Central Memory $CD8^+$ T Cells Appear to Have a Shorter Lifespan and Reduced Abundance As a Function of HIV Disease Progression

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Immunodeficiency in HIV Infection

- **Quantitative loss of $CD4^+$ T cells**
- **Qualitative changes in cell populations that persist**

  Grossman Z et al. *Nat Med* (2006)
  McCune JM. *Cell* (1991)

- **Loss of recall, or “memory”, responses to antigen**

  Clerici M et al. *J Clin Invest* (1989)

- **Loss of polyfunctional $CD8^+$ “memory” T cells that are able to control HIV viral load**

  Betts MR et al. Blood (2006)

- **Such memory responses represent the bedrock upon which the adaptive immune system is based**

  Kaech SM et al. Nat Immunol. (2003)
  Schluns KS & Lefrancois L. Nat Rev Immunol (2003)
  Seder RA & Ahmed R. Nat Immunol (2003)
  Tough DF & Sprent J. Stem Cells (1995)

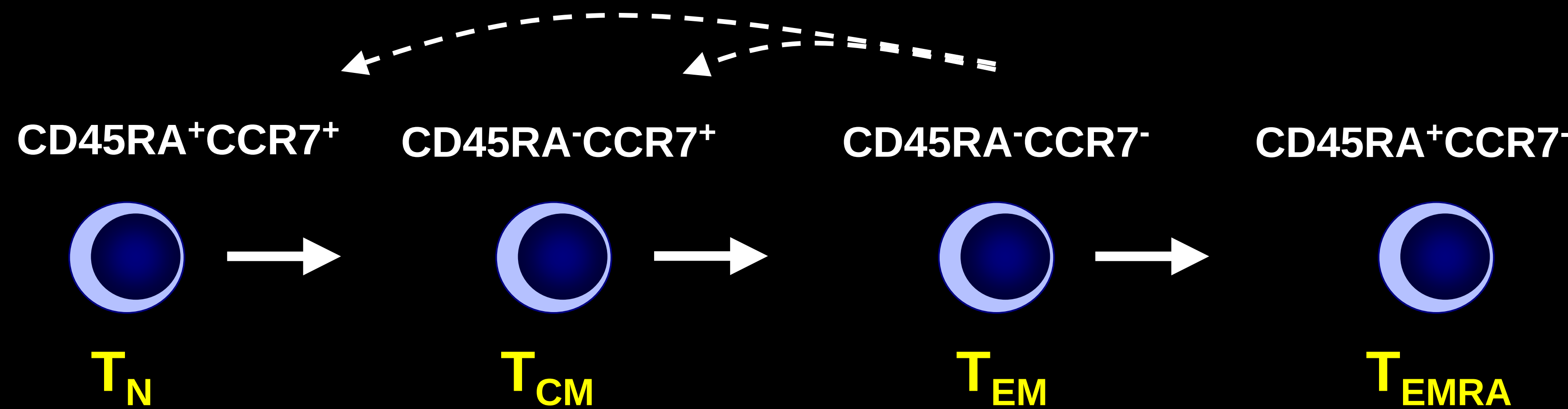
"Memory" in the T Cell Lineage and Generation and Maintenance of Memory CD8$^+$ T Cells

- Memory T cells are thought to differentiate from naïve T cells after exposure to antigen
- .. persist for very long periods of time
- .. rapidly proliferate and differentiate into effector T cells after secondary contact with cognate antigen

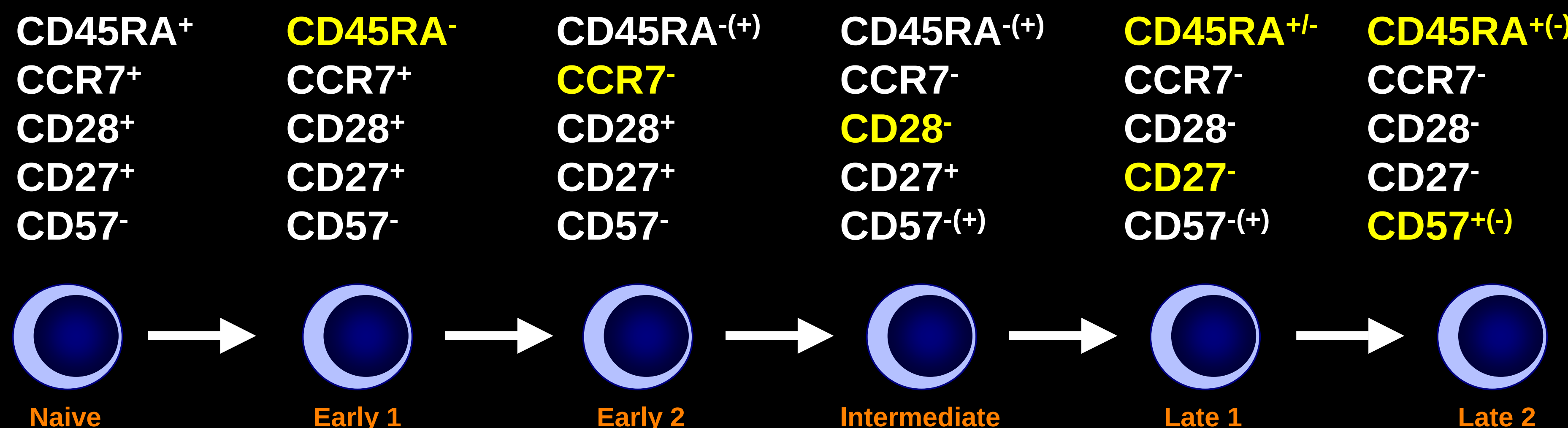
- Requirements for generation and maintenance of memory CD8$^+$ T cells include:
  - CD4$^+$ T cell help
  - a cellular machinery that provides for either ***self-renewal*** (e.g., through arrested development mediated by transcriptional repressors) or for ***limited homeostatic proliferation*** (e.g. mediated by IL-7 through the IL-7Rα).

Kaech SM et al. Nat Immunol. (2003)
Fearon DT et al. Science (2001)
Luckey CJ et al. PNAS (2006)

Linear Differentiation or Post-thymic CD8^{+} T Cell Development*



Lanzavecchia A & Sallusto F. Nat Rev Immunol (2002)
Champagne P et al. Nature (2001)



* Appay V & Rowland-Jones SL. Sem Immunol (2004)

In vivo ^2H_2O or ^{2}H-glusoce Labelling showed that Higher Proportions of T Cells Are Short-lived in Advanced HIV Infection Compared to Healthy Controls

- These short-lived cells have a memory/effector phenotype
- Long-lived potential progenitor T cells may be reduced in advanced HIV infection

Hellerstein MK et al. J Clin Invest (2003)

These Findings Led Us to Ask the Following Questions:

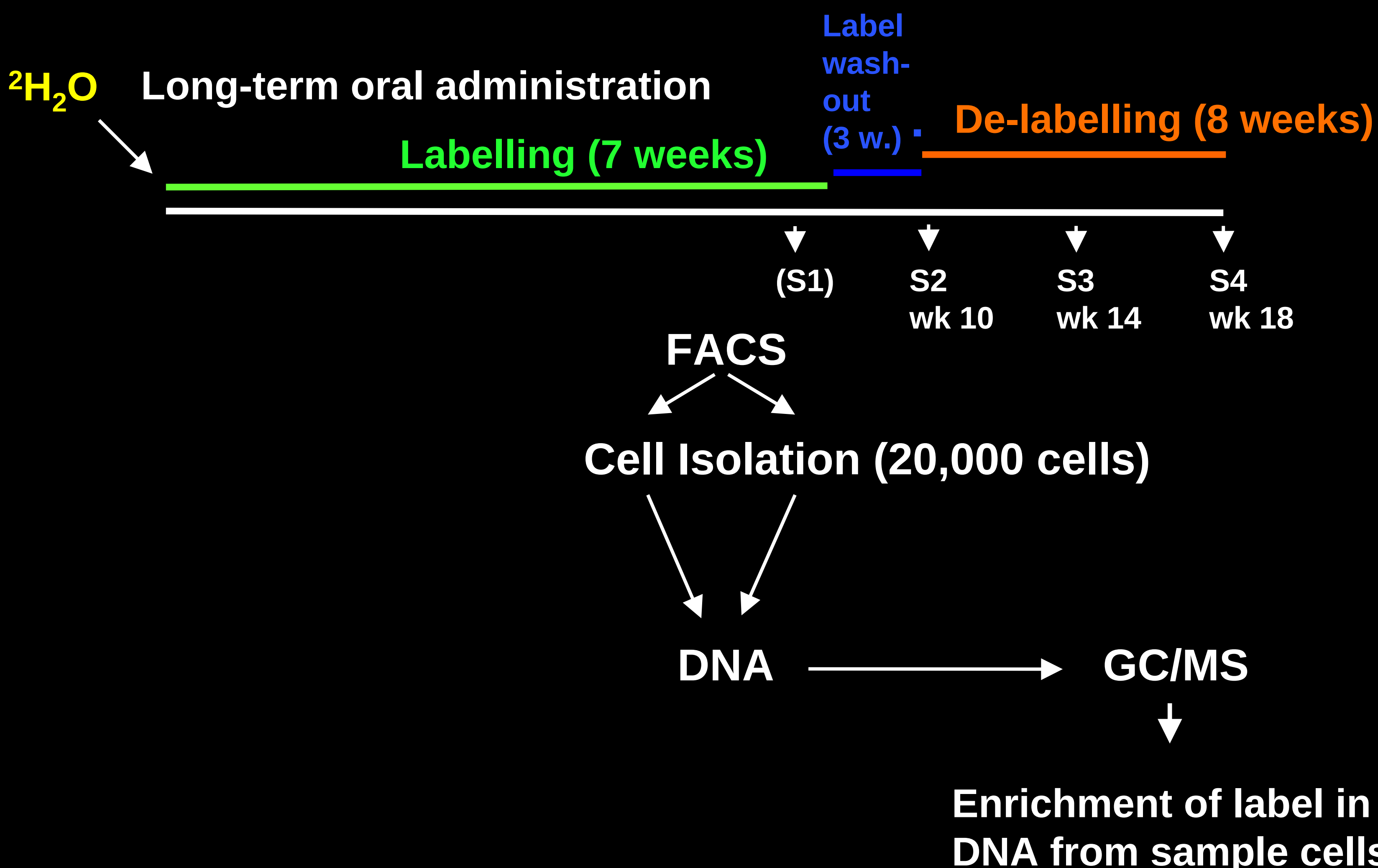
- **What is the phenotype and the lifespan of long-lived memory $CD8^+$ T cells in HIV-negative subjects?**

- **Does this phenotype and/or its lifespan change in the context of progressive HIV disease?**

We also Wanted..

.. to evaluate the applicability of the stable isotope (^2H_2O) / FACS / mass spectrometric method for the analysis of low abundance T cell subpopulations

Stable Isotope *In Vivo* Labelling with ^2H_2O



Calculations of Decay Constants, Half-lives, and Percentages of $CD8^+$ T Cell Subpopulations Remaining after 7 Weeks of ^2H_2O Labelling

- **Loss of label from cellular DNA of each subset was quantified between S2 (week 10) and S4 (week 18)**
- **The decay constant (k) was calculated using the equation for exponential decay:**

$$k = -[\ln(S2/S4)]/Dt$$

- **The half-life was calculated as:**

$$t_{1/2} = \ln(2)/k$$

- **Some $CD8^+$ T cell subpopulations did not loose label exponentially, which is why we also calculated the percentage of initially incorporated label:**

$$[S4/S2]$$

Phenotypes of Sorted $CD3^{+}CD8^{+}$ T Cell Subpopulations

MEMORY & EFFECTOR

T_{CM}

- $CD45RA^{-}CD28^{+}CCR7^{+}$

T_{EM1}

- $CD45RA^{-}CCR7^{-}$ ($CD28^{+/-}$)

T_{EM2}

- $CD45RA^{-}CD28^{-}CCR7^{-}$

NAÏVE & “RA” EFFECTOR

T_{N}

- $CD45RA^{high}CD28^{+}CCR7^{+}$

T_{EMRA}

- $CD45RA^{high}CD28^{-}CCR7^{-}$

Characteristics of Study Subjects

Group	Subject ID	Age	VL (copies/ml)	Years HIV-infected	CD4 count / ul of blood	CD8 count / ul of blood	Weeks of ^2H_2O labeling
HIV-negative	A	27	Negative	-	586	376	7
	B	56	Negative	-	513	292	7
	C	59	Negative	-	555	488	7
	D	64	Negative	-	903	258	7
	E	35	Negative	-	1289	563	ND
	F	35	Negative	-	832	246	ND
HIV-infected (untreated)	L	41	< 75	5	1028	998	ND
	M	40	8278	9	973	1500	ND
	G	40	2,059	NA	852	3388	7
	N	52	713	5	784	1052	ND
	O	52	< 75	17	730	929	ND
	P	52	8469	16	657	1960	ND
	Q	52	< 75	11	642	1067	ND
	R	59	87	15	615	1143	ND
	S	38	392	13	594	2079	ND
	H	41	2,220	NA	532	1764	7
	T	42	6,897	11	493	884	ND
	I	38	30,851	NA	431	1646	7
	U	37	16,212	6	360	578	ND
	K	36	448,343	NA	349	2445	7
	V	41	17,090	11	331	1067	ND
	J	53	74,199	NA	330	1397	7
	W	46	32,236	11	277	1207	ND
	X	40	15,534	15	170	1133	ND
	Y	60	222,000	17	78	586	ND
	Z	47	467,160	21	66	1281	ND

ND not determined; NA not available

Median CD8 count:

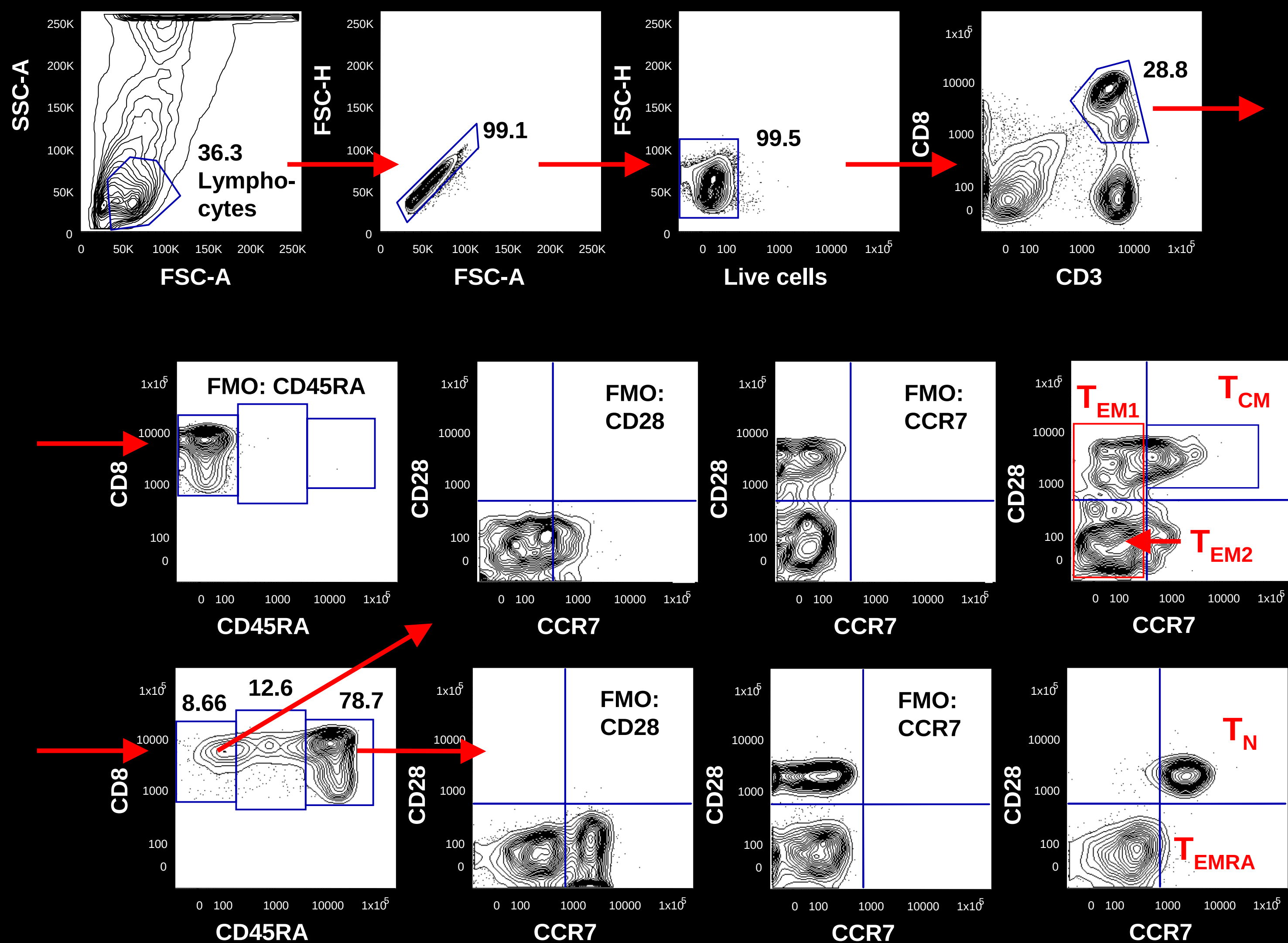
HIV-negative subjects:
334 CD8 T cells/μl; range 246 – 563)

versus

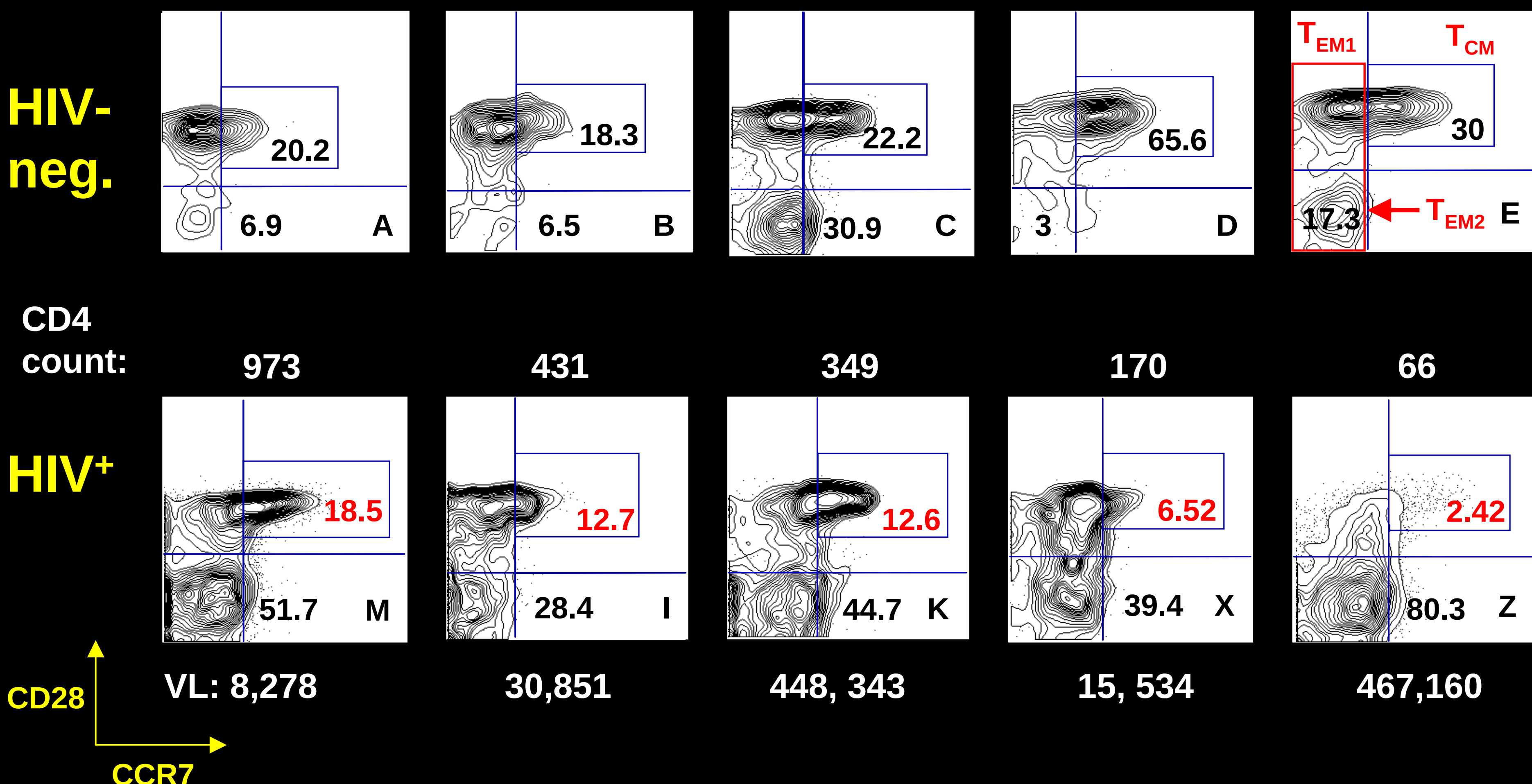
HIV-infected subjects:
1175 CD8 T cells/μl; range 578-3388)

($p<0.002$ HIV-negative versus HIV-infected)

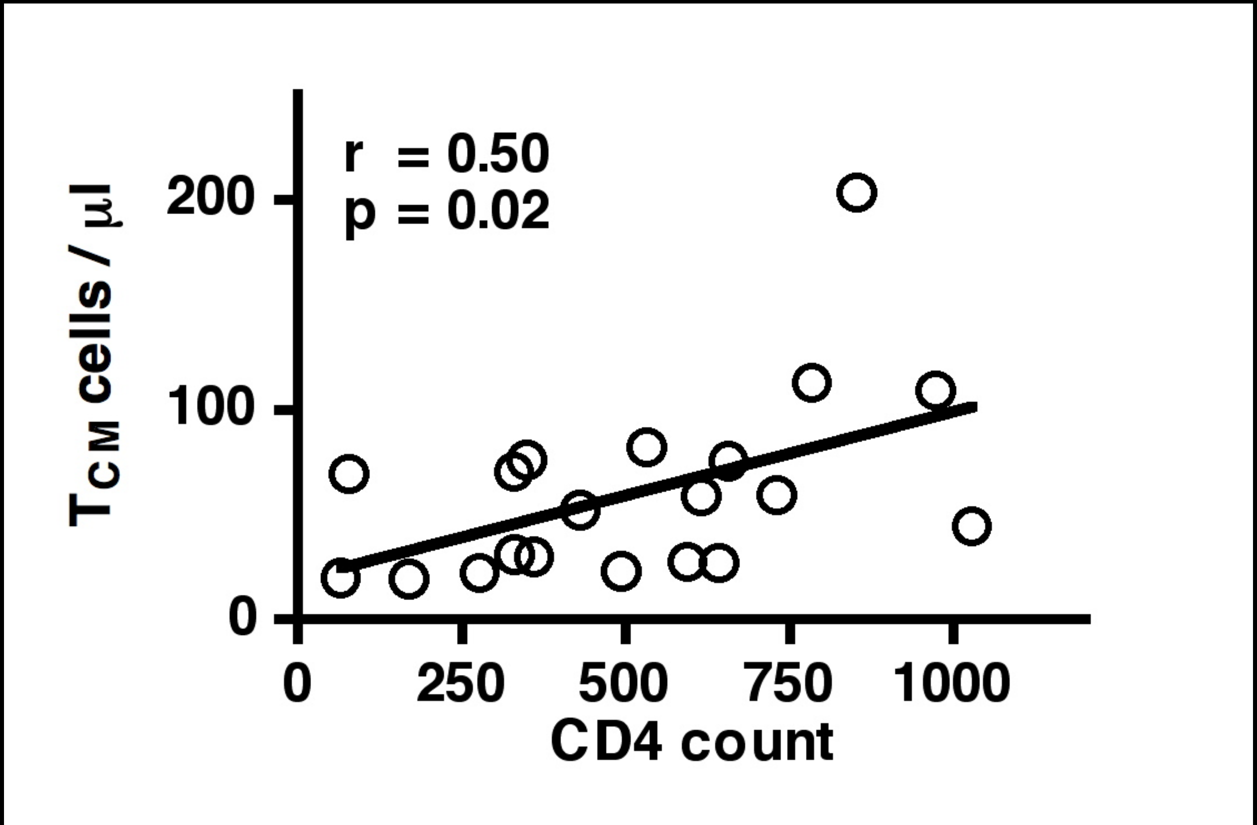
Gating Strategy for Analysis & Sorting



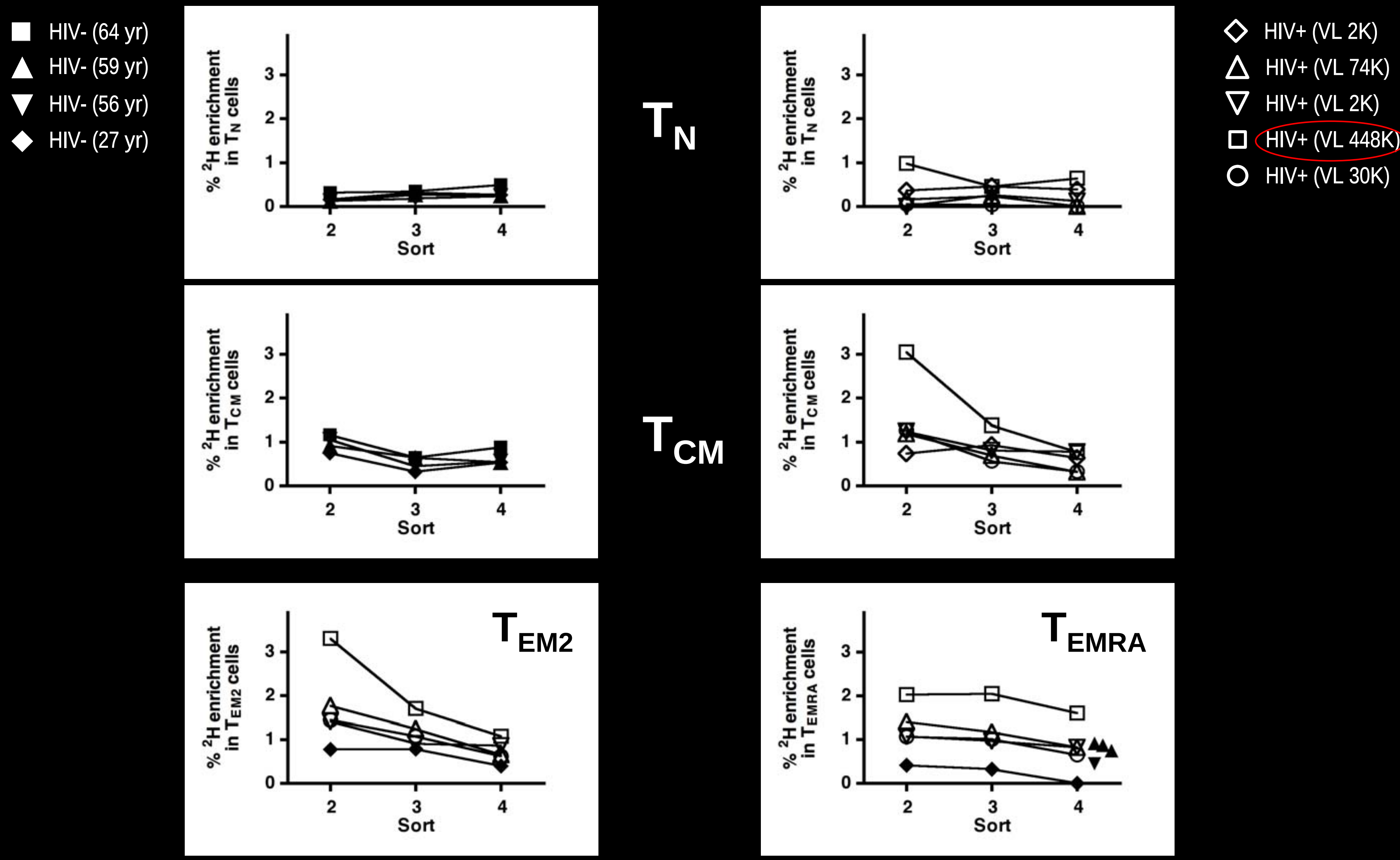
T_{CM} Cells Are Lost with Decreasing CD4 Counts



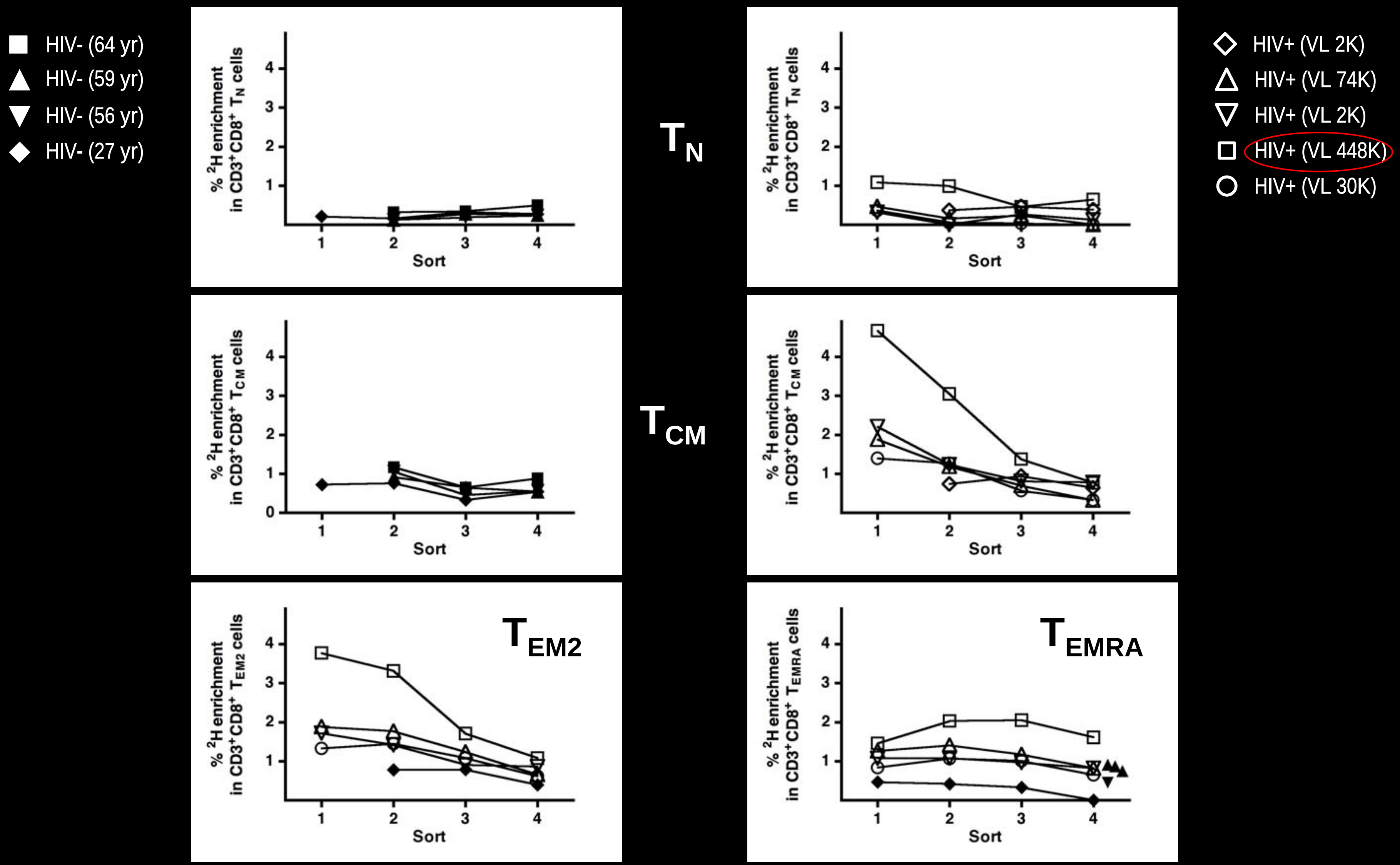
Decreased Numbers of CD8$^+$ T_{CM} cells / µl of Blood Correlate with Decreasing CD4 Counts



Label Die-away Curves of CD8^{+} T Cell Subpopulations



^2H Enrichment in $CD8^+$ T Cell Subpopulations

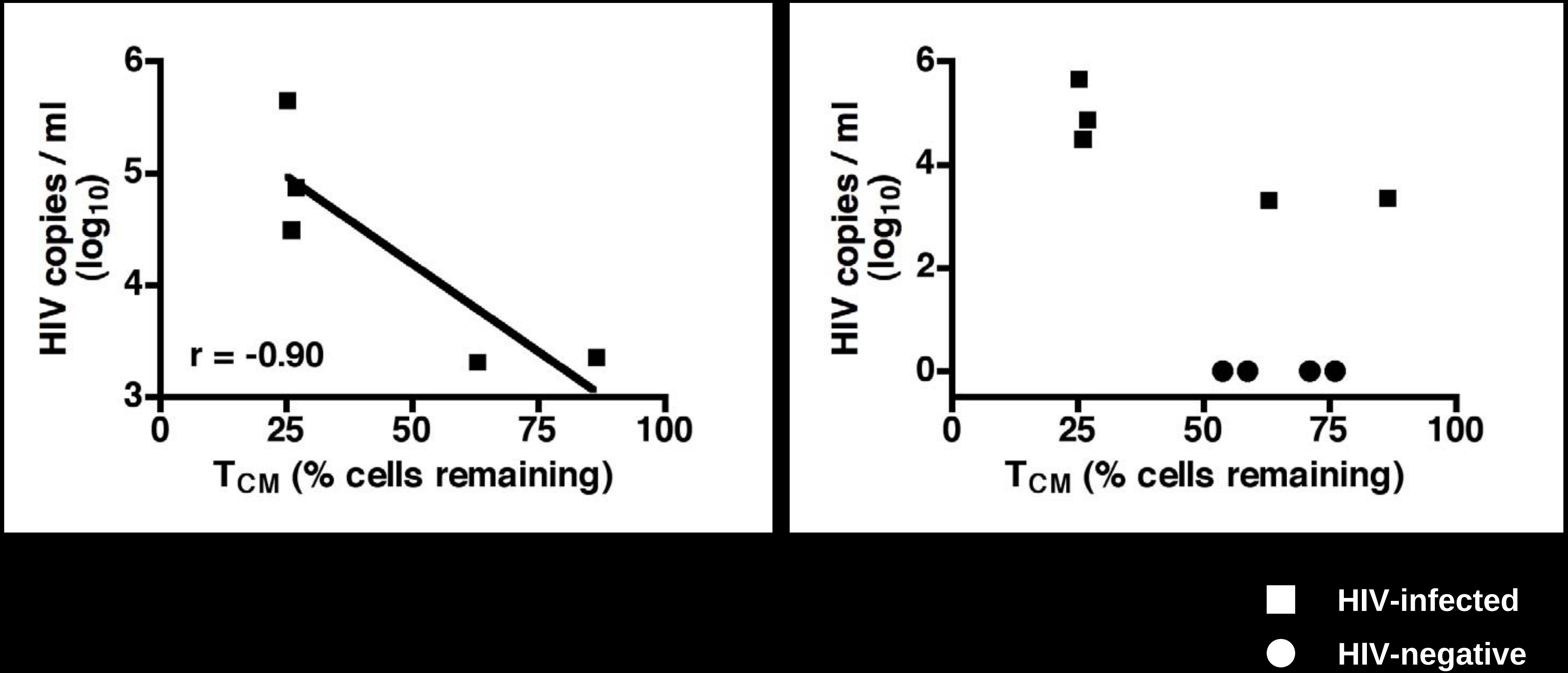


Shorter Half-life of CD8$^+$ T_{CM} Cells in HIV-infected Subjects with High Viral Load

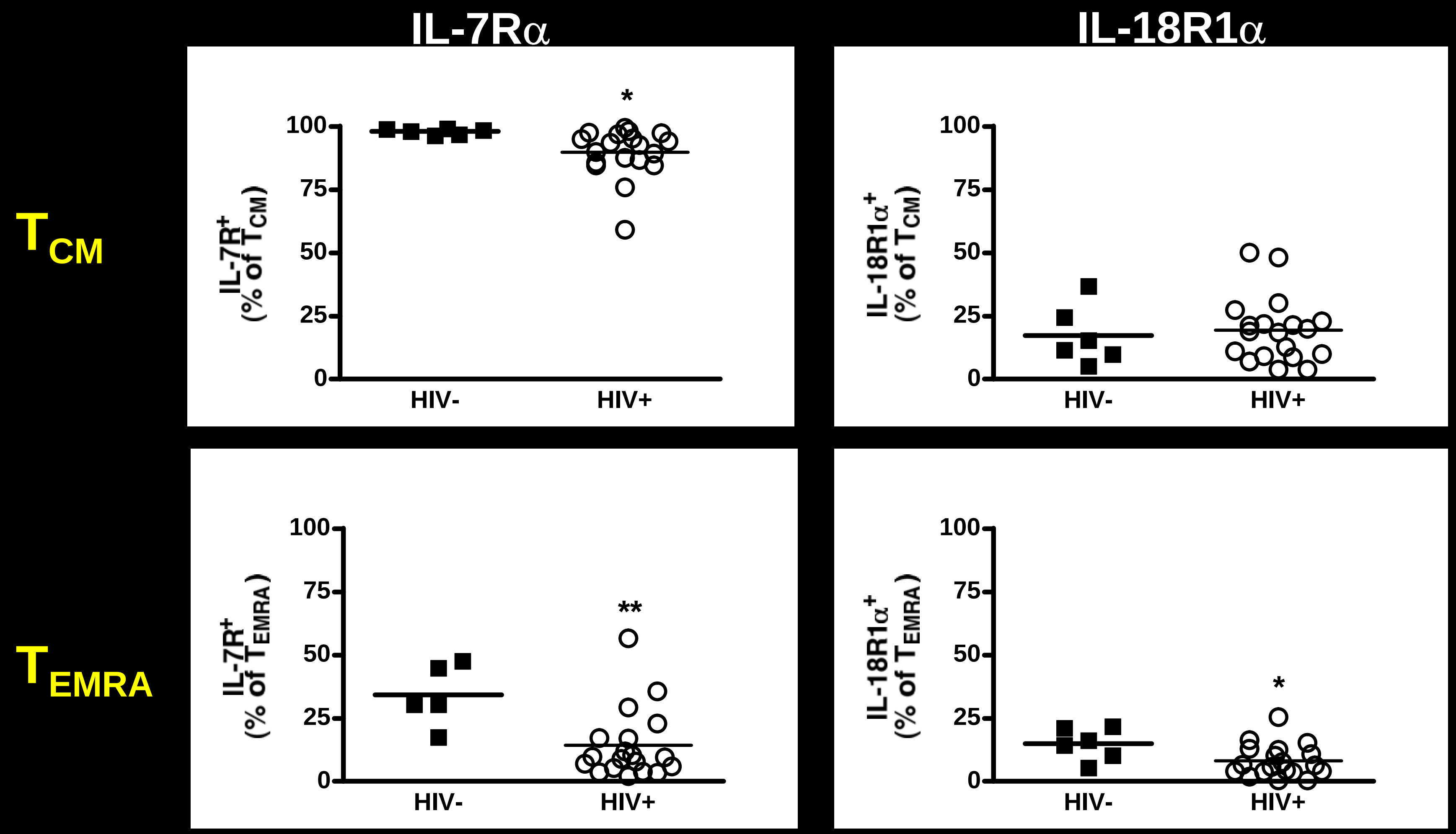
$CD3^+CD8^+$ T cell subset	Group	HIV copies/ml) (log_{10})	k (decay constant)	$T_{1/2}$ (half-life in days)	Percentage of cells remaining	Cells/ul	% of $CD3^+CD8^+$ T cells
$CD45RA^-CCR7^-CD28^-$ (T_{EM2})							
	HIV-negative	-	0.0119	58.1	51.3	8.3 (± 8.7)	2.2 (± 1.9)
	HIV-infected	4.3 (± 1.0)	0.0154 (± 0.0047)	49.3 (± 19.3)	43.3 (± 12.2)	241.4 (± 208.5)	9.9 (± 5.6)*
$CD45RA^+CCR7^-CD28^-$ (T_{EMRA})							
	HIV-negative	-	0.00007	9762	99.6	33.2 (± 27.3)	8.9 (± 6.4)
	HIV-infected	4.3 (± 1.0)	0.0069 (± 0.0028)	114.5 (± 47.2)	68.5 (± 10.6)	515.9 (± 162.3)***	25.0 (± 5.3)**
$CD45RA^-CCR7^+CD28^+$ (T_{CM})							
	HIV-negative	**-**	**0.0079** (± 0.0029)	**97.7** (± 36.8)	**64.9** (± 10.4)	30.5 (± 21.7)	9.15 (± 7.5)
	HIV-infected	**4.3** (± 1.0)	**0.0166** (± 0.0087)^A	**51.0** (± 30.4)^A	**45.5** (± 27.9)^A	96.7 (± 60.6)	4.38 (± 1.2)
	HIV+ High VL	**5.0** (± 0.6)	**0.0240** (± 0.0006)^A	**28.8** (± 0.7)^A	**26.0** (± 0.8)^A	66.1 (± 12.4)	3.8 (± 1.1)
$CD45RA^+CCR7^+CD28^+$ (T_N)							
	HIV-negative	-	-	-	175.5 (± 16.1)	159.4 (± 82)	44.0 (± 18.6)
	HIV-infected	4.3 (± 1.0)	-	-	42.8 (± 52.0)**	294.7 (± 198.9)	15.3 (± 11.6)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ HIV-negative versus HIV-infected;
^{A} $p < 0.05$ HIV-negative versus HIV-infected high VL

Lower Percentages of CD8+ T_{CM} Cells Remaining Correlate with Higher HIV VL



Expression of IL-7Rα or IL-18R1α on T_{CM} or T_{EMRA} $CD8^+$ T Cells



* p<0.05, ** p<0.01

Summary

- **The different kinetic die-away patterns in different $CD8^+$ T cell subpopulations demonstrate that the turnover of low-abundance T cell subpopulations can now be studied using the refined stable isotope / FACS / mass spectrometric method.**
- **Two long-lived $CD8^+$ memory/effector T cell subpopulations were found: T_{CM} cells expressing IL-7Rα and T_{EMRA} cells, of which a high fraction expresses CD57.**
- **T_{CM} cells appear to have a shorter half-life in HIV-infected subjects than HIV-negative subjects and decline numerically with progressive HIV disease.**
- **T_{EMRA} cells had a long half-life in both HIV-infected and HIV-negative subjects and were significantly increased in all HIV-infected subjects irrespective of their VL.**

Summary

- **Accepted traits of hematopoietic stem cells (such as higher expression of the transcriptional repressor, bcl6b*, or cell surface expression of IL-18R1α**) could not be ascribed to these human $CD8^+$ memory T cell subpopulations.**

- **However, a lower fraction of T_{CM} cells in HIV-infected individuals expressed IL-7Rα and the fraction of T_{CM} cells that expressed IL-7Rα trended to decrease with declining CD4 counts.**

- **The fraction of T_{EMRA} cells expressing IL-7Rα, IL-18Rα, or CD57 was also lower in HIV-infected individuals.**

* MandersPM. et al. PNAS (2005)
** Luckey CJ. et al. PNAS (2006)

Conclusions

- These data are consistent with the hypothesis that IL-7Rα^+ T_{CM} cells represent “true” memory $CD8^+$ T cells, the loss of which may be responsible in part for the progressive loss of T cell memory function during progressive HIV infection.

- Further exploration of these observations may provide a more complete understanding of the manner in which the $CD8^+$ T cell compartment is eroded, both numerically and functionally, as HIV disease advances.

Ladell K. et al. JI (2008) *in press*

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