V SIMPOSIUM DEL GRUPO DE ESTUDIO LATINOAMERICANO DE **LINFOPROLIFERATIVOS**

COMITÉ ORGANIZADOR: JUNTA DIRECTIVA GELL 2021 -2023

GELL
Grupo de Estudio Latinoamericano de Linfoproliferativos

DESARROLLO DE ENSAYOS CLÍNICOS EN EL MANEJO DE ATLL

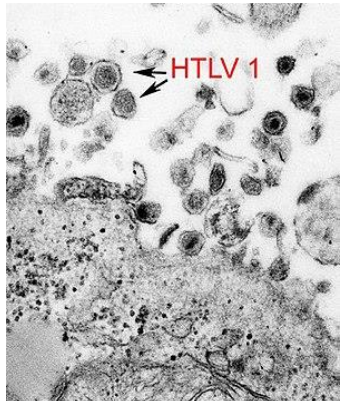
Juan Carlos Ramos, MD
Professor
Division of Hematology
Sylvester Comprehensive Cancer Center
University of Miami Miller School of Medicine

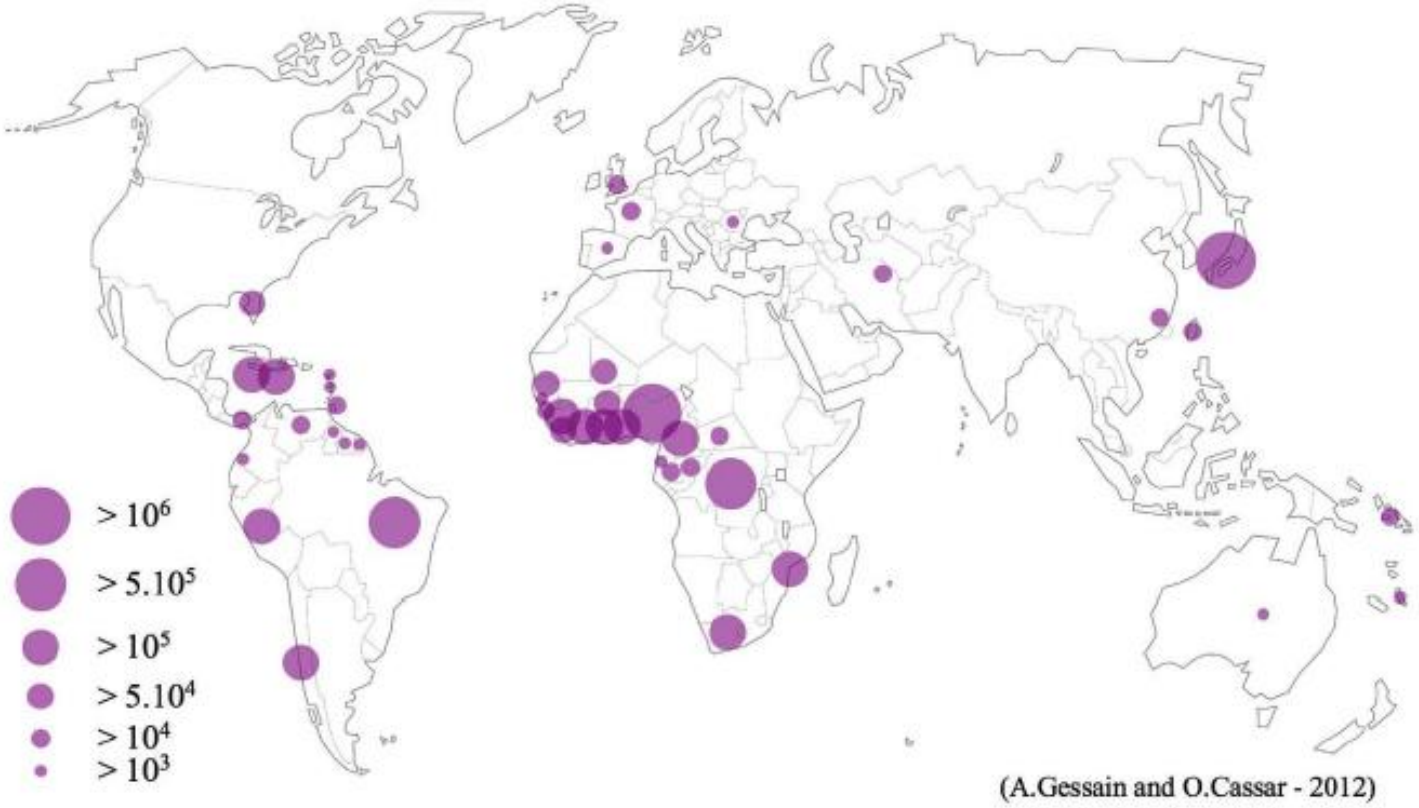
Disclosure

- Acrotech Biopharma supports this investigator-initiated trial (Ramos) by supplying belinostat at no cost

Adult T-cell leukemia-lymphoma (ATLL)

- Caused by human T-cell leukemia virus, type 1 (HTLV-1): Poiesz et al. 1980, Hinuma et al. 1981
  - 10-20 million people infected worldwide (Southern Japan, Central Africa, Caribbean, Brazil and Peru)
  - Transmission: Breast feeding, sexual intercourse, blood transfusion
- ATLL develops in 2-7% of HTLV-1 infected during 6th-7th decades
- Usually fatal with poor survival with low median survival
- Immunophenotype: **$CD4^+$ CD25+ $CD7^-$ $CD26^-$ $CCR4^+$** $CADM1^+$ $FOXP3^{+/-}$ $CD30^{+/-}$ $IRF4/MUM1^{+/-}$



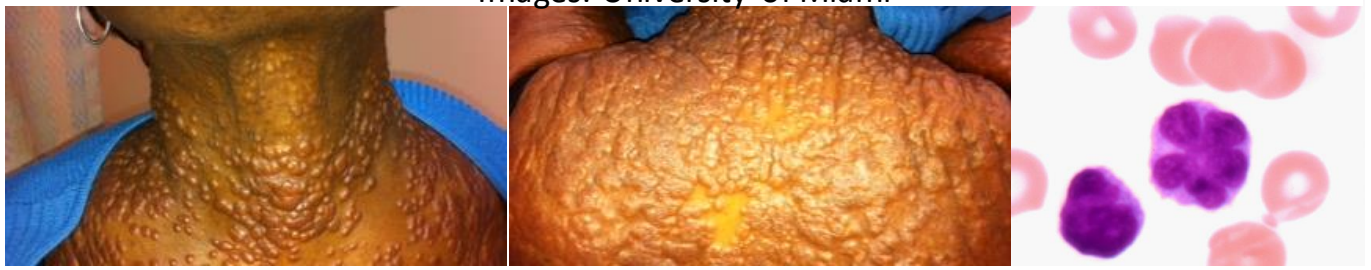


ATLL Sub-classification: *Shimoyama Criteria*

- **Aggressive subtypes**: frequent hypercalcemia and high LDH

  - **Acute type**:
    - Leukemia phase
    - Multi-organ involvement

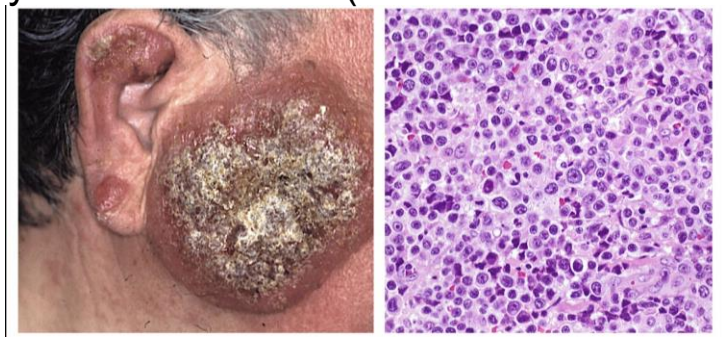
Images: University of Miami



  - **Lymphomatous type**: <1% leukemic cells

  - *Extranodal primary cutaneous variant*:
    - High-grade pathologic features
    - Nodules or tumors > 1 cm

Primary cutaneous ATL (Cook et al. JCO Jan 2009)



- **"Indolent" subtypes**: least common presentation

  - **Smoldering**: <5% leukemic cells, LDH < 1.5 x normal, +/- skin and lung involvement

  - **Chronic**: leukemic phase, normal LDH, +/- lymph nodes, skin, or lung involvement
    - *Unfavorable chronic type variant*: ↑LDH (< 2x normal) behaves more aggressive

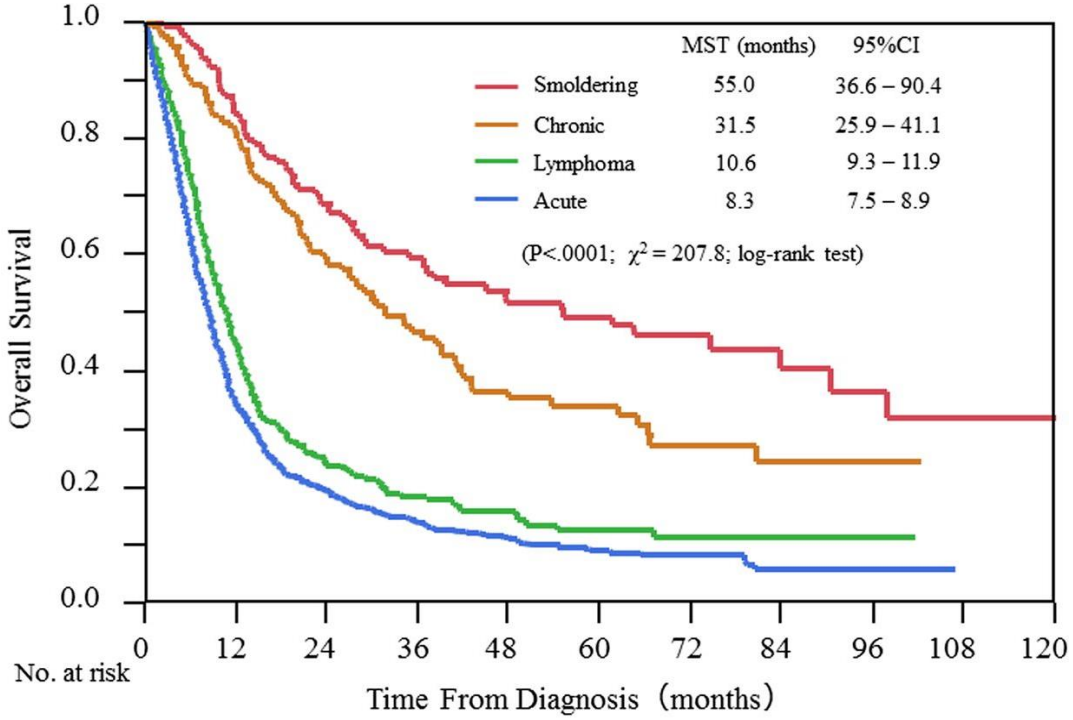
Standard modern therapies for ATLL only yield modest results

- **Median survival:**
  - Acute 4-8 months
  - Lymphomatous 10-11 months
  - After allogenic transplant (Japan): < 6 months

Japan:

Katsuya et al. *Blood* 2015

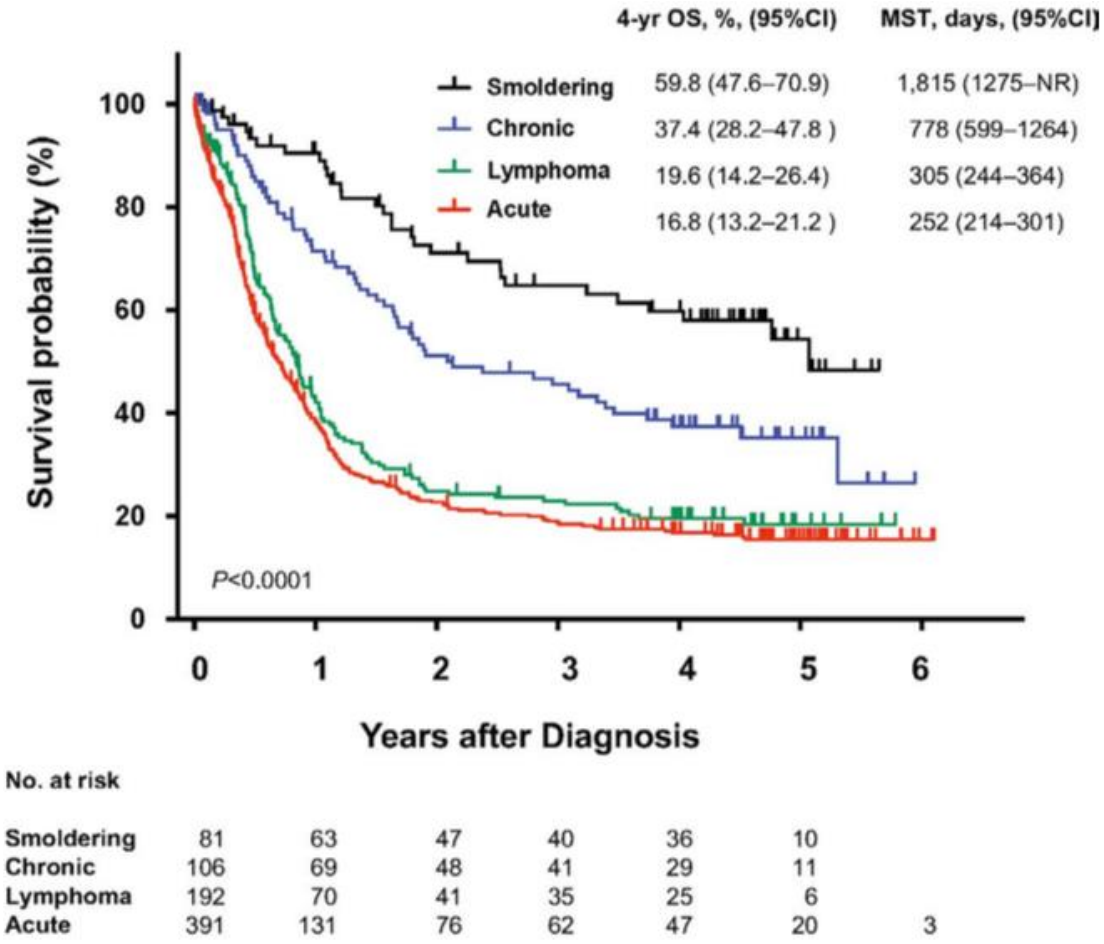
N=1594 (Years 2000-2009)



Japan:

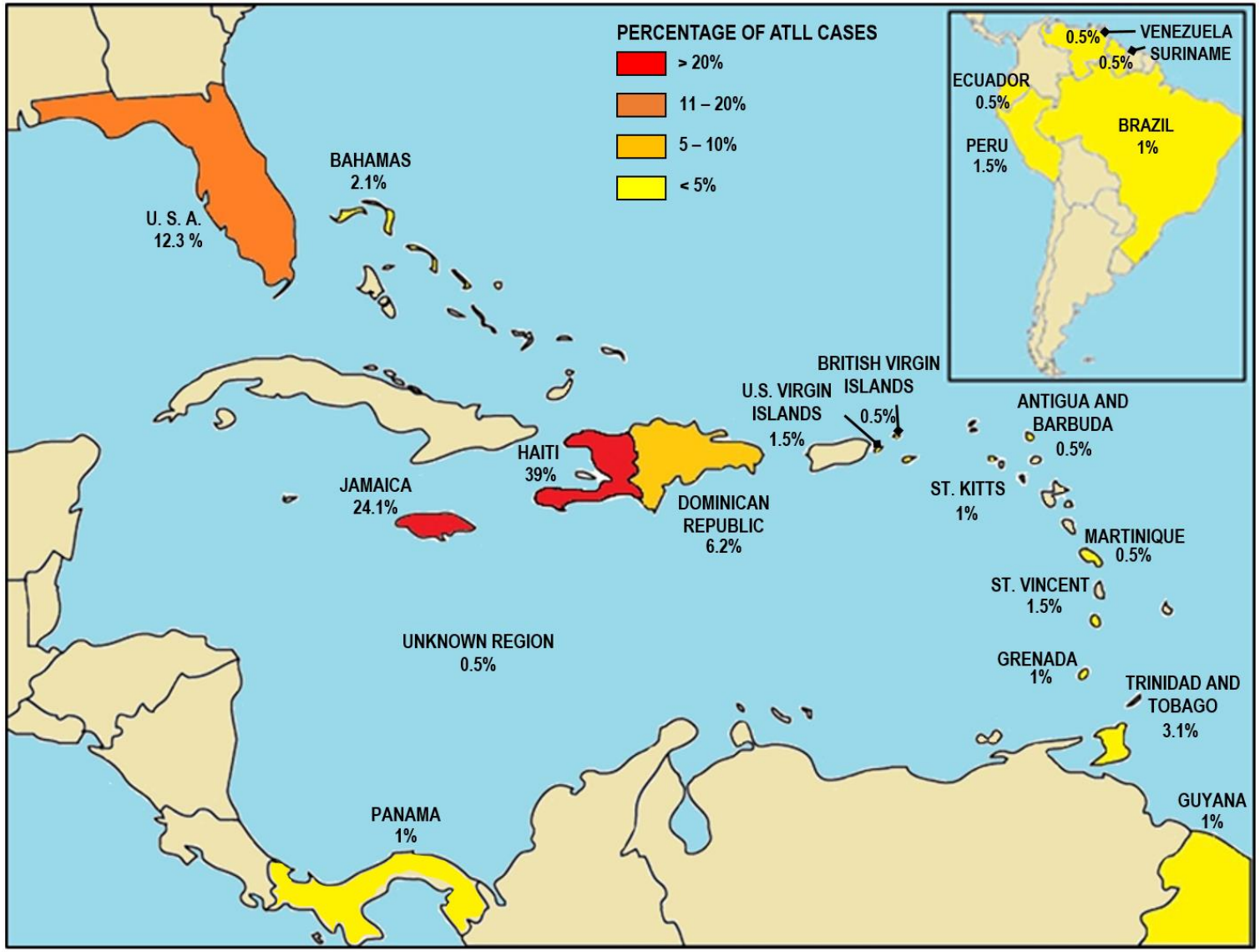
Imaizumi et al. *Cancer Science* 2020

N= 770 (Years 2010-2017)

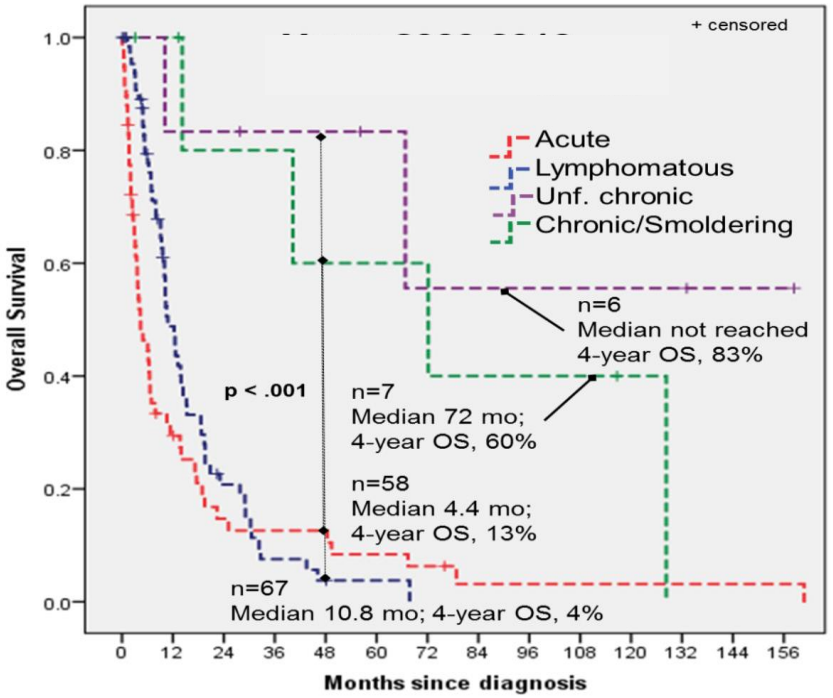


ATLL Encountered in Miami

N= 195 patients (Median age 52, HIV co-infection in 9%)



N=138 (Years 2000-2016)



Malpica et al. *Blood Advances* 2018

Treatment Options for ATLL

- **First line options:**
  - **Chemotherapy: Followed by allogenic stem cell transplant when feasible!**
  - Standard combination chemotherapy: CHOEP, EPOCH, CHOP-like, hyper cVAD, IVAC/MTX-ARA-C (Magrath)
  - VCAP-AMP-VECP +/- mogamulizumab (anti-CCR4 antibody, Japan standard)
    - ❖ Mogamulizumab may improve CR rates but without clear survival benefit, is not too effective treating lymph node compartment, and may increase GVHD (morbidity/mortality) in patients who go allo-transplant!
  - CHP-brentuximab (anti-CD30 ab-MMAE) (Approved in the U.S. for CD30+ PTCL, efficacy has not been established yet)
  - Single agents: Oral etoposide (in debilitated patients)
  - **Zidovudine-interferon-α (AZT-IFN): for non-lymphomatous types!**
  - AZT-IFN + $As0_3$: Highly effective in chronic ATLL (Kchour et al. Blood. 2009)
- **Second line options:**
  - **Standard lymphoma regimens:** i.e. DHAP, ICE, GEMOX
  - **Mogamulizumab** (Approved in the U.S. for CTCL):
  - **Brentuximab vedotin:** for CD30+ (ongoing trials, but lack of published data)
  - **Lenalidomide** (Approved in Japan, little positive experience in U.S.)
  - **Alemtuzumab** (anti-CD-52 ab): available as compassionate use in U.S
  - **Single agent chemotherapy:** Oral etoposide, pralatrexate (lack of efficacy data)
  - **HDAC inhibitors:** i.e. romidepsin, belinostat (often used, lack of efficacy data)

Standard Chemotherapy vs. Zidovudine-Interferonα (AZT-IFN) for ATLL

Zidovudine (ZDV) plus interferon (IFNα) can be efficacious in patients with aggressive leukemic ATL with longer progression-free survival as compared to chemotherapy in patients who achieve a complete response, but responses rates are still suboptimal

University of Miami Experience

Complete response (CR) rates

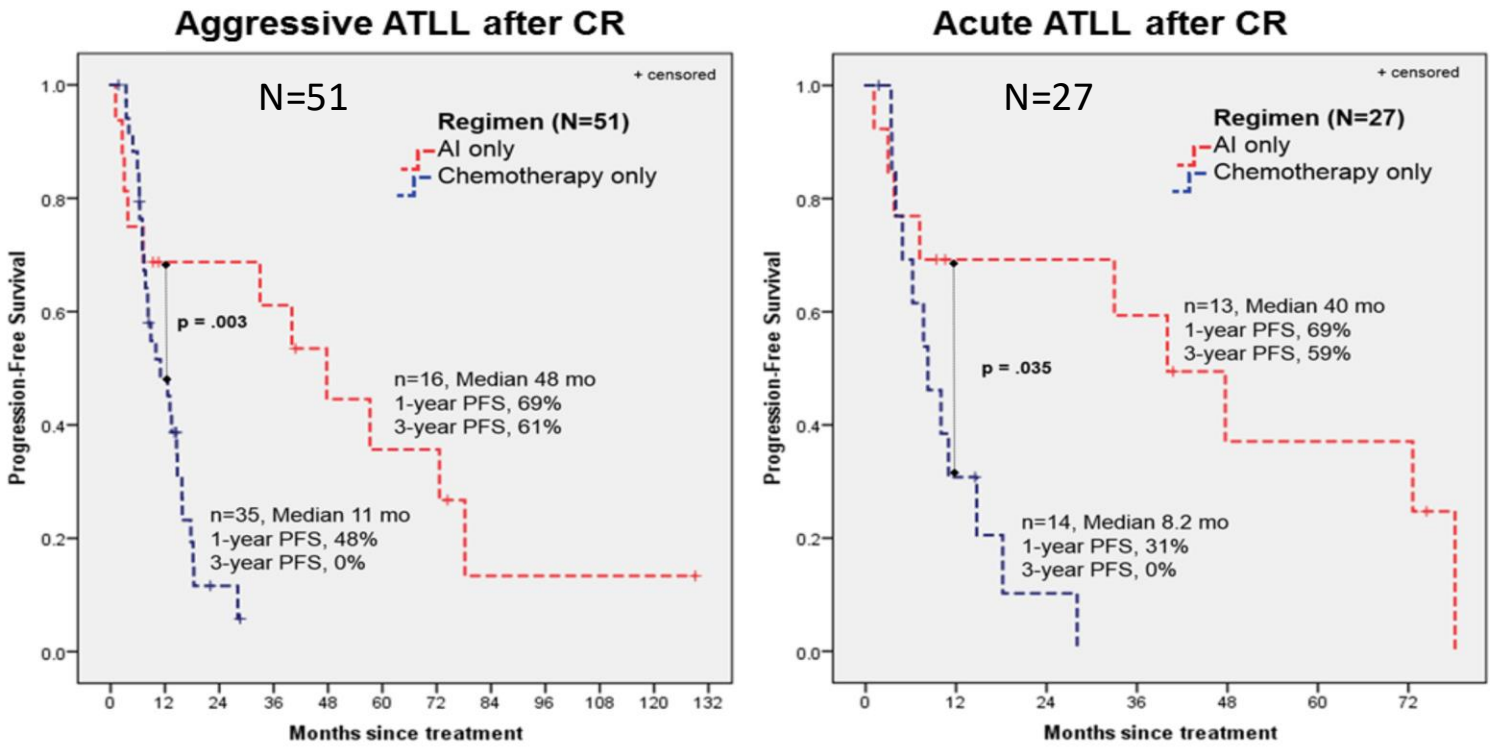
First line chemotherapy:

- Acute: 6/18= 33%
- Lymphomatous: 18/50= 36%

First line AZT-IFN:

- Acute: 10/42= 24%
- Lymphomatous: 1/10= 10%

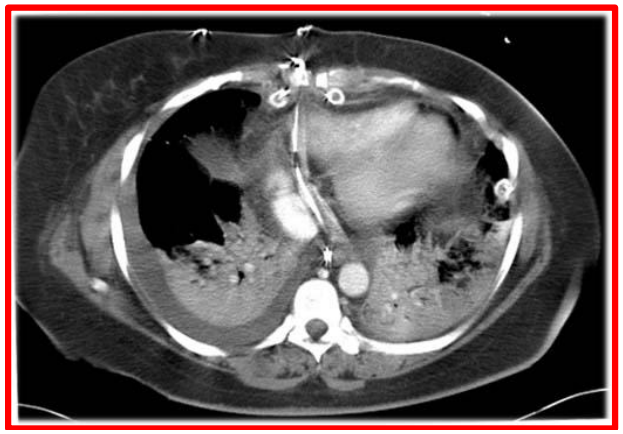
Progression-free survival (PFS) (after first CR)



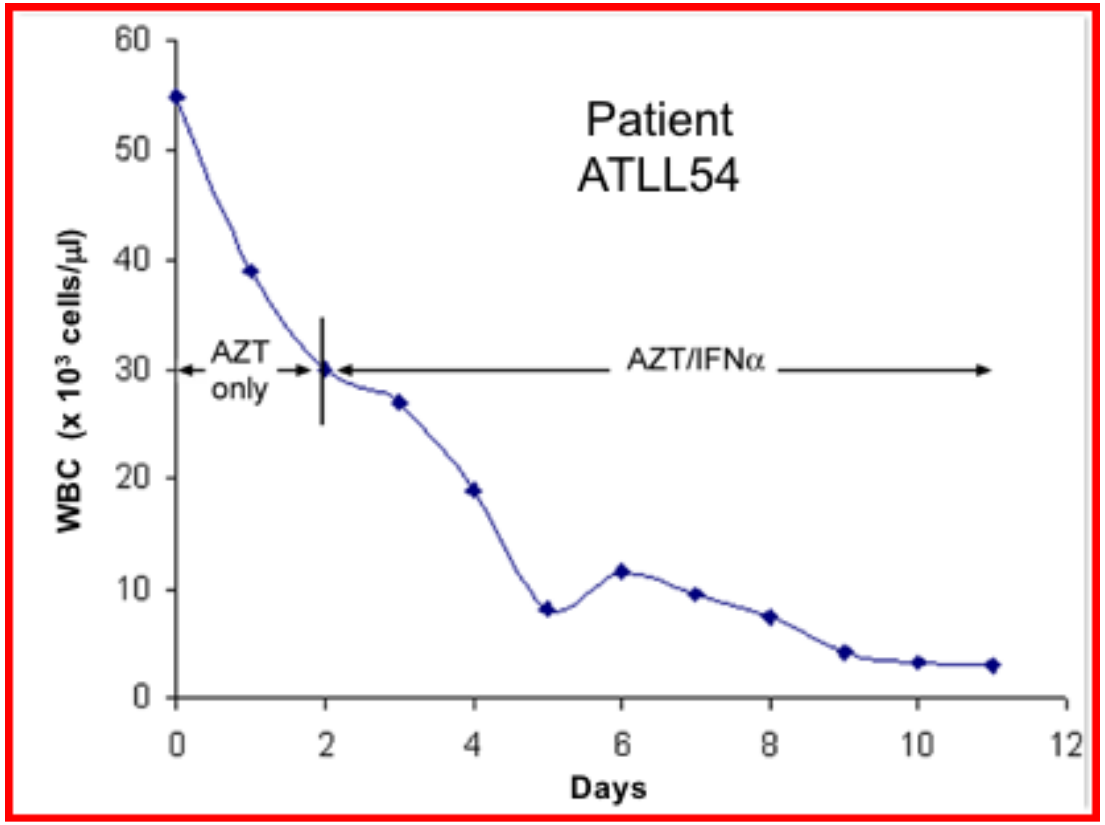
Malpica et al. *Blood Advances* 2018

AZT-IFN can be effective in acute type ATLL

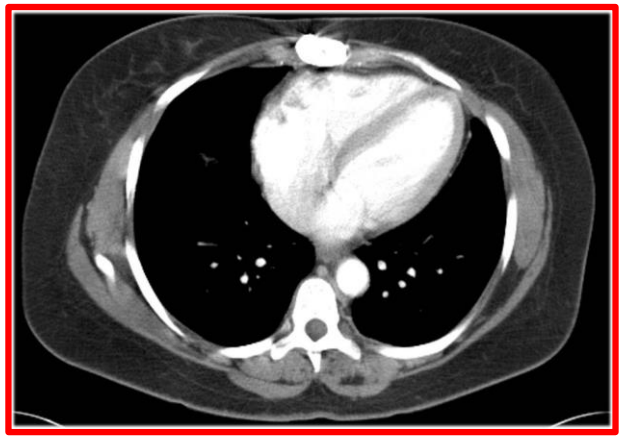
45 y/o Jamaican woman presented with WBC 55,000 x 10^3 cells/µl, hypercalcemia, high LDH, and large pleural and pericardial effusions causing tamponade



Response after AZT-IFN



Post treatment

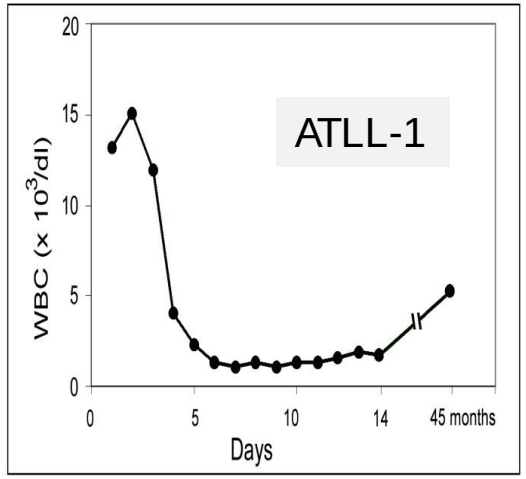


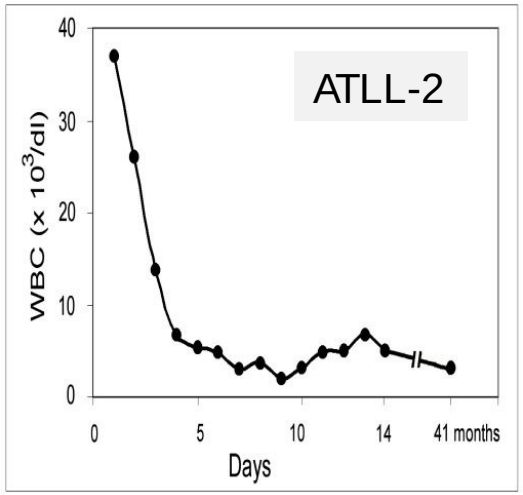
PFS= 4 years

Malpica et al. *Blood Advances* 2018

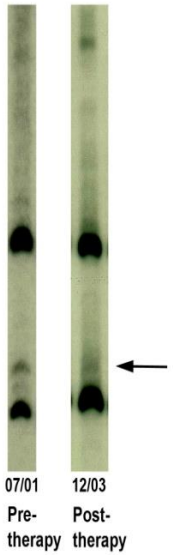
Long-term Responses to AZT/IFN → Persistent molecular disease

Hematologic responses



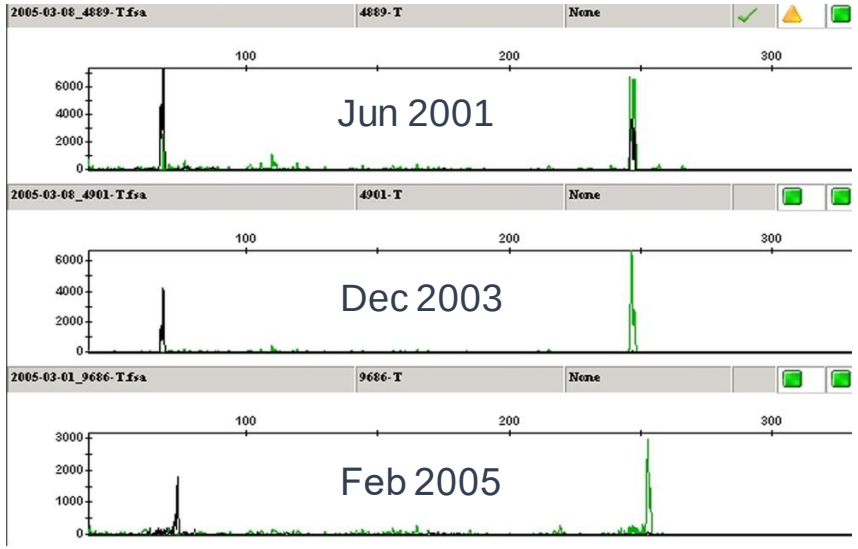


Southern blot

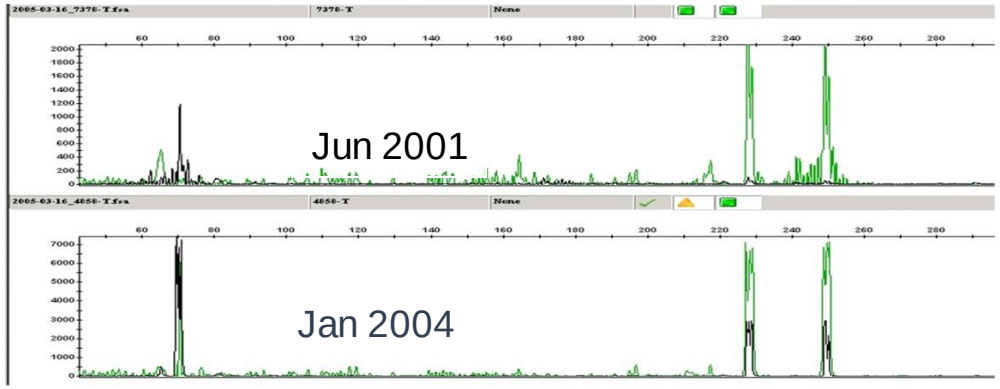


ATLL-1

Multiplex PCR for TCR gene rearrangement



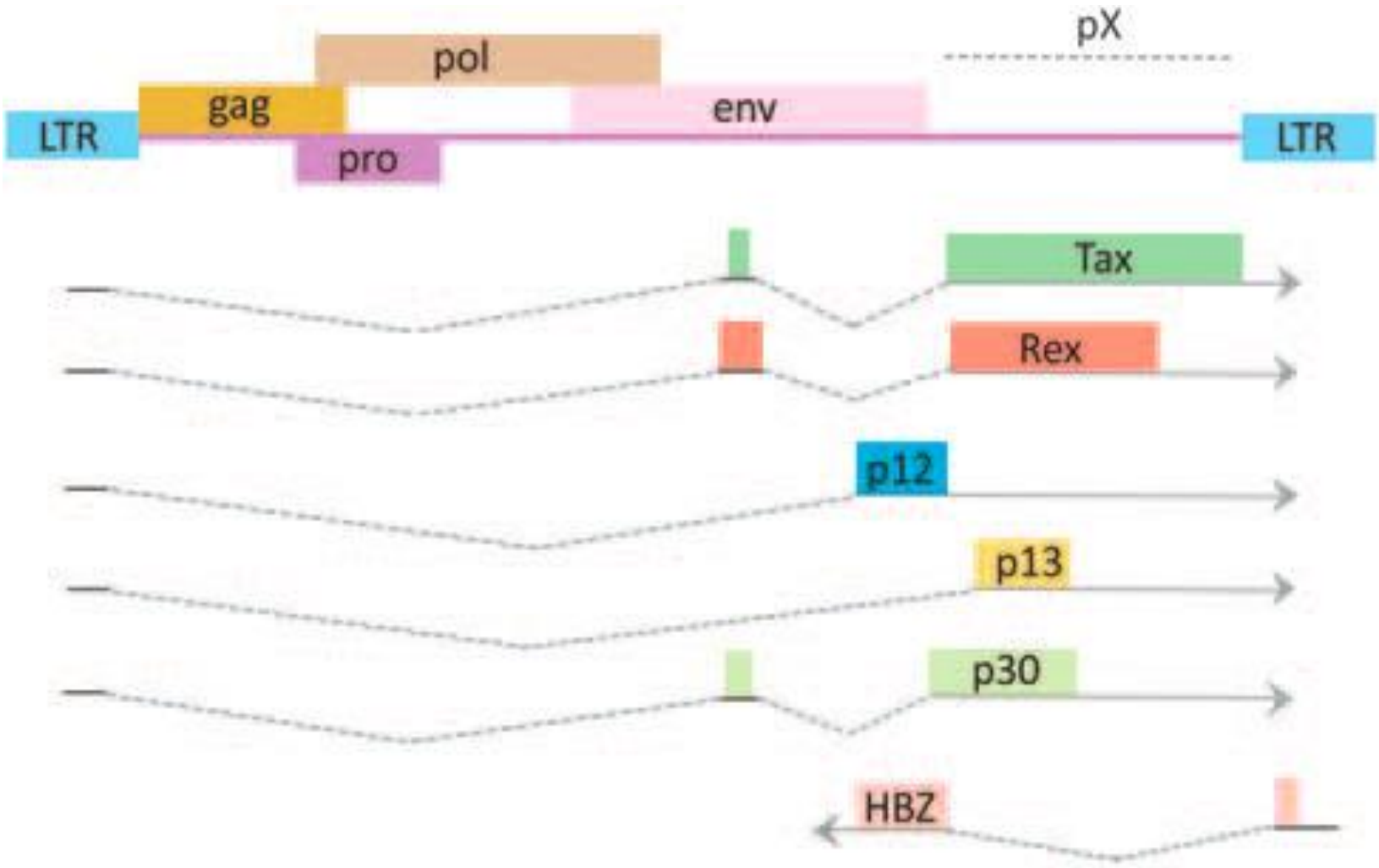
ATLL-2



ATLL-2

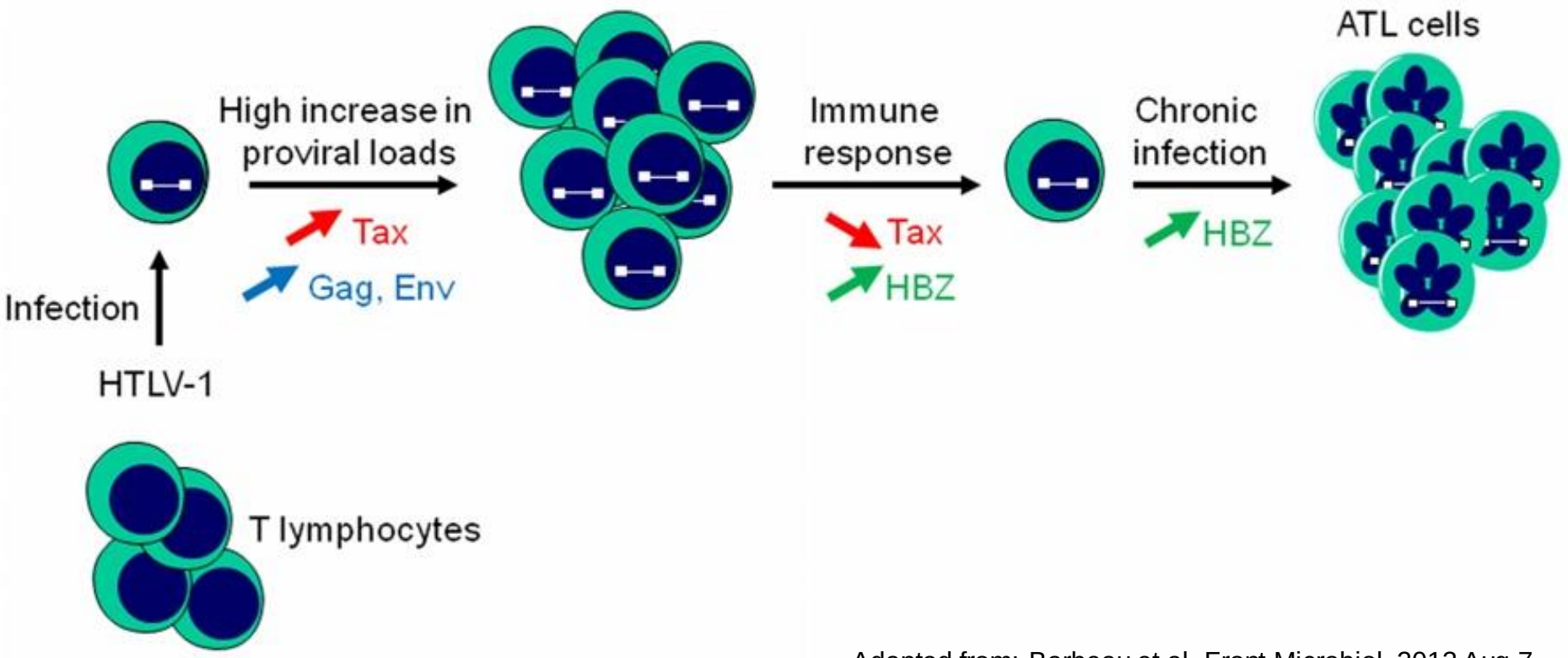
Ramos et al. Blood 2007 Apr 1;109(7)

HTLV-1 Provirus



Adapted from: M Matsuoka and K-T Jeang; *Oncogene* 2011

Clonal Evolution of Infected T-cells Leading to ATLL: Loss of Tax but not HBZ



Adapted from: Barbeau et al. Front Microbiol. 2013 Aug 7

HBZ:

- Expressed from negative strand at 3'end
- Expressed in chronically infected T-cells and ATLL cells

Tax:

- Not expressed in ATLL (due defective provirus at the 5' or epigenetic repression)

Immune-based Therapies Targeting HTLV-1/ATLL Developed at University of Miami

Immune-based Approaches Have Been Suboptimal for ATLL

➢ Allogeneic Stem cell transplant (mostly Japan experience)

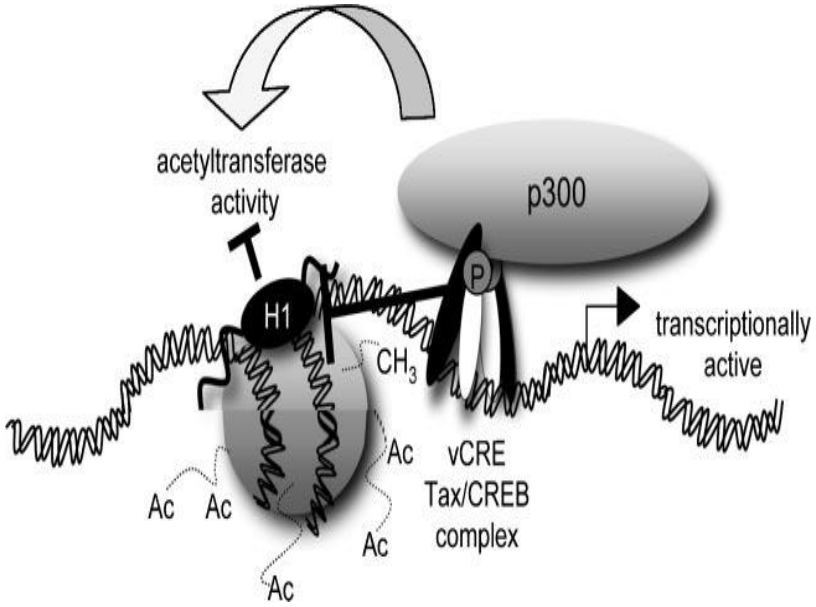
- About one-third of patients who reach this therapy can be cured
- Relatively high incidence of toxicity/GVHD and relapsed rates
- Limited availability in poor resource setting

➢ Check point inhibitors

- Nivolumab (NCT02631746): “Rapid progression of adult T-cell leukemia/lymphoma as tumor-infiltrating Tregs after PD-1 blockade”
- Trial discontinued due to fulminant progression of 3 patients enrolled
- Another trial is currently being conducted in Japan

Targeting HTLV-1/ATLL Using Histone Deacetylase (HDAC) Inhibitors

5' LTR Regulation by HDACs



Adapted from Konesky et al. JVI 2006

➢ HDAC inhibitors

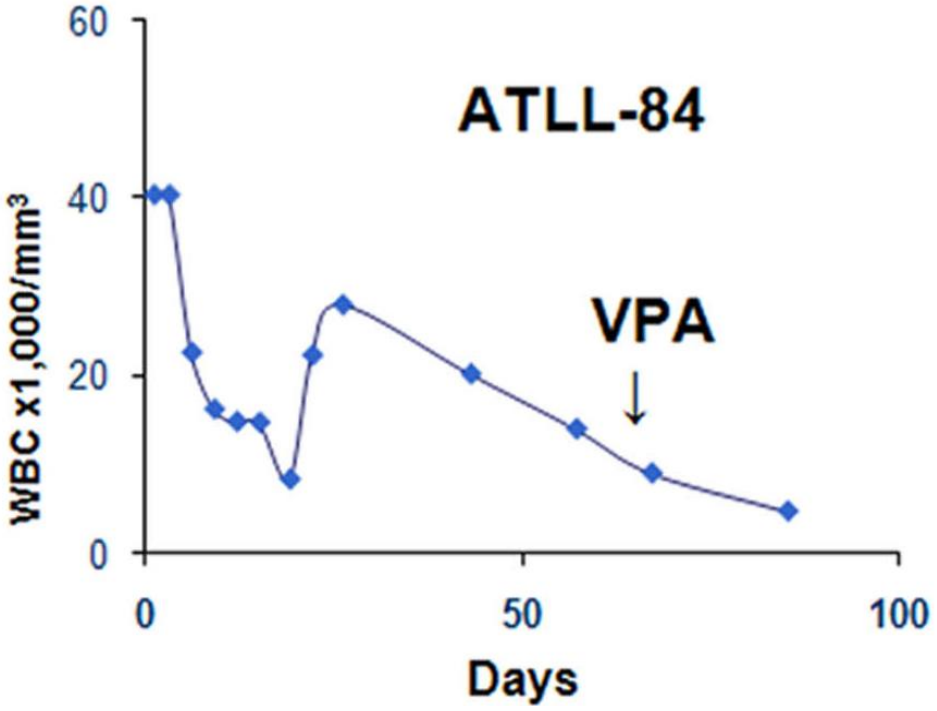
- Induce HTLV-1 (Tax) expression
- Pre-clinical activity in ATL/HTLV-1 transfected cells and or animal models
  - Desipeptide (Mori et al. 2004, Chen et al. 2009)
  - MS-275, SAHA (vorinostat) (Nishiokita et al. 2008)
  - LBH589 (panabinostat) (Hasegawa et al. 2011)
  - Valproic acid (VPA): **VPA + AZT decreased STLV-1 proviral loads in baboons (Afonso et al. 2010)**

Molecular Remission in Acute ATLL After Adding Valproic Acid (VPA) to AZT-IFN (ClinicalTrials.gov ID NCT00854581)

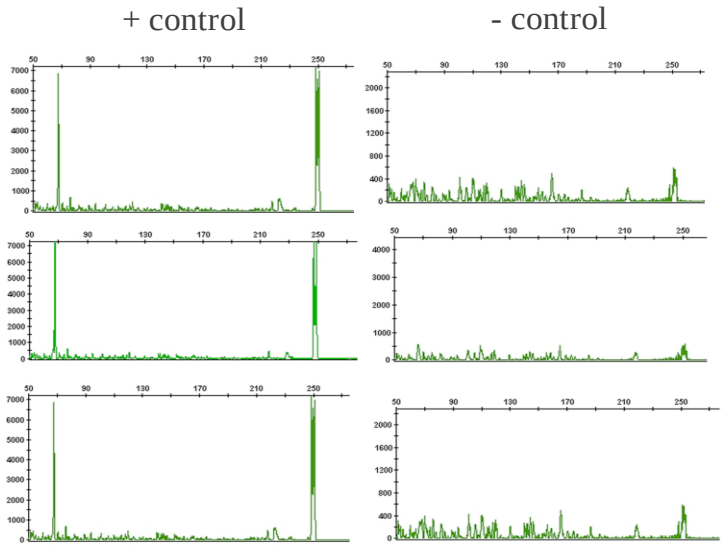
52 y/o Afro-Brazilian presenting with WBC= 240,000, hypercalcemia → Leukopheresis

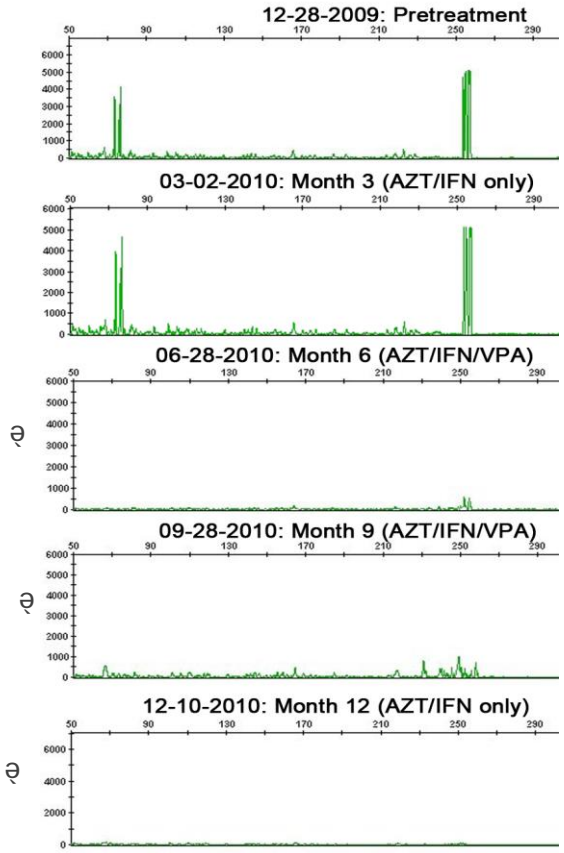
Treatment: High-dose ZDV/IFN → maintenance ZDV/IFN plus start VPA at day 60

Hematology response



Multiplex PCR for TCR gene rearrangement in CD4+ cells





HDAC inhibitors block HBZ expression, induce HTLV-1 Tax and apoptosis in ATLL cells

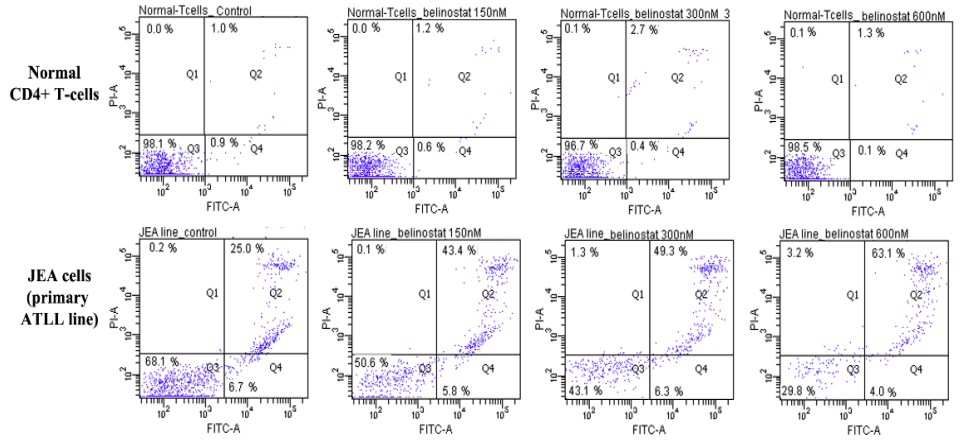
Belinostat:

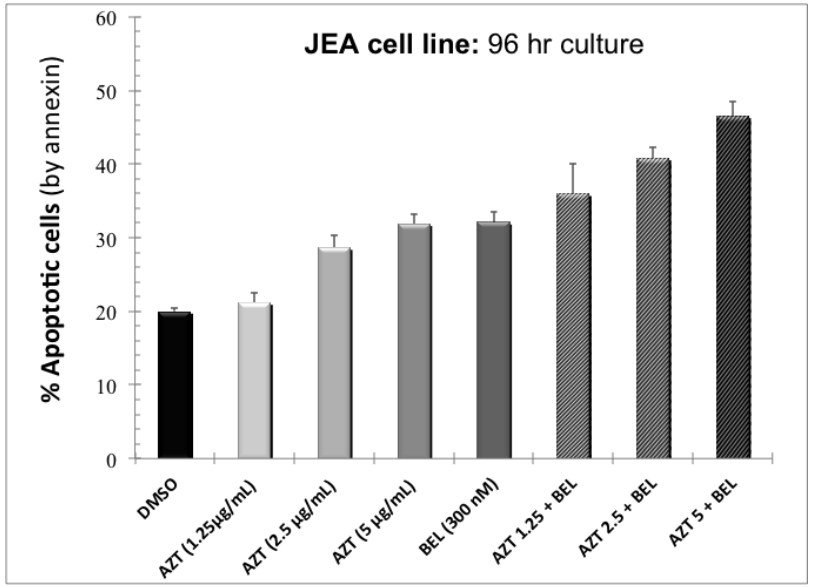
- Pan-HDAC inhibitor that obtained accelerated approval in the U.S. for the treatment of relapsed/refractory PTCL based on efficacy and duration of response

- Increases apoptosis in ATLL cells in the presence of AZT in dose-dependent manner, while it has no effect in normal CD4+ cells

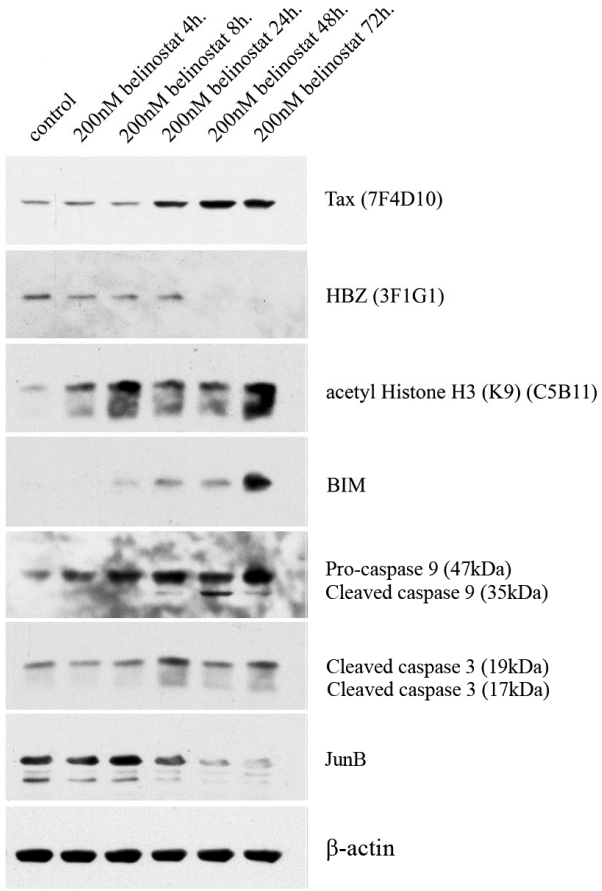
Apoptosis after fixed dose of belinostat (BEL) with increasing concentration of AZT after 4 days in culture (Control cells treated with DMSO)

Belinostat 150-600 nM





H3 acetylation
↓ HBZ
↑ Tax
↑ BIM
↓ JunB



Belinostat as Consolidation Therapy with Zidovudine for HTLV-1 Related ATLL:

ClinicalTrials.gov Identifier: NCT02737046

Primary Objectives:

- Determine the complete molecular response
- Determine the safety

Secondary objectives

- Study epigenetic effects
- CTL responses
- Impact on HTLV-1 proviral loads

Induction Therapy

AZT-IFN or chemotherapy > 2 weeks

↓

Complete or partial response

(with detectable clonal disease)

↓

Study enrollment

↓

Consolidation Therapy

AZT 300 mg orally three times daily

Belinostat 1,000 mg/m² on Days 1-5 every 3 weeks x 6 months

Optional: Continuation of IFNα in patients responding

↓

Molecular Assessments

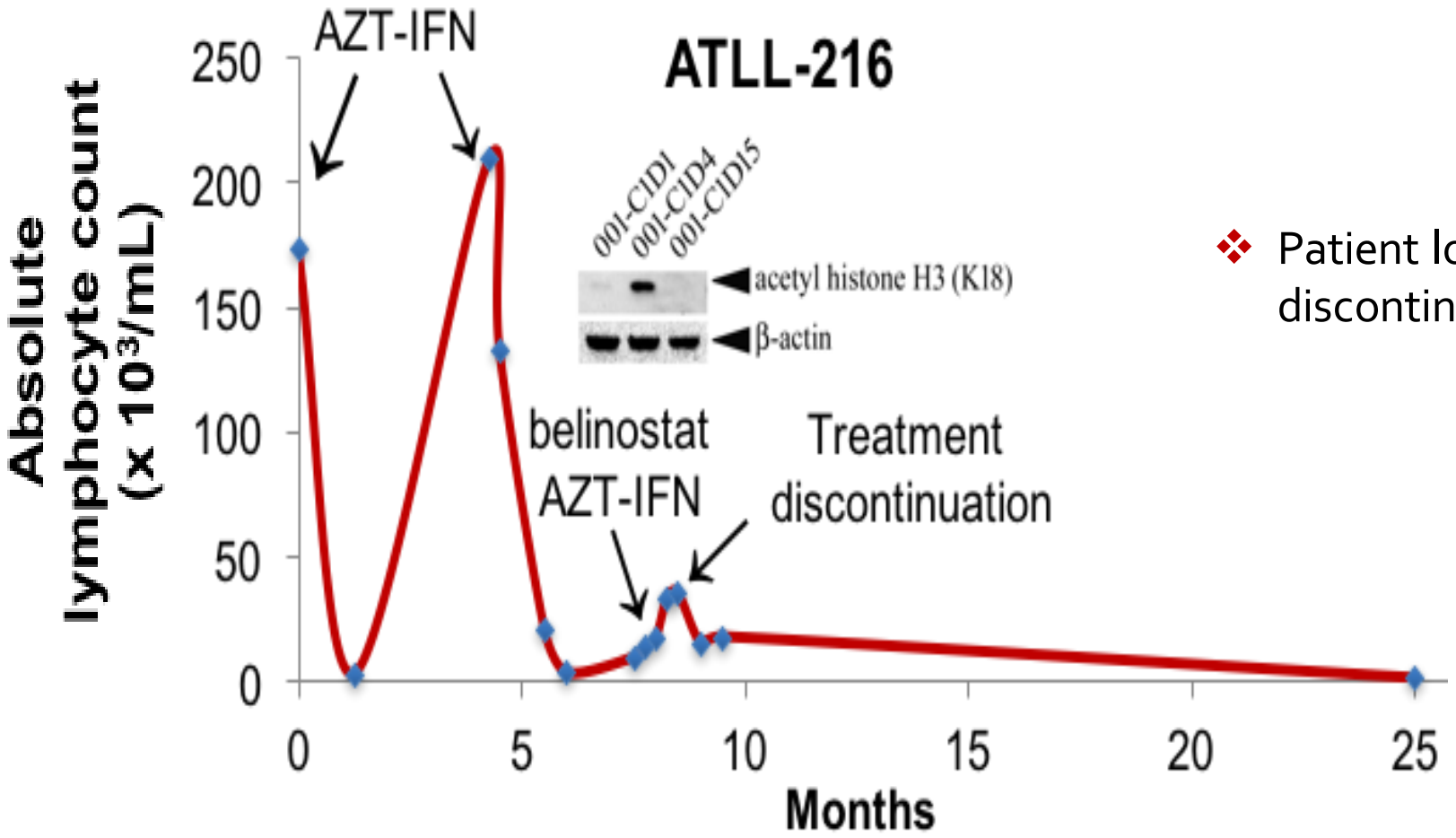
T-cell clonality , HTLV-1 PVLs, CTL responses, molecular and epigenetic effects

Interim Results

PATIENT ID	ATLL TYPE	AGE/ SEX	PRIOR THERAPIES	PROTOCOL THERAPY	# CYCLES	BEST RESPONSE	GRADE 3-4 ADVERSE EVENTS	PFS (mo.)	OS (mo.)	STATUS
001	Acute	32 F	AZT-IFNα x 4 mo	BEL/ZDV/IFNa2b	1	PD >> Hematologic CR	None	16	16	Dead
002	Acute	67 M	AZT-IFNα x 2wk > ZDV-peg IFNα2b x 6 mo	BEL/ZDV/peg IFNα2b	3	SD (Maintained PR)	Gr 4 neutropenia Gr 3 thrombocytopenia	12	15	Dead
003	Acute	60 M	**Relapsed after** AZT-IFNα x 13 yr, VCAP x 1	BEL/ZDV/IFNα2b	1	PD	None	0.5	8	Dead
004	Acute	45 F	**Relapsed after** AZT-IFNα x 2 wk, VCAP, ICE, oral etoposide	BEL/ZDV	6	SD Maintained PR	Gr 3 neutropenia Gr 3 thrombocytopenia	5	38	Alive
005	Acute	55 F	AZT-peg IFNα2α x 3 wk	BEL/ZDV/peg IFNa2α	3	CR (Molecular CR)	Gr 4 neutropenia Gr 4 thrombocytopenia	28	28	Alive
006	Acute	71 M	AZT-peg IFNα2a x 3 wk, vincristine x 1	BEL/ZDV/peg IFNa2α	4	PR >> Hematologic CR	Gr 4 neutropenia	5	19	Alive
008	Acute	47 M	**Relapsed after** CHOP/CHOEP x 6	BEL/ZDV/peg IFNa2α	2	CR (Molecular CR)	Gr 4 neutropenia Gr 4 thrombocytopenia	9	11	Alive
009	Acute	75 F	**Relapsed after** CHOP x 6	BEL/ZDV/peg IFNa2α	1	PD	Gr 4 neutropenia Gr 4 thrombocytopenia	1	`1	Dead
010	Acute	36 M	AZT-peg IFNα2a	BEL/ZDV/peg IFNa2α	8	PR >> Near Hematologic CR	Gr 4 neutropenia Gr 4 thrombocytopenia	5	5	Alive
011	Acute	41 M	Vincristine/cyclophos/dex x 1 AZT-peg IFNα2a	BEL/ZDV/peg IFNa2α	2	---	---	2	2	Alive

Results

Patient 001: 33 y/o Haitian woman with acute type ATLL, WBC=187,200, hypercalcemia (Ca= 17.5) and bone lesions

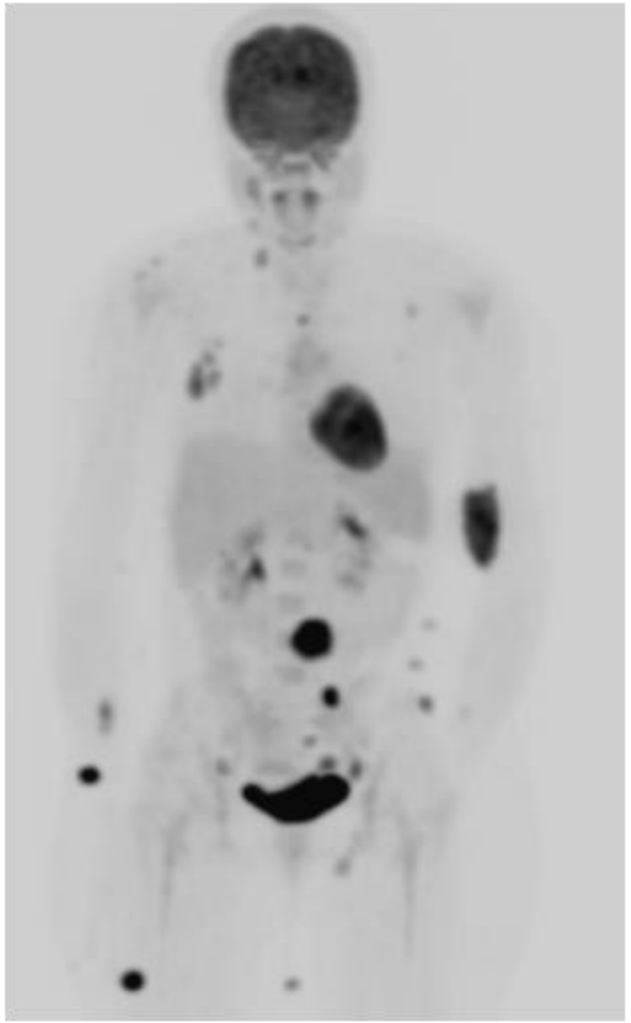


- Patient lost follow up after discontinuation of therapy at 3 weeks

Results **Patient 001:** No molecular evidence of disease in blood compartment (peripheral blood or bone marrow)

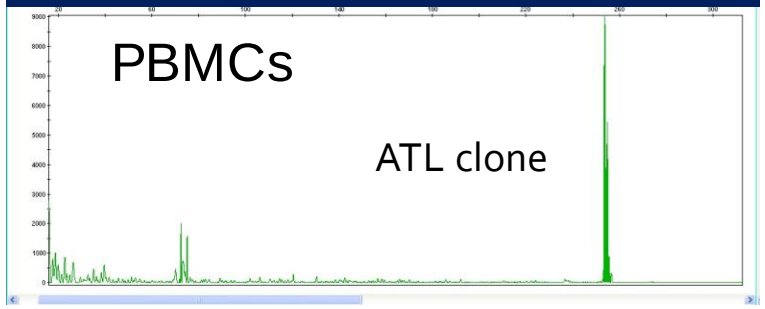
Lymphomatous relapse 16 months later

CT-PET
Left biceps mass and bone lesions

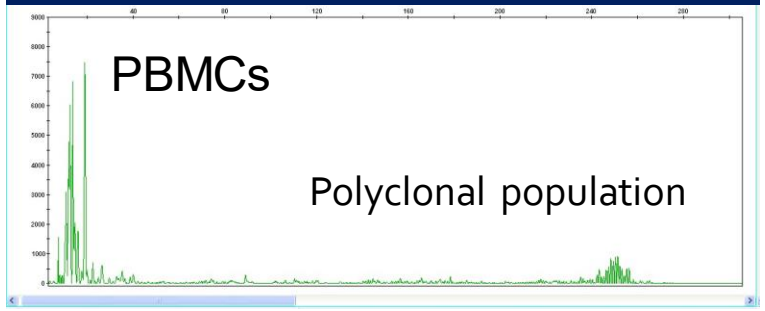


T-cell clonality: Multiplex PCR

Prior to belinostat



After 16 months



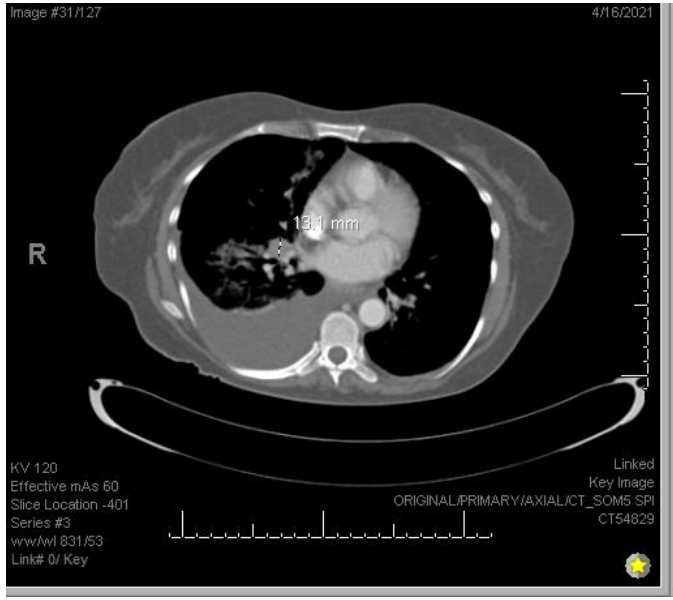
Patient 005: Complete Molecular response

- Discontinuation of therapy after 3 cycles due to cytopenias
- Persistent bone marrow involvement) (10%) at Month 4

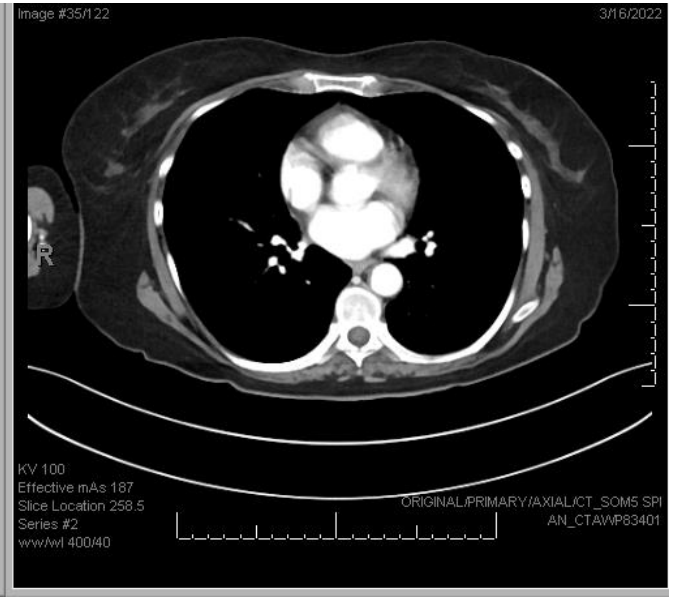
Spontaneous molecular remission!

- Repeat bone marrow biopsy at Month 5 showed no evidence of molecular disease
- Recovery from cytopenias (Month 8: re-started peg-interferon only)
- Continues to be disease free at 27 months

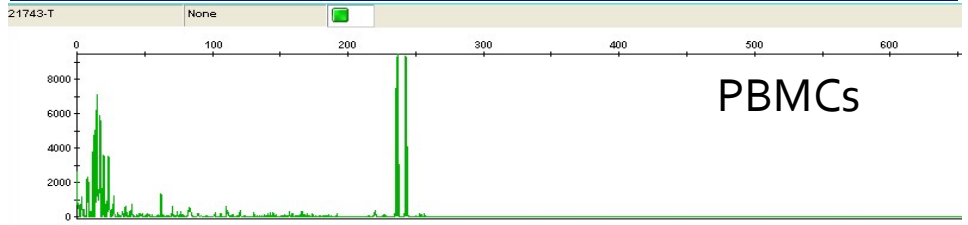
Baseline



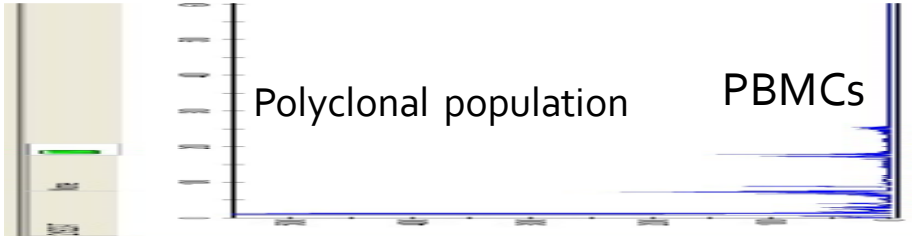
Month 12



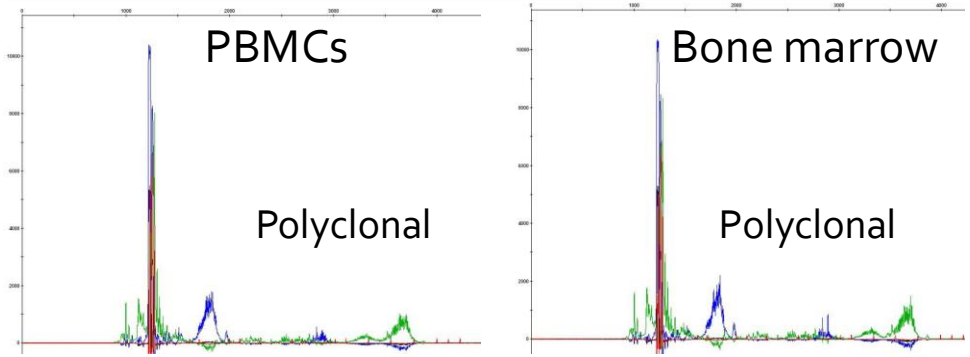
Prior to belinostat



Month 5



Month 12



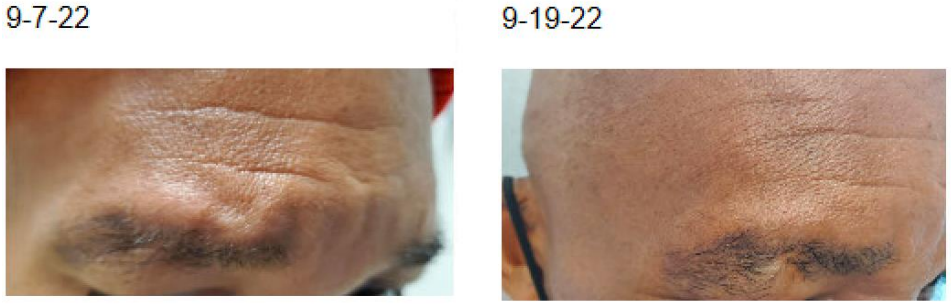
Patient 008: Complete Molecular response

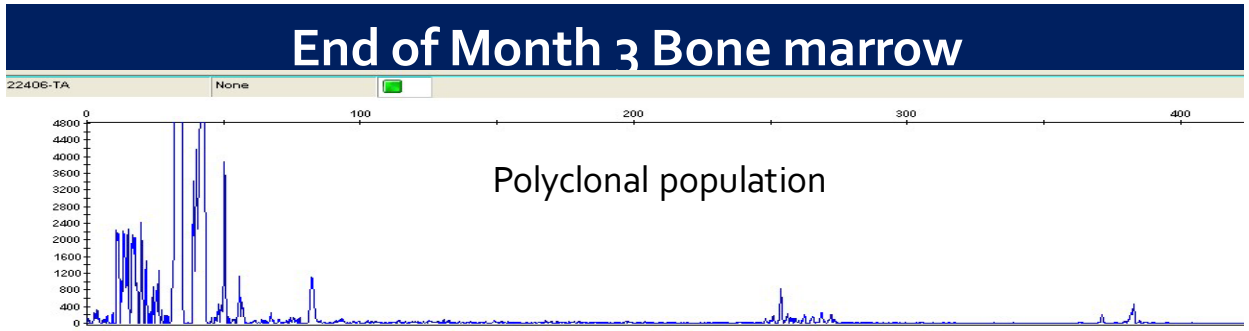
43 y/o man from Trinidad initially presenting with WBC 222,0000 (acute type ATLL), skin lumps, who relapsed after CHOEP chemotherapy

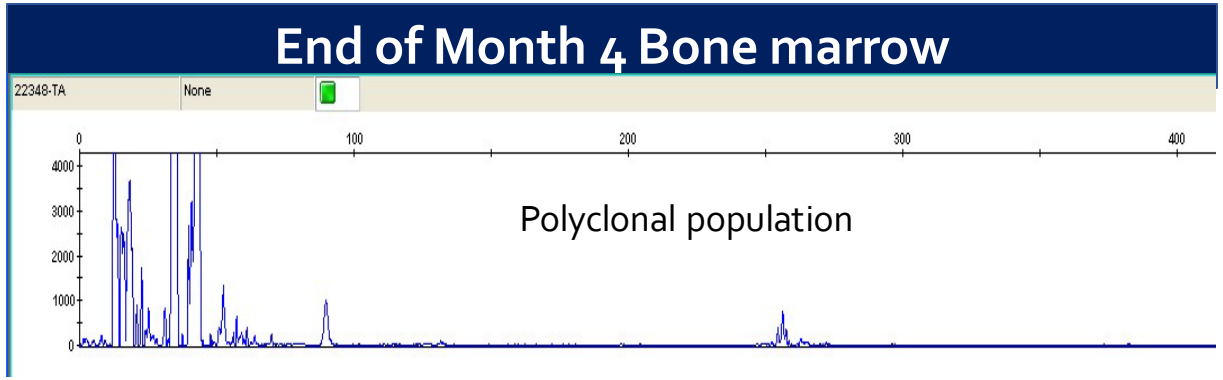
- Discontinuation of therapy after 2 cycles due to recurrent cytopenias
- Persistent ATLL in blood (7% of leukocytes, 27 % of T-cells) and bone marrow (2.4% involvement, 28% of T-cell population) at end of month 3

Spontaneous molecular remission!

- Repeat bone marrow biopsy at the end of Month 4 showed no evidence of molecular disease
- After recovery of cytopenias re-started peg-interferon only



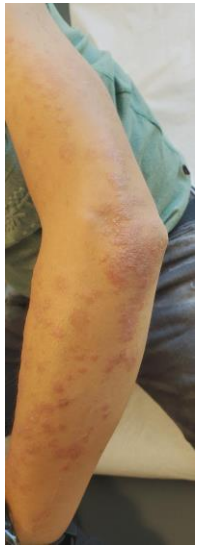




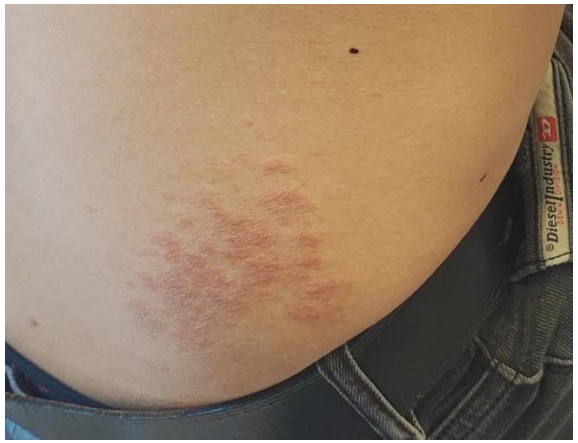
Patient 010: Near Complete Hematologic response

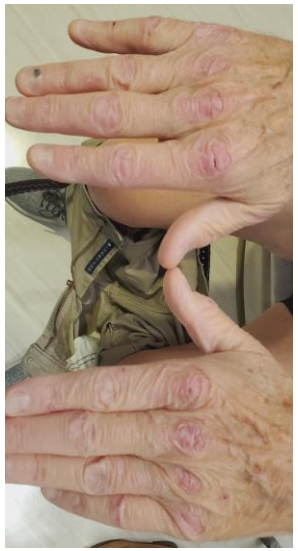
36 y/o Colombian male with ATLL, acute type, presented in February 3023 with WBC 180k, hypercalcemia and hyperuricemia, initially treated with leukopheresis followed pegylated interferon (Pegasys)

- March 2024: Belinostat-zidovudine-pegylated interferon, then started developing skin rash
- May 9, 2023 skin biopsies left upper extremities showed " Abnormal lymphoid infiltrate of cells positive for CD3 and a subset coexpress CD4 (most are negative), the atypical T cell infiltrate was positive for CD8 and a subset of atypical lymphoid cells coexpressed CD4.
- Skin lesion spontaneously resolved of skin plaques!
- After cycle 3, WBC, lymphocyte count, LDH were normal
- On 5-30-23, peripheral blood flow cytometry revealed only 0.16% involvement by ATLL, and bone marrow showed RESIDUAL ADULT T-CELL LEUKEMIA / LYMPHOMA COMPRISING ~2% OF TOTAL CELLULARITY.

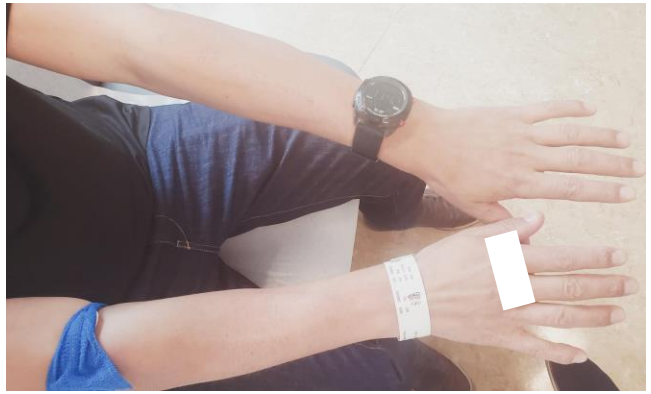


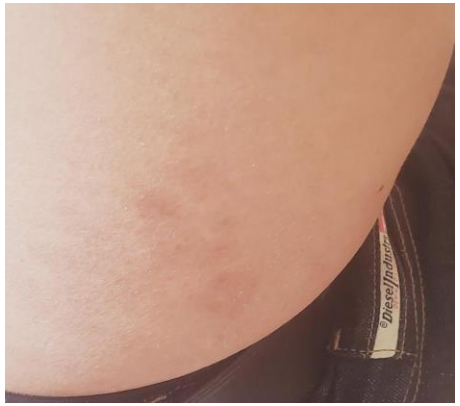
May 2023



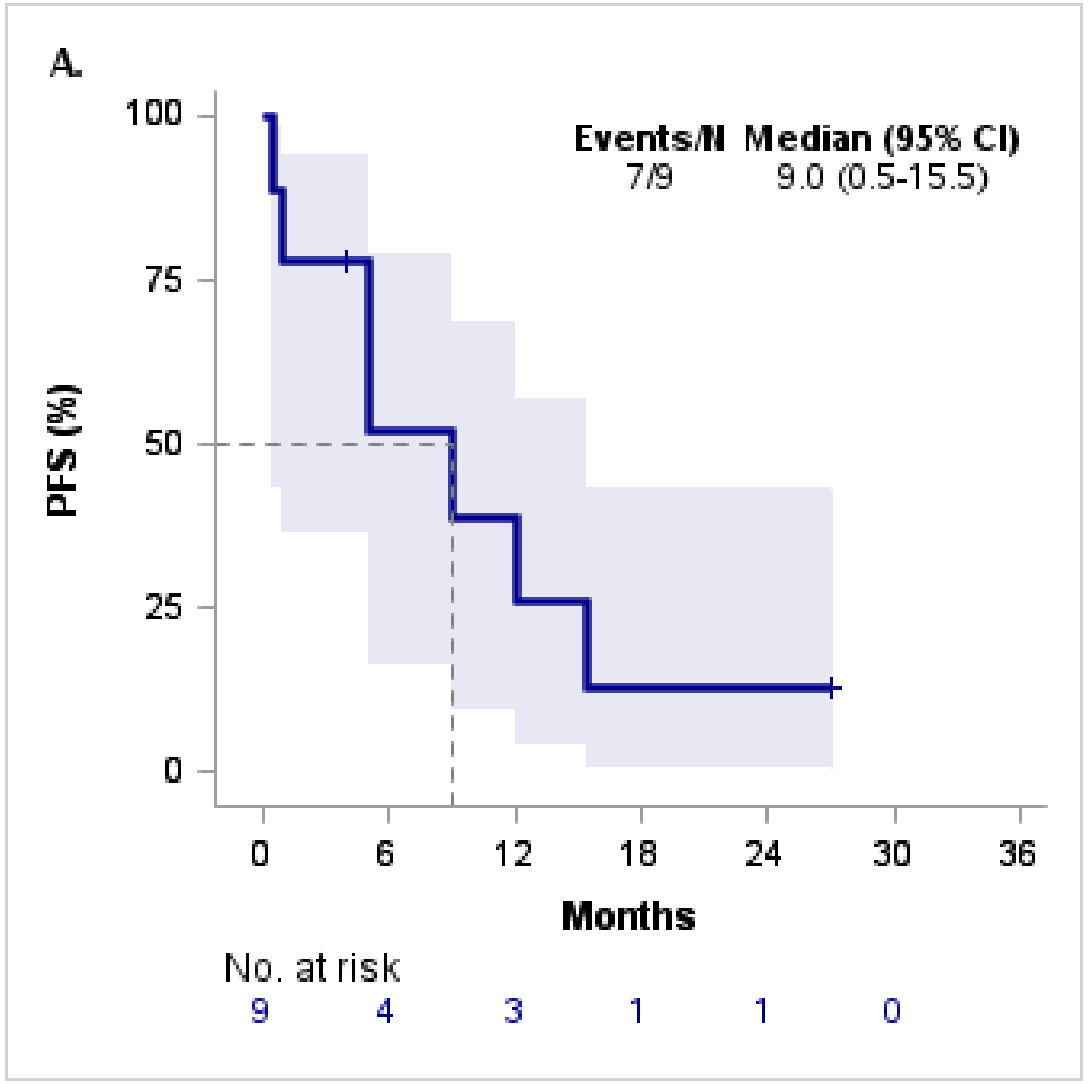


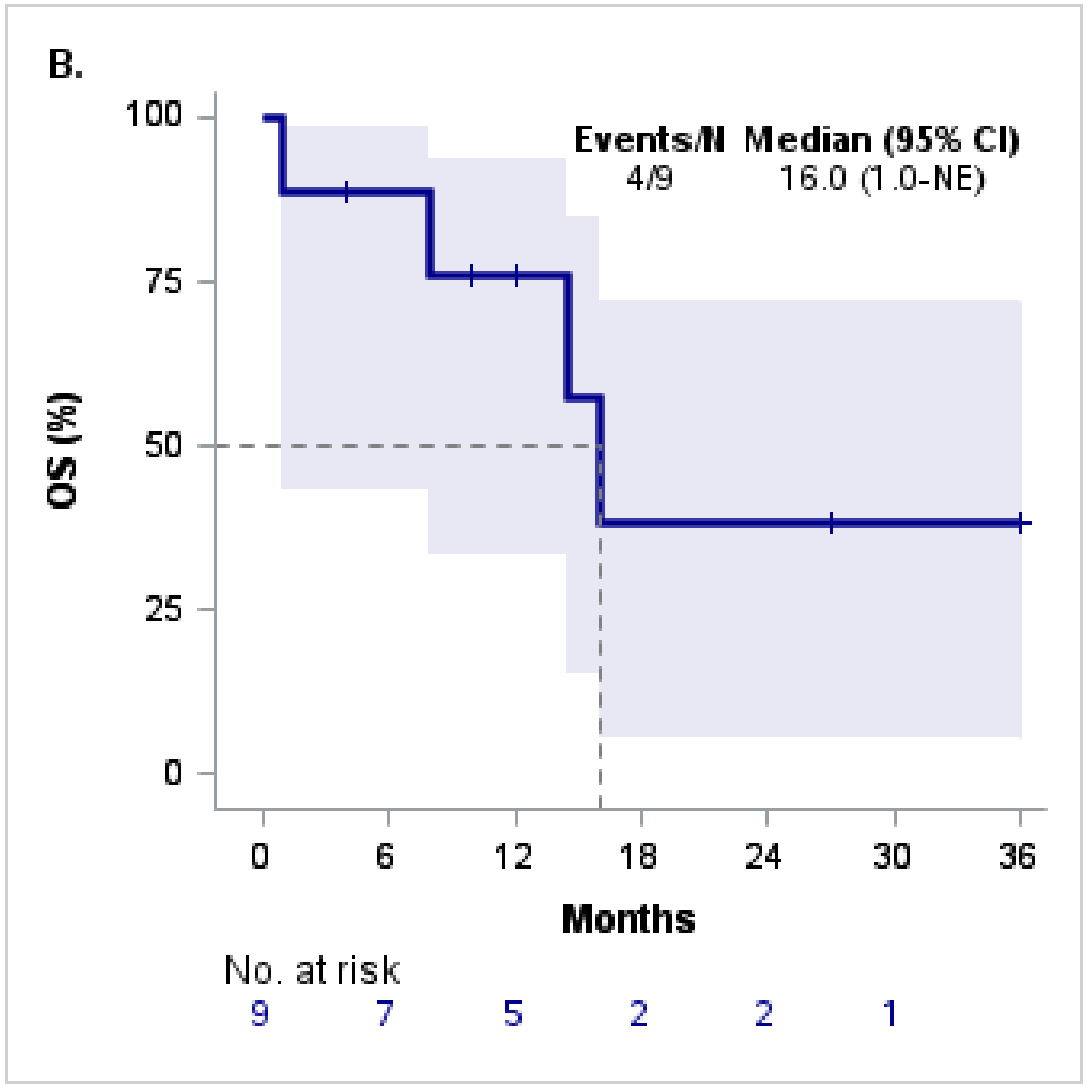
Jun 12, 2023





Interim: Survival Rates





Summary on Efficacy

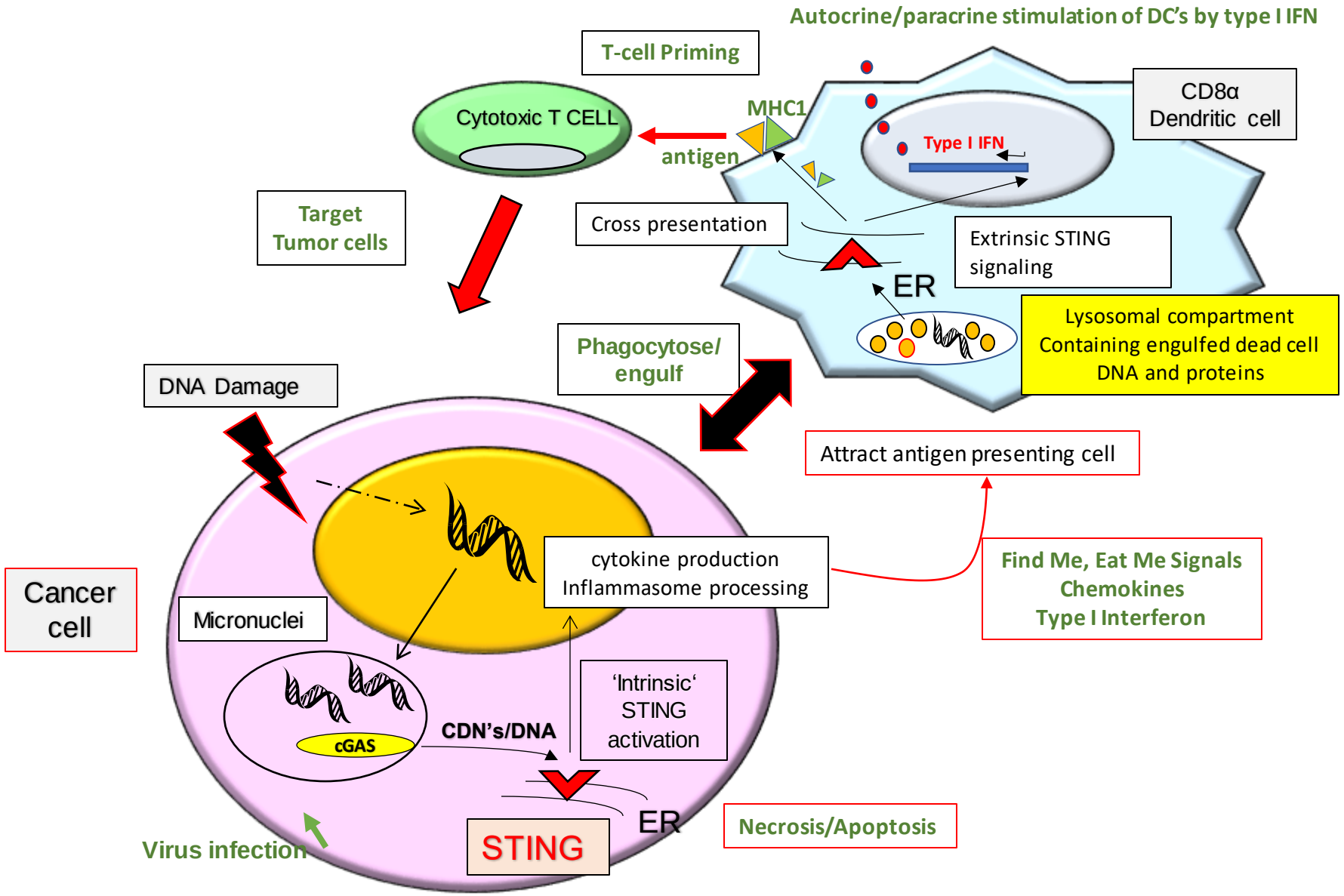
- Response rates: 2 complete responses (CR) + 2 partial responses (PR) = 44% + 2 stable disease
    - ❖ Hematologic CR 4/9 (44%)
- Complete hematologic molecular responses: 3/9 evaluable patients (33%)
    - ❖ Spontaneous after treatment discontinuation!
- Median PFS: 9 months
- Overall survival: 16 months

A Pilot Safety Trial of STING-dependent Adjuvants (STAVs) and Antigen-stimulated Dendritic Cells for Aggressive Relapsed/Refractory Leukemias

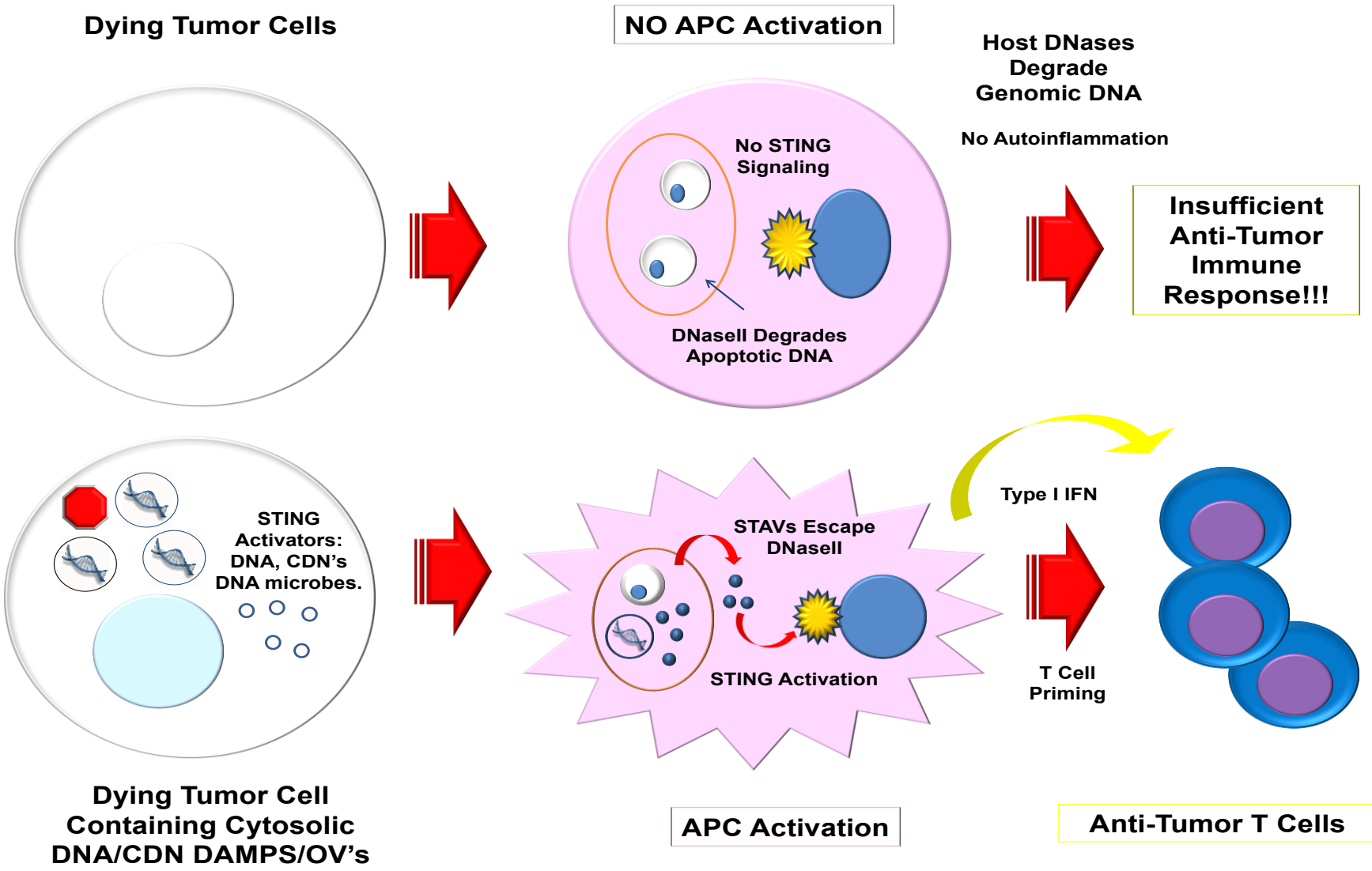
Role of STING (Stimulator of Interferon Genes) in Cancer

Glen N. Barber, Ph.D.

Professor
Chairman of Cell Biology
University of Miami

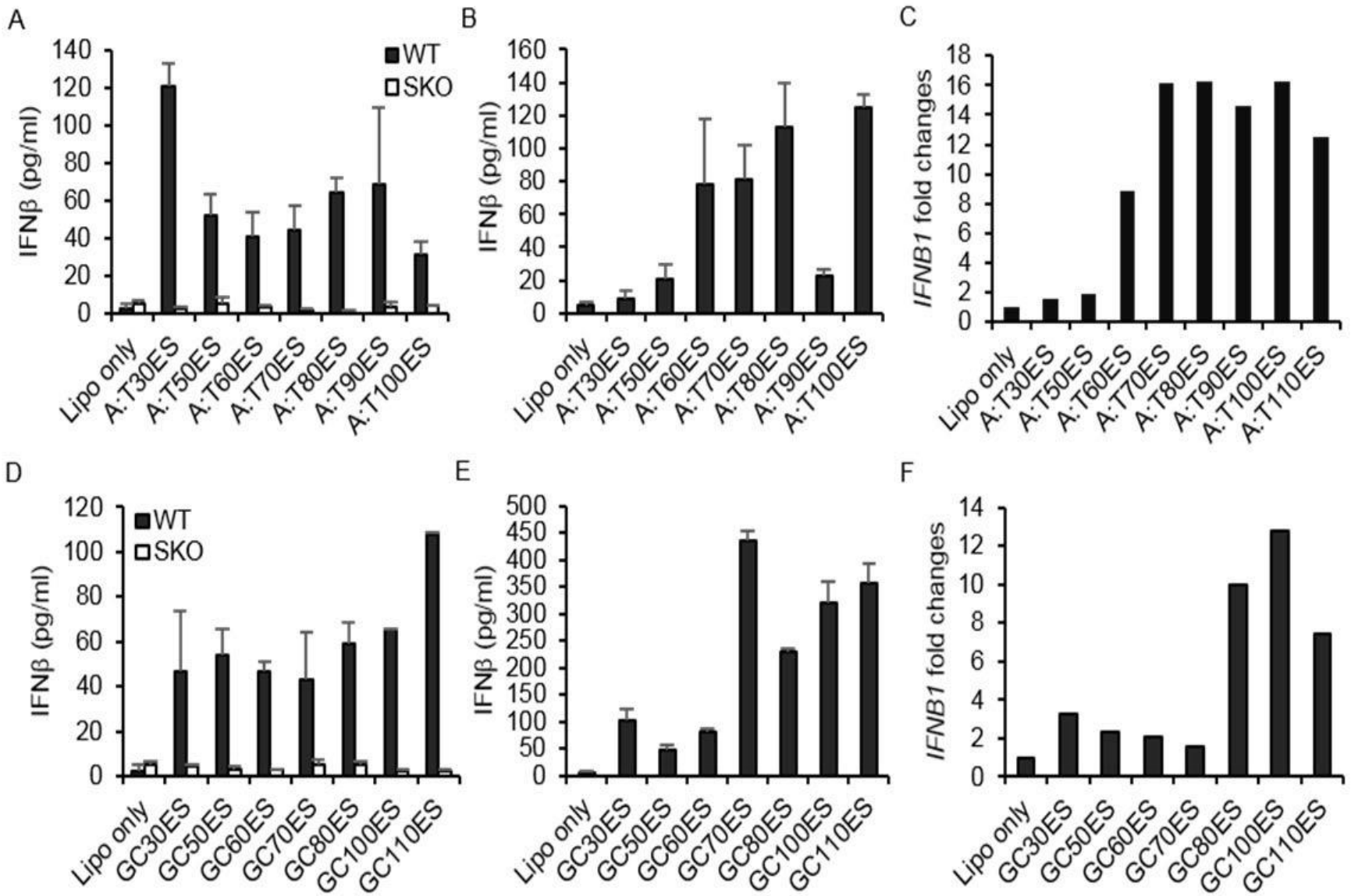


Effects of STING Activators (STAVs) in Dying Tumor Cells



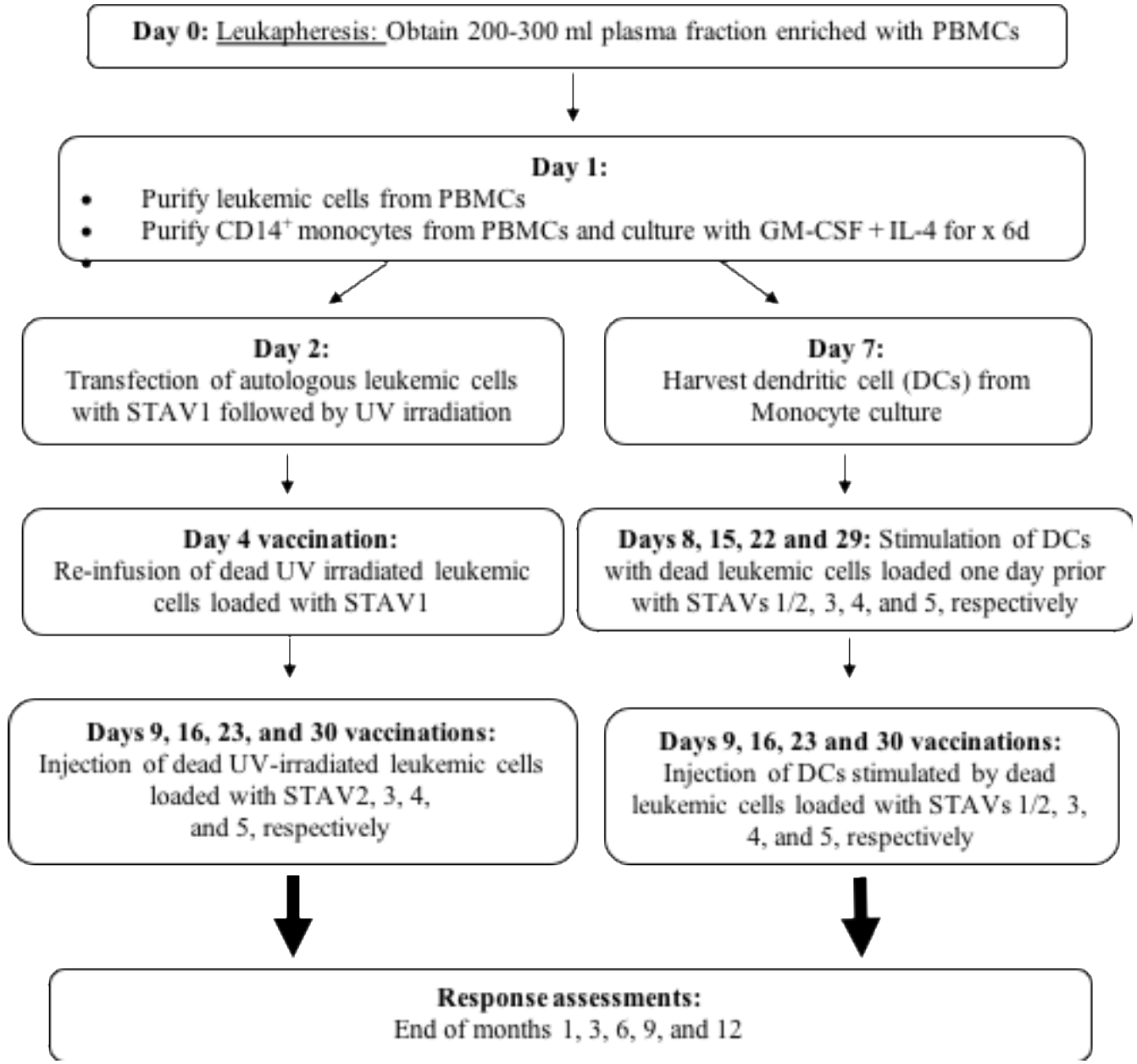
Ahn et al. Cell 2018

Effect of STING ligands with various sequences and length



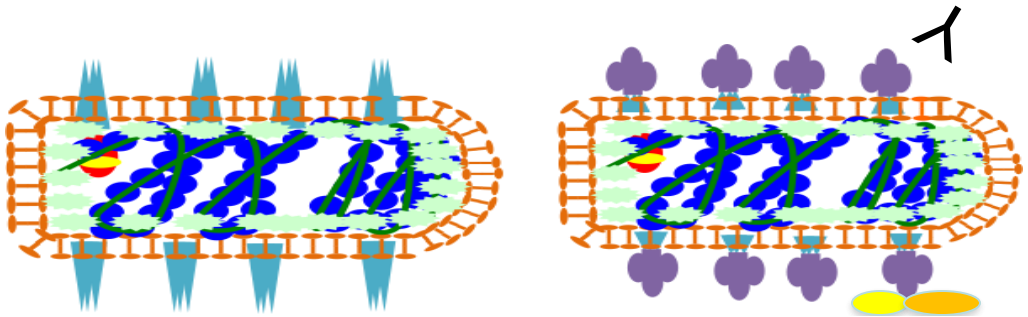
A Pilot Safety Trial of Autologous Leukemic Cells loaded with STING-dependent Activators (STAVs) and Stimulated Dendritic Cells for Aggeesive Leukemias (Clinicaltrials.gov NCT05321940)

Objectives	
Primary	
To test the safety and tolerability of administering autologous leukemic cells transfected with STAVs ex vivo along autologous dendritic cells (DCs) stimulated exogenously by STAVs loaded autologous tumor cells in subjects with aggressive leukemias.	
Secondary	
To evaluate the clinical and molecular response rates, and at minimum 1-year failure-free survival (FFS) and 1-year overall survival (OS).	
Exploratory	
To measure CTL responses	
To examine baseline expression of cGAS–STING cytosolic DNA sensing pathway molecules, and the immunologic effects induced by STAVs in patient-derived leukemic cells and in human macrophages after phagocytosis	
To investigate effect of on HTLV-1 in patients with ATLL	
To measure MRD and by TCR gene rearrangement PCR and HTLV-1 proviral loads longitudinally in patients with ATLL	



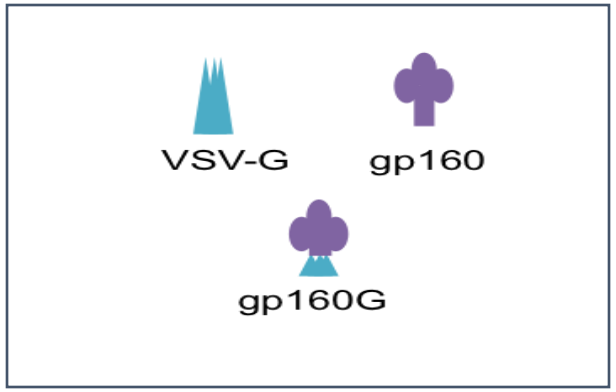
First-in-Human VSV-gp62-ΔHT HTLV-1 vaccination phase 1 study in patients with HTLV-1 infection

HTLV1 VSV-based vaccine (preventive, therapeutic)

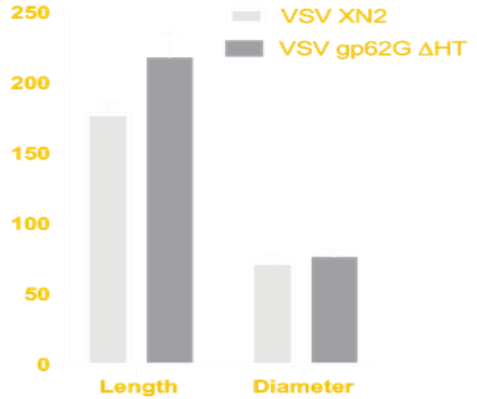


Wild Type VSV

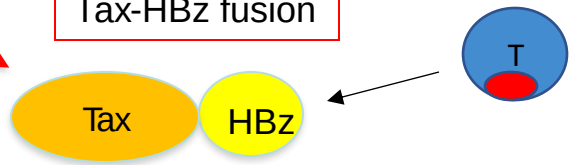
VSV HTLV1 gp62 Tax-HBz



HTLV1 Env gp62



Tax-HBz fusion



Tax and HBz are defective:
they cannot impair innate immune signaling

- The VSV envelope protein (G) has been replaced with the HTLV1 envelope protein (gp62).
- The recombinant virus also expresses the HTLV1 Tax and HBz proteins.

Objective:

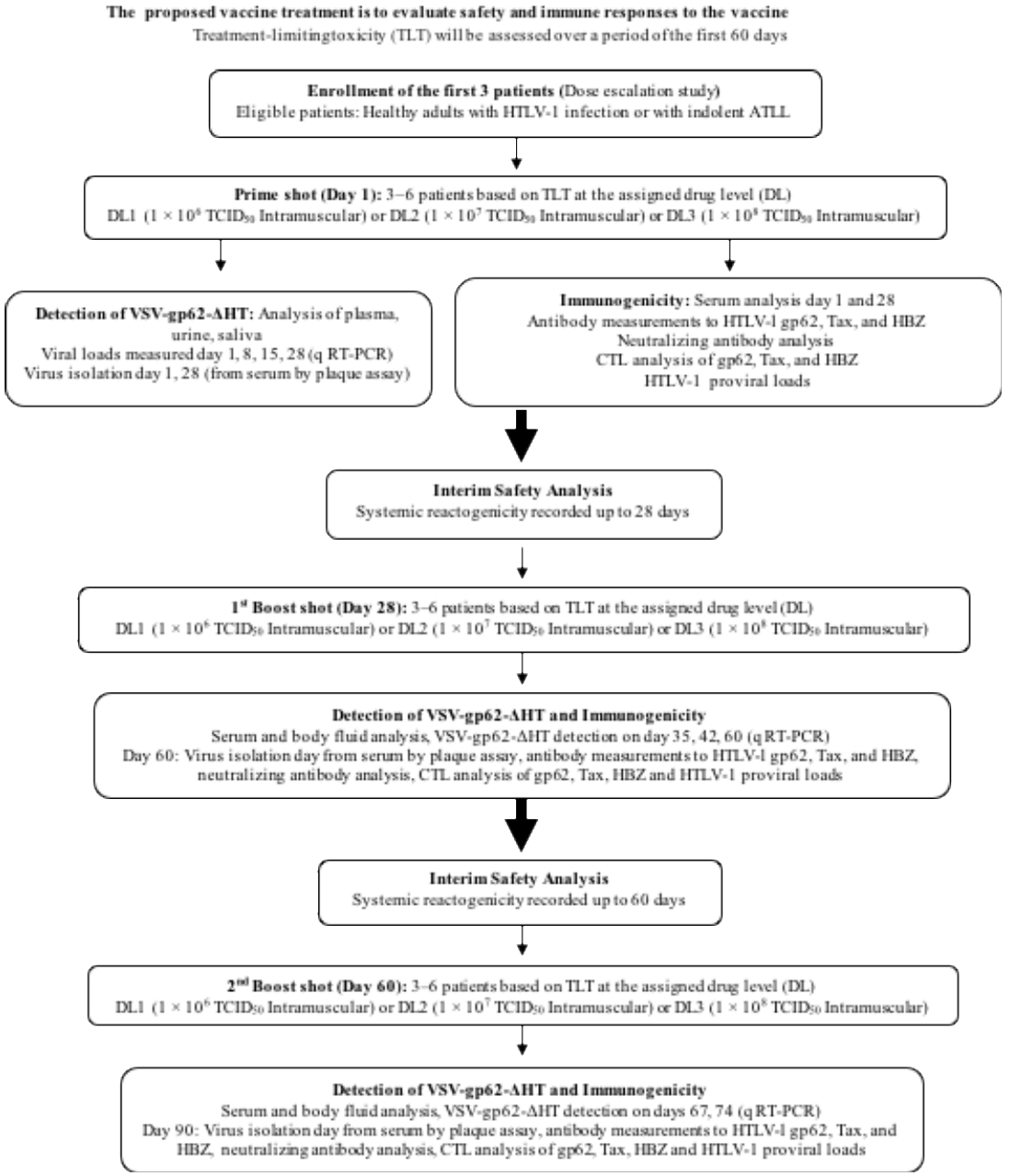
To generate neutralizing antibodies against gp62, to prevent syncytia formation and viral cell-cell spread

To generate CTL activity to both Tax and HBz, to clear infected cell populations

Barber et al.

First-in-Human VSV-gp62-ΔHT HTLV-1 vaccination phase 1 study in patients with HTLV-1 infection

	Objectives
Primary	
	[To determine the safety and tolerability of VSV-gp62-ΔHT in HTLV-1-positive adults]
	[To determine the recommended phase 2 dose (RP2D) of VSV-gp62-ΔHT based on treatment-limiting toxicity (TLT) evaluation during the first 60 days (after the 1st and 2nd vaccine doses)]
Secondary	
	To evaluate the pharmacokinetics (PK) of VSV-gp62-ΔHT in HTLV-1-positive adults
	To understand the immunogenicity of VSV-gp62-ΔHT by evaluating host antibody and cytotoxic T-cell (CTL) responses to HTLV-1 proteins (gp62, HBZ, and Tax) and evaluating the ability of neutralizing antibodies
	To evaluate the effect of VSV-gp62-ΔHT on overall survival (OS) of HTLV-1-positive adults at 1 year
	To evaluate treatment efficacy in HTLV-1-positive adults who have indolent or chronic ATLL
Exploratory	
	To evaluate the impact of VSV-gp62-ΔHT on HTLV-1 proviral loads (PVL)



Envisioned HTLV-1 Program at UM

- HTLV-1 related diseases disparately affect individuals of African or Afro-Caribbean descent in the U.S., Caribbean and South American regions.
- At our institution, we probably encounter the most patients with ATLL in North America.
- Our laboratories are uniquely equipped with rare and difficult to establish patient derived ATLL models which serve as invaluable tools for our studies
- Our goal is to establish highly efficacious and novel immune-based therapies for ATLL, a fatal disease, and to pro-actively eradicate HTLV-1 infection

1. 5R01CA223232 $1,748,000 ***(****Ramos*)
Project Title: *Epigenetic Targeting of Afro-Caribbean Variant of HTLV-1 Related Adult T-Cell Leukemia-Lymphoma*

2. NCI/STTR 2R42CA250629-02A1. $2,300,000 (Yeong, Ramos, Barber)
Project Title: *STING Activators as Therapy for Cancer*

3. NCI R01CA252049 ***$3,200,000,000*** (Barber)
Project Title: *Development of a HTLV-1 Vaccine*

4. NIAID R41AI165061 ***$600,000 (***Barber)
Project Title: *Human T cell Lymphotropic Virus Vaccine development (GMP manufacture).*