

DF/HCC Protocol 08-160
Serious Adverse Event Analysis

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Overview

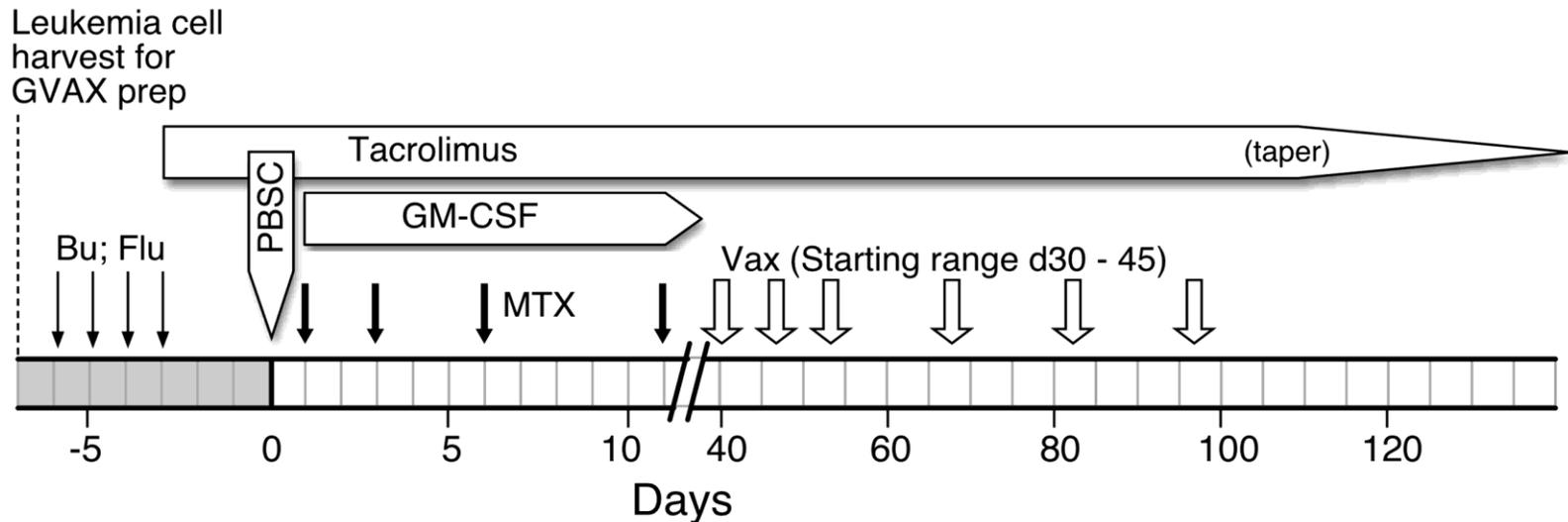
- **Background to clinical trial**
- **Synopsis of patient course**
- **Autopsy findings**
- **Laboratory investigations**
- **Implications**

Integrating tumor cell vaccines with allogeneic hematopoietic stem cell transplantation

- **HSCT is curative immunotherapy for some hematologic malignancies (graft-versus-leukemia effects through donor lymphocytes)**
- **Early post-transplant period provides advantages for cancer vaccination**
 - **Homeostatic lymphoid expansion favors T effectors over Tregs**
 - **Increased levels of IL-7, IL-12, IL-15**
 - **Endogenous activators of TLRs and NODs**

DF/HCC Protocol 04-023

GVAX s/p allo-HSCT for high risk acute myeloid leukemias and advanced myelodysplasias



Adenoviral GM-CSF transduction of autologous leukemic cells

AML/MDS GVAX s/p HSCT clinical summary

- **24 patients enrolled**
 - 20% marrow blasts - median
- **15 initiated vaccination**
 - Nine withdrawn for disease-related issues
- **7 with mild chronic GVHD**
- **10 received all six vaccines**
 - One withdrawn for acute GVHD, 4 for progression
- **9/10 finishing vaccination achieved **durable** complete responses (minimum 2.5 yrs)**

Clinical grade GM-CSF secreting K562 cells

- **GM-CSF may be provided in trans by a “bystander” line admixed with autologous tumor**
- **K562 cells lack steady-state MHC class I and II expression**
- **Plasmid expression vector (CMV promoter) for GM-CSF production (5-13 $\mu\text{g}/10^6$ cells/24 hrs)**
- **1,000 rads sufficient for cell cycle arrest; 2,000 rads induce lethality; 10,000 rads for clinical use**
- **Clinical trials in CML (K562 alone), glioblastoma, follicular lymphoma, AML and CLL s/p allo-HSCT**

GM-CSF K562 cell vaccines at DFCI

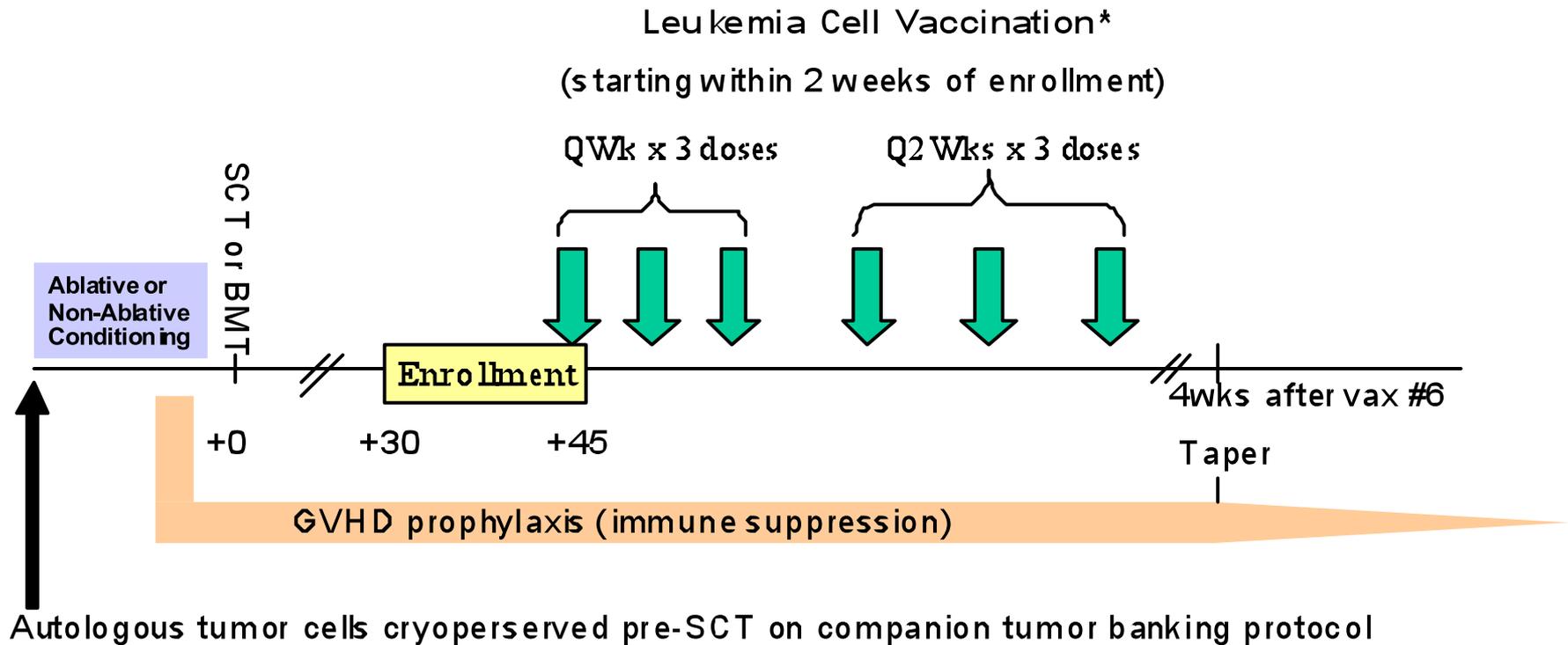
- **Initiated 5/15/06**
- **Five clinical trials**
- **459 individual vaccines administered to 84 patients**
- **32 other AML/MDS patients early after allogeneic HSCT**
- **17 patients with CLL early after allogeneic HSCT**

GM-CSF K562 cell vaccines at DFCI

- **Favorable safety profile**
- **Grade 1-2, transient local VAX reactions common**
- **Occasional modest increase in WBC counts**
- **One episode of urticaria related to VAX**
- **One episode of thrombocytopenia/anemia several months after VAX, felt possibly related to immunization**

DF/HCC 08-160 Protocol Schema

GM-K562/Leukemia cell Vaccination after AlloSCT



CS clinical course (I)

- **52 years old**
- **Diagnosed with AML (M2) on 7/3/09**
- **Initially treated with daunorubicin, cytarabine, etoposide**
- **Underwent autologous HSCT on 11/3/09**
- **Decitabine consolidation**
- **Relapse 11/22/10**

CS clinical course (II)

- **Myeloblasts harvested from bone marrow 1/20/11**
- **Allogeneic HSCT 2/11/11**
 - **Intermediate intensity conditioning regimen (fludarabine and melphalan)**
 - **9/10 allele matched unrelated donor (Germany)**
 - **Rapamycin, FK-506, methotrexate as GVHD prophylaxis**
 - **Minor early complications (Hickman line infection)**
 - **Complete donor chimerism beginning at four weeks after HSCT and maintained thereafter**

CS clinical course (III)

- **Vaccines administered on 3/14/11, 3/21/11, 3/28/11, and 4/11/11 (out of a planned six)**
- **Each vaccine was composed of an admixture of 4.4×10^6 autologous myeloblasts and 1×10^7 GM-CSF K562 cells that were irradiated (10,000 rads)**
- **0.5 ml subcutaneous/0.5 ml intradermal injections into the limbs on a rotating basis**

CS clinical course (IV)

- **Strong and persistent local reaction to VAX #1**
- **Minimal reaction to VAX #2-4**
- **Progressive elevation in WBC (28,900 on 4/11/11)**
- **Work-up for infection negative**
- **Punch biopsy of VAX #1 reaction revealed typical GM-CSF triggered cellular infiltrate**

CS clinical course (V)

- **5/9/11: WBC 127,900 with 22% eosinophils; steroids initiated**
- **5/12/11: bone marrow showed no AML; tri-lineage maturation with myeloid hyperplasia**
- **FIP1L1-PDGR α , BCR-ABL, JAK2 V617F negative**
- **Donor-derived hematopoietic cells**

CS clinical course (VI)

- **5/16/11: WBC 207,500 with 46% eos; decreasing platelets and red blood cells**
- **Intermittent shortness of breath**
- **Admitted to Brigham and Women's Hospital**
- **Hydroxyurea initiated**
- **Echocardiogram and PE CT scan normal**

CS clinical course (VII)

- **5/20/11: Deep, focused biopsy of VAX #1 site showed blasts in subcutaneous tissue**
- **Immature cells identified as K562 by surface phenotype and genetic analysis**
- **Ki-67 positivity in a minority of viable blasts (10-20%); cannot discriminate arrested from cycling cells; proliferation primarily noted in adjacent inflammatory cells**

CS clinical course (VIII)

- **5/27/11: Deeper wedge resection of vaccination site**
- **Similar pathologic features: 80-90% of blasts necrotic**
- **Specimen processed to single cells; after several weeks of culture, replicating K562 cells obtained for laboratory analysis**

CS clinical course (IX)

- **5/27/11: Nilotinib initiated, as K562 cells harbor a BCR-ABL fusion**
- **Progressive decrease in WBC counts**
 - **5/27/11: 132,600**
 - **5/30/11: 81,400**
 - **6/2/11: 51,500**
 - **6/6/11: 20,100**
- **Blood cultures positive for enterococcus and coagulase negative staphylococcus; vancomycin begun**

CS clinical course (X)

- **6/2/11: worsening shortness of breath**
- **Bilateral pulmonary infiltrates**
- **Broad spectrum antibiotics and anti-fungals added**
- **EKG abnormalities**
- **Troponin-T leak**

CS clinical course (XI)

- **6/6/11: Respiratory failure requiring intubation**
- **Valganciclovir for possible cytomegalovirus (CMV) pneumonitis**
- **Doxycycline for possible atypical pneumonia**

CS clinical course (XII)

- **Respiratory syncytial virus (RSV) detected by PCR in nasopharynx**
- **Blood cultures again positive for coagulase negative staphylococcus**
- **Acute oliguric renal failure**
- **Echocardiogram showed global hypokinesis**
- **6/7/11: shock liver, rise in Troponin-T, death**

Overall clinical assessment

Death due to complications of hemodynamic and respiratory failure within a setting of immune suppression-associated RSV pneumonia, sepsis, acute respiratory distress syndrome, and multi-organ failure

Autopsy results (I)

- **Acute, extensive, circumferential myocardial infarction**
- **Intra-myocardial small vessel thrombi containing eosinophilic debris**
- **Extensive cardiac mural thrombi (left and right ventricles) with numerous eosinophils and Charcot-Leyden crystals**

Autopsy results (II)

- **Diffuse, bilateral alveolar damage, with arteriolar and capillary thrombi containing eosinophilic debris, likely from the cardiac mural thrombi**
- **No evidence for infection**
- **Systemic small vessel emboli containing eosinophilic debris in spleen, cecum, ileum, bladder, glomeruli, and brain**

Autopsy results (III)

- **Bone marrow hypercellular, with myeloid predominance; no AML**
- **Soft tissues adjacent to VAX #1 site and 5 cm distal show K562 cells. Features are similar to prior two biopsies, with 80-90% of infiltrate necrotic, 10-20% of viable cells Ki-67+**
- **No K562 cells elsewhere**

Overall post-mortem evaluation

Death primarily resulted from thromboembolic complications of eosinophil-associated cardiac damage, with extensive mural thrombi

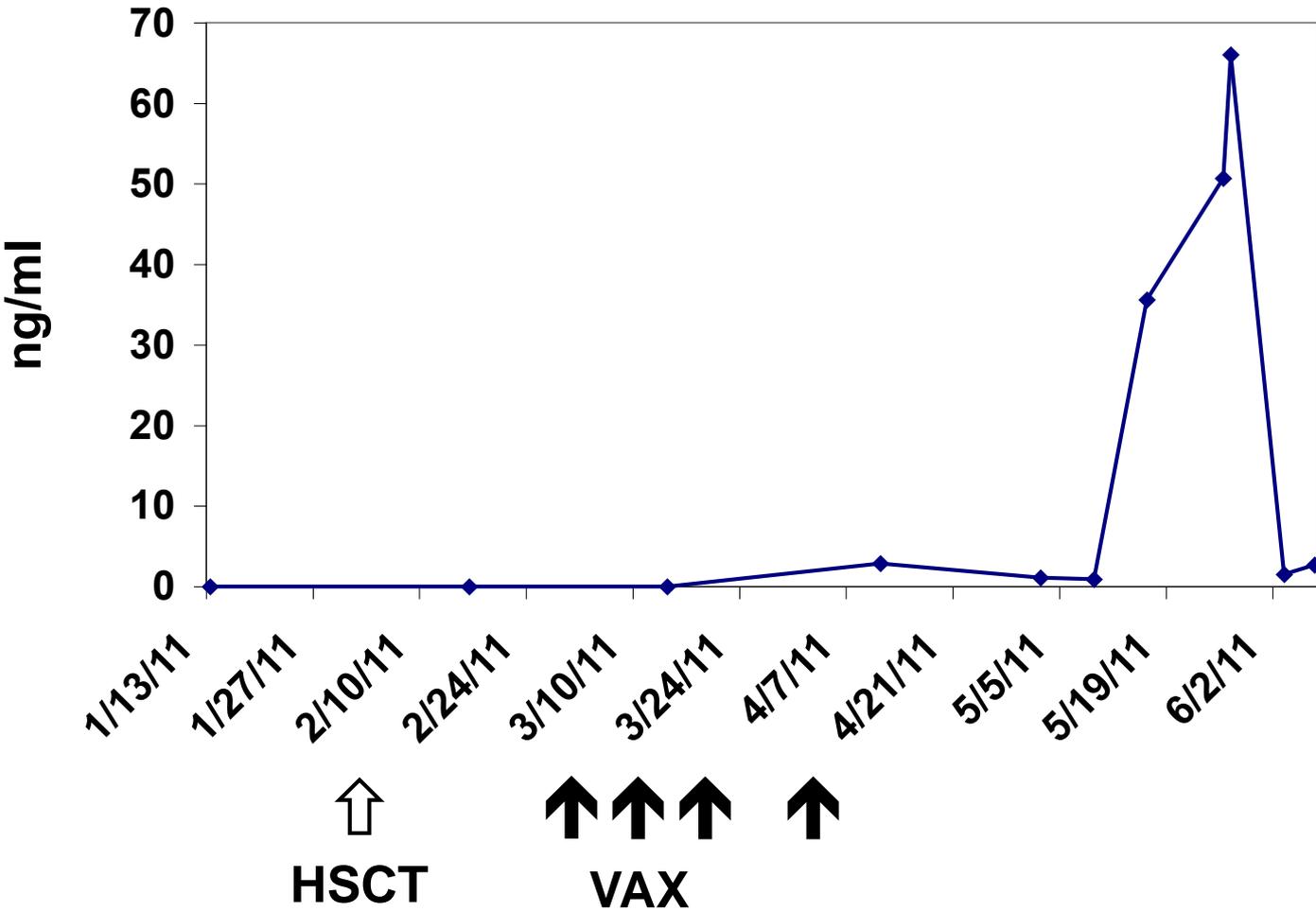
(Loeffler's endomyocarditis)

Major questions

- **What caused the leukocytosis?**
- **Why did the irradiated GM-CSF K562 cells persist?**
- **Why did CS deteriorate in the context of decreasing leukocyte counts?**

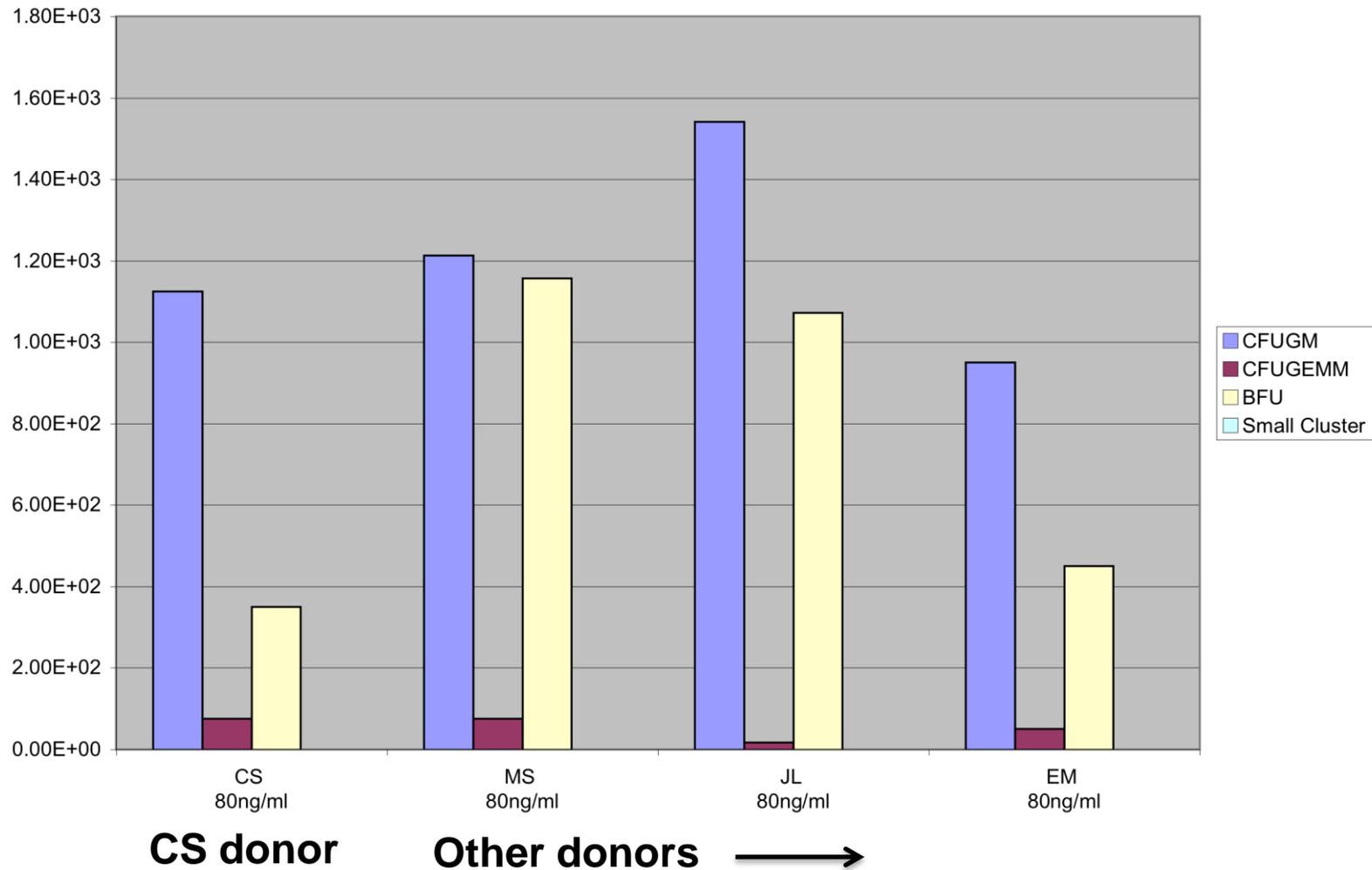
What caused the leukocytosis?

Plasma GM-CSF levels increased in association with leukocytosis



Donor mobilized stem cells show normal sensitivity to GM-CSF in vitro

Standard Methocult with 80ng/ml GMCSF



GM-CSF was likely the primary stimulus for leukocytosis (IL-3 and IL-5 not detected)

Single case report of Loeffler's endomyocarditis with recombinant GM-CSF protein as adjunct to anti-microbial therapy (Blood 1992)

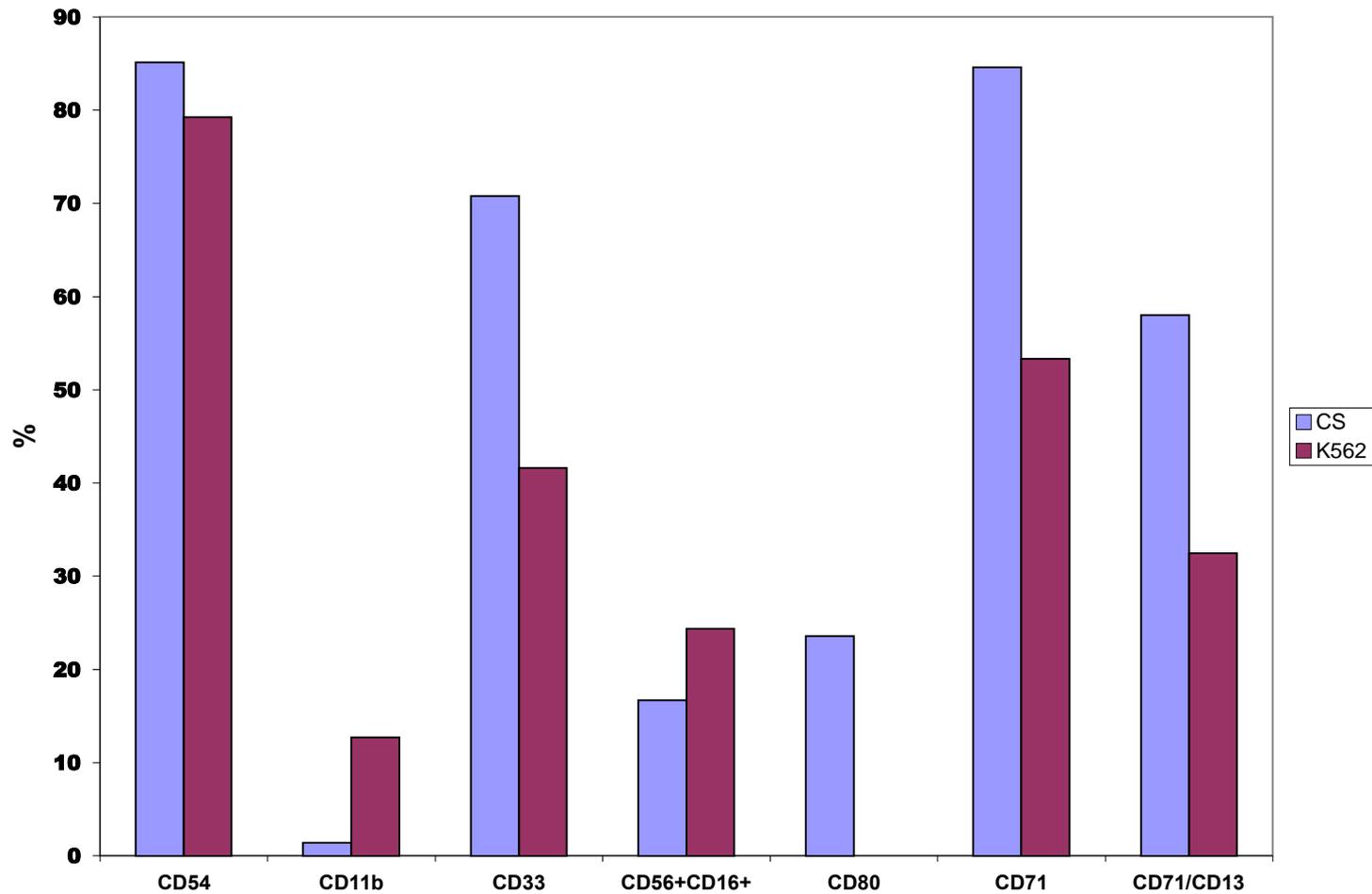
**Why did the irradiated GM-CSF
K562 cells persist?**

Irradiation of CS vaccines

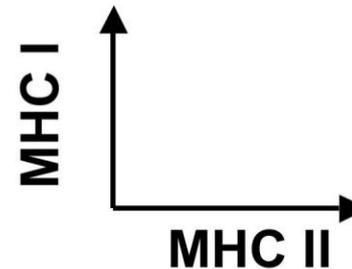
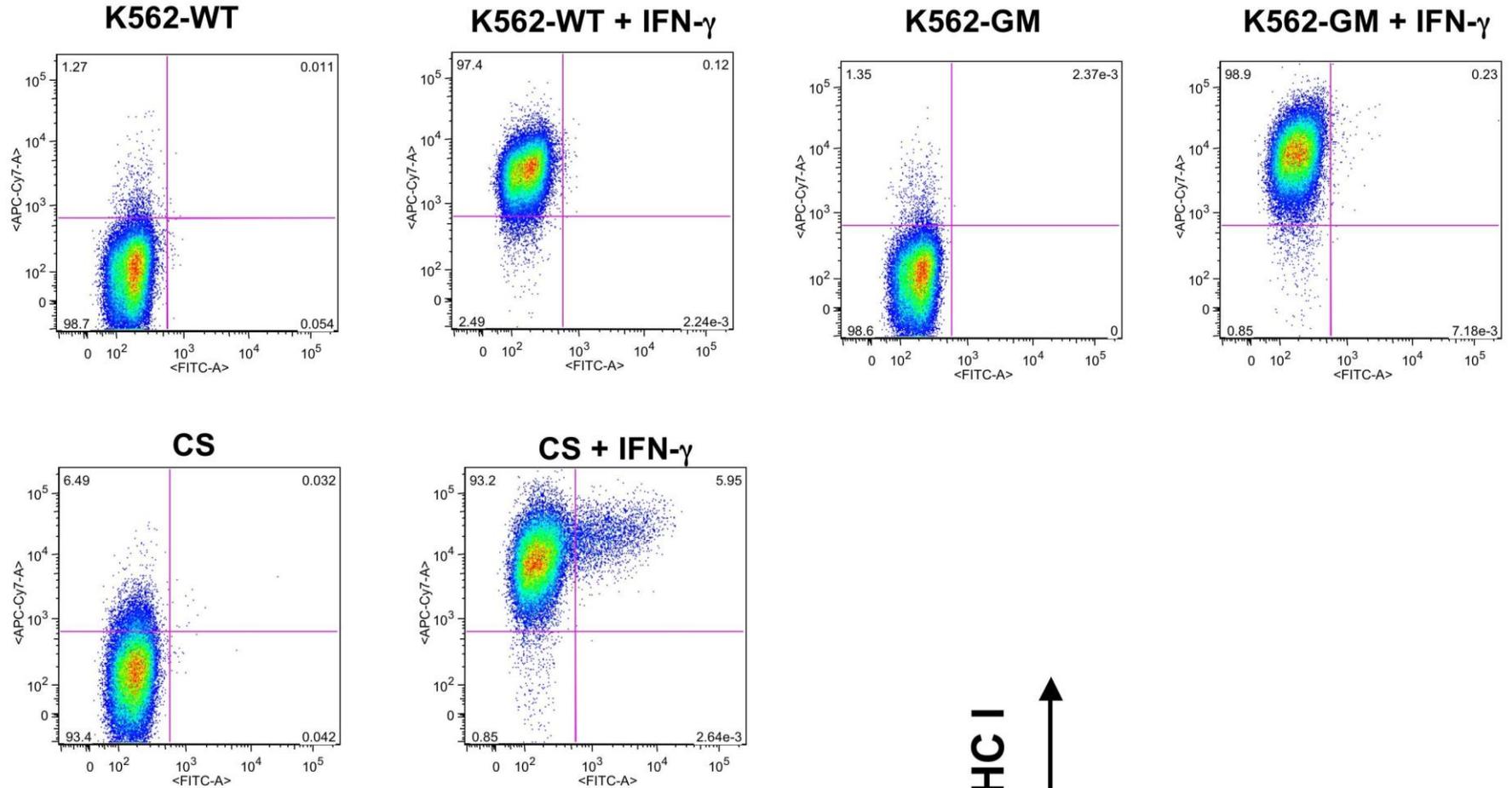
- **Manufacturing records reviewed**
- **Irradiator inspected**
- **Videotapes of irradiation procedures examined**
- **No evidence for an error in manufacture**

**Was there an aberrant response of
the K562 cells to irradiation?**

Altered surface phenotype of recovered CS K562 cells



Enhanced IFN- γ response of CS K562 cells

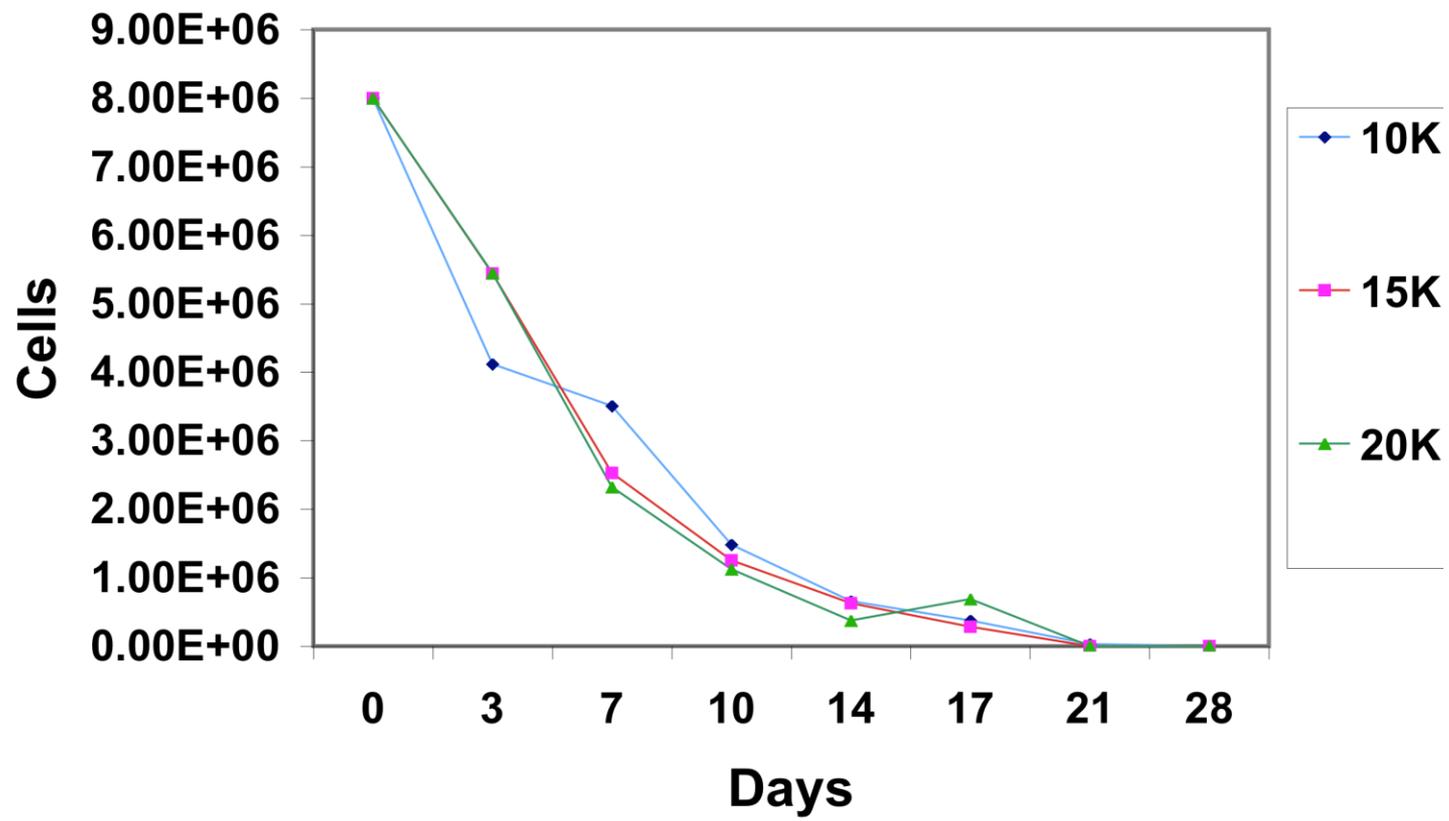


Cytogenetic analysis of CS K562 cells

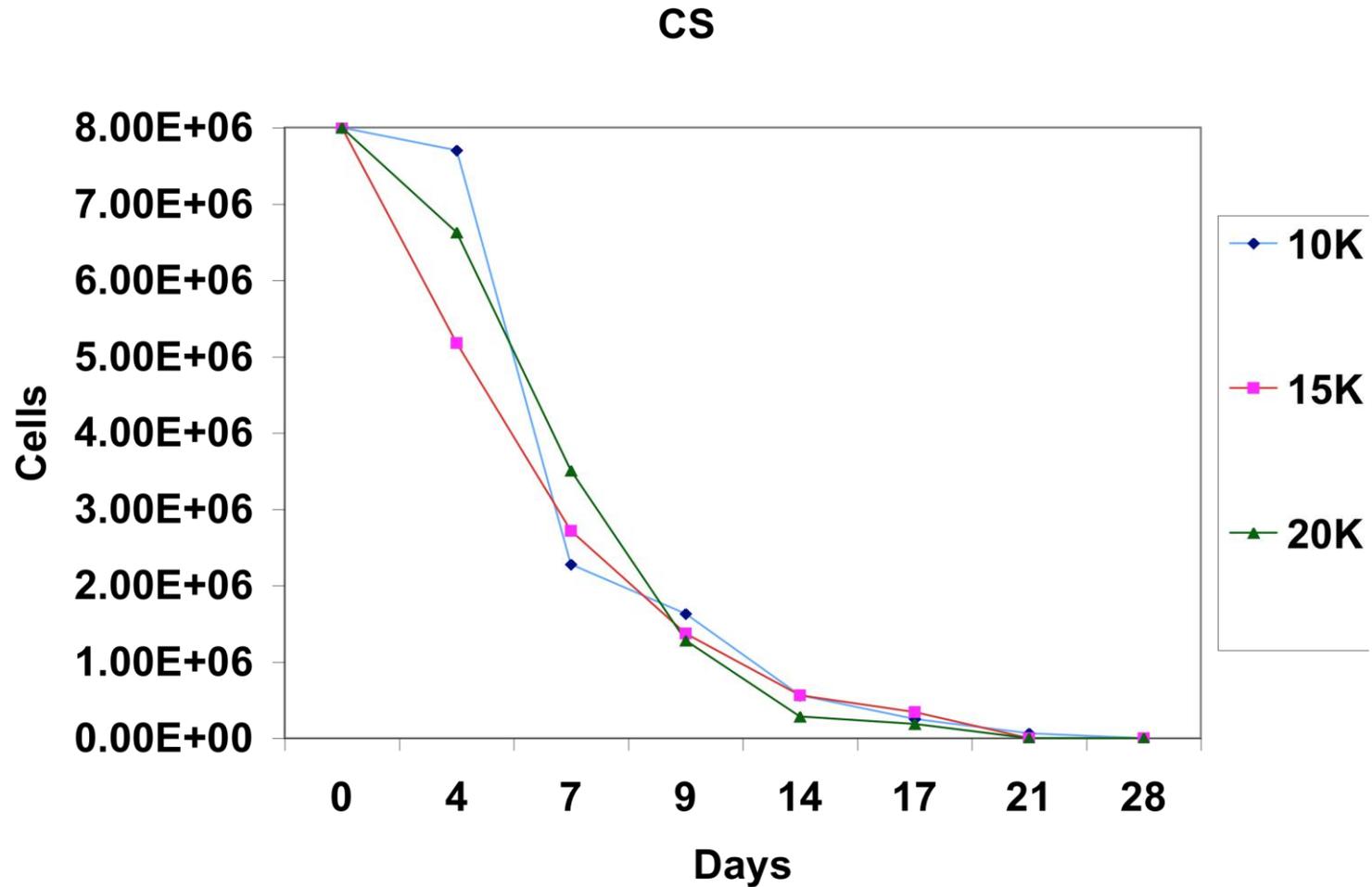
- **GTG banding revealed complex karyotypes of both GM-CSF K562 cells and CS K562 cells**
- **Numerous chromosomal translocations**
- **Amplification of BCR-ABL locus**
- **CS K562 cells show three additional chromosomal abnormalities compared to GM-CSF K562 cells**
- **Changes consistent with radiation induced double stranded DNA breaks and aberrant DNA repair**

Radiation sensitivity of GM-CSF K562 clinical lot

K562-GM



Radiation sensitivity of CS-K562 cells

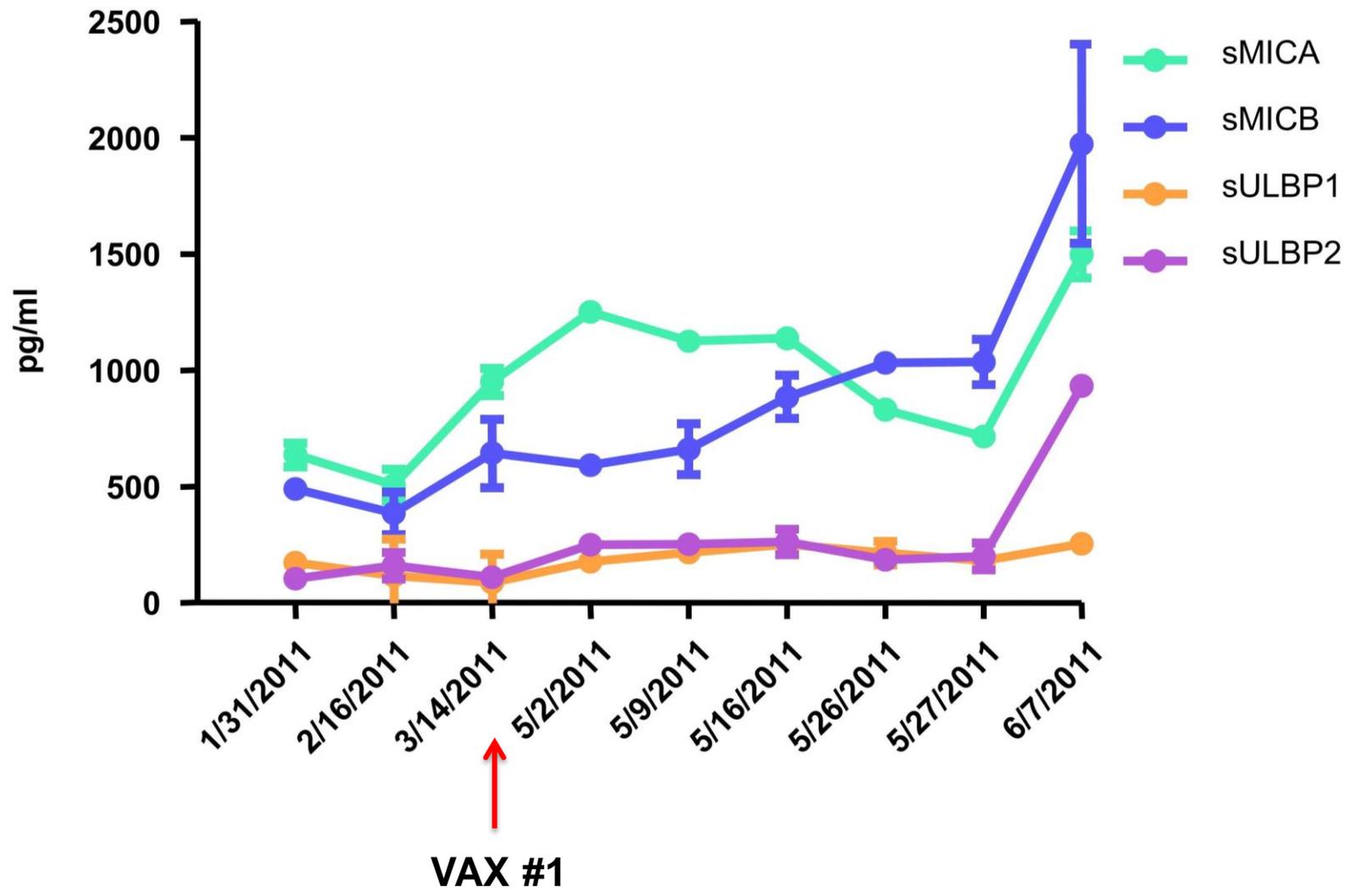


CS cells show genetic and phenotypic changes compared to GM-CSF K562 cells and manifest replicative potential in vitro, but retain radiation sensitivity in vitro

Does the host have a role in killing irradiated K562 cells?

NK cells efficiently lyse K562 cells in vitro through a mechanism involving the NKG2D activating receptor

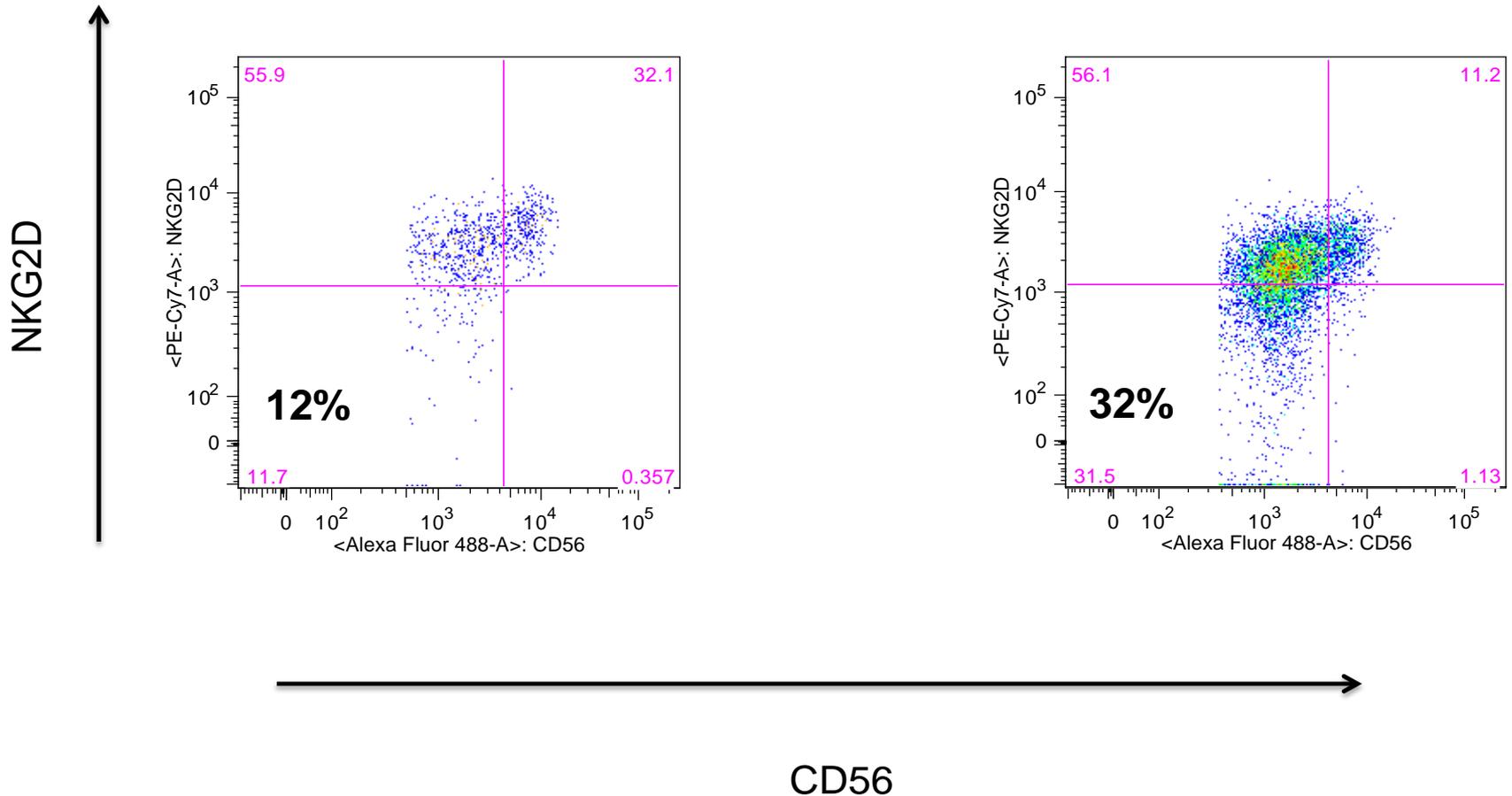
High levels of soluble NKG2D ligands in CS at the time of *beginning* VAX



Soluble NKG2D ligands trigger NK cell dysfunction at the time of VAX initiation

Healthy Donor

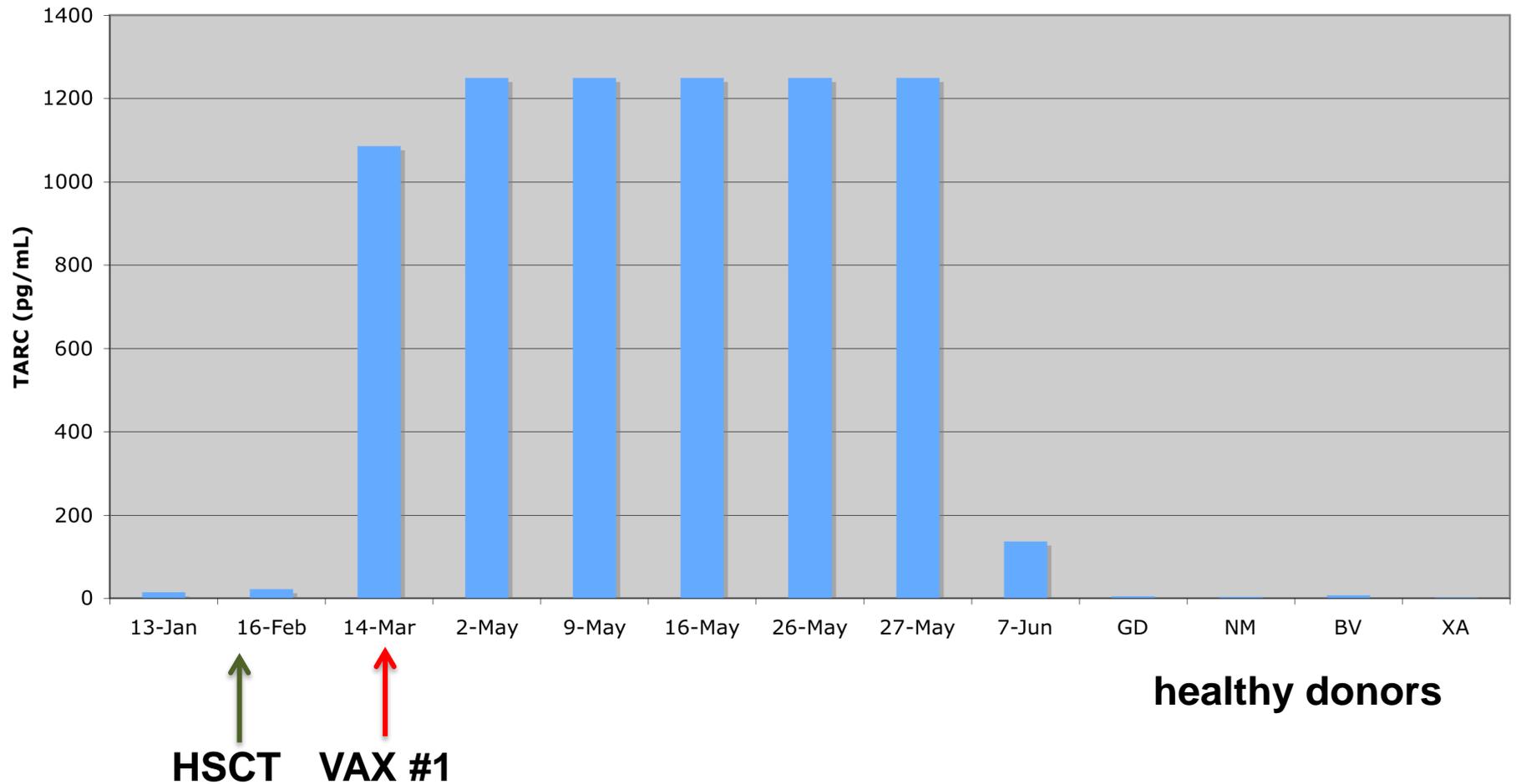
CS 3/14/11



**In addition to soluble NKG2D ligands,
rapamycin impairs NK cell killing of
K562 cells in vitro**

**Were there other factors that impaired
immune-mediated killing
of K562 cells?**

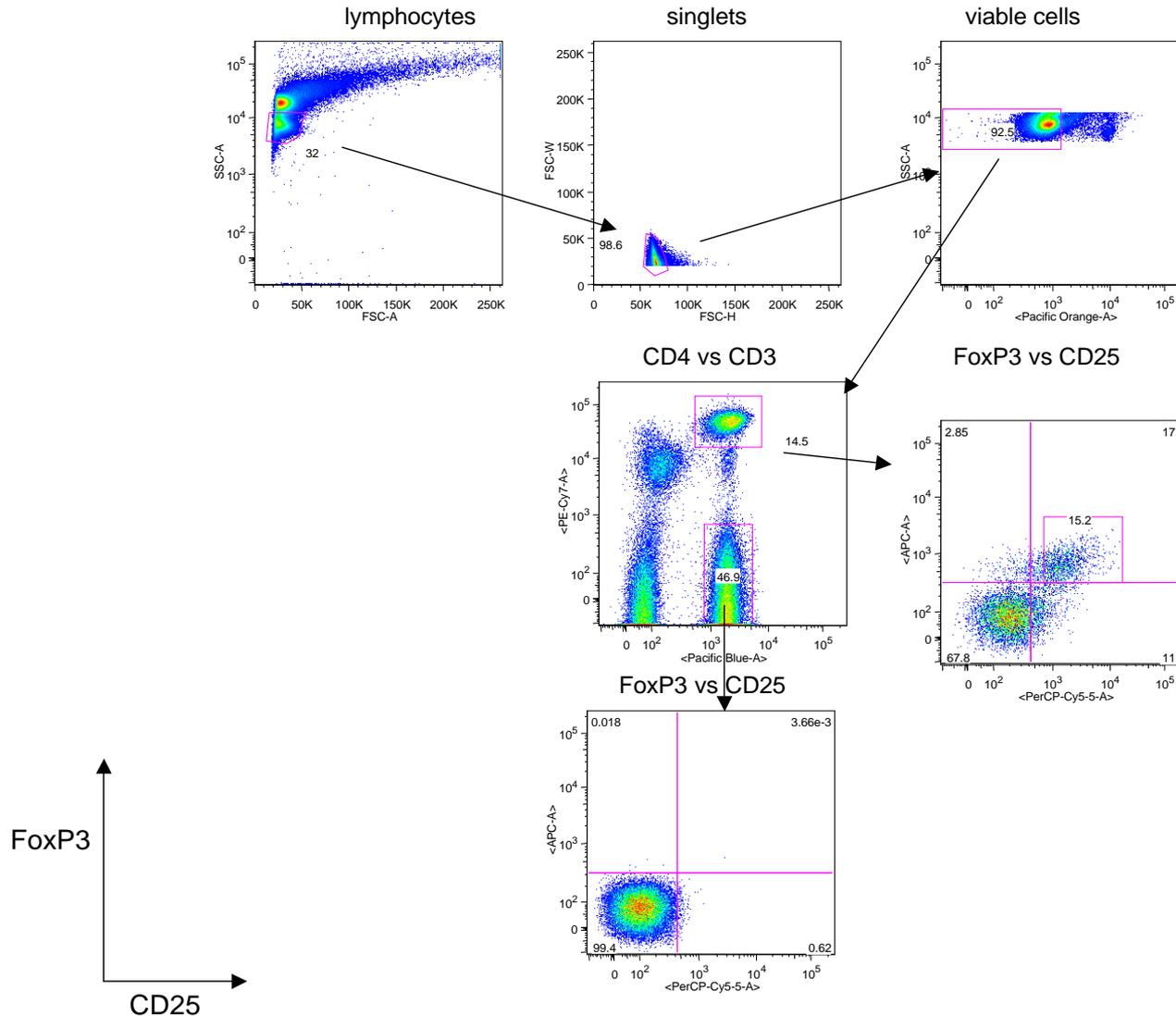
CCL17 (TARC) plasma levels rose after HSCT but *before* VAX



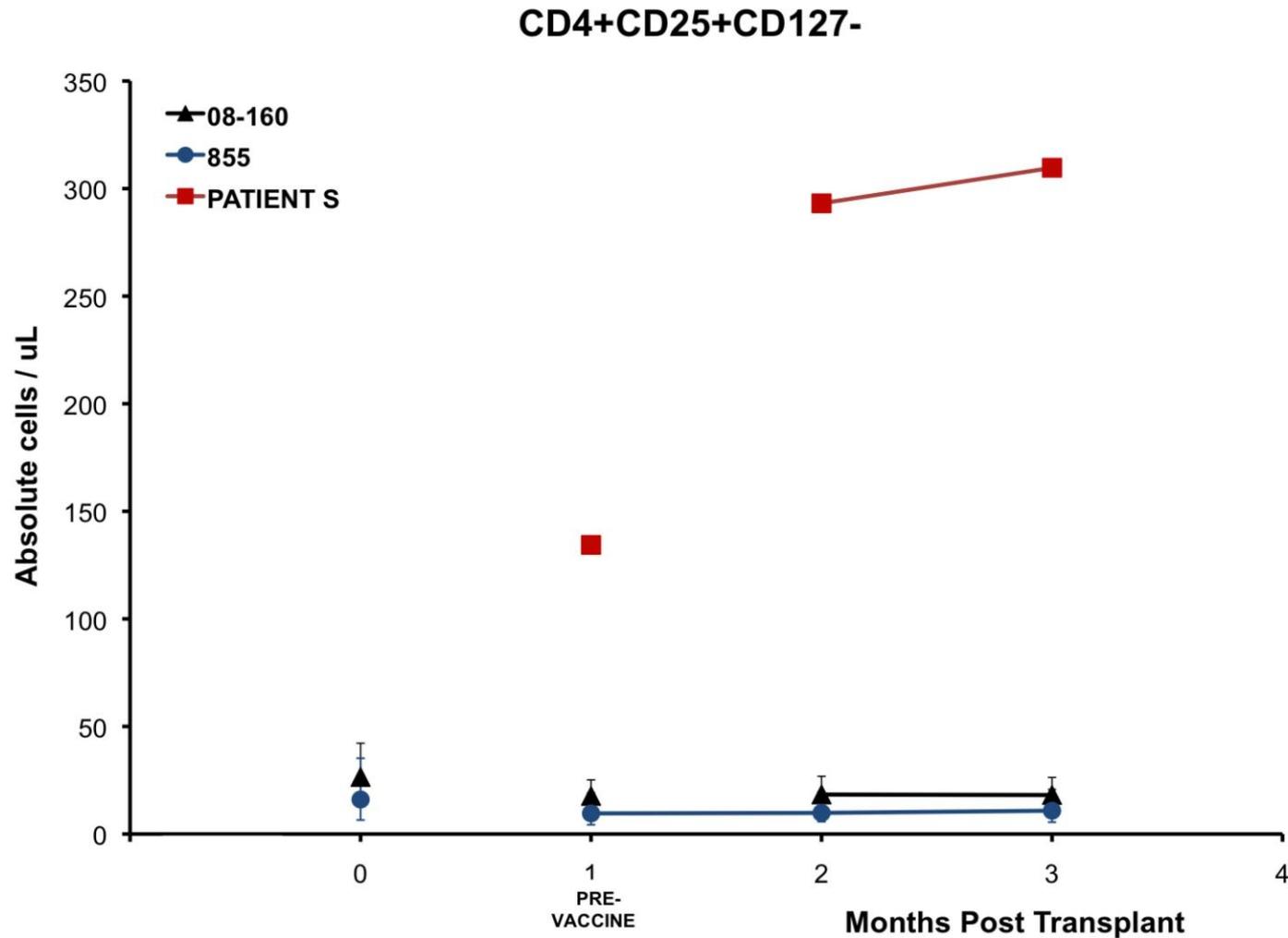
Luminex assays of 70 soluble factors

**CCL17 is associated with FoxP3⁺
Tregs, which inhibit both innate and
adaptive anti-tumor cytotoxic
responses**

FoxP3⁺ Tregs were increased in CS at the time of *beginning* VAX

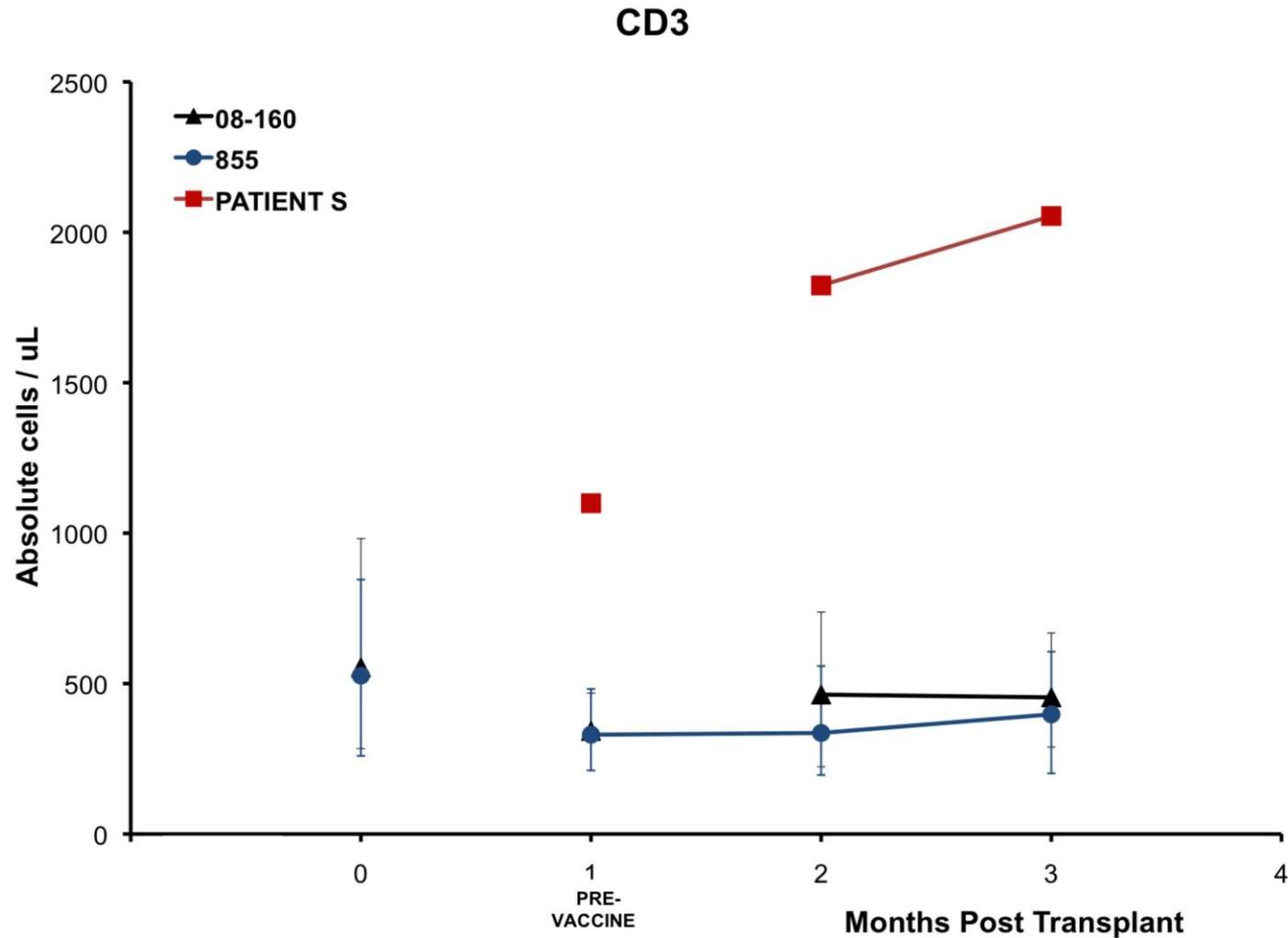


Aberrant Treg reconstitution in CS compared to other allo-HSCT patients

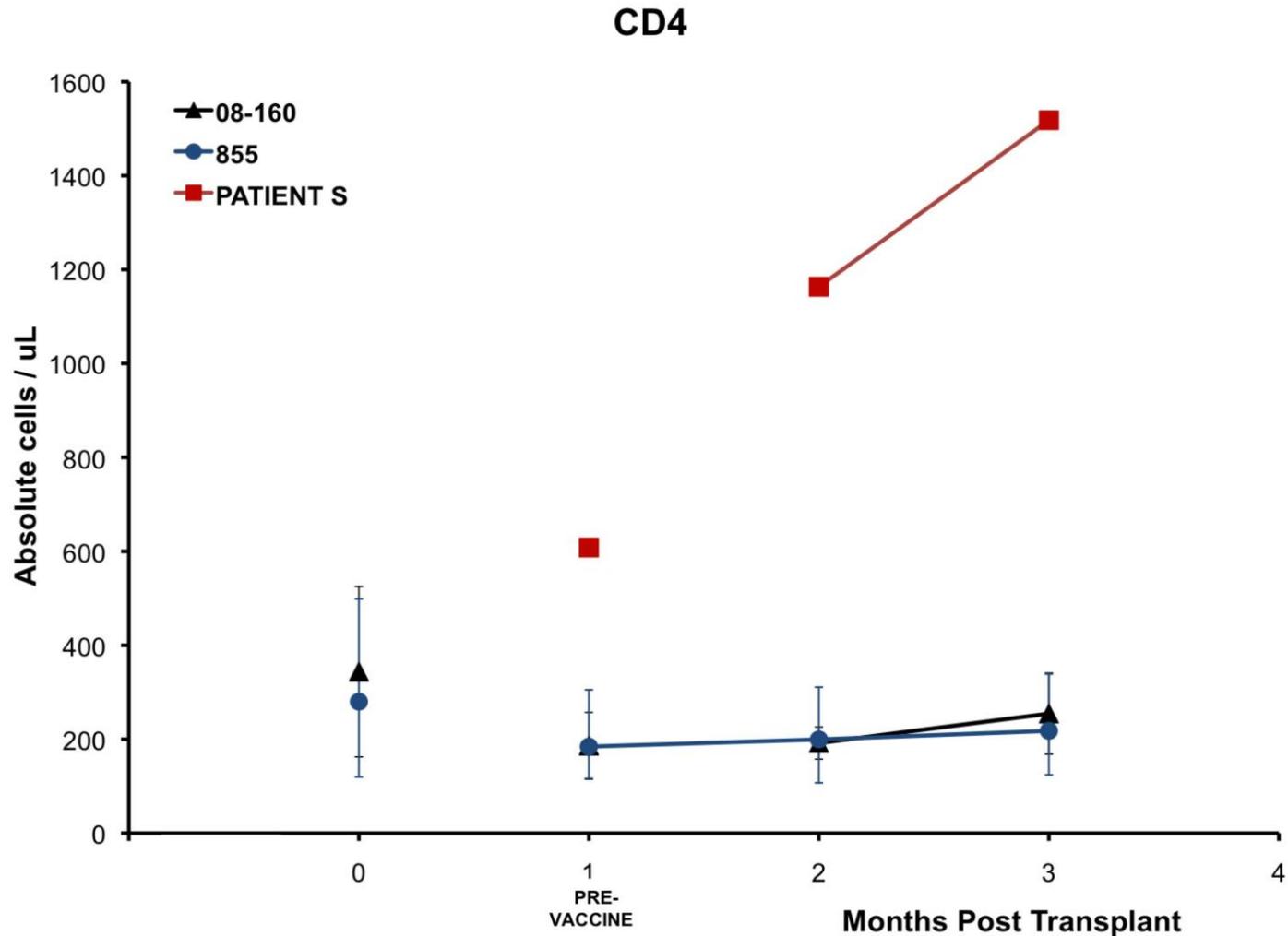


N = 254

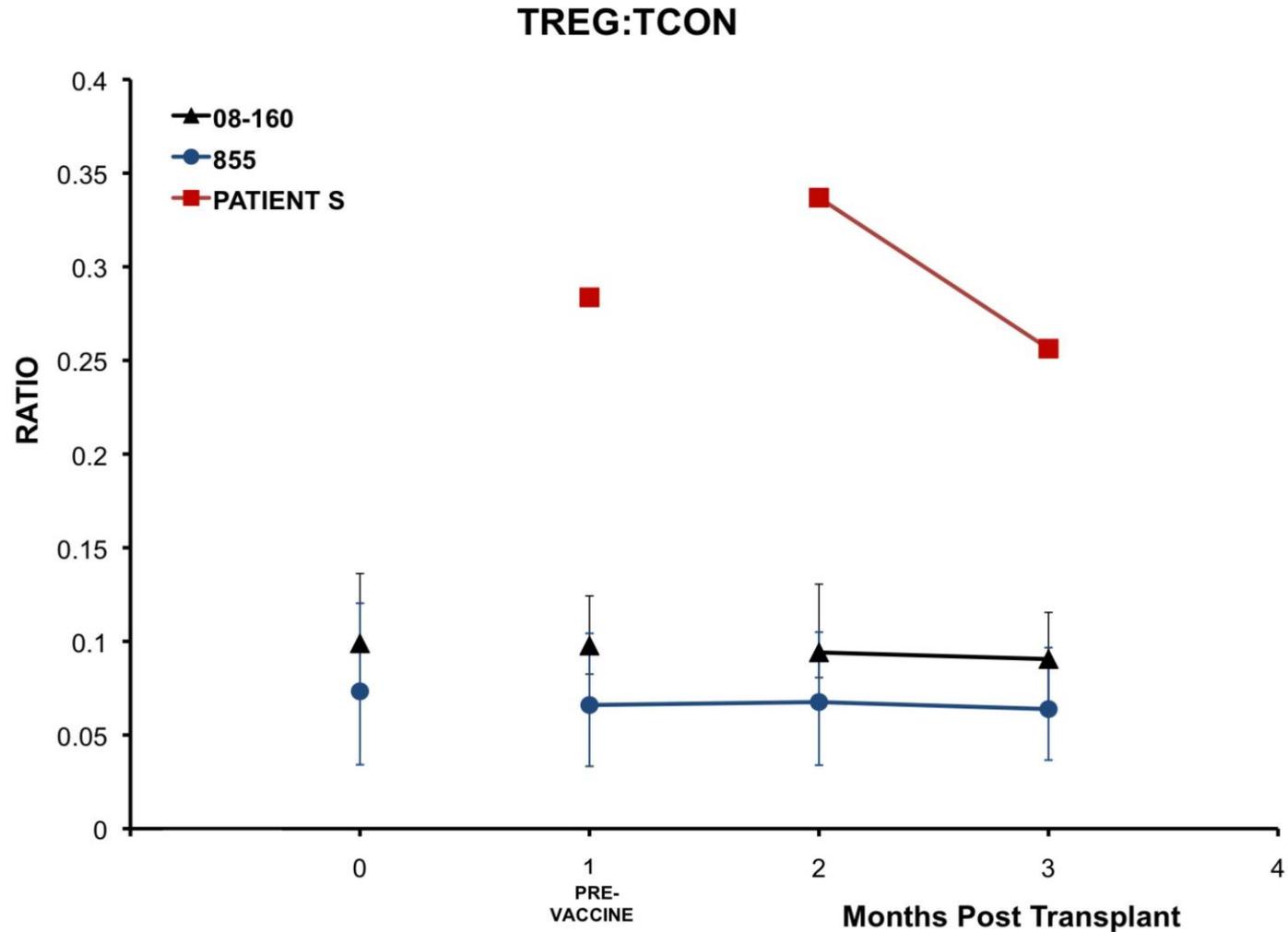
Altered CD3⁺ T cell reconstitution in CS



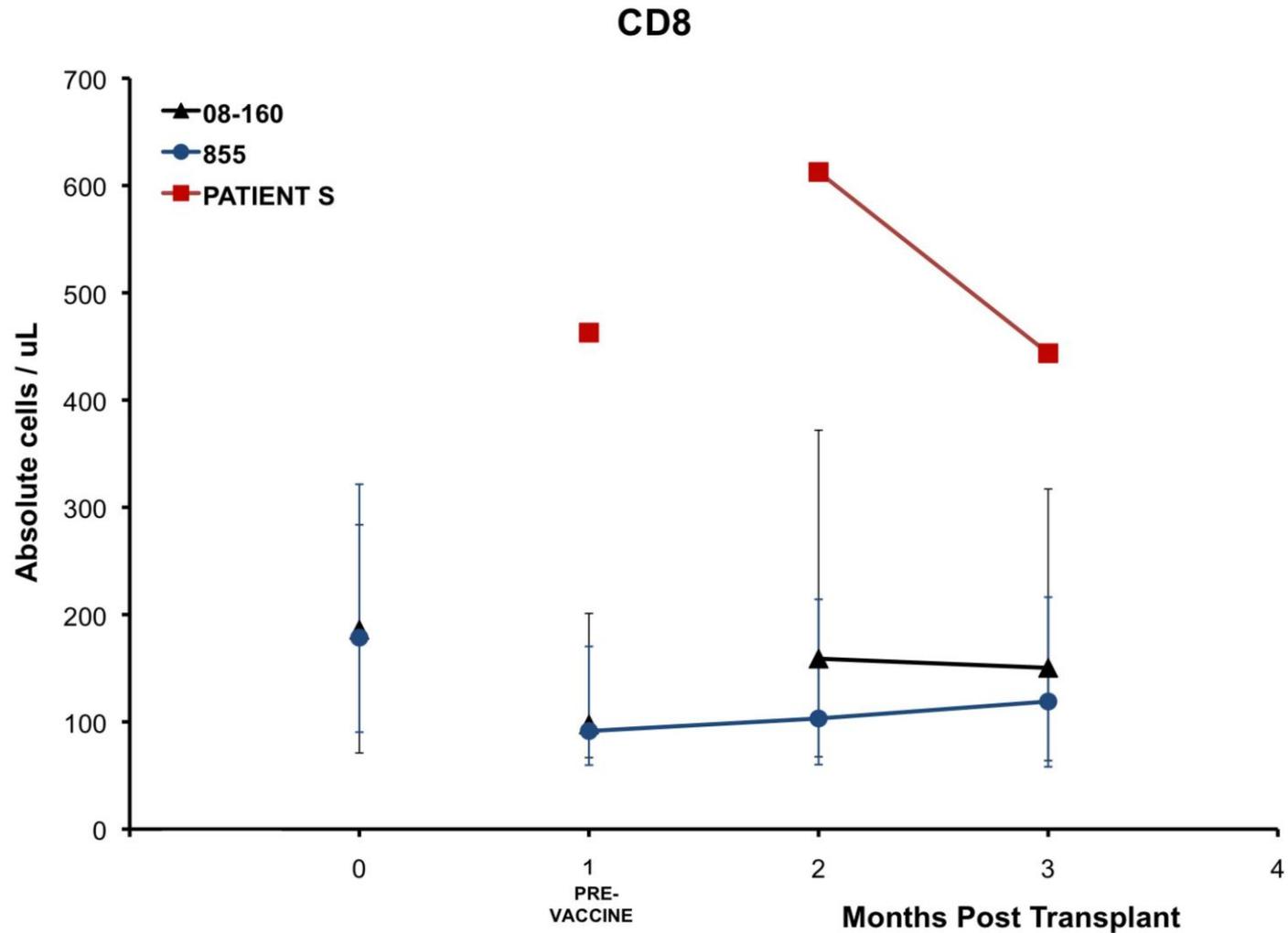
Altered CD4⁺ T cell reconstitution in CS



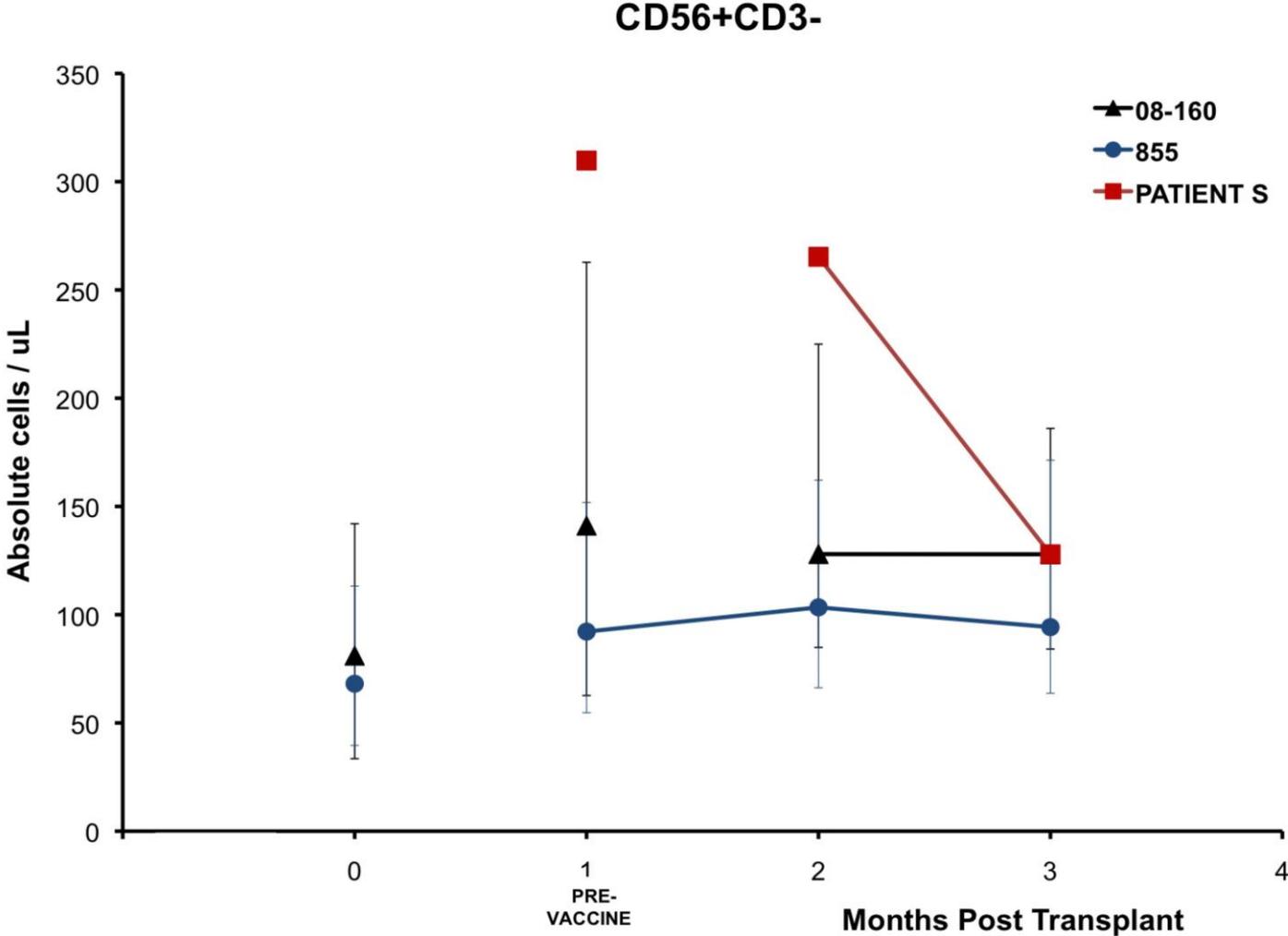
Altered CD4⁺ T cell subset ratios in CS



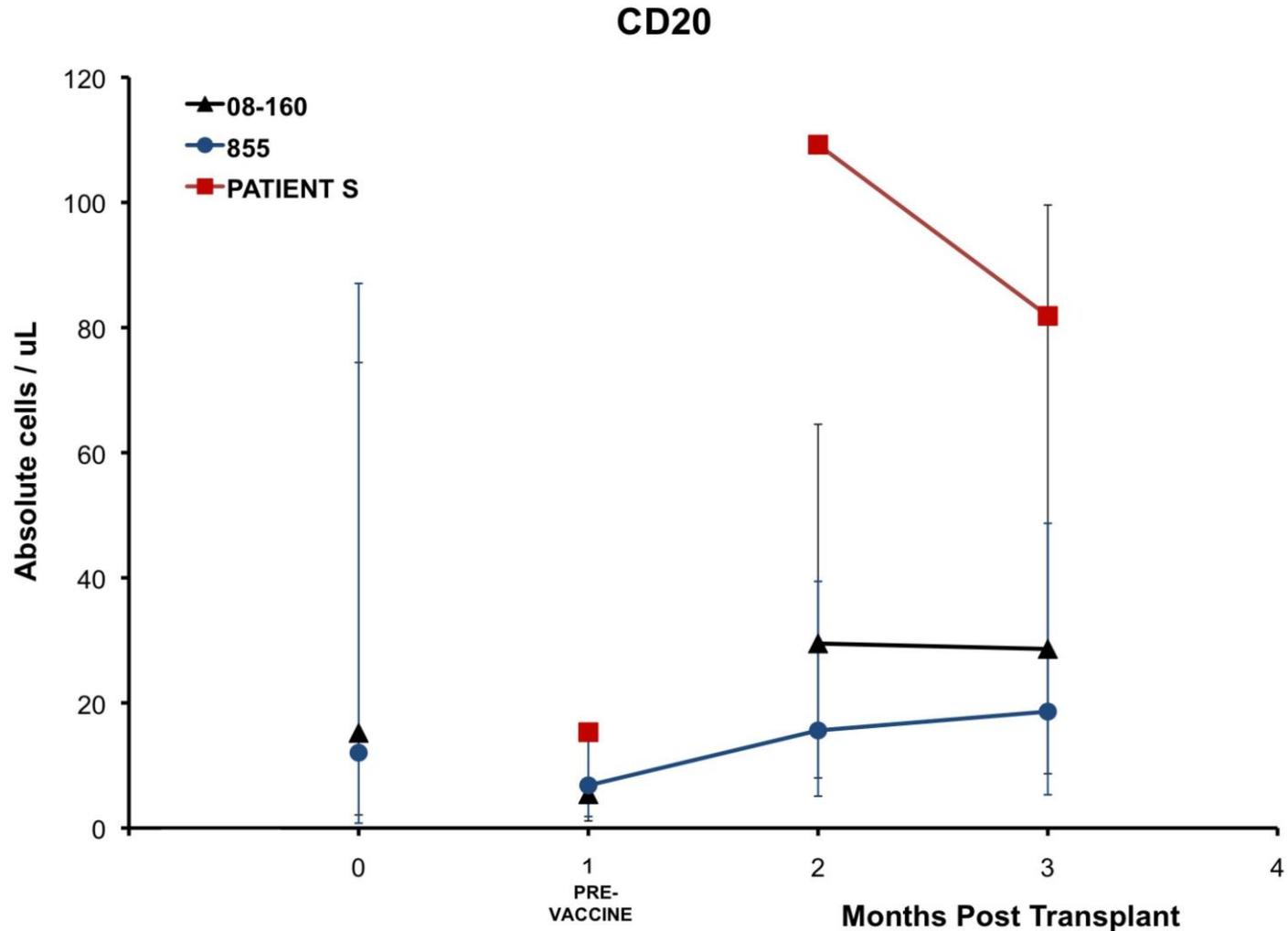
Altered CD8⁺ T cell reconstitution in CS



Altered NK cell reconstitution in CS

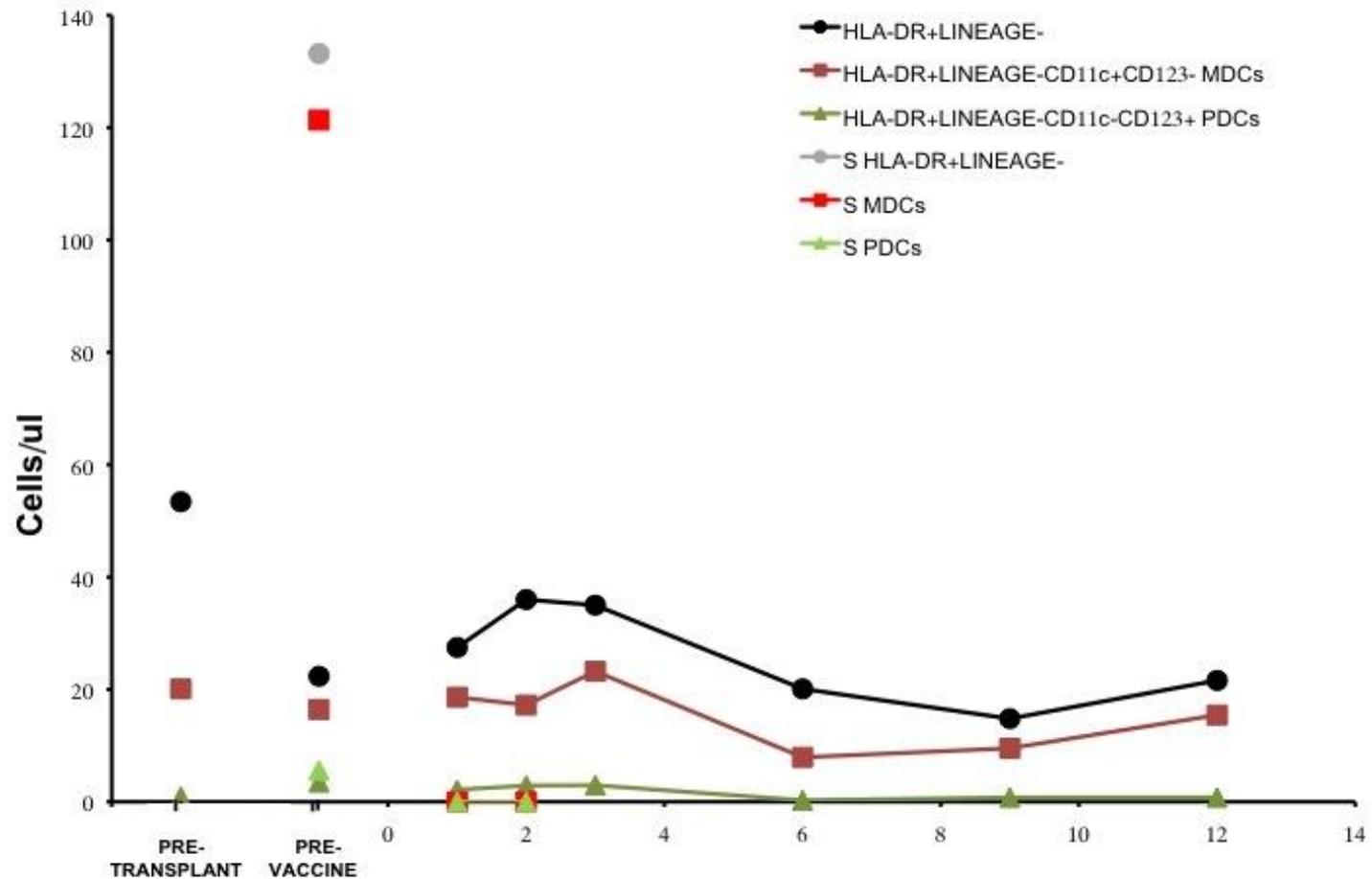


Altered B cell reconstitution in CS



Altered dendritic cell reconstitution in CS

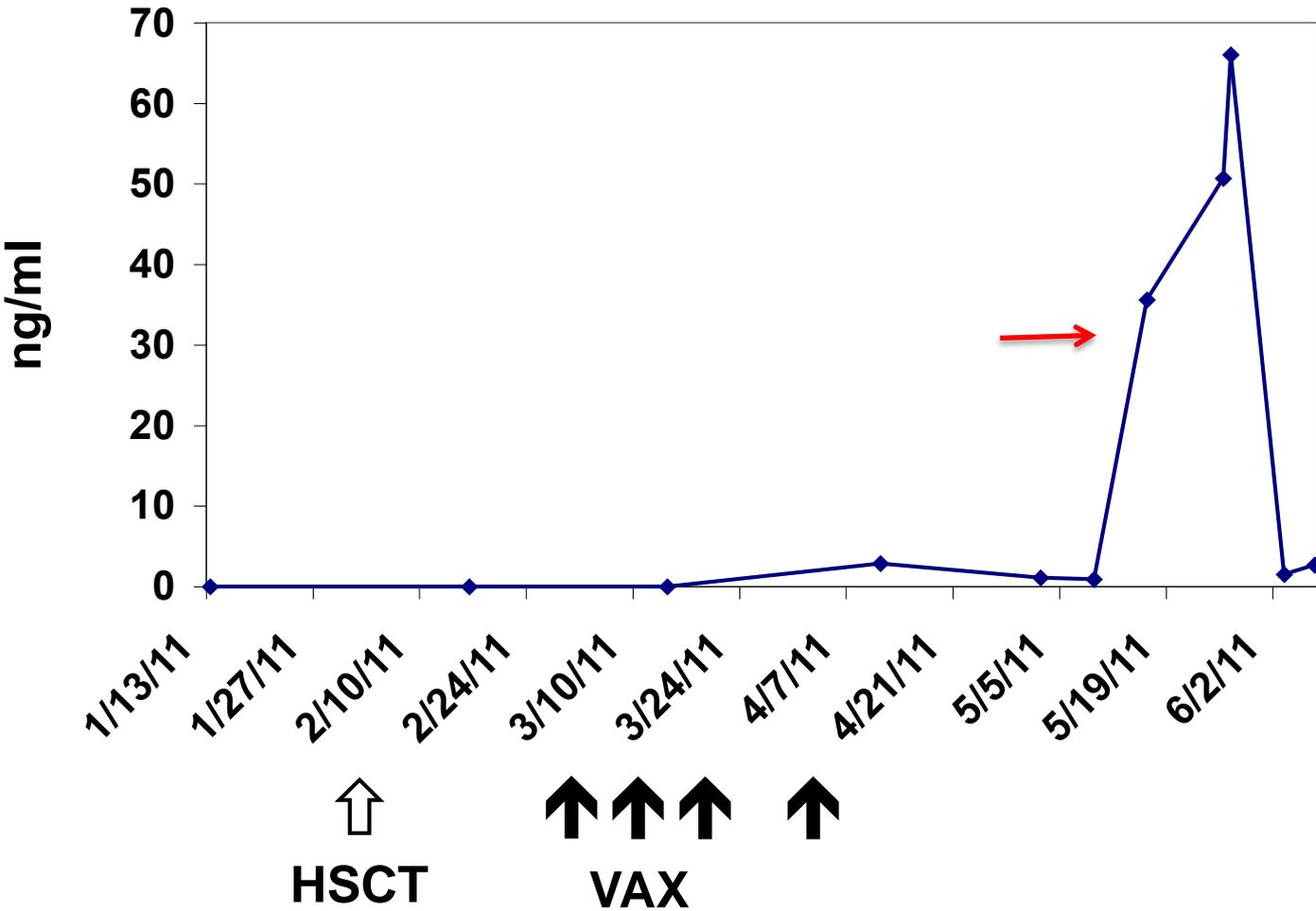
Dendritic cells



In CS, aberrant donor immune reconstitution, soluble NKG2D ligands, and rapamycin/FK-506 together compromised innate and adaptive immune mechanisms with the capacity to kill residual, irradiated K562 cells

**Were K562 cells the only source of
GM-CSF in CS?**

Plasma GM-CSF levels increased in association with leukocytosis



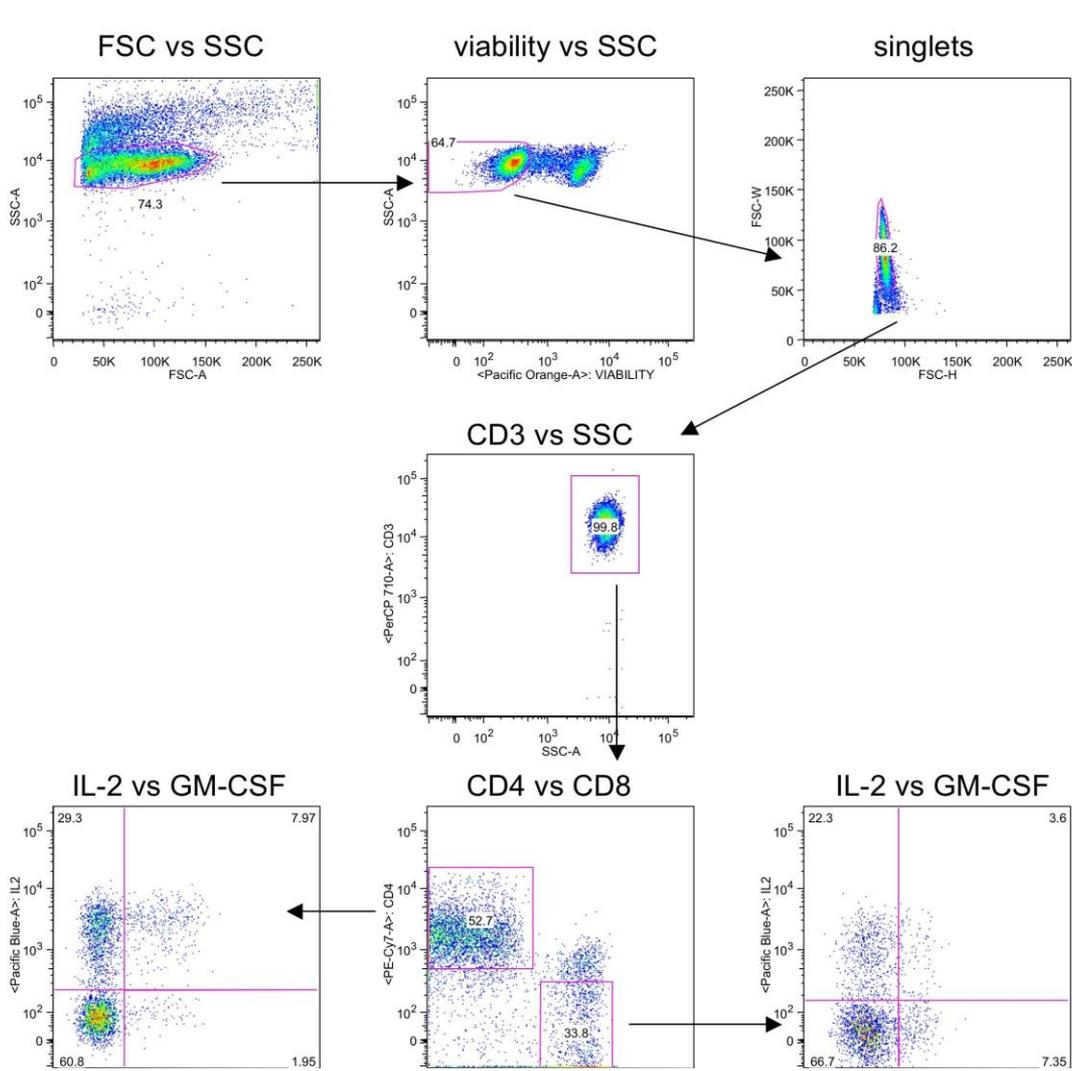
CS K562 cells show reduced GM-CSF secretion in vitro

Cell Type	Irradiation Dose	GM-CSF Secretion ng/2x10 ⁵ cells
K562WCB D1	0 Rads	3258
K562WCB D1	10K	1033
K562WCB D1	15K	2933
K562WCB D1	20K	1089
CS D1	0 Rads	1658
CS D1	10K	3184
CS D1	15K	1148
CS D1	20K	516
K562WCB D3	0 Rads	5255
K562WCB D3	10K	4769
K562WCB D3	15K	2327
K562WCB D3	20K	5900
CS D3	0 Rads	1150
CS D3	10K	471
CS D3	15K	655
CS D3	20K	95

**Mathematical modeling of K562 GM-CSF
production rates coupled with the
pathologic findings of 80-90% necrotic
cells and 10-20% Ki-67+ viable cells failed
to match experimentally determined
plasma GM-CSF levels**

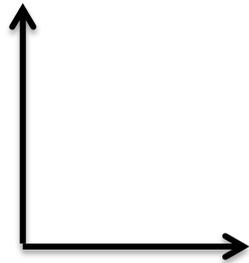
What other cells might release GM-CSF?

Donor derived T cells in CS can produce GM-CSF



Sorted cells stained from 5.9.11

IL-2

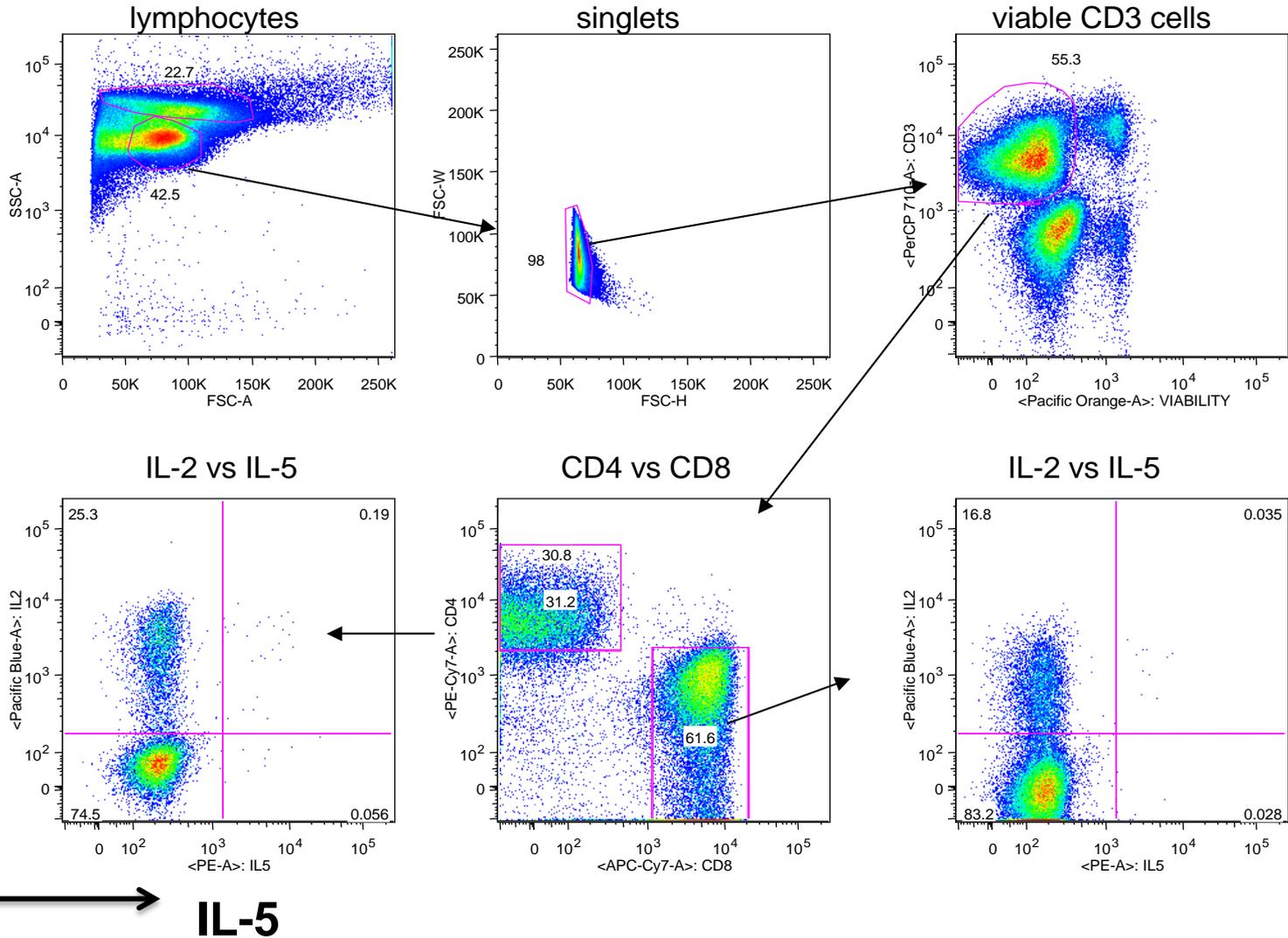


GM-CSF

**Further studies are required to
characterize GM-CSF production by donor
derived eosinophils, neutrophils,
monocytes, etc.**

**CCL17 is also associated with some cases
of T lymphocyte-driven hypereosinophilia**

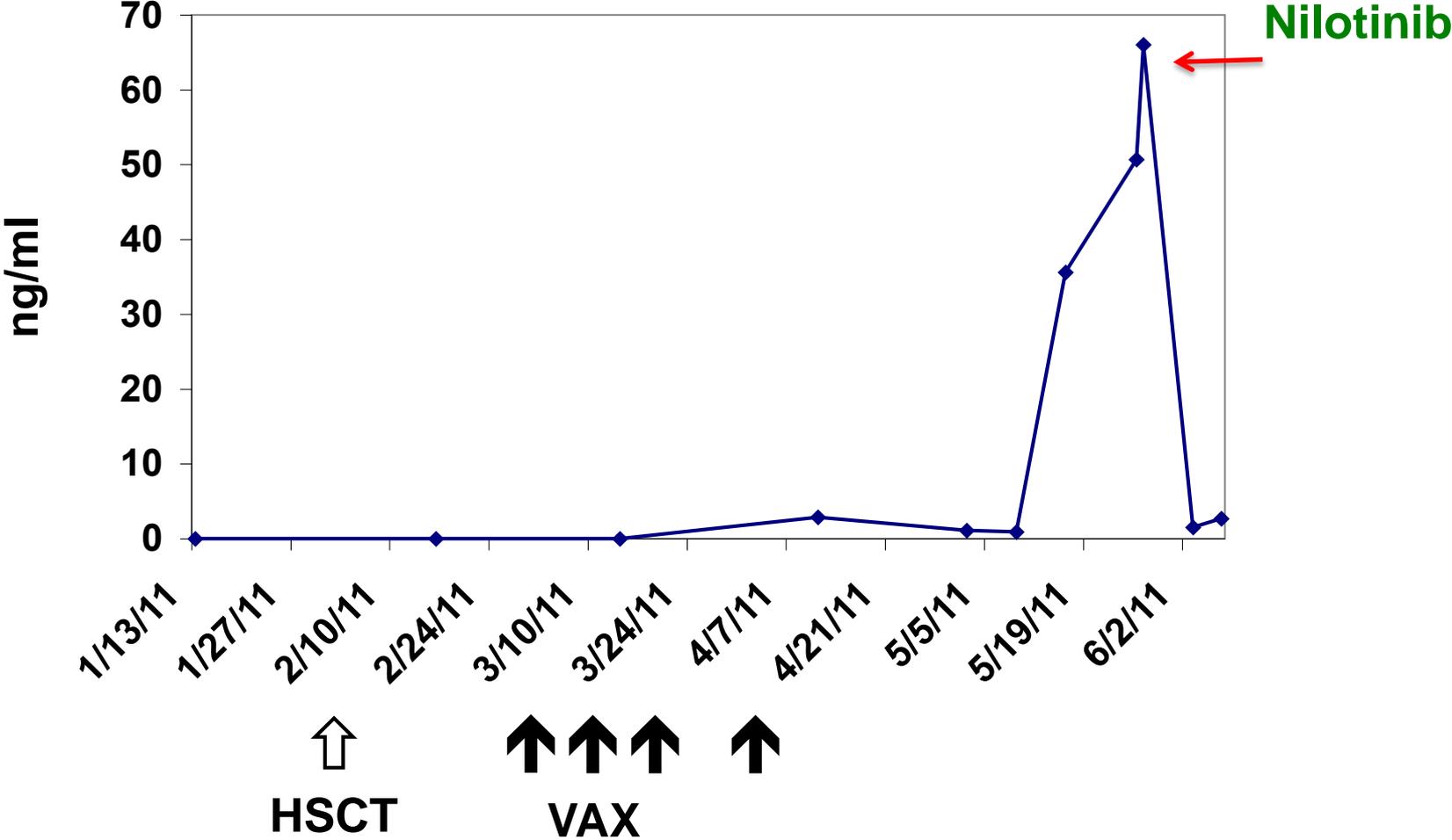
CS T cells produce minimal IL-5



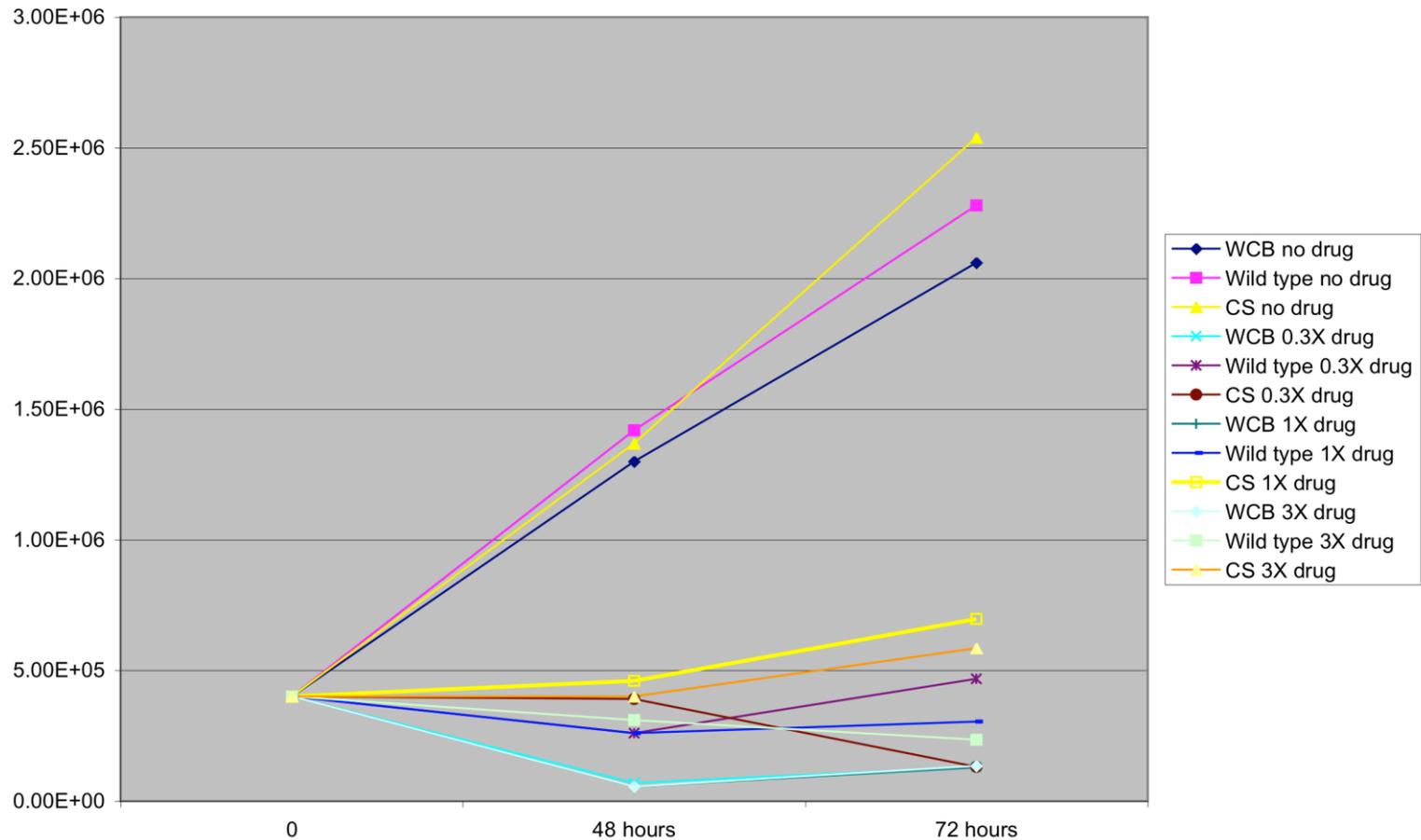
**Additional studies are required to elucidate
the interplay of CCL17 and GM-CSF in
hypereosinophilia**

**What led to the late reduction in
GM-CSF plasma levels and
white blood cell counts?**

Plasma GM-CSF levels increased in association with leukocytosis



CS K562 cells manifest sensitivity to nilotinib in vitro



- Pathologic analysis did not reveal differences in K562 cell viability or Ki-67 staining between pre-nilotinib and post-mortem samples (*only 11 days of therapy*)
- Impact of nilotinib on K562 GM-CSF expression *in situ* remains to be clarified
- Potential effects of nilotinib on GM-CSF driven leukocytosis and/or leukocyte GM-CSF production need to be investigated

Nilotinib/imatinib for hypereosinophilia

- **Major advance for cases driven by oncogenic lesions such as FIP1L1-PDGFR α**
- **Several reports of acute cardiac deterioration shortly after initiation of therapy, particularly when troponin-T elevated**
- **Corticosteroids might reduce this toxicity**
- **Prior autologous HSCT may have increased the risk for thrombo-embolic complications in CS**

Summary

- **Unique serious adverse event with no precedent in our experience**
- **Prolonged survival of irradiated K562 cells in vivo (3 months)**
- **Some surviving K562 cells manifested replicative capacity but retained radiation sensitivity in vitro**
- **Multiple immune defects related to allo-HSCT and AML may have contributed to K562 cell persistence**
- **Unusual immune reconstitution may have supported the development of hypereosinophilia**

Implications

- **Frequency of irradiated tumor cell vaccine persistence should be defined more rigorously**
- **“Recall reactions” at vaccination sites years after treatment raise the possibility of immune equilibrium in long-term responders**
- **Persistence might be important for the maintenance of protective tumor immunity**
- **Persistence might increase the risk of toxicity if sufficient immune defects are present**
- **Nilotinib might serve as a specific safeguard against persisting K562 cells**

DF/HCC Protocol 06-196 GM-K562/CLL s/p allo-HSCT

- **Similar design as DF/HCC 08-160**
- **17 patients vaccinated to date**
- **Median follow-up of 32.5 months (as of 9/11)**
- **13 patients achieved complete response
(12 ongoing)**
- **3 patients achieved ongoing partial response**
- **1 patient shows stable disease**