ISF35 Active Immunotherapy for Blood Cancer Intranodal Studies

National Institutes of Health (NIH)

Office of Biotechnology Activities
Recombinant DNA Advisory Committee

121st Meeting

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Januario E. Castro, M.D. Associate Clinical Professor
UCSD Moores Cancer Center
Department of Medicine
Division of Bone Marrow & Stem Cell Transplantation

UNIVERSITY of CALIFORNIA, SAN DIEGO
MEDICAL CENTER MOORES CANCER CENTER

memgen
active immune therapies

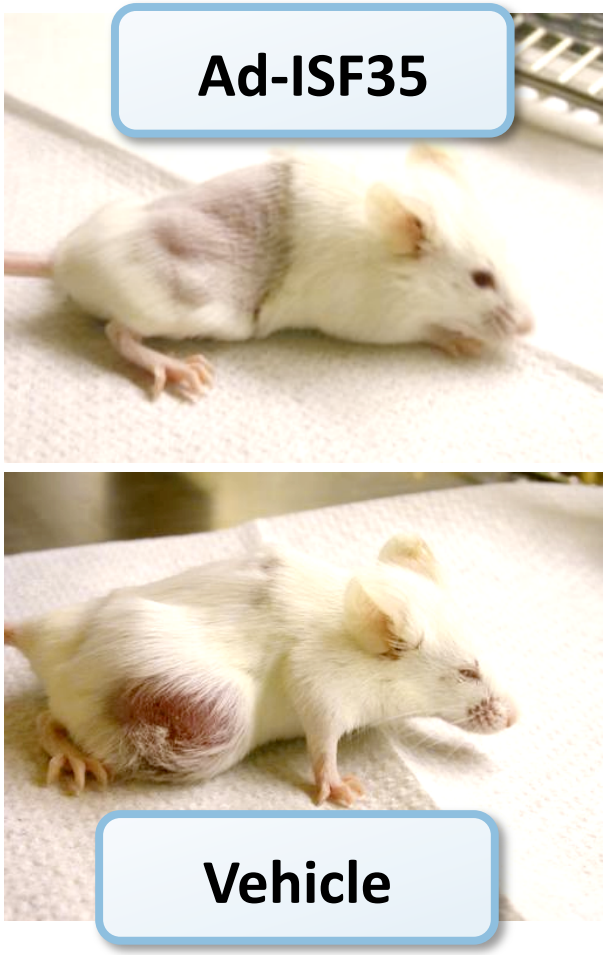
RAC Comments/Questions

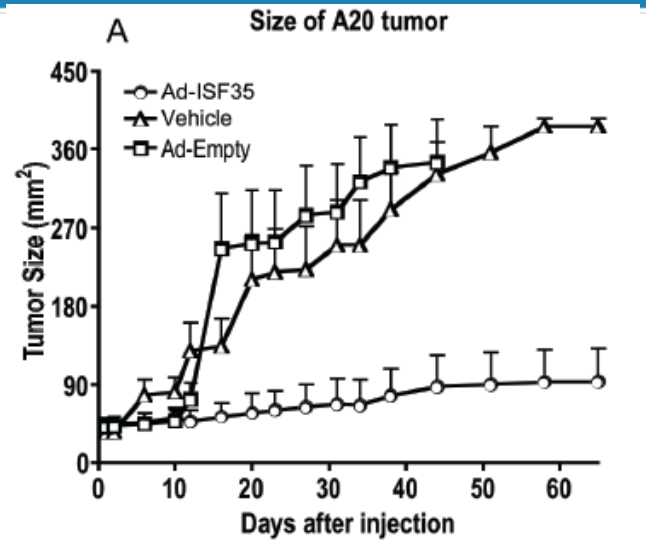
Reviewers: *Hildegund Ertl, M.D. ; Jane Flint, Ph.D. ; Jeffrey Kahn, Ph.D.*

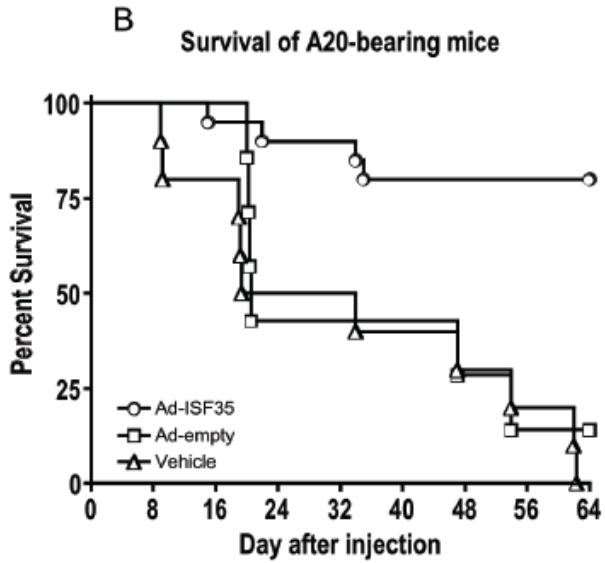
- *ISF35 mechanisms of action and rationale for intranodal / intratumoral injection ?*
- *Immunological Effects mediated by Intranodal / Intratumoral ISF35 injection in mice ?*
- *Equivalence of Ad-ISF35 Intratumoral / Intranodal Doses in Mice vs. Humans ?*
- *Effect of Transducing Non-Leukemia cells. Bystander Effect ?*

A20 – NHL Animal Model of Intratumoral Injection Ad-ISF35

Week 5



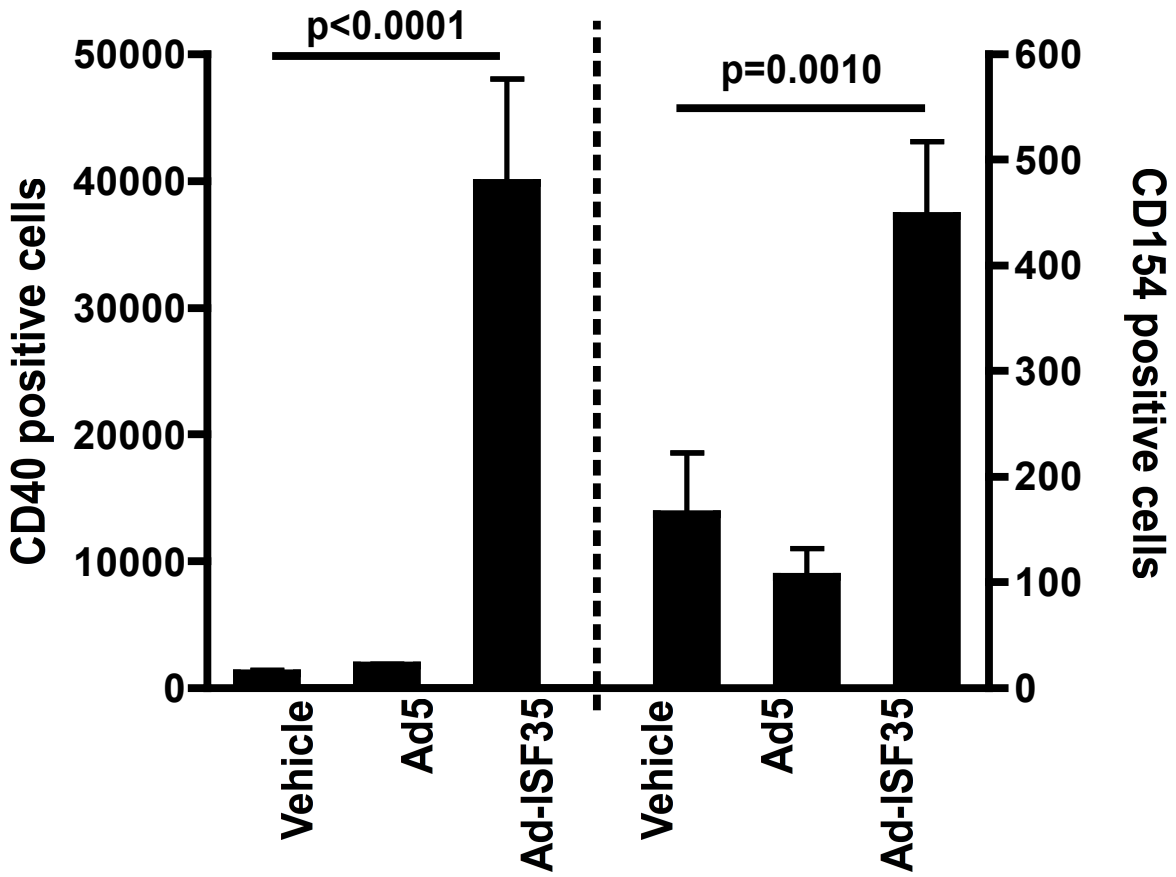




Ad-ISF35 Dose 1x10^{10} vp

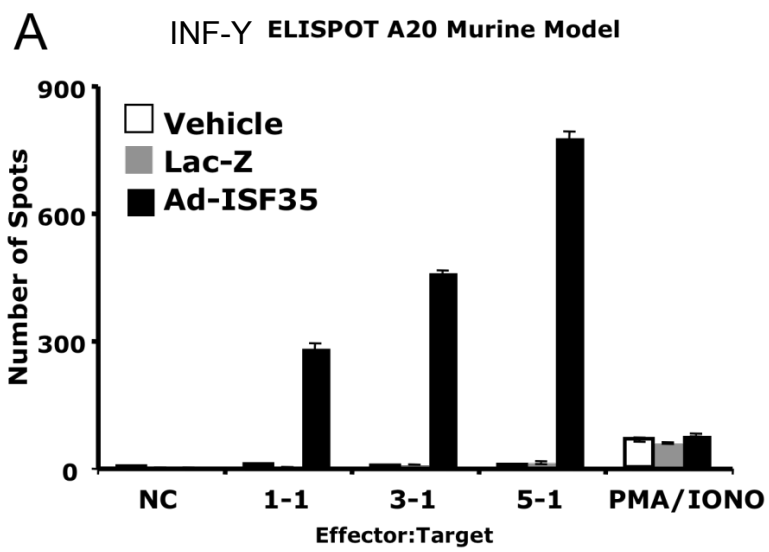
[The equivalent w/w dose in humans is 2x10^{13} vp]

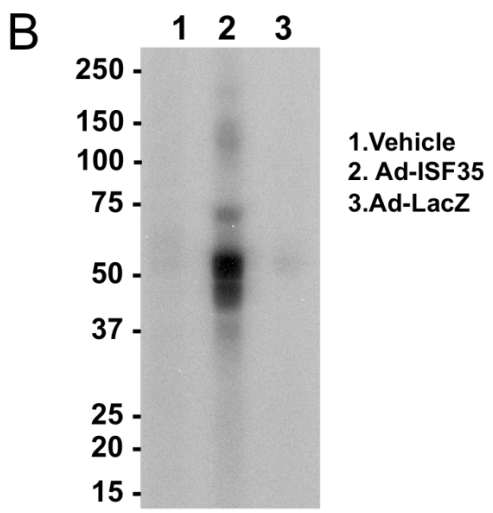
Ad-ISF35 Intratumoral A20 NHL Mouse Model
Bystander Effect

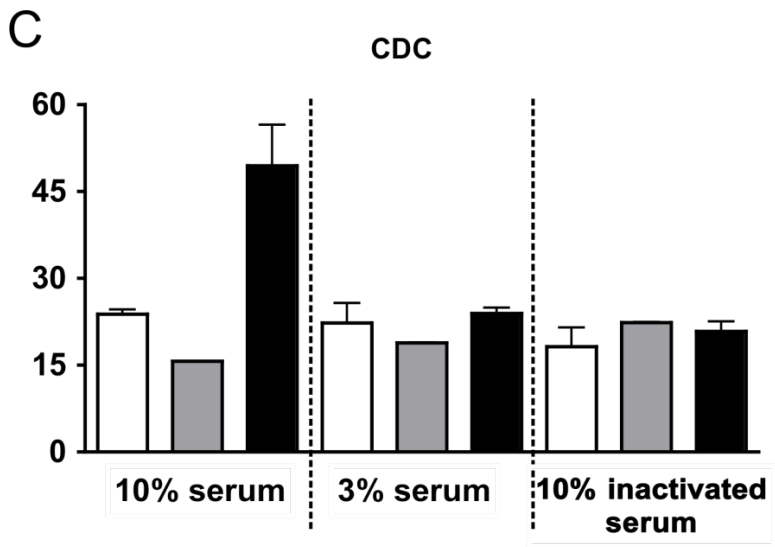


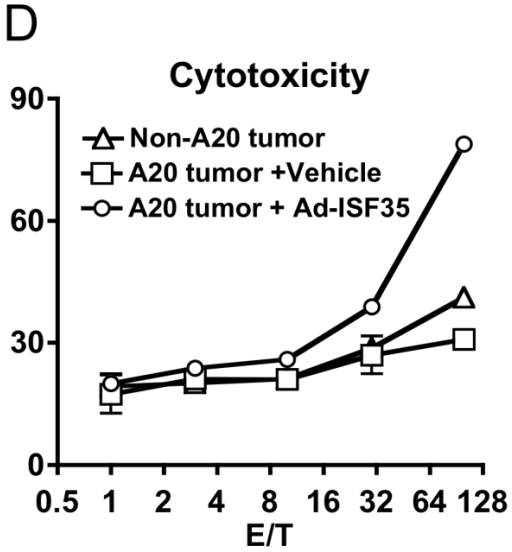
20-25% CD40⁺ Cells **0.25% CD154⁺ Cells**

Ad-ISF35 Intratumoral A20 NHL Mouse Model
Immunological Effects

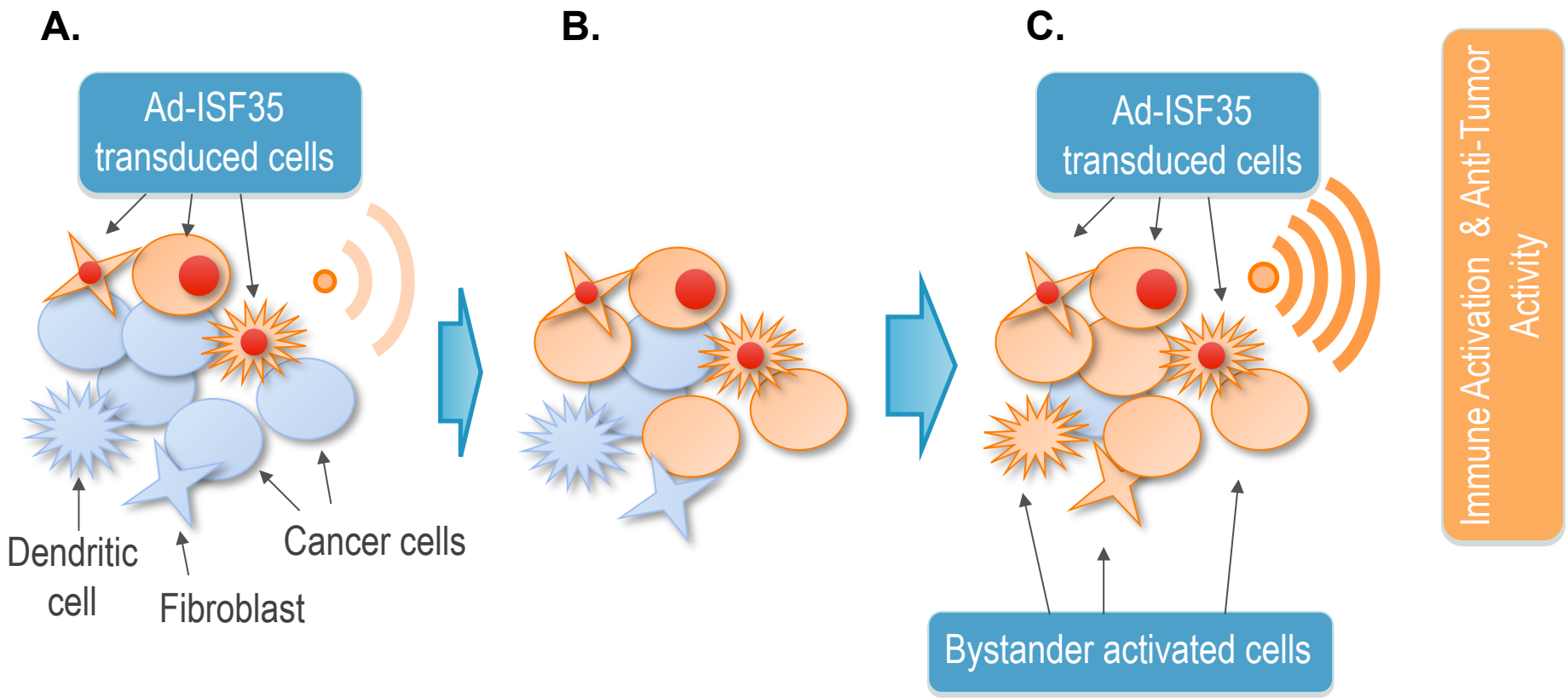








Bystander Effect Hypothesis



90% cells express

RAC Comments/Questions

Reviewers: *Hildegund Ertl, M.D. ; Jane Flint, Ph.D. ; Jeffrey Kahn, Ph.D.*

- *Correlative Studies Phase I / II Trials ?*
- *Vector characteristics, IU vs. vp ratio. Test for RCA ?*
- *Clarifications about AE phase I study.*
- *Potential to induce cross-reactive anti-human CD40L (CD154) antibodies?*
- *Future Plans after Phase II studies are completed ?*

Clinical Trial Summary

Study Title: A phase I study of immune gene therapy for patients with Chronic Lymphocytic Leukemia using intranodal injection of Ad-ISF35, a membrane stable CD40 binding protein

NIH OBA #: 0607-784

Investigator: Januario E. Castro, M.D.

Date: June 17, 2010

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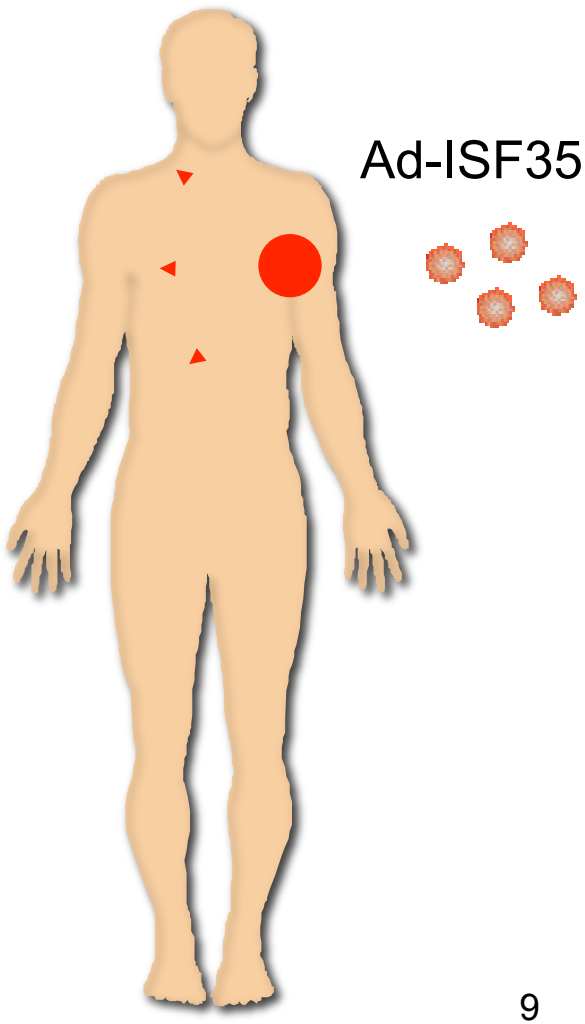
Rationale for Intratumoral Injection of Ad-ISF35

- Not all patients have circulating cancer cells that can be harvested for *Ex vivo* transduction.
- **Transduction *in vivo*** offers the possibility to effect cells from the Tumor micro-environment.
- Potential application in other cancers:
  - *NHL*
  - *Breast cancer*
  - *Melanoma*
  - *Prostate cancer*
  - *Pancreatic cancer*
  - *Lung cancer*
  - *Endometrial cancer*
  - *Ovarian*



- Ad-ISF35 (MEM-ADV-FP-008)

  4.46×10^{11} vp / ml - 7.69×10^{10} IU / ml

  vp / IU ratio of 5.8

  (RCA) test using the A549 cell line inoculation method. Sensitivity of ≤ 1 RCA per 3×10^{10} vp.

Demographics and Characteristics

Cohort (dose)	Pt #	Age	Sex	IgV_H (% Hom)	%ZAP70+	%CD38+	Prior Treatments	Cytogenetics	FISH	ECOG	Rai	β2M (g/dL)
1 *$1x10^{10}$ vp*	1	49	M	92.2	29.8	0	Idiotype vaccine conjugated with KLH	46,XY	del13q	0	II	1.6
	2	55	M	96.8	36.7	0	None	46,XY	del13q	0	II	4
	3	67	M	100	28.1	0	AT-101, HDMP+R, A	46,XY	del17p, del13q	0	II	1.3
2 *$3.3x10^{10}$ vp*	4	73	M	100	10.3	0	GT, X-cyte therapy, HDMP+R, AT-101+R	NA	del11q, del13q	0	IV	2.5
	5	52	M	94	5.2	44	HDMP+R	48,XY	tri12,del13q	0	II	2.1
	6	53	M	NA	NA	NA	FM, FCR, R, A, O	46,XY	normal	0	II	2.5
3 *$1x10^{11}$ vp*	7	53	F	98.6	4.8	NA	None	46,XX	del11q	1	II	2.9
	8	48	M	100	33.7	NA	CP, C, F, FCR, HDMP +R, AT-101+R	46,XY	del11q, del13q	0	II	3.7
	9	61	M	99.3	98.7	98.7	FR, R	47,XY	Tri12	0	II	2.9
4 *$3.3x10^{11}$ vp*	10	55	F	95.7	81.5	0	FR, A, Anti-CD40 antibody, AT-101+R	46,XX	normal	1	IV	1.2
	11	54	F	100	8.1	82	None	NA	normal	0	II	2.2
	12	45	M	NA	32.8	0	FCR, R	47,XY	tri12, del17p	0	IV	3.4
3E *$1x10^{10}$ vp*	13	57	M	100	92.5	19.3	HDMP+R	46,XY	del11q	0	IV	2.9
	14	54	F	NA	NA	NA	None	46,XX	del13q	0	III	2.6
	15	63	F	96.1	83.9	8.7	HDMP+R, A, HSP90	46,XX	del13q	0	II	2.8

* ***A*** *= Alemtuzumab;* ***AT-101*** *= BCL-2 family member inhibitor;* ***C*** *= Chlorambucil;* ***CP*** *= Chlorambucil and Prednisone;* ***F*** *= fludarabine;* ***FCR*** *= Fludarabine, Cyclophosphamide and Rituximab;* ***FM*** *= Fludarabine and Mitoxantrone;* ***FR*** *= Fludarabine and Rituximab;* ***GT*** *= gene therapy (CD154);* ***HDMP*** *= High-Dose Methylprednisolone;* ***HSP90*** *= Heat Shock Protein 90 Inhibitor;* ***NA*** *= not available;* ***O*** *= Ofatumumab;* ***R*** *= Rituximab;* ***X-cyte*** *= T-Cell Therapy*

Adverse Events – First 21 Days

Event	Grade I / II				Grade III / IV			
	Cohort 1 *(n=3)*	Cohort 2 *(n=3)*	Cohort 3 *(n=6)*	Cohort 4 *(n=3)*	Cohort 1 *(n=3)*	Cohort 2 *(n=3)*	Cohort 3 *(n=6)*	Cohort 4 *(n=3)*
Neutropenia*	1 (33%)	2 (67%)	2 (33%)			1 (33%)	1 (17%)	2 (67%)
Hypophosphatemia		1 (33%)	1 (17%)				1 (17%)	2 (67%)
SGOT elevation	1 (33%)	0	1 (17%)	1 (33%)				1 (33%)
Fatigue	3 (100%)	3 (100%)	6 (100%)	3 (100%)				
Injection site reaction	3 (100%)	3 (100%)	6 (100%)	3 (100%)				
Flu-like symptoms	1 (33%)	3 (100%)	6 (100%)	3 (100%)				
Hyperglycemia**	1 (33%)	3 (100%)	5 (83%)	3 (100%)				
Thrombocytopenia*	1 (33%)	3 (100%)	5 (83%)	2 (67%)				
Hyperuricemia	2 (67%)	2 (67%)	4 (67%)	1 (33%)				
Sweating		2 (67%)	4 (67%)	2 (67%)				
Hypocalcemia	1 (33%)	2 (67%)	3 (50%)	1 (33%)				
Nausea	1 (33%)		1 (17%)	2 (67%)				
SGPT elevation			2 (33%)	2 (67%)				
Constipation			2 (33%)	1 (33%)				
Insomnia			1 (17%)	2 (67%)				
Anorexia			2 (33%)					
Arthritis (non-septic)		1 (33%)	1 (17%)					
Diarrhea		1 (33%)		1 (33%)				
Hypotension	1 (33%)			1 (33%)				
TOTAL Events / Pt	**5.3**	**8.6**	**8.6**	**9.3**	**0**	**0.3**	**0.3**	**1.7**
TOTAL Events	**16**	**26**	**52**	**28**	**0**	**1**	**2**	**5**

** Based on 1996 NCIWG criteria; ** Patient 3 with h/of Insulin dependent diabetes mellitus (ID-DM) was not included.*

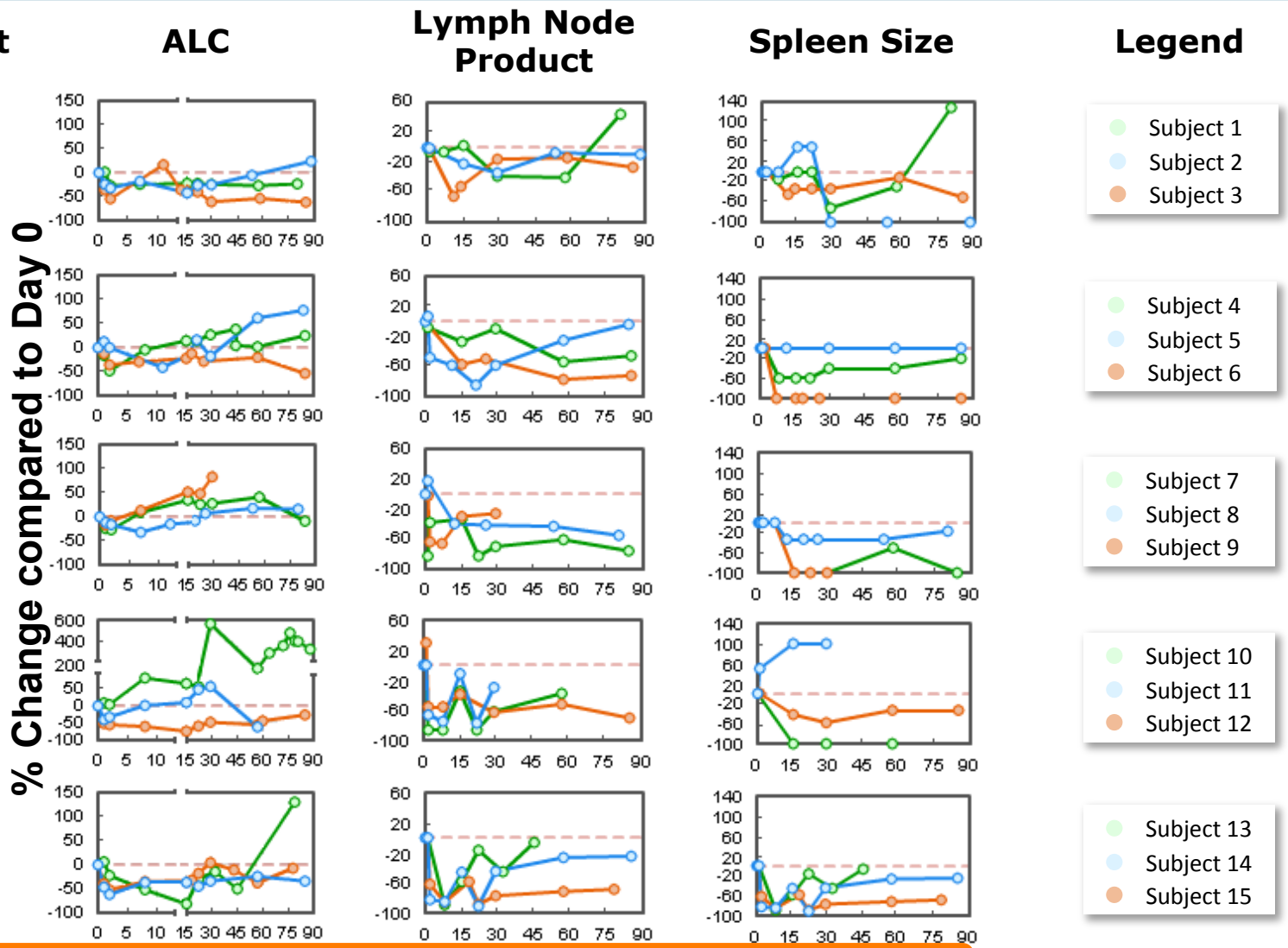
Dose-Limiting Toxicities (DLTs)

- **DLTs**: based ONLY on the first 21 days of treatment AE grade ≥ 3
- Exceptions:
  - Grade ≥3 nausea, vomiting or diarrhea uncontrolled with appropriate treatment
  - Grade ≥4 neutropenia / thrombocytopenia LASTING ≥7 days
- **Maximum tolerated dose (MTD)**: highest dose at which no more than 1/6 have DLTs

Event	Cohort	Grade	Patient #
Hypophosphatemia	4	3	11, 12
SGOT elevation	4	3	11
Hypophosphatemia	3E	3	14
Neutropenia	3E	4	13

MTD:
3.3×10^{10} vp

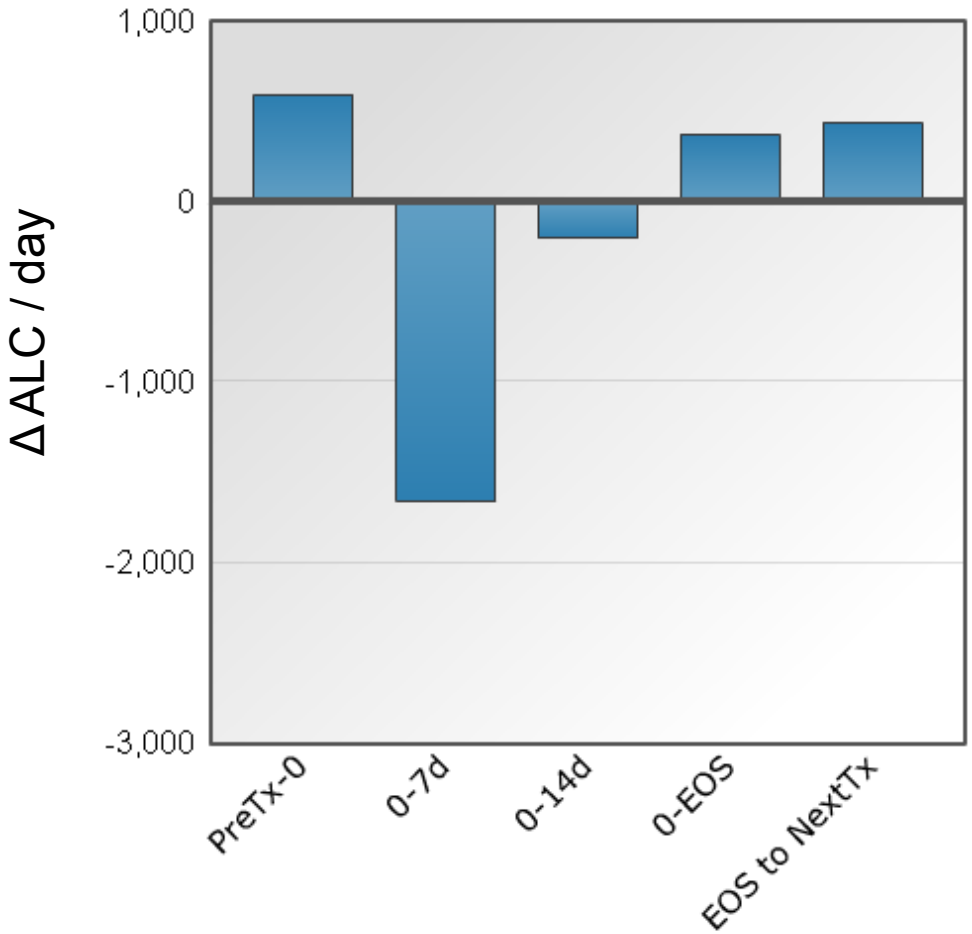
Clinical Parameters



> 50% Reduction 66% 80% 73%

Clinical Parameters

Change Absolute Lymphocyte Count (ALC) per Day

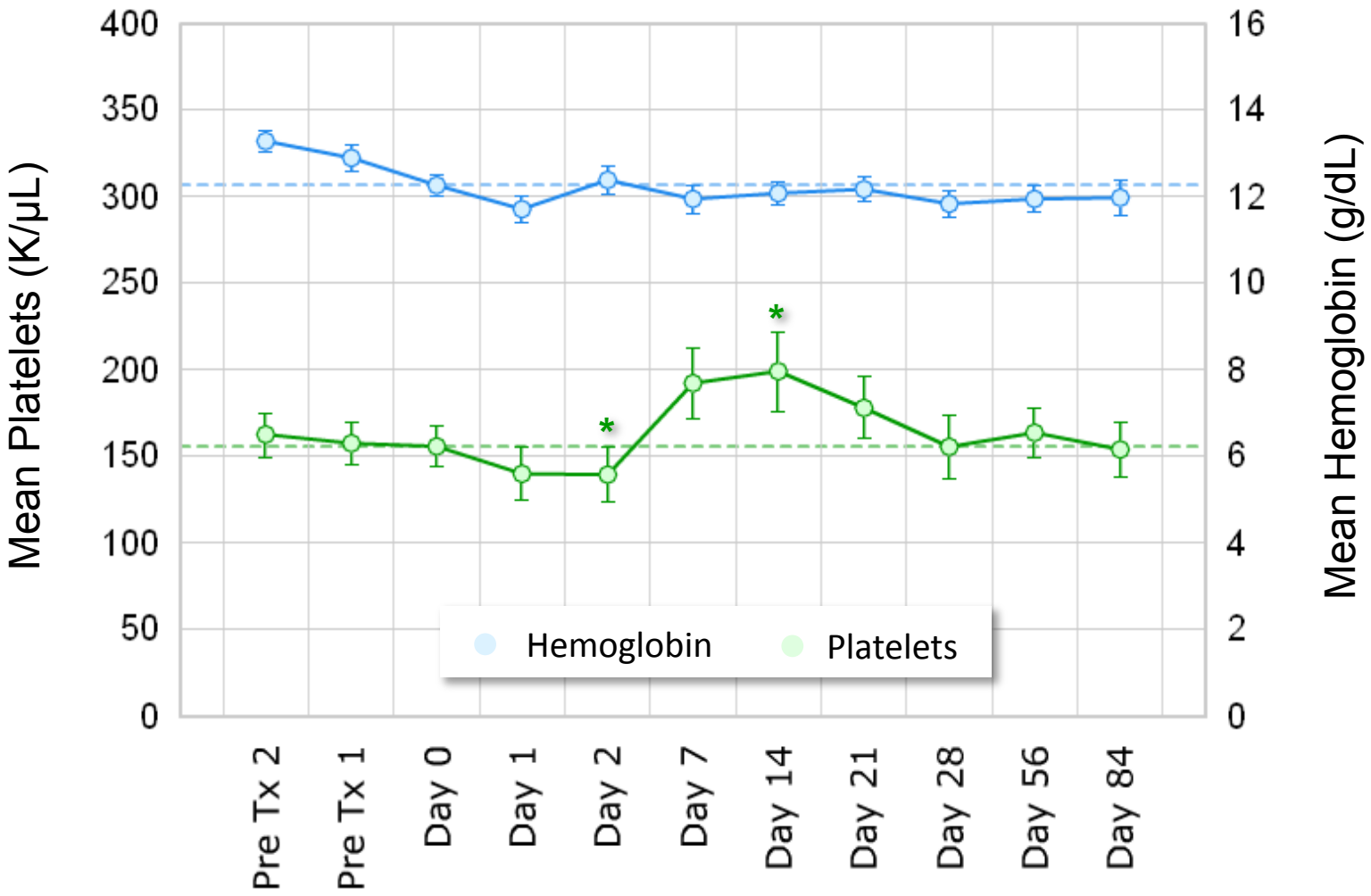


	Median	Mean
PreTx-0	407	592
0-7d	-321	-1654
0-14d	-33	-207
0-EOS	140	373
EOS-Next Tx	100	438

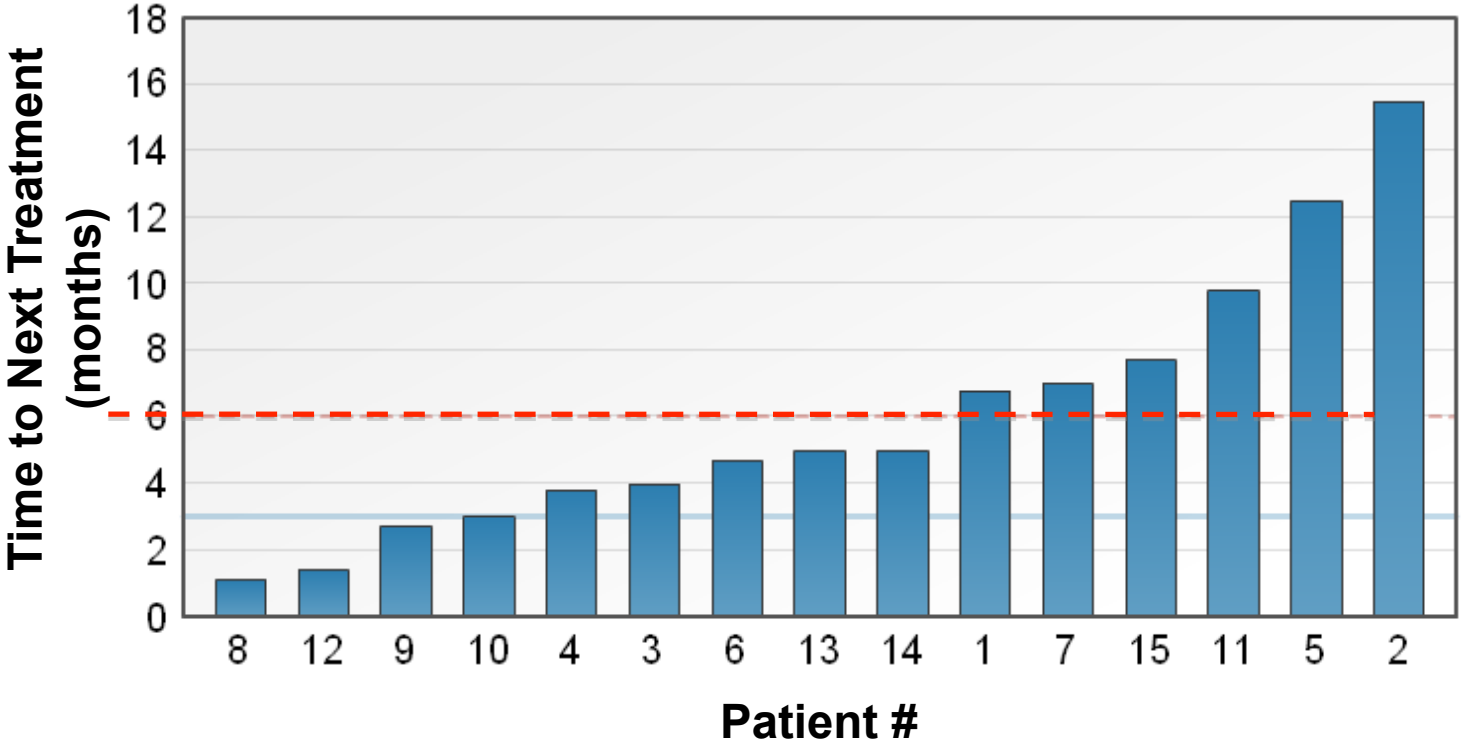
- No evidence of lymphocyte proliferation
- > 50% patients achieved more than 50% decrease in AL

Clinical Parameters

Mean Hemoglobin and Platelets



Response - Time to Next Treatment



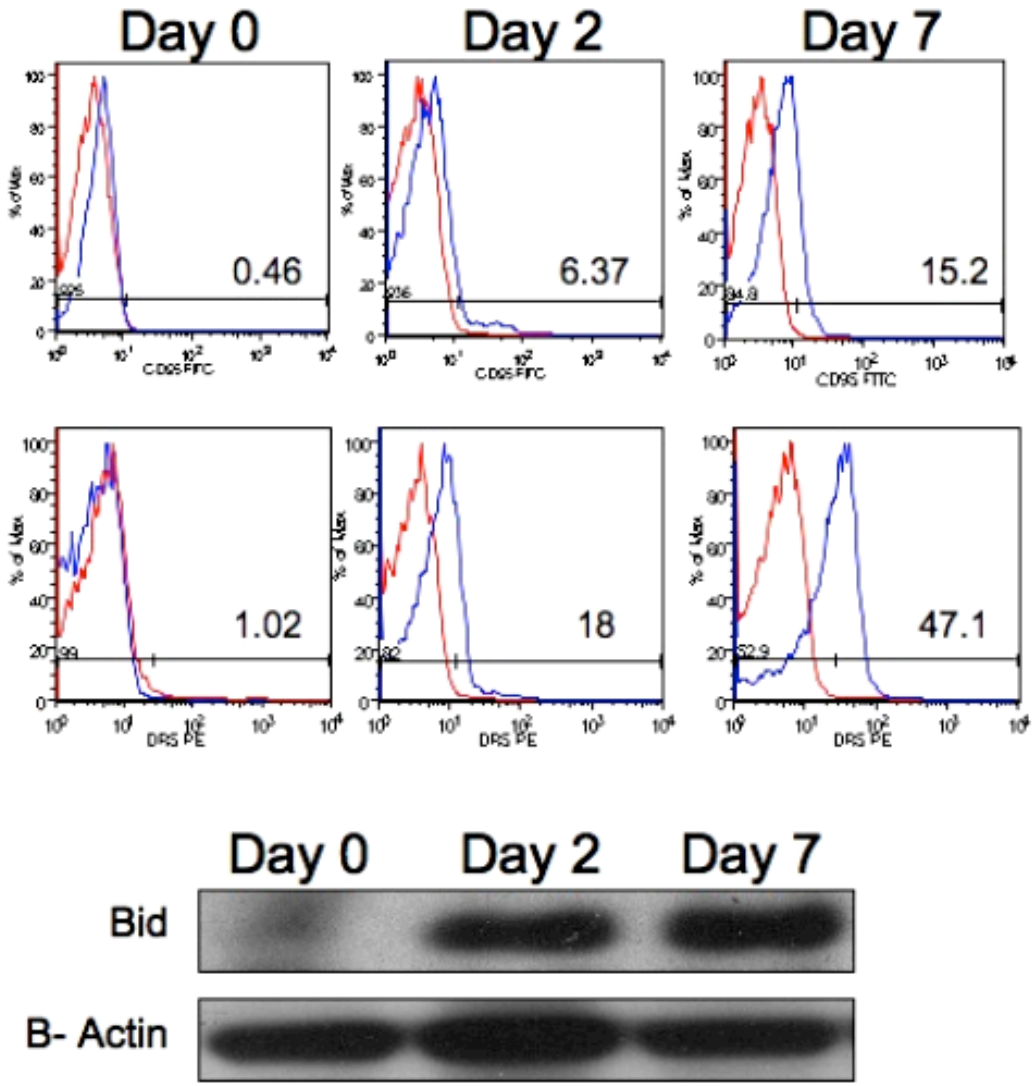
- Clinical response (NCI '96): PR = 2; SD = 8; PD = 5
- Cut-off point of 6 months based on definition of fludarabine-refractory patients

Ad-ISF35 Intranodal CLL Phase I
Bystander Effect

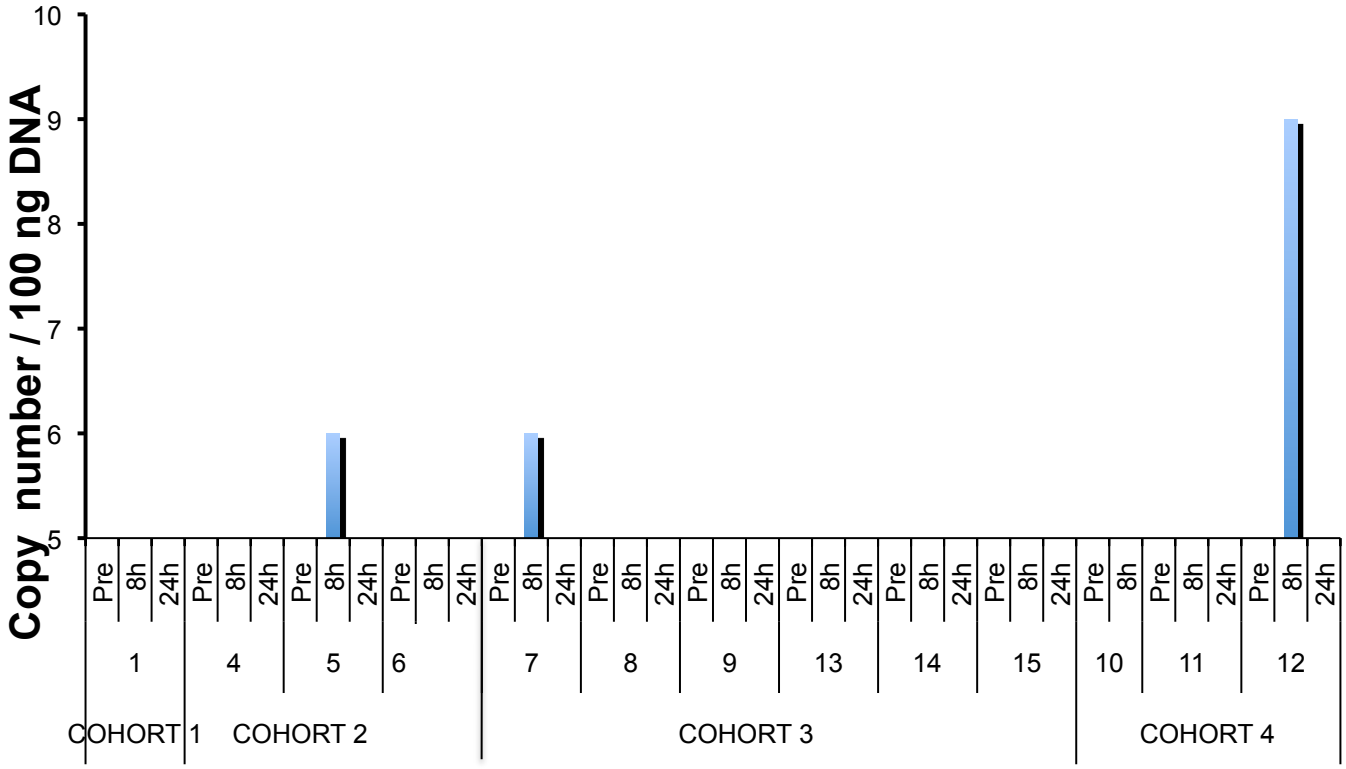
A. CD95

B. DR5

C. Bid



Ad-ISF35 Intranodal CLL Phase I
Evidence of Ad-ISF35 in PBMCs



- Ad-ISF35 DNA detected in 3 / 13 patients

Conclusions Phase I Study

- Intranodal injection of Ad-ISF35 was safe and well-tolerated
- MTD and recommended Phase II dose :

  3.3×10^{10} Ad-ISF35 vp
- Systemic biologic and clinical responses in CLL patients (***Bystander Effect in non-transduced peripheral blood leukemia cells***).
- Durable, significant reductions in tumor burden. Two patients / 15 achieved a Partial Response (PR) based on NCI-IWCLL criteria.
- These data provides the rationale for clinical studies in other types of cancer including lymphoma and solid tumors.

Phase II Clinical Trials Summary

Study Title: Phase II-A study of immune gene therapy for patients with CLL using repeat intranodal injections of Ad-ISF35

NIH OBA #: 1004-1028

Study Title: Phase II-A study of immune gene therapy for patients with CLL and NHL using repeat intranodal injections of Ad-ISF35

NIH OBA #: 1002-1029

Investigator: Januario E. Castro, M.D.

Date: June 17, 2010

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Study Objectives

Primary Objective (***Phase II – A***)

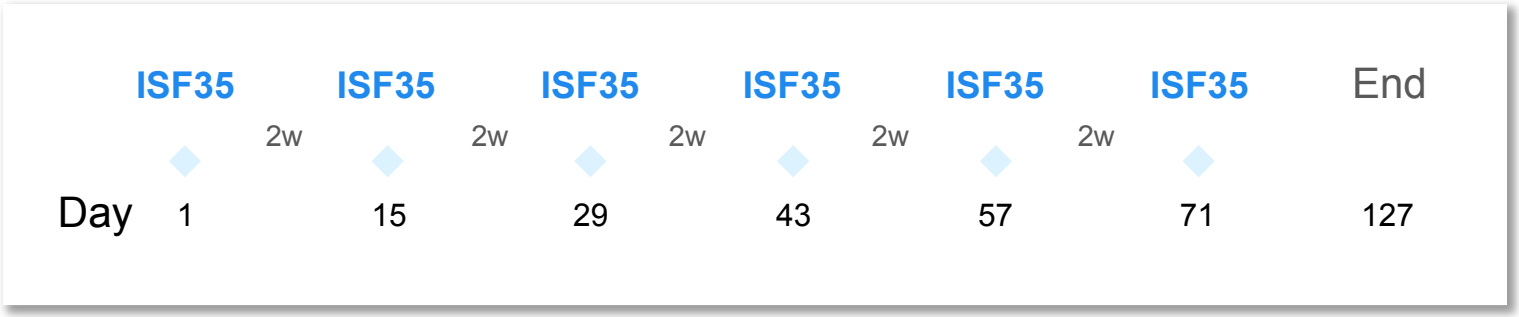
- Determine and monitor clinical and biological responses in patients treated with repeat intranodal injections of Ad-ISF35

Secondary Objectives

- Determine the safety of repeat administration of Ad-ISF35 injected directly into lymph nodes of patients with CLL / NHL
- Determine pharmacodynamic (PD) parameters in patients treated with repeat intranodal injections of Ad-ISF35

Study Design

- Phase II-A, open-label, fixed-dose (3.3 x 10^{10} vp), single institution study
- Six intranodal injections of ISF35 at two-week intervals
- Continued follow-up for 12 months after last injection



Eligibility

Inclusion Criteria

- Males and females 18 years of age and older
- ECOG performance status ≤ 2
- Anticipated survival of at least 3 months
- At least ONE single accessible AND palpable lymph ≥ 2 x 2 cm
- Negative test results for current/active infection ≤ 30 days of registration:
  - CMV, HIV-1, HIV-2, HTLV-1, HTLV-2, hepatitis A, B, C

CLL / SLL subjects

- Indication for treatment of B-cell CLL/SLL by NCI Working Group guidelines, such as:
  - Massive/progressive lymphadenopathy or splenomegaly
  - Grade ≥ 2 fatigue
  - Fever ≥ 100.5°F or night sweats
  - ≥10% weight loss
  - Lymphocytosis
  - Progressive marrow failure: anemia or thrombocytopenia
- Intermediate or high risk, poor prognosis CLL/SLL

NHL subjects

- At least one prior treatment
- One of the following NHL subtypes:
  - Follicular lymphoma
  - Diffuse large cell lymphoma
  - Mantle cell lymphoma
  - CLL / SLL
- Stage III or IV disease at any time in the past

Demographics and Characteristics

Pt #	Age	Sex	IgV_H *(% Hom)*	%ZAP70+	%CD38+	Prior Treatments	FISH	LDT *(mo)*	ECOG	Rai / Ann Arbor	β2M *(g/dL)*
CLL-1	56	M	96.8	36.7	14.9	GT-2	del(13q)	6	1	II	5.4
CLL-2	63	F	96.1	89.3	8.7	HDMP+R, HSP90, A, GT-2	del(13q)	> 6	0	II	5.5
CLL-3	73	M	100	10.3	75.2	GT, X-Cyte, HDMP+R(2), GT-2, AT-101+R	del(13q), del(11q)	4	0	II	5.4
CLL-4	71	M	100	43.2	75.7	HDMP+R, A	del(13q)	6	0	II	5.0
CLL-5	61	M	100	56.6	n/a	My -Vax	tri(12)	> 6	0	II	3.2
CLL-6	71	M	100	45.2	41.4	C+P, CR+D, HDMP+R, FR	del(13q)	> 6	0	I	5.5
CLL-NHL-1	63	F	97.9	1.9	3.5	(none)	del(13q), del(11q)	> 6	2	II	5.1
CLL-NHL-2	56	M	97.2	68.5	n/a	AT-101(2), HDMP+R(2)	normal	3	0	II	4.3
CLL-NHL-3	74	M	94.3	0.7	99.1	CP, FCR, A	del(13q)	4	0	IV	5.2
CLL-NHL-4	70	M	100	n/a	n/a	CVP, Spleen XRT, FD, Hyper CVAD, Auto-HSCT	normal	> 6	0	III	2.3
CLL-NHL-5	69	M	98	36.9	0.6	(none)	del(13q)	> 6	0	II	4.9

* ***A*** *= Alemtuzumab;* ***AT-101*** *= BCL-2 family member inhibitor;* ***CP*** *= Chlorambucil and Prednisone;* ***CR + D*** *= Cyclophosphamide, Rituximab, and Dexamethasone;* ***CVP*** *= Cyclophosphamide, vincristine, and prednisone;* ***FCR*** *= Fludarabine, Cyclophosphamide and Rituximab;* ***FD*** *= Fludarabine and Dexamethasone;* ***GT*** *= gene therapy (CD154);* ***GT-2*** *= gene therapy (ISF35);* ***HDMP*** *= High-Dose Methylprednisolone;* ***HSP90*** *= Heat Shock Protein 90 Inhibitor;* ***R*** *= Rituximab;* ***X-cyte*** *= T-Cell Therapy*

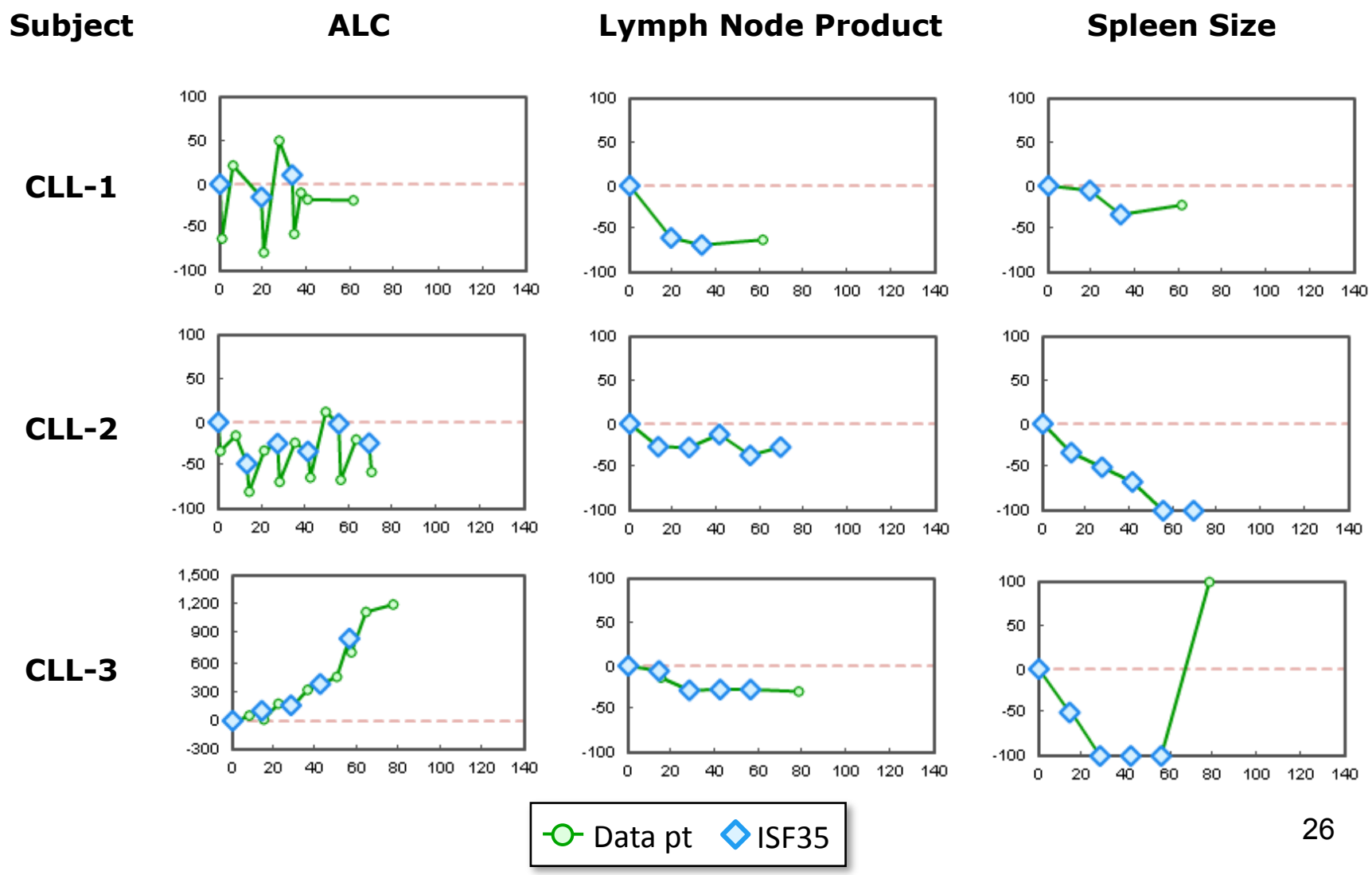
Adverse Events

Chronic Lymphocytic Leukemia (CLL) Patients

Event	Grade I / II						Grade III / IV					
	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6
Flu-like symptoms	1	1	1	1		1					1 ¶	
Vomiting				1				1 *				
Hyponatremia												1 §
Arthritis (non-septic)	1	1			1							
Injection site reaction	1	1	1	1	1	1						
Sweating	1	1	1	1	1	1						
Fatigue	1	1	1	1	1	1						
Nausea	1	1		1		1						
Dyspnea				1	1							
Anorexia	1			1		1						
Urinary frequency				1		1						
Nausea	1	1		1		1						
Constipation						1						
TOTAL Events	**8**	**7**	**4**	**10**	**5**	**9**	**0**	**1**	**0**	**0**	**1**	**1**

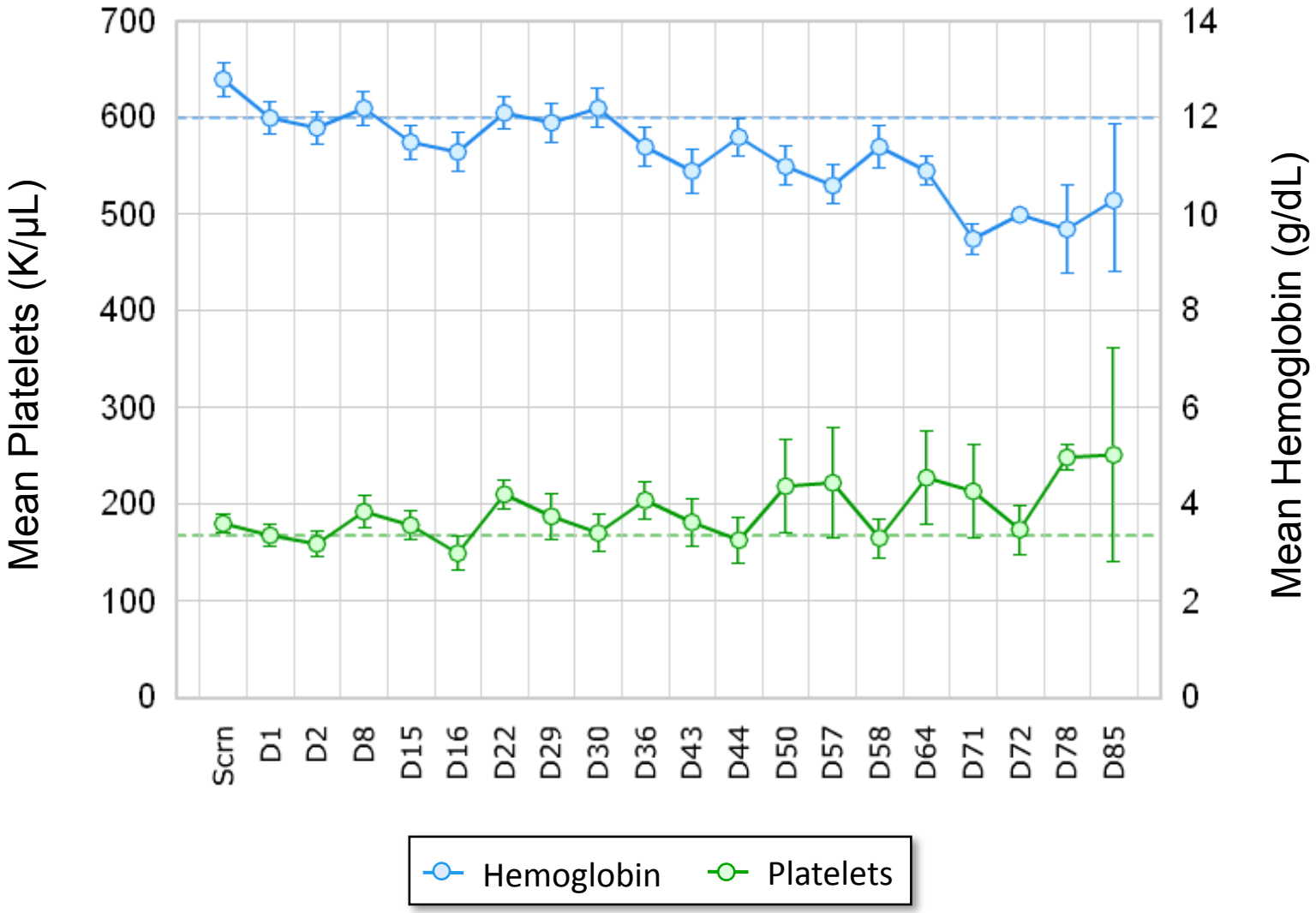
*Patient was hospitalized and his symptoms improved within 24 hours
¶ Fever >103 ˚F detected by the patient at home but not my nursing personnel.
§ Hyponatremia G-III (DLT – AE possibly related to study drug), patient asymptomatic, improved within 7 days.

Clinical Parameters



Clinical Parameters

Chronic Lymphocytic Leukemia (CLL) Patients



Response to Treatment

Pt #	Age	Sex	IgV_H (% Hom)	%ZAP70+	%CD38+	Prior Treatments	FISH	LDT (mo)	ECOG	Rai / Ann Arbor	β2M (g/dL)	Resp
CLL-1	56	M	96.8	36.7	14.9	GT-2	del(13q)	6	1	II	5.4	SD
CLL-2	63	F	96.1	89.3	8.7	HDMP+R, HSP90, A, GT-2	del(13q)	> 6	0	II	5.5	SD
CLL-3	73	M	100	10.3	75.2	GT, X-Cyte, HDMP+R (2), GT-2, AT-101+R	del(13q), del (11q)	4	0	II	5.4	PD
CLL-4	71	M	100	43.2	75.7	HDMP+R, A	del(13q)	6	0	II	5.0	PR
CLL-5	61	M	100	56.6	n/a	My -Vax	tri(12)	> 6	0	II	3.2	SD
CLL-6	71	M	100	45.2	41.4	C+P, CR+D, HDMP+R, FR	del(13q)	> 6	0	I	5.5	SD
CLL-NHL-1	63	F	97.9	1.9	3.5	(none)	del(13q), del (11q)	> 6	2	II	5.1	PR
CLL-NHL-2	56	M	97.2	68.5	n/a	AT-101(2), HDMP+R(2)	normal	3	0	II	4.3	PD
CLL-NHL-3	74	M	94.3	0.7	99.1	CP, FCR, A	del(13q)	4	0	IV	5.2	PD
CLL-NHL-4	70	M	100	n/a	n/a	CVP, Spleen XRT, FD, Hyper CVAD, Auto-HSCT	normal	> 6	0	III	2.3	SD
CLL-NHL-5	69	M	98	36.9	0.6	(none)	del(13q)	> 6	0	II	4.9	PR

* ***Partial response (PR): 3*** *Stable disease (SD): 5* *Progressive disease (PD): 3*

Ad-ISF35 Intranodal Phase I / II Studies
Correlative Studies

Lab Study	Assays
Evaluation of Cell Surface Receptors by Flow Cytometry	• CD3; 4; 5; 8; 16; 19; 20; 23; 28; 54; 56; 80; 86; 95 • DR5
Apoptosis analysis	• Western blot: p21, p73, Bid • Annexin V or similar apoptosis assay • MLPA
Antibody responses	• Anti-adenovirus • Anti-ISF35 • Anti-human CD154
Cytokine analysis	• IL-2 • TNF-α
Anti-tumor immune response	• ELISPOT • MLR

Ad-ISF35 Intranodal CLL-NHL Phase II

Anti-Adenovirus / Anti-hCD154 / Ad-ISF35 Neutralizing Ab-Titers

Trial	Patient #	Anti-Adenovirus Antibodies Titer[1]			Anti-hCD154 Antibodies[2]		Ad-ISF35 Neutralizing Antibody Titers		
		Pre	Post	Fold Change	Pre	Post	Pre	Post	Fold Change
Phase II CLL	1	531	2326	4	ND*	ND	< 10	247	25
	2	501	308	< 1	ND	ND	< 10	< 10	0
	3	1377	1306	< 1	ND	ND	429	317	< 1
	4	51	28	< 1	ND	ND	< 10	< 10	0
	5	56	143	3	ND	ND	61	9174	150
	6	189	79	< 1	ND	ND	1390	855	< 1
Phase II NHL-CLL	1	86	362	4	ND	ND	4290	13331	3
	2	< 10	19	2	ND	ND	< 10	< 10	0
	3	33	19	< 1	ND	ND	< 10	< 10	0
	4	< 10	52	5	ND	ND	341	668	3
	5	31	234	8	ND	ND	< 10	329	33

* ***ND*** *= Non-detectable*

1. Anti-Adenovirus titers were measured by ELISA assay in plasma samples obtained before and after the last injection of Ad-ISF35
2. Anti-hCD40L antibodies were measured by ELISA assay (binding of antibodies to recombinant hCD154 coated on ELISA plates) and flow cytometry (binding of antibodies on HeLa cells expressing hCD154) in plasma samples obtained before and after the last injection of Ad-ISF35

Conclusions

- Repeat intranodal dose administration of Ad-ISF35 has been well tolerated by the majority of patients. Only one patient developed Grade III hyponatremia after injection #4 considered **DLT** (This patient received no additional injections of Ad-ISF35). No dose adjustments have been required on the remaining patients

- We have not observed long-term toxicities (Median follow up 12 months)

- We have observed 3 / 10 CLL patients with PR based on IW-CLL-08 criteria (Patient CLL-4, CLL-NHL-1, CLL-NHL– 5).

- We have observed antibody production against Ad-ISF35 with the ability to neutralize vector transduction but not against h-CD154.

Informed Consent Form (ICF) Questions

Reviewer: *Jeffrey Kahn, Ph.D.*

Topic	Questions	Responses
Language	Complex, confusing, ambiguous	New ICFs were created and sent to RAC on 6/15/10 as Addendum#1 to initial response.
Study Benefits	Overstates the results of a Phase I trial	Section was revised. Please see Addendum#1 (p.10).
Ad-ISF35 Administration Phase	Different ICFs for inpatient and outpatient groups	Developing 2 different ICFs is being considered.
Optional blood draws / release of excess tissue	Purpose(s), tests, whom, tissue bank housing samples identification, boilerplate language	ICFs revised and "optional procedures section" was inserted to clarify these questions (p.12).
General	ISF35 description, eligibilty, acronyms, table of tests, CR definition, follow up phase treatment, "other medications" listed as a study risk, request for autopsy, media interest	ICFs were amended to incorporate these suggestions.

Informed Consent Form (ICF) Questions

Reviewer: *Jeffrey Kahn, Ph.D.*

Topic	Questions	Responses
Conflict of Interest	Dr. Kipps' role	• Serves as Co-Investigator • Reviews Study Data; Participates in Publications • The University of California holds the patent, Dr. Kipps is an inventor (clarified on ICF) **Dr. Kipps DOES NOT (for any Ad-ISF35 study):** • Serve as PI • Advise patients re: suitability for trial • Consent patients to participate as subjects • Evaluate eligibility • Participate in the supervision or monitoring of the study

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