

# ISF35 Active Immunotherapy for Blood Cancer

## NIH Protocol #0810-952

Phase Ib Study of Autologous Ad-ISF35-Transduced CLL B Cells and Fludarabine, Cyclophosphamide, and Rituximab (FCR) in Subjects with Fludarabine-Refractory and/or del(17p) Chronic Lymphocytic Leukemia (CLL)

---

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

---

June 17, 2010



UNIVERSITY of CALIFORNIA, SAN DIEGO  
MEDICAL CENTER MOORES CANCER CENTER



# RAC Comments/Questions

Reviewers: *Hildegund Ertl, M.D. ; John A. Zaia, M.D. ; David A. Williams, M.D.*

- *ISF35 mechanisms of action and rationale for using an immunotherapy followed by a chemotherapy regimen?*
- *Preclinical toxicology study dosing based on viral particles?*
- *Dosage and route of ISF35 for previous human studies?*
- *Rationale for use of non-irradiated cells?*

# Chronic Lymphocytic Leukemia (CLL)

- Most common leukemia in Western countries
- Incurable disease
- High-risk CLL populations:

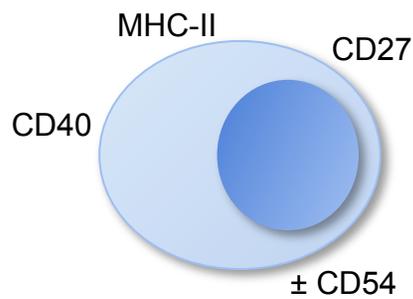
## Fludarabine-refractory CLL

- Did not respond to fludarabine, or relapsed within 6 months
- Median survival about 9-16 months after treatment
- Approved therapies:  
Campath® (anti-CD52 mAb). OR 30%  
Ofatumumab (anti-CD20 mAb).  
Refractory Fludarabine / Campath  
OR 42% (No CRs- TTP 6.5 months)

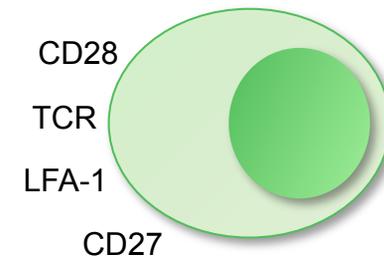
## del(17p), or “17p-deleted” CLL

- CLL with cytogenetic changes in short arm of chromosome 17
- Predicts poor response and shorter survival
- EBMT recommends stem cell transplant at disease progression

# CD154 Immune Activation of CLL Cells



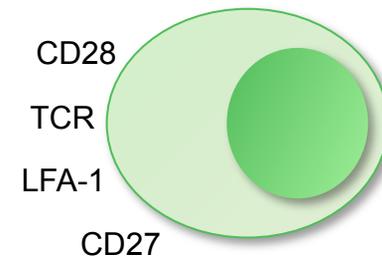
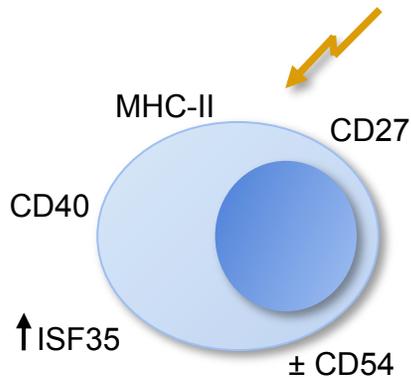
"Stealth" APC



T cell

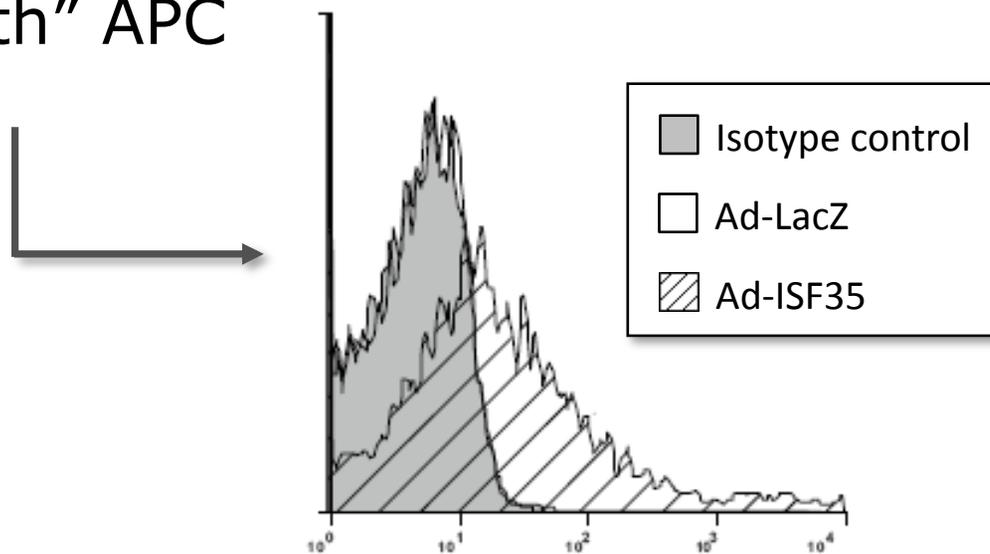
# CD154 Immune Activation of CLL Cells

Transduction with vector encoding CD154



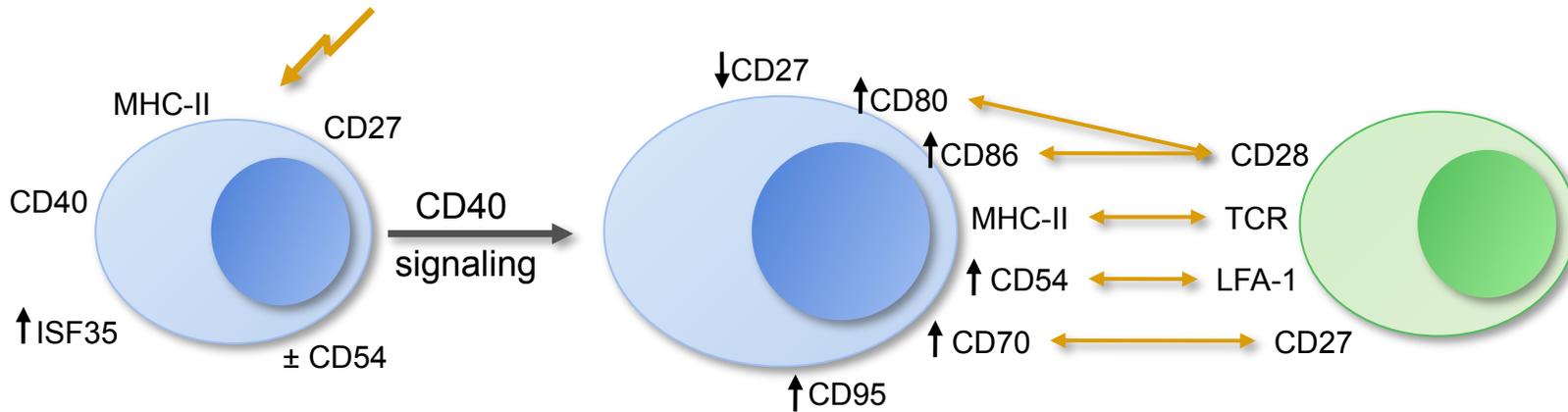
"Stealth" APC

T cell



# CD154 Immune Activation of CLL Cells

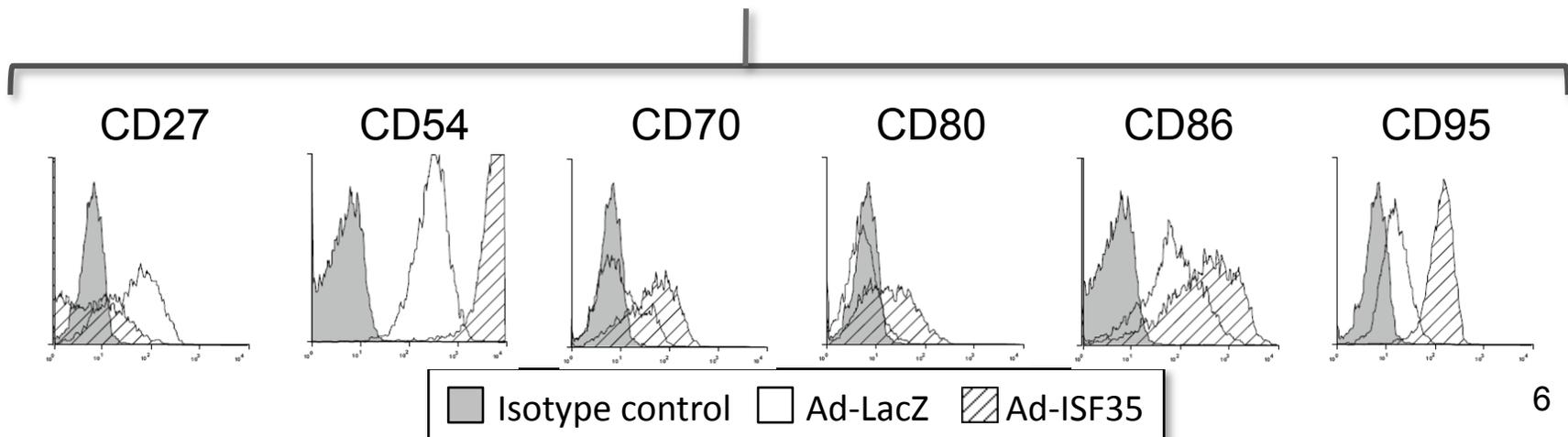
Transduction with vector encoding CD154



"Stealth" APC

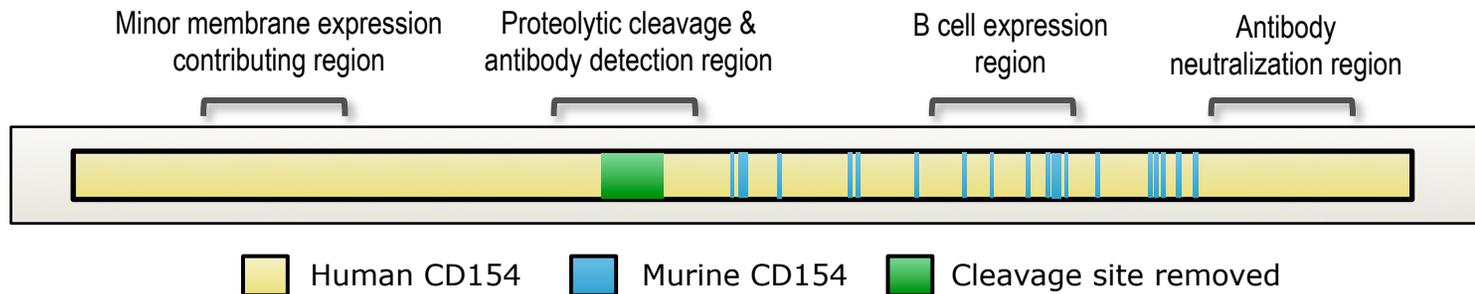
**"Alarm" APC**

T cell

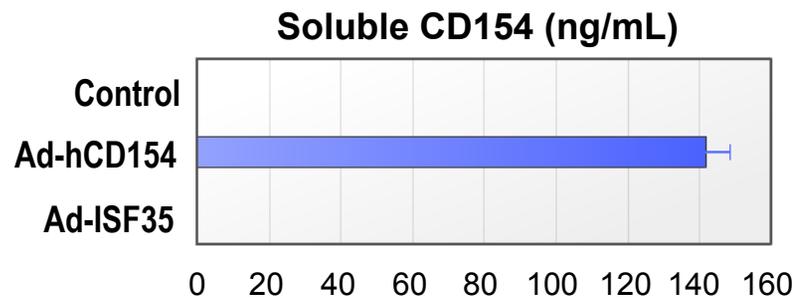
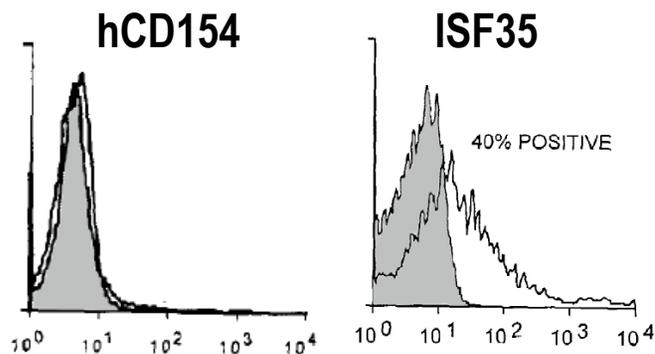


# ISF35: Active Immunotherapy

## ISF35: 92% AA Homology to Human CD154



- Human CD154 expression precluded on CLL cells
- ISF35 stably expressed on CLL cells
- ISF35 resistant to proteolytic cleavage



# Preclinical and Pharmacology/Toxicology Studies

Ad-ISF35 and Ad-ISF154

Study #	Design	Dose, Vector	Main Finding
EB06-02202 (2008)	Repeat intratumoral injection of Ad-ISF35 in tumor-bearing mice	<ul style="list-style-type: none"> <li>• 0 (control)</li> <li>• <math>3 \times 10^9</math> vp Ad-ISF35</li> <li>• <math>3 \times 10^{10}</math> vp Ad-ISF35</li> </ul>	<ul style="list-style-type: none"> <li>• Well tolerated</li> <li>• Rough coats at high dose after second injection</li> <li>• Transient increase in testis weight</li> <li>• Transient increase in ovarian weight high dose</li> <li>• ISF35 transgene distribution mainly in tumors for up to 5 days following injection</li> </ul>
EB06-002-1 (2007)	Single intratumoral injection of Ad-ISF35 in tumor-bearing mice	<ul style="list-style-type: none"> <li>• 0 (control)</li> <li>• <math>3 \times 10^9</math> vp Ad-ISF35</li> <li>• <math>3 \times 10^{10}</math> vp Ad-ISF35</li> </ul>	<ul style="list-style-type: none"> <li>• Well tolerated</li> <li>• Transient elevation white blood cells and platelet counts</li> <li>• Slight decreases in hemoglobin and mean corpuscular volumes</li> </ul>
Intratumoral Injection Study (2007)	Intratumoral injection of Ad-ISF35 in B cell lymphoma model in mice	<ul style="list-style-type: none"> <li>• Saline (control)</li> <li>• <math>2 \times 10^{10}</math> vp Ad-LacZ</li> <li>• <math>2 \times 10^{10}</math> vp Ad-ISF35</li> </ul>	<ul style="list-style-type: none"> <li>• Tumor rejection and long-term survival of &gt;80% of treated animals in Ad-ISF35 treated animals compared to 0% survival in mice treated with saline or control Ad-LacZ vector</li> </ul>
3-H66 (1997)	Single IV inj of Ad-mCD154 in mice	<ul style="list-style-type: none"> <li>• <math>2 \times 10^{10}</math> vp Ad-mCD154</li> <li>• <math>6.6 \times 10^{11}</math> vp Ad-mCD154</li> </ul>	<ul style="list-style-type: none"> <li>• Abnormal clinical observations:                             <ul style="list-style-type: none"> <li>– Dose-related changes in red blood cells</li> <li>– Liver serum chemistry</li> <li>– Microscopic changes in spleen, liver, lungs, and lymph nodes</li> </ul> </li> </ul>
3-K41 (1997)	Single IV inj of Ad-mCD154 in mice	<ul style="list-style-type: none"> <li>• <math>2 \times 10^8</math> vp Ad-mCD154</li> <li>• <math>2 \times 10^9</math> vp Ad-mCD154</li> <li>• <math>2 \times 10^{10}</math> vp Ad-mCD154</li> <li>• <math>2 \times 10^{11}</math> vp Ad-mCD154</li> </ul>	<ul style="list-style-type: none"> <li>• MTD determined as <math>2 \times 10^9</math> vp</li> <li>• <math>2 \times 10^{10}</math> and <math>2 \times 10^{11}</math> vp resulted in euthanization of mice</li> </ul>

# Previous / Active Human mCD154 / ISF35 Studies

## Dosage and Route of Administration

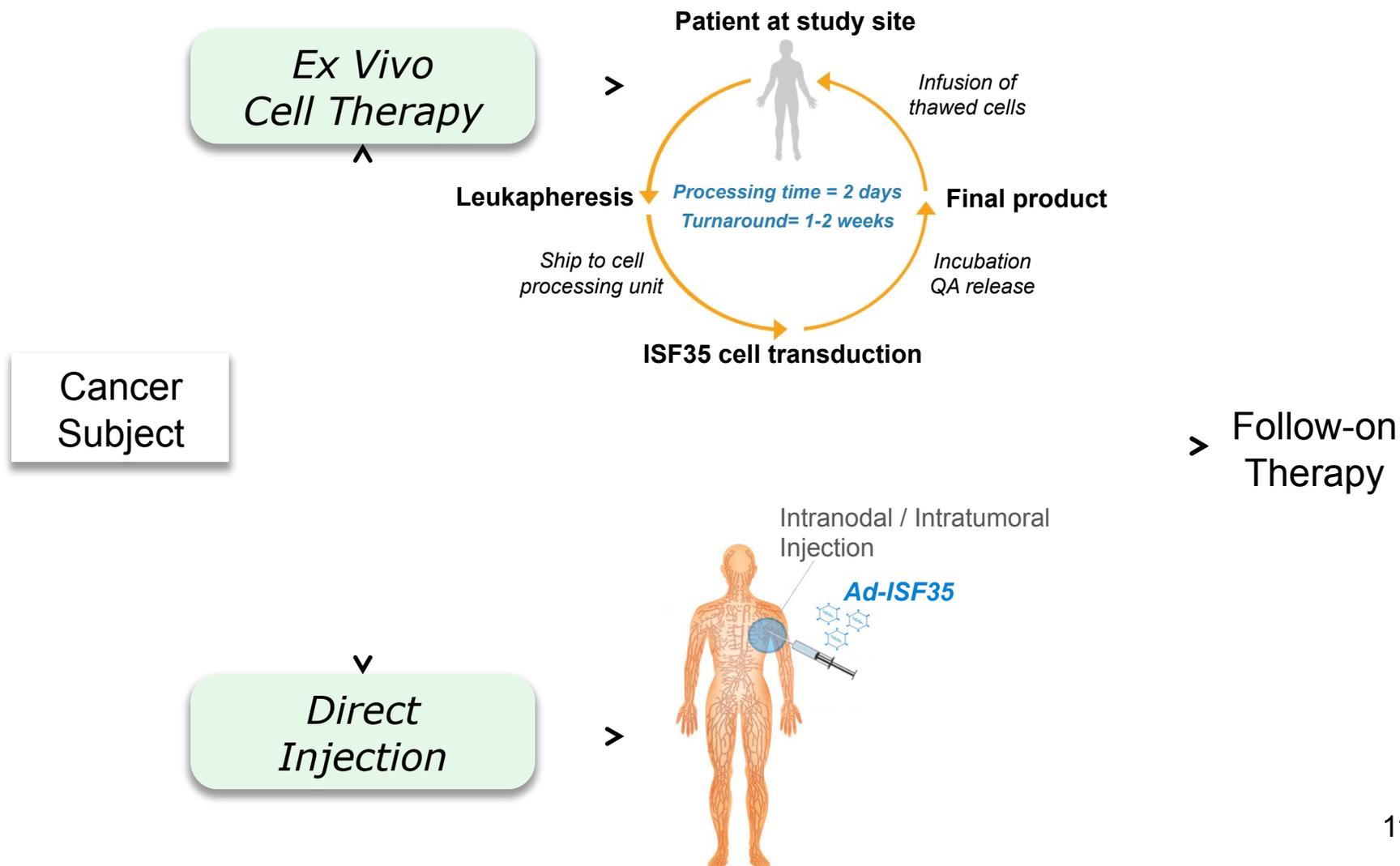
NIH ID	Target	Intervention	Route	Dosages	Phase	Start	End	Size
9803-242	CLL	mCD154 x 1	Infusion	<ul style="list-style-type: none"> <li>• 3 x 10<sup>8</sup> cells</li> <li>• 1 x 10<sup>9</sup> cells</li> </ul>	1	1997	2001	11
0005-401	CLL	mCD154 x 2	Infusion	<ul style="list-style-type: none"> <li>• 3 x 10<sup>8</sup> cells</li> <li>• 1 x 10<sup>9</sup> cells</li> </ul>	2	2002	2004	7
0601-757	CLL	ISF35 x 1	Infusion	<ul style="list-style-type: none"> <li>• 1 x 10<sup>8</sup> cells</li> <li>• 3 x 10<sup>8</sup> cells</li> <li>• 1 x 10<sup>9</sup> cells</li> </ul>	1	Jun 2006	Mar 2007	9
0601-757	CLL	ISF35 x 2	Infusion	<ul style="list-style-type: none"> <li>• 1 x 10<sup>8</sup> cells</li> <li>• 3 x 10<sup>8</sup> cells</li> <li>• 1 x 10<sup>9</sup> cells</li> </ul>	1b	Apr 2007	Nov 2007	4
0607-784	CLL	ISF35 x 1	Dir inj	<ul style="list-style-type: none"> <li>• 1.0 x 10<sup>10</sup> vp</li> <li>• 3.3 x 10<sup>10</sup> vp</li> <li>• 1.0 x 10<sup>11</sup> vp</li> <li>• 3.3 x 10<sup>11</sup> vp</li> </ul>	1	Aug 2007	Nov 2008	15
0810-952	CLL	ISF35 x 3 + FCR x 3	Infusion	3 x 10 <sup>8</sup> cells	1b	Sept 2008	(ongoing)	12*
1004-1028	CLL	ISF35 x 6	Dir inj	3.3 x 10 <sup>10</sup> vp	2	Jan 2009	(ongoing)	28*
1004-1029	NHL / CLL	ISF35 x 6	Dir inj	3.3 x 10 <sup>10</sup> vp	2	Jan 2009	(ongoing)	28*

\* Anticipated sample size of ongoing trial.

# Rationale for Use of Non-Irradiated Cells

- The transduced CLL cells that are transferred back into a patient are constitute <1% of the total leukemic burden.
- Since CLL cells are known to be slowly dividing and non-metastatic in nature, the use of non-irradiated cells was not considered a high risk for CLL patients.
- It was considered irradiation could limit the survival of cells and mitigate their functional activity. Since Ad-ISF35 transduced CLL cells are frozen prior to re-administration to patients, long-term storage of irradiated cells may negatively impact their quality and viability upon thawing and infusion.
- Since 1997 the use of non-irradiated cells has been approved / recommended by the FDA / RAC as an acceptable autologous CLL product.
- Since 1997, a total of 35 human Subjects (CLL patients) have received non-irradiated CLL cells transduced with Ad-ISF154/ Ad-ISF35. There has been no evidence of uncontrolled proliferation, shorter survival, persistence of transduced cells, or conversion of the transduced cells into aggressive leukemia / lymphoma *in vivo*.

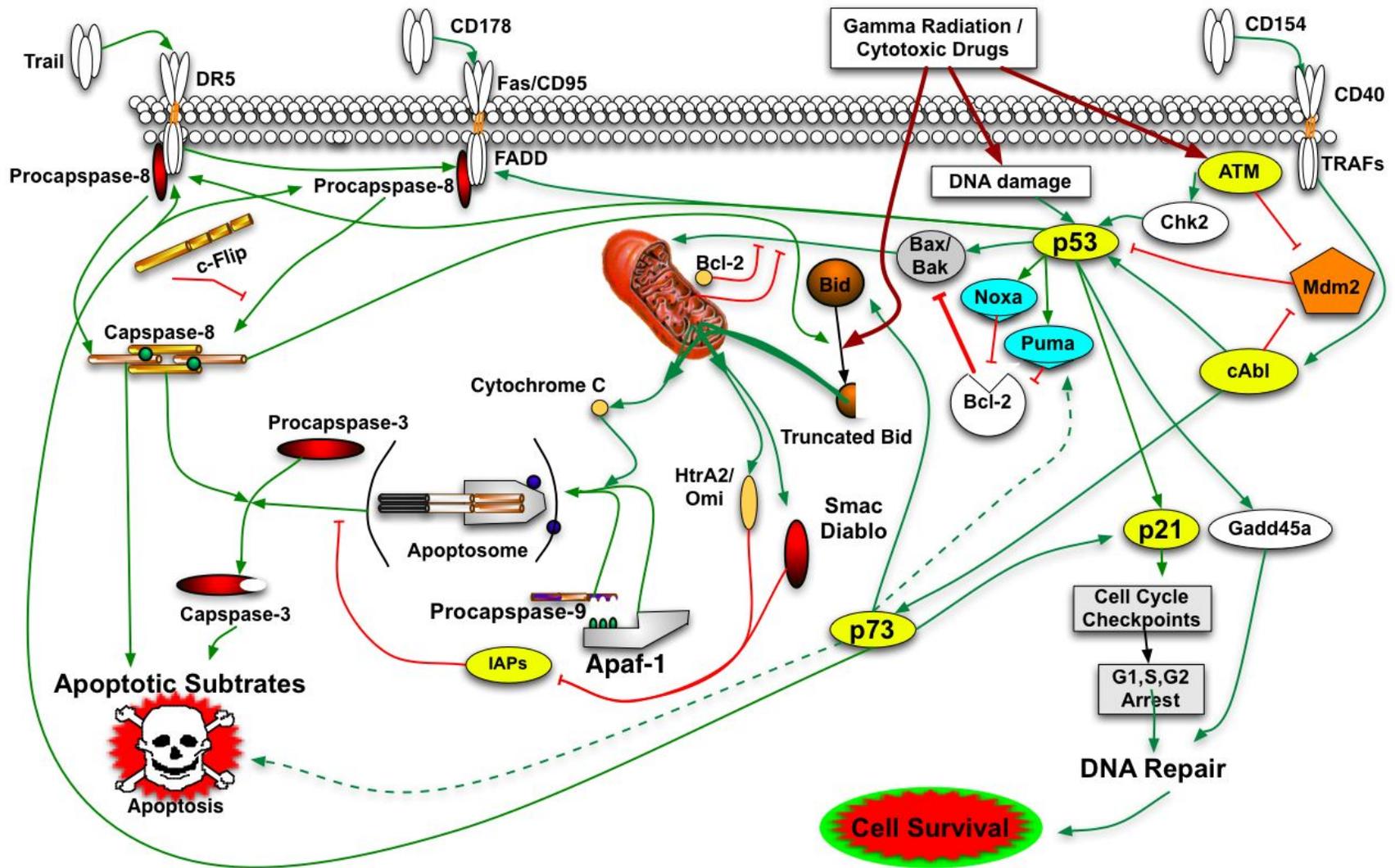
# ISF35: Routes of Administration



# ISF35: Mechanisms of Action

- **Anti-Tumor Immunity**
  - Cellular
    - Autologous T cell responses
    - Bystander effect activation of CLL cells
  - Humoral
    - Antibody generation against leukemic self antigens (ROR1)
  - Innate Immunity
    - Rapid leukemic cells reductions *in vivo* (Fas and DR5 death receptor killing)
- **Pro-Apoptotic Upregulation**
  - Death receptors (Fas and DR5)
  - Bcl-2 family members (Bid, Bax, Noxa, Puma)
  - Growth arrest proteins (p21)
- **Activation of p53 Independent Pathways**
  - p63, p73 pathways
  - Chemotherapy re-sensitization

# p73 – Ancillary Death Pathway



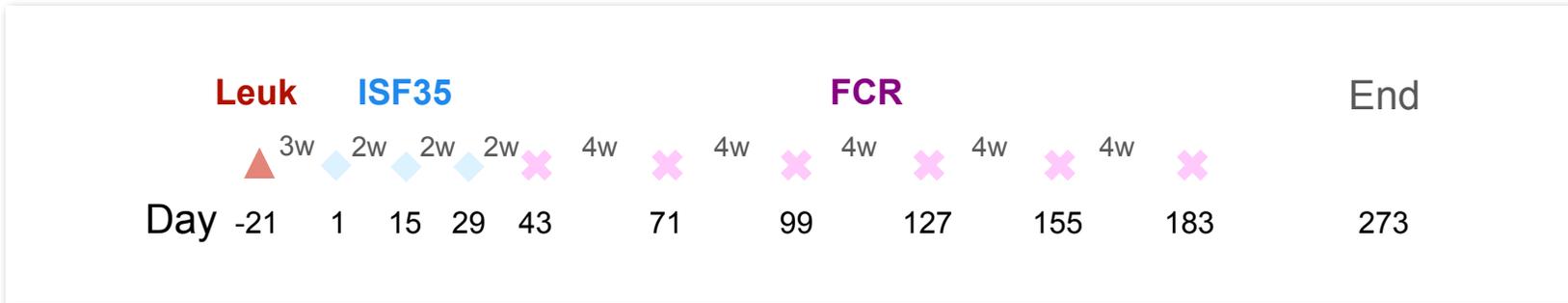
# RAC Comments/Questions

Reviewers: *Hildegund Ertl, M.D. ; John A. Zaia, M.D. ; David A. Williams, M.D.*

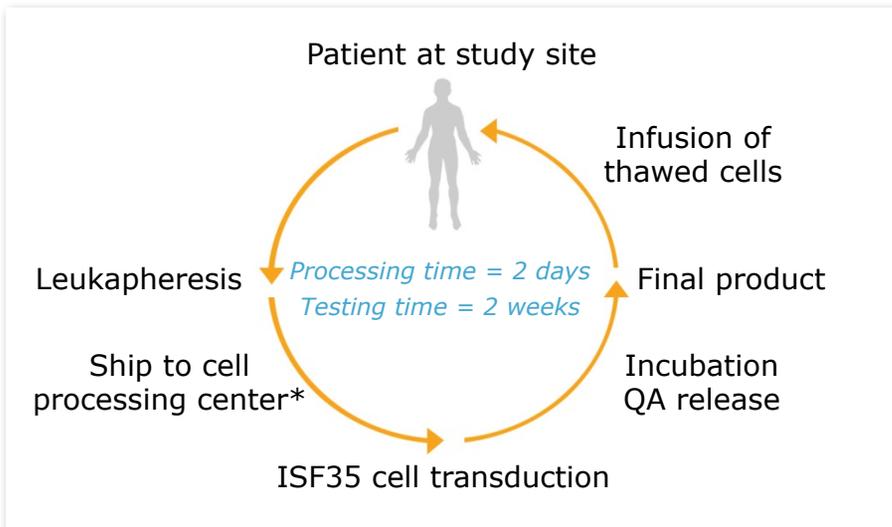
- *Updates for patients already enrolled in protocol?*
- *Proposed pharmacodynamic studies that are part of secondary study objectives?*
- *Defined criteria for continued infusion of cells or repeat cycles of FCR?*
- *Defined premeds prior to cell infusion?*
- *Potential to induce cross-reactive anti-human CD40L (CD154) antibodies?*

# ISF35 + FCR Trial Design

## Schema



## Treatment Cycle



- Leukapheresis
- 3 infusions of Ad-ISF35-transduced cells
- Up to 6 cycles of FCR
- One leukapheresis sufficient for three infusions Ad-ISF35

\* Cell transduction and QA done at Waisman Clinical BioManufacturing Facility

# Criteria to Continue FCR

The protocol will be amended to address this recommendation. The bullet points in Section 5.4 will read:

- Starting on Day 43, up to six cycles of fludarabine, cyclophosphamide, and rituximab (FCR) will be given every 28 days, unless the principal investigator decides at any time that continued treatment is not in the best interest of the patient. ***Reasons to be considered to discontinue or delay FCR treatment are the following:***
- Any non-hematological toxicity Grade III - IV that does not resolved to Grade  $\leq 1$  within 14 days.
- Hematological toxicities (Cytopenias) Grade III - IV (Based on IW-CLL criteria) that does not resolve within 14 days despite of growth factor administration.
- No response or disease progression
- Secondary malignancy or disease
- Patient preference

# Pre-medications to be Administered

- These clinical protocol documents will be amended to reflect the description of premedication. Subjects enrolled in this study will receive the following pre-medications:
- ***30 minutes prior to infusion of Ad-ISF35 transduced CLL cells:***
- Acetaminophen 650 mg oral
- Diphenhydramine 25 mg IV
- Prochlorperazine 10 mg oral.
  
- ***30 minutes prior to FCR administration:***
- Prochlorperazine 10 mg oral
- Ondansetron 8 mg IV
- Decadron 10 mg IV
- Diphenhydramine 25 mg IV (only prior to Rituximab administration)
- Acetaminophen 650 mg oral (only prior to Rituximab administration)

# Study Objectives

## Primary Objective

- Assess **toxicity**, **tolerability**, and **safety** of autologous Ad-ISF35-transduced cells followed by FCR in subjects with fludarabine-refractory and/or del(17p) CLL

---

## Secondary Objectives

- Explore **anti-leukemia activity**, including:
  - Reduction in leukemia count, lymphadenopathy, and splenomegaly
  - Improvement in bone marrow function
  - Objective response rate and response duration
- Assess **pharmacodynamic endpoints**, including:
  - B and T cell anti-leukemia immune responses
  - Antibody production against Ad-ISF35-transduced cells
  - Changes in bystander leukemia cell phenotype

# Pharmacodynamic Analysis

Lab Study	Assays
<i>In vitro</i> chemosensitivity assays	<ul style="list-style-type: none"><li>• F-ara-A (fludarabine)</li><li>• Bendamustine</li><li>• Lenalidomide</li></ul>
Flow cytometry	<ul style="list-style-type: none"><li>• CD3; 4; 5; 8; 16; 19; 20; 23; 28; 54; 56; 80; 86; 95</li><li>• DR5</li></ul>
Apoptosis analysis	<ul style="list-style-type: none"><li>• Western blot: p21, p73, Bid</li><li>• Annexin V or similar apoptosis assay</li><li>• MLPA (Multiplex Ligation-dependent Probe Amplification)</li></ul>
Antibody responses	<ul style="list-style-type: none"><li>• Anti-adenovirus</li><li>• Anti-ISF35</li><li>• Anti-human CD154</li></ul>
Cytokine analysis	<ul style="list-style-type: none"><li>• IL-2</li><li>• TNF-<math>\alpha</math></li></ul>
Anti-tumor immune response	<ul style="list-style-type: none"><li>• ELISPOT</li><li>• ROR1</li><li>• MLR</li></ul>

# Demographics and Characteristics

Pt #	Age	Sex	% del(17p)	IgV <sub>H</sub> (% Hom)	%ZAP70+	%CD38+	Prior Treatments	LDT (mo)	ECOG	Rai	β2M (g/dL)	Intervention
1	65	M	65.5	85.6	< 20	0	CLL vaccine, IPT (Cyt+Dex+Dox)	> 6	2	II	3.2	ISF35 x 3 FCR x 3
2	69	F	70.5	100.0	59.4	0	F, FR, R (2), FCR, AT-101+R, CNF2024, OFAR	< 3	0	I	2.2	(none)
3	56	M	56.5	99.7	74.3	0	(none)	11	0	II	2.1	ISF35 x 3 FCR x 3
4	63	M	89.0	88.0	n/a	0	Leuk (2)	< 6	n/a	II	2.2	(none)
5	58	F	71.0	94.0	10.7	55	(none)	< 6	0	II	2.0	ISF35 x 3 FCR x 3
6	68	M	92.5	98.3	n/a	67	F, R, CVP, A, O	< 6	n/a	III	8.5	ISF35 x 2 FCR x 1†

\* **A** = alemtuzumab; **AT-101** = BCL-2 family member inhibitor; **CNF2024** = heat shock protein 90 inhibitor; **CVP** = cyclophosphamide, vincristine, prednisolone; **F** = fludarabine; **FR** = fludarabine, rituximab; **FCR** = fludarabine, cyclophosphamide, rituximab; **IPT (Cyt+Dex+Dox)** = insulin potentiation therapy: cytarabine, dexamethasone, and doxorubicin; **O** = ofatumumab; **OFAR** = oxaliplatin, fludarabine, cytarabine, rituximab; **R** = rituximab

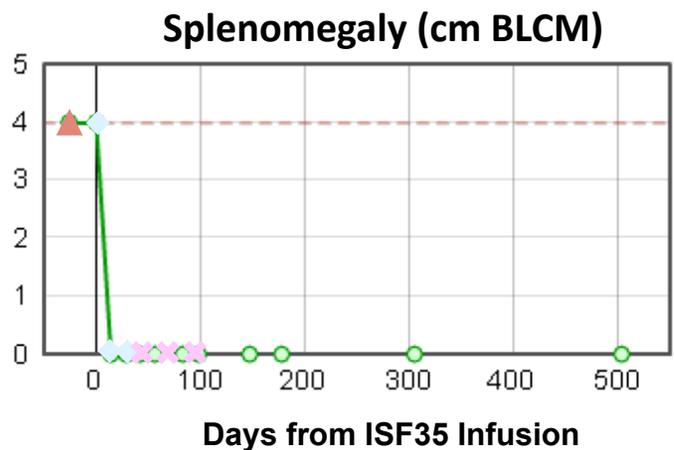
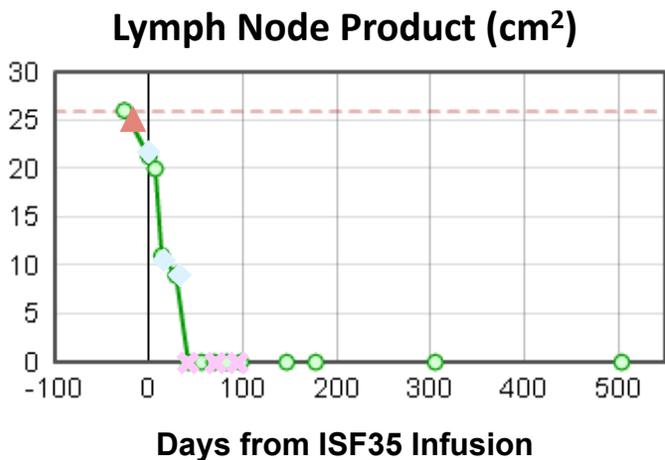
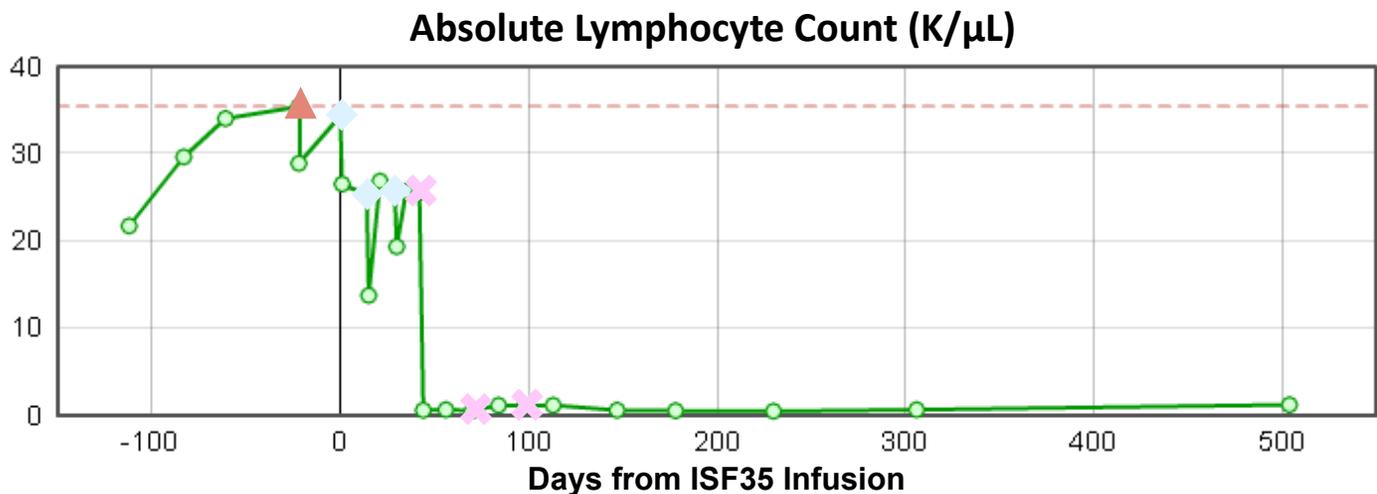
† Patient 6 received ofatumumab + HDMP after being withdrawn from study due to progressive disease

# Adverse Events Related to ISF35 + FCR

Preferred Term*	Total (N = 4)	
	Grade I/II (%)	Grade III/IV (%)
Hypophosphatemia	25	50
Fatigue	75	25
Neutropenia	50	25
Hyperbilirubinemia	25	25
Chills	100	0
Fever	100	0
Nausea	100	0
Constipation	75	0
Dizziness	75	0
Headache	75	0
Myalgia	75	0
Night sweats	75	0

\* Includes events that occurred in at least 3 of 4 patients, related to ISF35 **or** FCR.

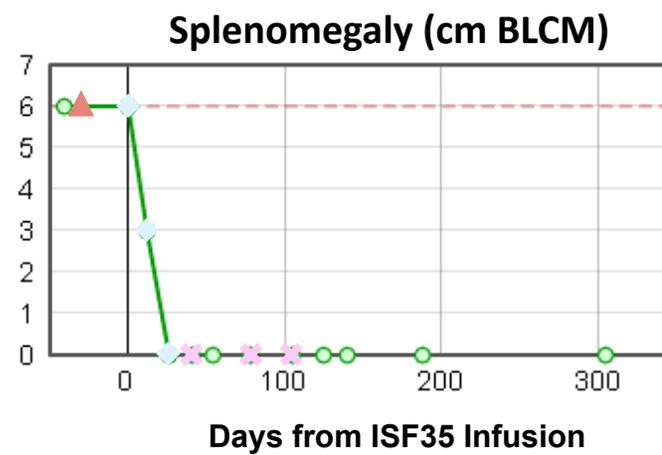
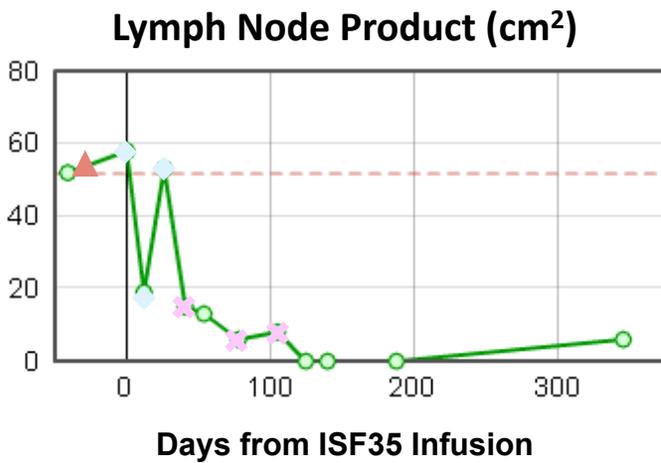
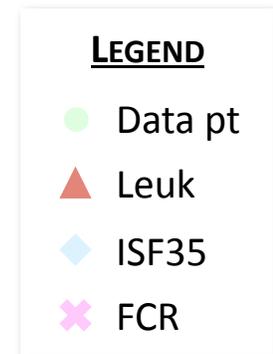
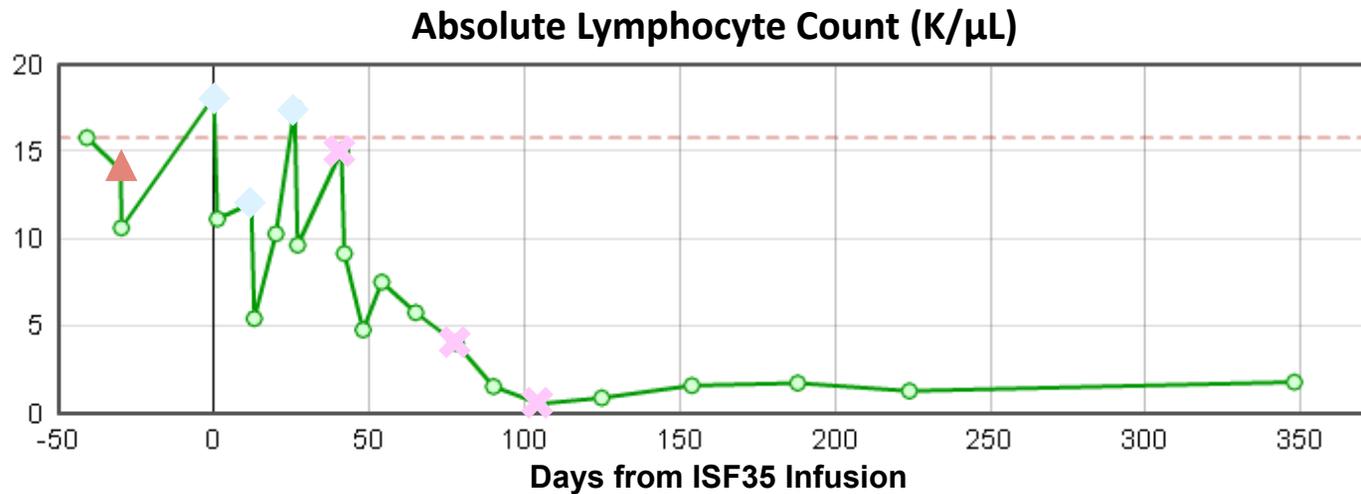
# Patient 1: Complete Response



**LEGEND**

- Data pt
- ▲ Leuk
- ◆ ISF35
- ✕ FCR

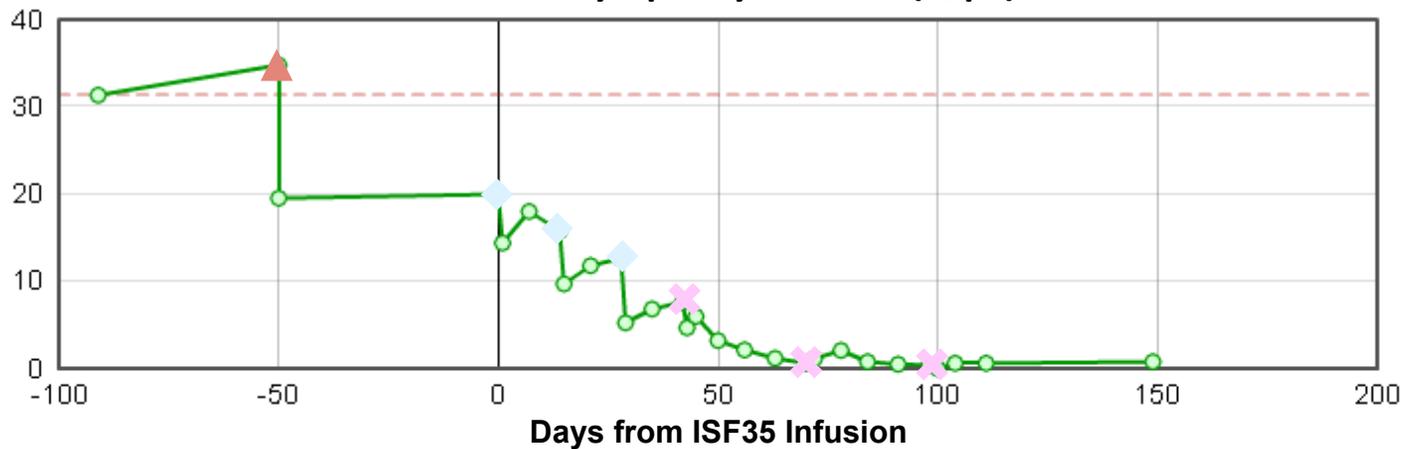
# Patient 3: CR with Incomplete Marrow Recovery



\* Persistent neutropenia → CR with incomplete marrow recovery

# Patient 5: Clinical Complete Response

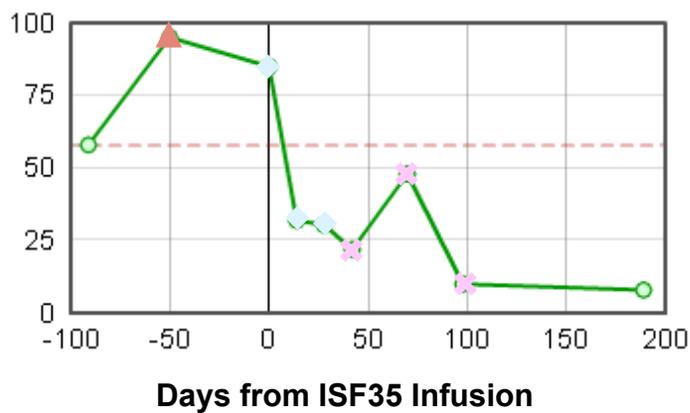
**Absolute Lymphocyte Count (K/ $\mu$ L)**



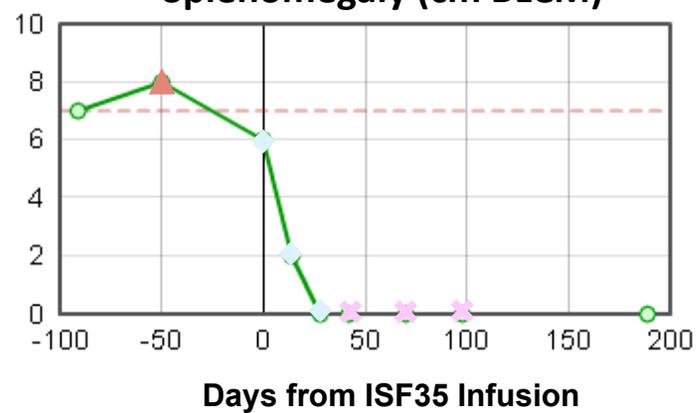
**LEGEND**

- Data pt
- ▲ Leuk
- ◆ ISF35
- ✖ FCR

**Lymph Node Product (cm<sup>2</sup>)**



**Splenomegaly (cm BLCM)**



# Patient 6: Progressive Disease

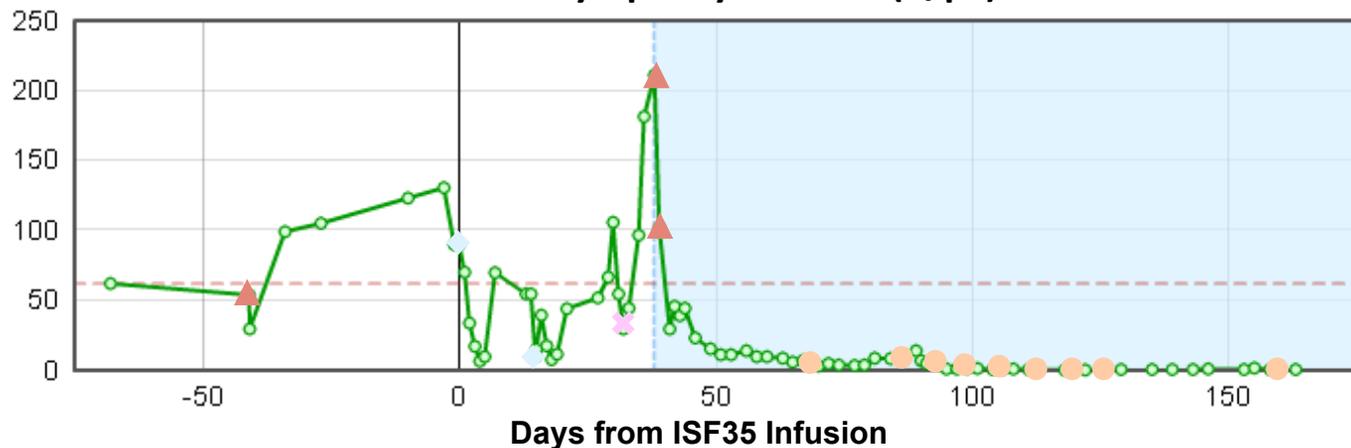
- **Refractory to fludarabine and alemtuzumab, 92.5% del(17p)**
- Prior therapies:

Therapy	Start	End	Response
Single-agent fludarabine	Dec 2006	Mar 2007	<b>PD</b>
Single-agent rituximab	Mar 2007	Apr 2007	SD
Cyclophosphamide, vincristine, prednisolone	Oct 2007	Nov 2007	<b>PD</b>
Single-agent alemtuzumab	Jan 2008	Apr 2008	<b>PD</b>
Single-agent ofatumumab	Jul 2008	Jan 2009	PR

- Received two infusions of ISF35, 1 course of FCR
- Two “possibly” drug-related, **serious adverse events**:
  - Grade 2 hypotension, dyspnea, syncope: hospitalization required
  - Grade 2 hypotension, dizziness, dyspnea, tachycardia: hospitalization required
  - A third series of events, unrelated to ISF35, resulted in another hospitalization
- Formally withdrawn from study January 2010 due to progressive disease

# Patient 6: Clinical Parameters

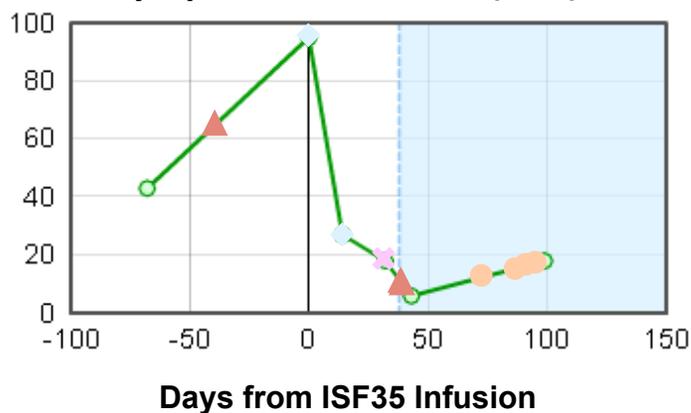
**Absolute Lymphocyte Count (K/ $\mu$ L)**



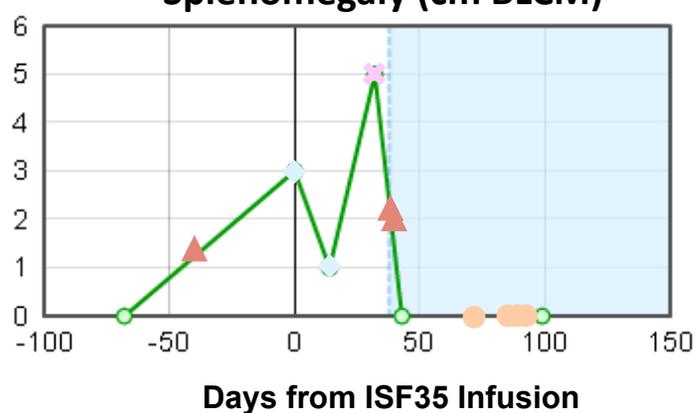
**LEGEND**

- Data pt
- ▲ Leuk
- ◆ ISF35
- ✕ FCR
- O+HDMP
- Off Study

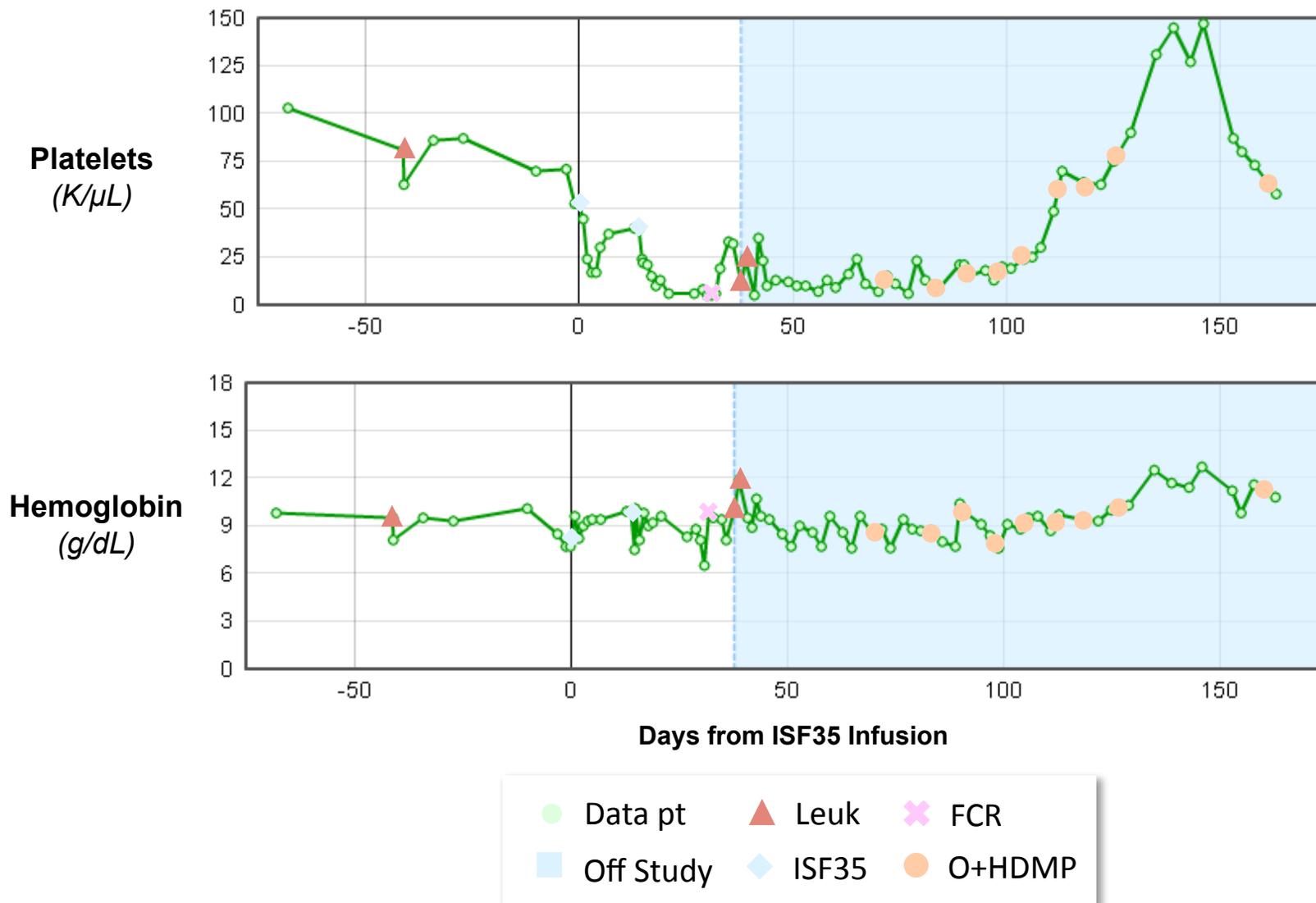
**Lymph Node Product (cm<sup>2</sup>)**



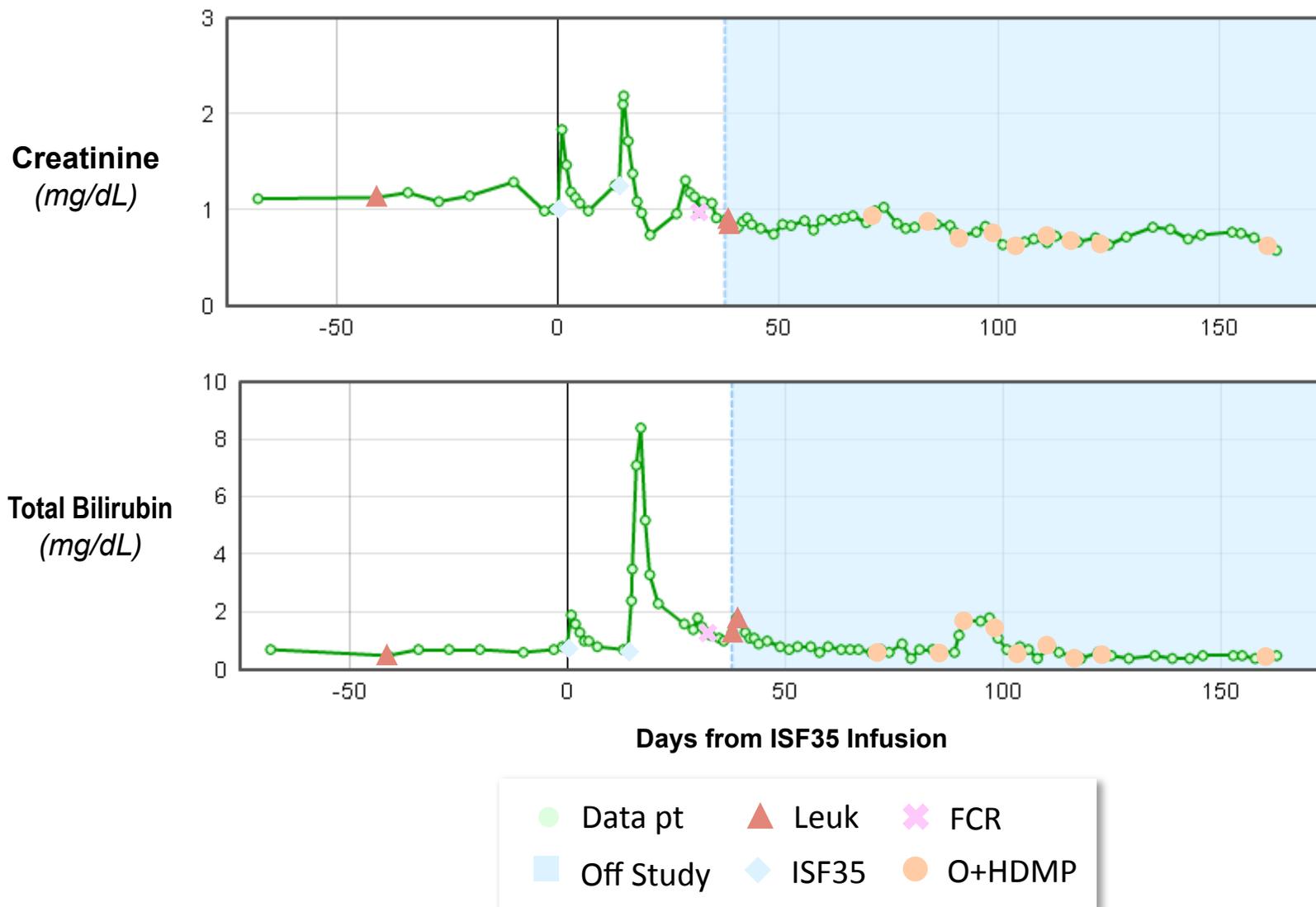
**Splenomegaly (cm BLCM)**



# Patient 6: Platelets and Hemoglobin

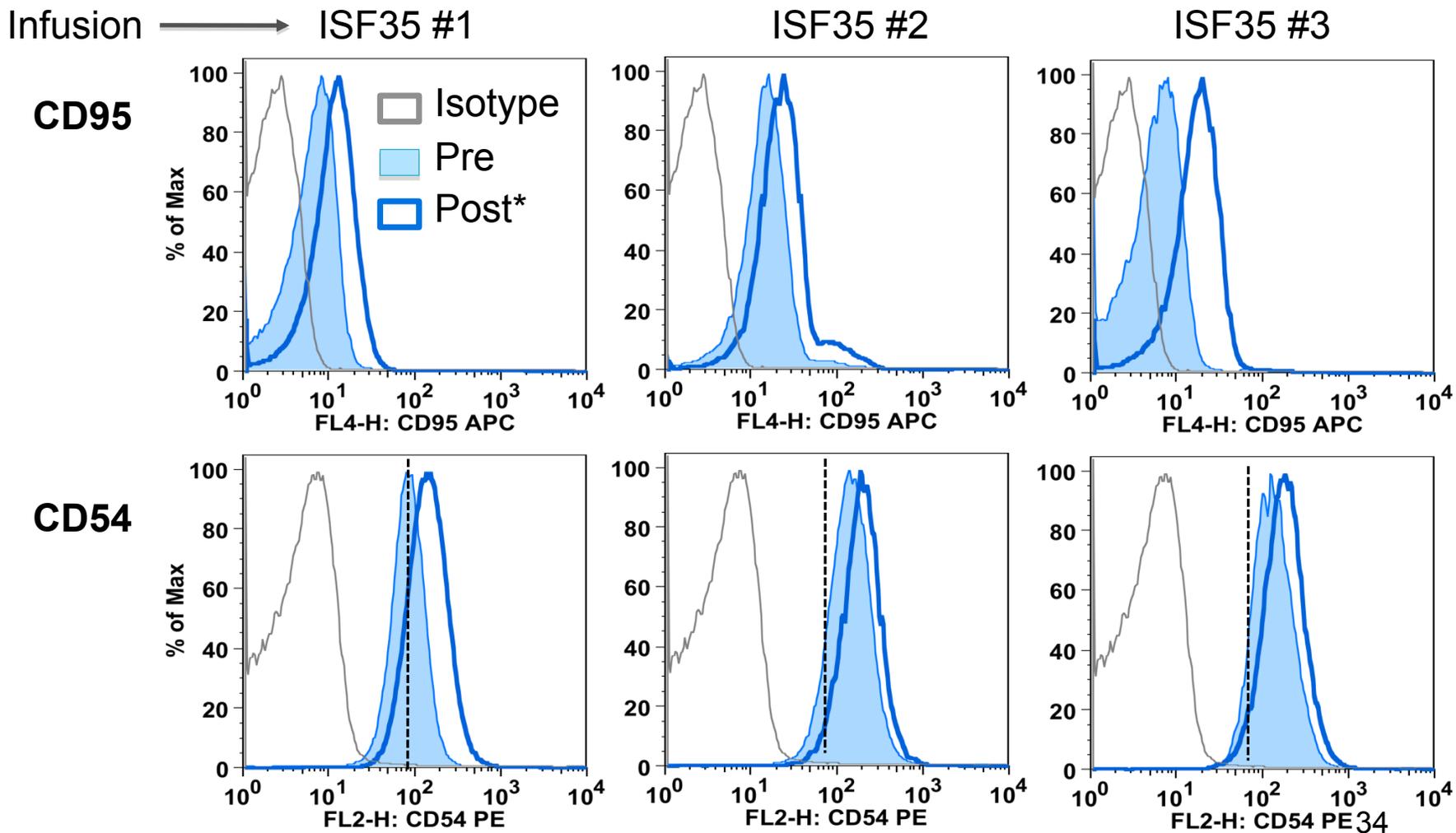


# Patient 6: Renal Function



# Bystander Effect: CD95 (Fas) and CD54

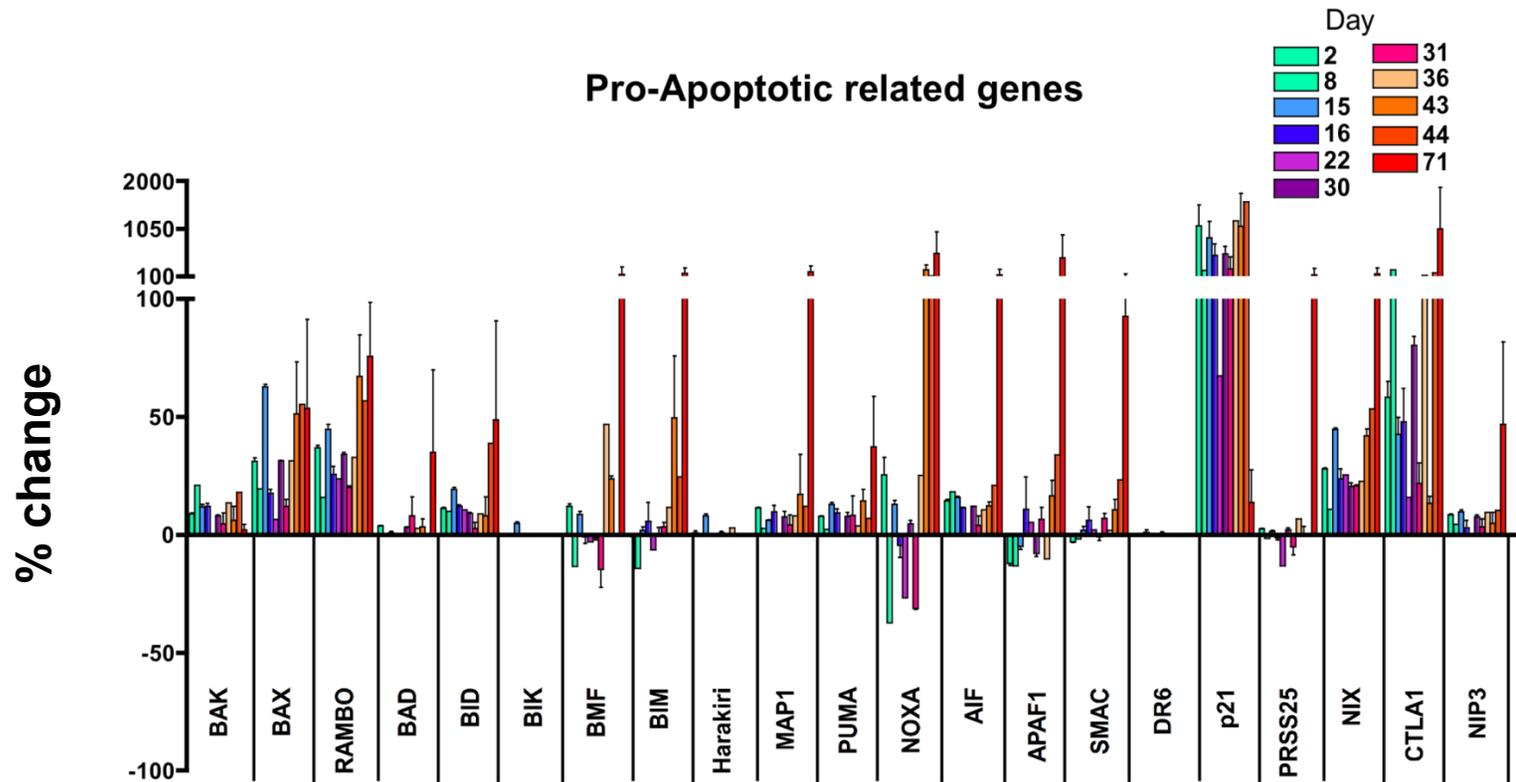
Subject 3, ISF35 + FCR



\* 1 day after the infusion

# Up-regulation of Pro-Apoptotic Genes

Subject 1, ISF35 + FCR

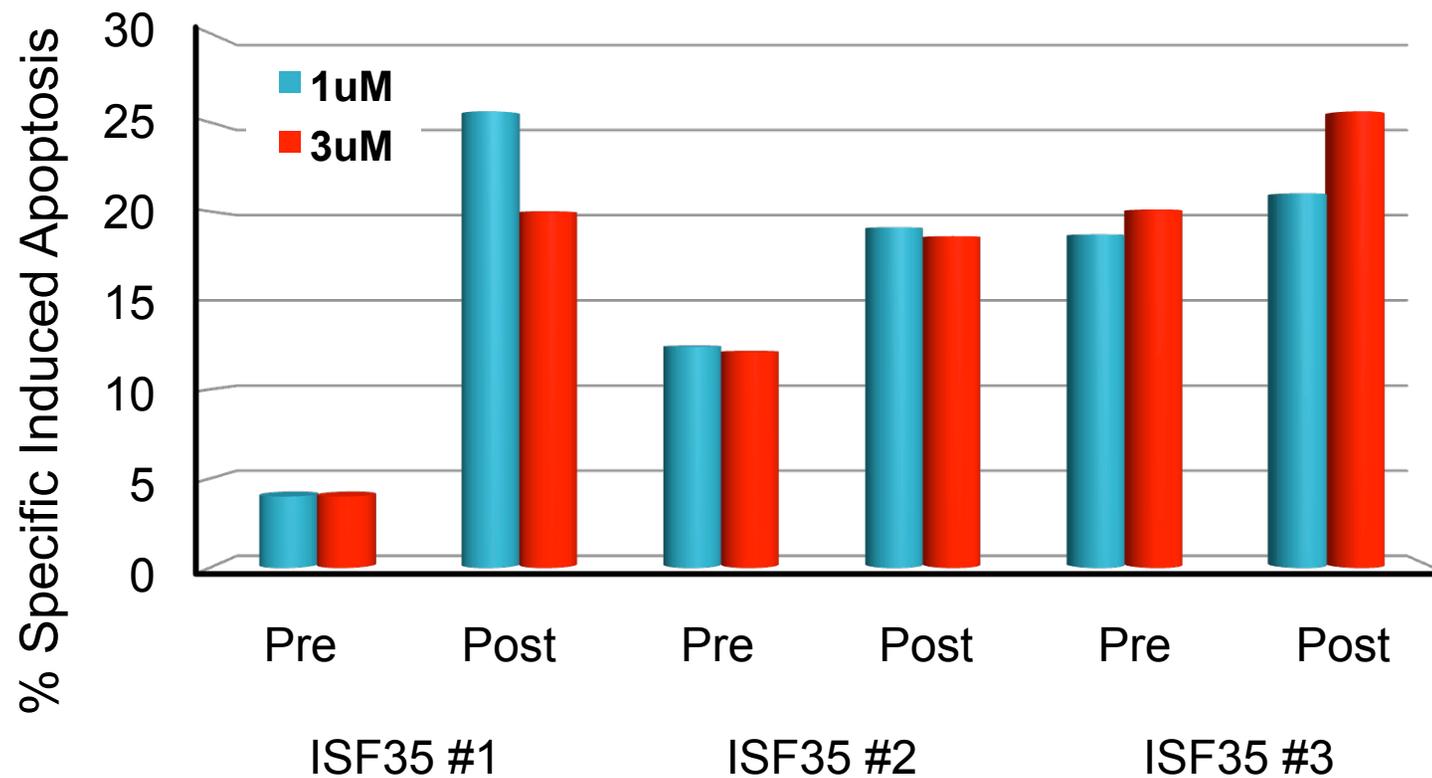


- **80%** of pro-apoptotic genes show up-regulation

\* Gene expression assessed by PCR assay

# ISF35 Sensitizes del(17p) CLL Cells to Fludarabine

Subject 1, ISF35 + FCR



# Anti-Adenovirus and Anti-hCD154 Antibodies

Pt #	Anti-Adenovirus Antibodies Titer <sup>1</sup>					Anti-hCD154 Antibodies Titer <sup>2</sup>	
	Pre	Post Ad-ISF35	Fold Change	Post FCR	Fold Change	Pre	Post
1	662	2244	3	2048	3	ND*	ND
3	975	16008	16	12763	13	ND	ND
5	424	-	-	430	1	ND	ND
6	28	34	1	-	-	ND	ND

\* **ND** = Non-detectable by ELISA or Hela-hCD154 Functional Binding Assay

- No anti-hCD154 antibodies generated after ISF35
- Anti-adenovirus antibodies increased

# RAC Comments/Questions

Reviewers: *Hildegund Ertl, M.D. ; John A. Zaia, M.D. ; David A. Williams, M.D.*

- Risk of death from severe anaphylactic reactions?
  - ✓ Our experience using the Ad-ISF35 and Ad-ISF154 vector in human Subjects indicates that AEs developed in these patients are ***transient*** and ***reversible***. (Until June -2010 a total of 35 human Subjects had received transduced cells and 26 had received intranodal injections).
  - ✓ We will amend the informed consent document to include this risk of death from anaphylactic reactions as a consequence of infusion of transduced cells with Ad-ISF35.

# RAC Comments/Questions

Reviewers: *Hildegund Ertl, M.D. ; John A. Zaia, M.D. ; David A. Williams, M.D.*

- *Conflict of interest determinations made?*

No COI identified for Dr. Castro

- *What is Dr. Kipps' Role on the Study?*

- ✓ Dr. Kipps 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

# Conclusions

- Combination of Ad-ISF35 Transduced CLL cells + FCR is feasible and well tolerated by the majority of patients.
- Only 1 patient out of four treated had AE that required dose / administration modification of Ad-ISF35 transduced CLL cells (Patient 6)
- We have observed objective responses in all patients. 2 CR, 1 Clinical / Hematological CR, 1 PD (Final Assessment pending). These data is very encouraging as responses in this high risk patient population are expected to be lower.
- Correlative studies suggest the presence of Bystander Effect in PBMCs. This include expression of costimulatory molecules, death receptors as well as upregulation of p53 dependent genes in non-transduced cells.
- Correlative studies suggest the presence of chemosensitization *in vitro* after infusion of Ad-ISF35 transduced CLL cells.
- Plans to continue enrolment and complete accrual of 12 patients in a multi-institutional study.

# Acknowledgments

- ***Memgen LLC***  
Mark Cantwell, Ph.D.  
Josh Allen
  
- ***UCSD***  
Roxana Phillips  
Theresa Bishop  
Johanna Melo  
Kenneth Smith  
Brenda Wong  
Rachel Nosowsky