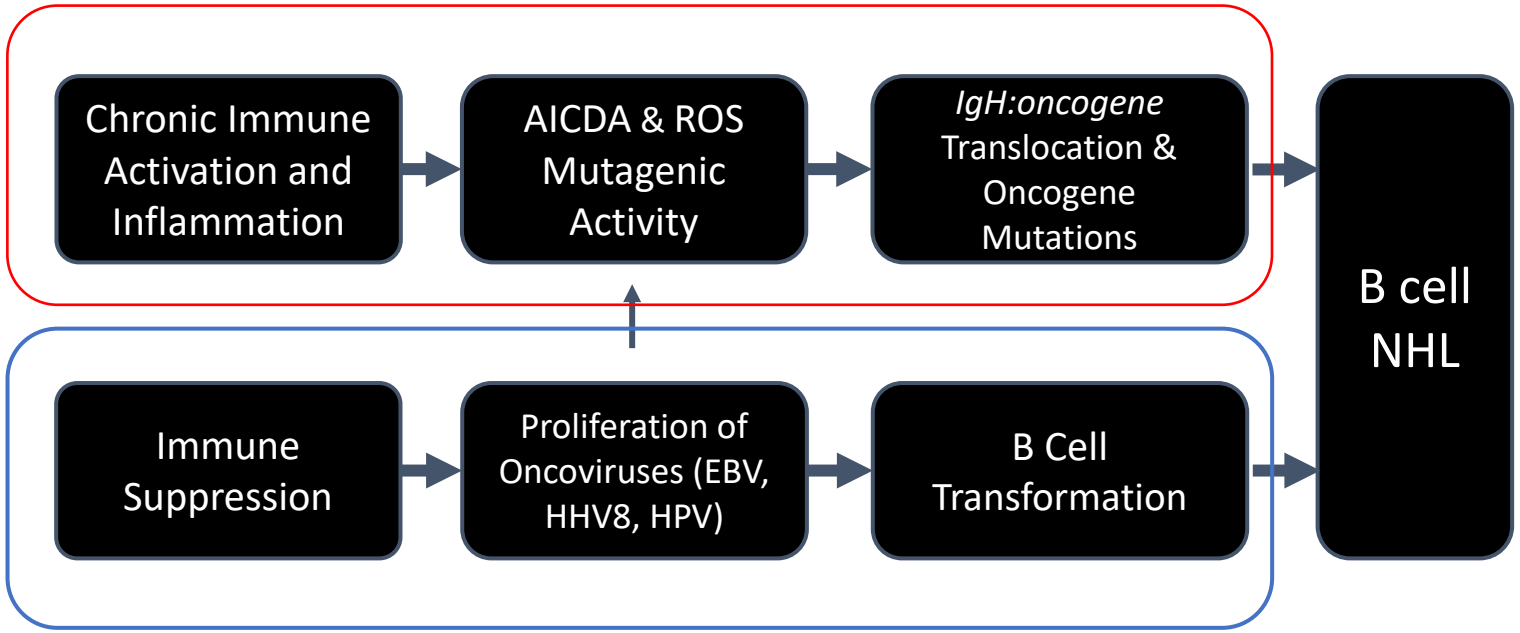
  1) loss of immunoregulatory control of EBV-infected B cells

  2) molecular lesions (oncogene mutations, *MYC:IgH* translocations) that result from errors in DNA modifying events (somatic hypermutation, class switch recombination) that occur during the process of B cell activation.

- Evidence that B cell activation is associated with AIDS-NHL comes from studies showing that:
  - serum levels of several cytokines (Breen, *et al*. CEBP 20:1303, 2011), and PBMC *AICDA* expression (Epeldegui, *et al*. AIDS 21:2265, 2007), are elevated prior to AIDS-NHL diagnosis
  - HIV can directly induce *AICDA* expression and production of cytokines, including IL10, in B cells (Epeldegui, *et al*. PLoS ONE 5:e11448, 2010).

Model for Lymphomagenesis in HIV infection

>500 CD4 count, mainly systemic DLBCL and BL
HAART exposed



<100 CD4 count, mainly EBV+ CNS lymphoma

Features of AIDS-NHL Subtypes

AIDS-NHL Subtype	Virus	%	CD4+ T cell count	Characteristic molecular lesions	
				Oncogene hyper-mutation	Translocations
Primary CNS lymphoma (25%-30%)	EBV	100	<50		
Primary effusion lymphoma (PEL) (rare)	EBV KSHV	50-80 100	<50		
Systemic diffuse large B cell lymphoma (DLBCL) (25%-30%)	EBV	40-60	>200	Majority	*BCL-6* mutations & translocation
Burkitt's lymphoma (25%-30%)	EBV	30-50	>200	Some	*c-MYC:IgH* translocation

Gopal, JCO, 2014; Chen, Oncolgist, 2007; Gaidano, Blood, 2003; Martinez, Opin Oncol, 2002

Hypothesis:

Elevated immune activation and inflammation (elevated serum cytokines and biomarkers) precede the diagnosis of AIDS-NHL

- We and others have shown that:
  - AICDA is elevated prior to AIDS-NHL and that CD40L HIV virions induced AICDA expression in B cells (Epeldegui et al. AIDS, 2007, Epeldegui PLoS Ibe 2011, Imbeault et al. J. Virol. 2011)
  - Chronic Immune activation is elevated prior AIDS-NHL (Vedrame et al. CEBP 2013)
  - Microbial translocation is elevated prior to AIDS-NHL (Epeldegui et al. AIDS 2018, Marks et al. AIDS 2014). Microbial translocation is the translocation of commensal microbial products from the intestinal lumen into de systemic circulation in the absence of overt bacteremia.

How do we study lymphomagenesis?

- By studying **biomarkers** as predictors of disease or as prognostic for treatment.
  - ✓**Immune biomarkers**, such as markers of inflammation , immune function (Th1, TH2, TFH, TH17), soluble receptors.
  - ✓Biomarkers **of microbial translocation**, Microbial translocation is present in different diseases: HCV, HBV and HIV infection, IBD, alcohol use, GVHD, Fatty liver disease.
  - ✓**Exosomes** as biomarkers, exosomes are extravesicles secreted by cells, that carry proteins in cell surface or as cargo, they can also carry DNA/RNA.
- By studying the role of regulatory B cells or Bregs, Bregs are characteristic for being $CD19^{+}CD38^{++}CD24^{++}$ and have regulatory function by secreting IL10 and expressing PDL1.Therefore having dual function in disease:
  1) Inhibiting T cell function through IL-10 and PDL1
  2) Activating B cells, since IL10 is a B cell stimulatory cytokine.

A prospective study of serum microbial translocation biomarkers and risk of AIDS-related non-Hodgkin lymphoma

Marta Epeldegui[a], Larry Magpantay[a], Yu Guo[a], Gordana Halec[a], William G. Cumberland[a], Priscilla K. Yen[a], Bernard Macatangay[b], Joseph B. Margolick[c], Anne F. Rositch[c], Steven Wolinsky[d], Otoniel Martinez-Maza[a] and Shehnaz K. Hussain[e]

Background: Chronic immune activation is a harbinger of AIDS-associated non-Hodgkin lymphoma (AIDS-NHL), yet the underlying basis is unclear. Microbial translocation, the passage of microbial components from the gastrointestinal tract into the systemic circulation, is a source of systemic immune activation in HIV infection and may be an important contributor to chronic B-cell activation and subsequent AIDS-NHL development.

Method: We measured biomarkers of microbial translocation including bacterial receptors/antibodies, intestinal barrier proteins, and macrophage activation-associated cytokines/chemokines, in serum from 200 HIV-infected men from the Multicenter AIDS Cohort Study prior to their AIDS-NHL diagnosis (mean = 3.9 years; SD = 1.6 years) and 200 controls. Controls were HIV-infected men who did not develop AIDS-NHL, individually matched to cases on $CD4^+$ T-cell count, prior antiretroviral drug use, and recruitment year into the cohort.

Results: Biomarkers of bacterial translocation and intestinal permeability were significantly increased prior to AIDS-NHL. Lipopolysaccharide-binding protein (LPB), fatty acid-binding protein 2 (FABP2), and soluble CD14 were associated with 1.6-fold, 2.9-fold, and 3.7-fold increases in AIDS-NHL risk for each unit increase on the natural log scale, respectively. Haptoglobin had a 2.1-fold increase and endotoxin-core antibody a 2.0-fold decrease risk for AIDS-NHL (fourth versus first quartile). Biomarkers of macrophage activation were significantly increased prior to AIDS-NHL: B-cell activation factor (BAFF), IL18, monocyote chemoattractant protein-1 (MCP1), tumor necrosis factor-α (TNFα), and CCL17 had 2.2-fold, 2.0-fold, 1.6-fold, 2.8-fold, and 1.7-fold increases in risk for each unit increase on the natural log scale, respectively.

Conclusion: These data provide evidence for microbial translocation as a cause of the systemic immune activation in chronic HIV infection preceding AIDS-NHL development.

AIDS 2018, **32**:945–954

Immune Activation and Microbial Translocation as Prognostic Biomarkers for AIDS-Related Non-Hodgkin Lymphoma in the AMC-034 Study

Laura E. Martínez[1,2], Shelly Lensing[3], Di Chang[3], Larry I. Magpantay[1,2], Ronald Mitsuyasu[1], Richard F. Ambinder[4], Joseph A. Sparano[5], Otoniel Martínez-Maza[1,2,6], and Marta Epeldegui[1,2,6]

ABSTRACT

Purpose: AIDS-related non-Hodgkin lymphoma (ARL) is the most common cancer in HIV-infected individuals in the United States and other countries in which HIV-positive persons have access to effective combination antiretroviral therapy (cART). Our prior work showed that pretreatment/postdiagnosis plasma levels of some cytokines, such as IL6, IL10, and CXCL13, have the potential to serve as indicators of clinical response to treatment and survival in ARL. The aims of this study were to identify novel prognostic biomarkers for response to treatment and/or survival in persons with ARL, including biomarkers of microbial translocation and inflammation.

Experimental Design: We quantified plasma levels of several biomarkers (sCD14, LBP, FABP2, EndoCab IgM, IL18, CCL2/MCP-1, sCD163, IP-10/CXCL10, TARC/CCL17, TNFα, BAFF/BLyS, sTNFRII, sCD44, and sIL2Rα/sCD25) by multiplexed immunometric assays (Luminex) or ELISA in plasma specimens obtained from ARL patients enrolled in the AMC-034 trial, which compared infusional combination chemotherapy (EPOCH: etoposide, vincristine, doxorubicin, cyclophosphamide, and prednisone) with concurrent or sequential rituximab. Plasma was collected prior to the initiation of therapy ($n = 57$) and after treatment initiation ($n = 55$).

Results: We found that several biomarkers decreased significantly after treatment, including TNFα, sCD25, LBP, and TARC (CCL17). Moreover, pretreatment plasma levels of BAFF, sCD14, sTNFRII, and CCL2/MCP-1 were univariately associated with overall survival, and pretreatment levels of BAFF, sTNFRII, and CCL2/MCP-1 were also associated with progression-free survival.

Conclusions: Our results suggest that patients with ARL who responded to therapy had lower pretreatment levels of inflammation and microbial translocation as compared with those who did not respond optimally.

SCIENTIFIC REPORTS

Corrected: Author Correction

OPEN

Elevated numbers of PD-L1 expressing B cells are associated with the development of AIDS-NHL

Received: 15 October 2018
Accepted: 5 June 2019
Published online: 28 June 2019

Marta Epeldegui[1,2,3], David V. Conti[4], Yu Guo[3], Wendy Cozen[4,5], Manuel L. Penichet[1,2,6,7,8] & Otoniel Martínez-Maza[1,2,3,7,9]

The risk for non-Hodgkin lymphoma (NHL) is markedly increased in persons living with human immunodeficiency virus (HIV) infection, and remains elevated in those on anti-retroviral therapy (cART). Both the loss of immunoregulation of Epstein-Barr virus (EBV) infected cells, as well as chronic B-cell activation, are believed to contribute to the genesis of AIDS-related NHL (AIDS-NHL). However, the mechanisms that lead to AIDS-NHL have not been completely defined. A subset of B cells that is characterized by the secretion of IL10, as well as the expression of the programmed cell death ligand-1 (PD-L1/CD274), was recently described. These PD-L1$^+$ B cells can exert regulatory function, including the dampening of T-cell activation, by interacting with the program cell death protein (PD1) on target cells. The role of PD-L1$^+$ B cells in the development of AIDS-NHL has not been explored. We assessed B cell PD-L1 expression on B cells preceding AIDS-NHL diagnosis in a nested case-control study of HIV+ subjects who went on to develop AIDS-NHL, as well as HIV+ subjects who did not, using multi-color flow cytometry. Archival frozen viable PBMC were obtained from the UCLA Multicenter AIDS Cohort Study (MACS). It was seen that the number of CD19$^+$CD24^{++}CD38^{++}and CD19$^+$PD-L1$^+$cells was significantly elevated in cases 1–4 years prior to AIDS-NHL diagnosis, compared to controls, raising the possibility that these cells may play a role in the etiology of AIDS-NHL. Interestingly, most PD-L1$^+$ expression on CD19$^+$ cells was seen on CD19$^+$CD24^{++}CD38^{++} cells. In addition, we showed that HIV can directly induce PD-L1 expression on B cells through interaction of virion-associated CD40L with CD40 on B cells.

How do we study lymphomagenesis?

- By studying follicular CD8 T cells ($CD8^+CXCR5^+BCL\text{-}6^+PD1^+$):

  - ✓fCD8 T cells are thought to be induced with inflammation and immune activation and are present in the B cell follicles.
  - ✓fCD8 T cells have the capability to interact with B cells and induce them to secrete antibodies, more like a helper T cell.
  - ✓fCD8 T cells may be important in diseases associated with B cell activation such as: autoimmune disease, HIV infection and cancer.

- By studying mouse models of lymphoma:

  - ✓Using the Humanize mouse model and immune deficient mice as models for lymphomagenesis.

Targeting TfR1 with the ch128.1/IgG1 Antibody Inhibits EBV-driven Lymphomagenesis in Immunosuppressed Mice Bearing EBV$^+$ Human Primary B-cells

Laura E. Martínez[1,2], Tracy R. Daniels-Wells[3], Yu Guo[1,2], Larry I. Magpantay[1,2], Pierre V. Candelaria[3], Manuel L. Penichet[2,3,4,5,6], Otoniel Martínez-Maza[1,2,4,5,7], and Marta Epeldegui[1,2,4]

ABSTRACT

Epstein–Barr virus (EBV) is a human gammaherpesvirus associated with the development of hematopoietic cancers of B-lymphocyte origin, including AIDS-related non-Hodgkin lymphoma (AIDS-NHL). Primary infection of B-cells with EBV results in their polyclonal activation and immortalization. The transferrin receptor 1 (TfR1), also known as CD71, is important for iron uptake and regulation of cellular proliferation. TfR1 is highly expressed in proliferating cells, including activated lymphocytes and malignant cells. We developed a mouse/human chimeric antibody targeting TfR1 (ch128.1/IgG1) that has previously shown significant antitumor activity in immunosuppressed mouse models bearing human malignant B-cells, including multiple myeloma and AIDS-NHL cells. In this article, we examined the effect of targeting TfR1 to inhibit EBV-driven activation and growth of human B-cells *in vivo* using an immunodeficient NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ [NOD/SCID gamma (NSG)] mouse model. Mice were implanted with T-cell–depleted, human peripheral blood mononuclear cells (PBMCs), either without EBV (EBV$^-$), or exposed to EBV *in vitro* (EBV$^+$), intravenously via the tail vein. Mice implanted with EBV$^+$ cells and treated with an IgG1 control antibody (400 μg/mouse) developed lymphoma-like growths of human B-cell origin that were EBV$^+$, whereas mice implanted with EBV$^+$ cells and treated with ch128.1/IgG1 (400 μg/mouse) showed increased survival and significantly reduced inflammation and B-cell activation. These results indicate that ch128.1/IgG1 is effective at preventing the growth of EBV$^+$ human B-cell tumors *in vivo*, thus, indicating that there is significant potential for agents targeting TfR1 as therapeutic strategies to prevent the development of EBV-associated B-cell malignancies.

Significance: An anti-TfR1 antibody, ch128.1/IgG1, effectively inhibits the activation, growth, and immortalization of EBV$^+$ human B-cells *in vivo*, as well as the development of these cells into lymphoma-like tumors in immunodeficient mice.

How it all started?

Early career

- Undergraduate in Biology at the Universidad Complutense de Madrid.
- Master's degree in Developmental Biology at the Universidad Complutense de Madrid.
- PhD at University of California Los Angeles in Martinez-Maza lab.
  - ✓UCLA AIDS Institute Esther Hays Student Research Award.
  - ✓University AIDS Research Program (UARP) now the California HIV/AIDS Research Program (now CHRP) Fellowship.
  - ✓NIAID Clinical Immunology Training Grant (T32)
- Postdoc at the University of California Los Angeles Uittenbogaart lab.
  - ✓ NRSA Rheumatology Training Grant (T32)
  - ✓ NRSA Tumor Immunology Training Grant (T32)

Career Development

- UCLA CFAR career development supplement Award.
- NCI CURE career development supplement Award
- R21- Exploratory Grant Award to Promote Workforce Diversity in Basic Cancer Research. R21-CA220475 (PI: Epeldegui)
- AIDS Malignancies Consortium (AMC) Lab Translational Fellowship.
- All this led to getting my first R01.

Things that helped me succeed

- Mentors- they are key to your success. Good mentors are invested in your success for you not for them.
- NIH/NCI programs and opportunities- NIH/NCI has lots of opportunities. Program officers are a great resource.
- Perseverance- don't let negative news get to you, dwell for a little then move on and always learn from your failures.
- Collaborators- they are also important on your success. Surround yourself with collaborators that complement your research. It is so much more fun to do science collaborating.
- Take a break- sometimes taking a break and doing something it makes you feel good may be the best medicine.

Things that I am working on

- Patience- I observe that senior faculty have much more patience.
- It's ok to say no- be realistic with what you can accomplish.
- Let go- it's ok to sometimes not to have everything under control.
- Mentoring- pass on your knowledge onto others, try to change things that don't work.
- Educate on Diversity- talk about it, why is it important, try to break the cycle.
- Be supportive- everyone is different.

Working on establishing my research group.

Organization of the Epeldegui lab:

1) Biomarker arm:

- ✓We collaborate with groups from all the US measuring biomarkers (mainly epidemiologist).
- ✓Serve as the biomarker core for the AIDS Malignancies Consortium.
- ✓Work with cohorts (MACS, AMC,…) on developing new biomarkers.

2) Basic research:

- ✓ Studying pathogenesis and how AIDS-NHL develops.

Working on establishing my research group.

How I am making it work:

- ✓I share resources with my mentor.
- ✓Have a lab manager that keeps all the lab maintenance and biomarker work moving.
- ✓My current postdoc was an undergraduate that worked with me when I was a graduate student.
- ✓Undergraduates are an important part of the lab (volunteers or work study)
- ✓Currently looking for another postdoc and a technician.
- ✓It’s hard to balance how much work needs to be done and how much you can afford

Plans for the future….

Keep it up!

Martinez-Maza/ Epeldegui Lab
Laura Martinez
Larry Magpantay
Yu Guo
Ruchal Patel

AMC
Shelly Lensing
Di Chang
Joseph Sparano
Ron Mitsuyasu
Richard Ambinder
Jeff Bethony

ACSR
Paige Bracci
Jeff Bethony
Mike McGrath

MACS
Roger Detels
Otoniel Martinez-Maza
Shehnaz Hussain

UCI
Wendy Cozen

Funding
NCI- R01CA228157-01
NCI- AMC Lab/Translational Fellowship
NCI- UCLA JCCC P30CA016042-S
NIH-NCI, R21-CA220475
UCLA Tumor Immunology Training Grant (T32), Individual Postdoctoral Fellowship for Dr. Martinez, 2T32CA009120-41A1
NCI- R01CA228157-S02

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