A Dose Escalation Study of Cyclophosphamide (CTX) to Enhance SB-728-T Engraftment

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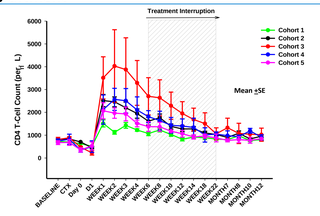
Sangamo BioSciences

SB-728-1101 Cytoxan Study Rationale

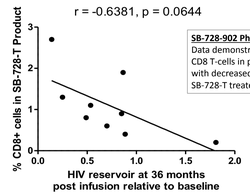
- **CCR5 is a major co-receptor for HIV entry**
  - Mutation produces a nonfunctional protein
    - Homozygotes are resistant to HIV infection
    - Heterozygotes have slower disease progression
  - Berlin Patient HIV-free off HAART for >5 years after $\Delta 32^{-/-}$ HSCT
- **Zinc Finger Nuclease enables the precise disruption of the CCR5 gene of autologous CD4+ T-cells (SB-728-T). Engraftment of biallelic modified CD4+ T-cells correlates with reduction in VL**
- **Cyclophosphamide has been used successfully to enhance adoptive T-cell transfer in oncology**
  - Homeostatic proliferation and production of T-cell growth factors
  - Elimination of regulatory T-cells
  - Expansion of dendritic cells
  - Create space in the bone marrow

Current study undertaken to increase SB-728-T engraftment

CTX at Doses up to 1 gm/m² Increases CD4 T-cell Engraftment

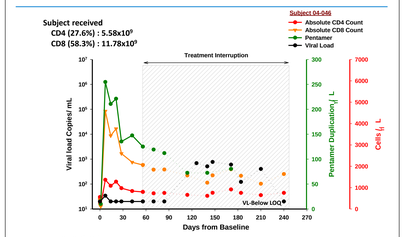


Rationale for Maintaining CD8 T-cells in SB-728-T



SB-728-902 Phase 1 Study: Cohorts 1-3
Data demonstrated that higher levels of CD8 T-cells in product were correlated with decreased Pro-viral HIV DNA in INR SB-728-T treated subjects at 36 months

Subject 04-046 (CTX 1000 mg/m² + CD8)



VL= Viral Load,
LOQ=Limit of Quantification (<20 Copies/mL)

SB-728-1101 Cytoxan Study Design

- **Open label, multicenter, cyclophosphamide dose escalation, with single dose SB-728-T infusion**
- **Study population**
  - Aviremic on stable HAART
  - R5 tropic virus
  - CD4 T-cells >500 cells/mm³
  - Platelets >200,000/mm³
  - PMN >2500 cells/mm³
- **Dose Escalation of CTX**
  - 100, 500, 1000, 1500 and 2000 mg/m²
- **Single infusion of SB-728-T**
  - 1.0 – 4.0 x 10^{10} cells
- **16-week ART treatment Interruption (TI) beginning 6 weeks after infusion of SB-728-T**
  - TI extended if VL<10,000 copies/mL and CD4>500 cells/mm³

CTX at Doses up to 1 gm/m² Increases CCR5 Modified Cell Engraftment

Activated CD8 and Low CCR5 are Associated with Elite Control in 92 Subjects (CR Ramirez et al. – CROI 2013)

"Important" variables that predict "elite" control (P < 0.00005)

Gag-Specific CD4+ T cells
- % 107+IFN+IL2+TNF+
- %IL21+ (higher)

Gag-Specific CD8+ T cells
- %107+IFN+IL2+TNF+ (higher)

} ↑HIV-specific T cells (poly-functional)

CD8+T cells
- % activated (CD38+DR+) total cells (higher)
- % activated (CD38+DR+) total, CM and TM that express CCR5 (lower)

} CD8 cells are activated, but they express low CCR5

CD4+ T cells
- % CM that are **CCR5**-CD38+HLADR+ (lower)
- % CM that express **CCR5**+CD38-HLADR- (higher)

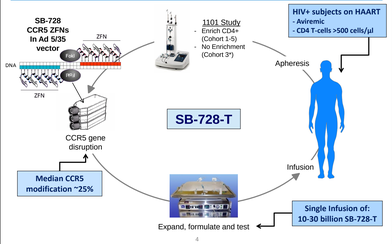
} Strong negative association between HLA-DR and CCR5

Overall misclassification rate was low (~10%)

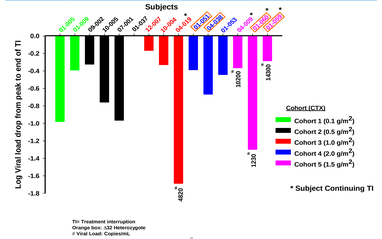
Subject 01-070 (CTX 1000 mg/m² + CD8)

VL= Viral Load

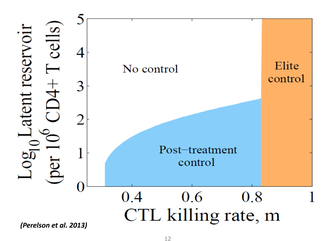
SB-728-T: Zinc Finger Nuclease Driven CCR5 Modified Autologous CD4⁺ T-cells



Viral Load Drop from Peak After CD4 SB-728-T Four Subjects Continue on Prolonged TI

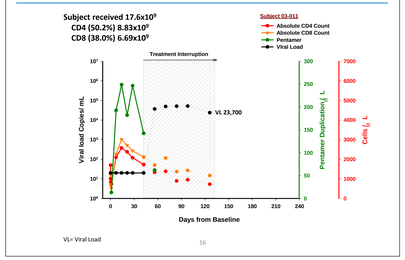


Post-Treatment Control Depends on Latent HIV Reservoir Size (L_0) and CTL Killing Rate (m)



(Perelson et al. 2013)

Subject 03-011(CTX 1000 mg/m² + CD8)



VL= Viral Load

SB-728-1101: Subject Demographics Treatment with SB-728-T Modified CD4 T-cells

Cohort	Sex	N	Race	Age* (yrs)	Years * HIV	Screen CD4* (per uL)	CTX* (mg)	SB-728-T* (billion)
1	M	3	2 CAU 1 BLACK	44 (45)	4 (3)	721 (696)	200 (200)	11.4 (12.5)
2	5M 1F	6	CAU	42 (43)	5 (4)	896 (868)	940 (938)	20.6 (16.8)
3	M	3	CAU	46 (38)	7 (8)	774 (827)	2077 (1930)	12.1 (10.1)
4	M	3	2 CAU 1 ASIAN	36 (31)	10 (12)	966 (870)	3880 (3800)	27.2 (29.7)
5	M	3	CAU	51 (50)	14 (12)	642 (819)	3163 (3600)	23.4 (23.5)

* Mean (Median)

SB-728-T Following CTX Administration is Safe

- **SB-728-T is safe and well tolerated**
  - Mild infusion reactions – low grade fever, chills
  - Garlic odor due to DMSO
- **Cytoxan at doses up to 1.0 gm/m² is safe and well tolerated in HIV infected patients**
  - No evidence of hematuria or hemorrhagic cystitis
  - No clinically significant effects on neutrophils, monocytes erythrocytes and platelets
  - Nausea and vomiting increases with increasing CTX dose
    - Prophylactic antiemetic is clearly needed
    - Aloxi/Emend regimen appears to be effective

Cohort 3* Subject Demographics CTX 1000 mg/m² + Modified CD4 and CD8 T-cells

Subj ID	Gender	Race	Age (yr)	Yrs HIV	Screen CD4 (per uL)	Screen CD4/8	CTX (mg)	SB-728-T Dose (x 10^9)
Cohort 3* (1000 mg/m² + CD8 Replete)								
04-046	M	Cauc	24	2011	531	1.72	1600	20.2
01-070	M	Am Indian	47	2007	721	0.90	2300	8.1
03-011	M	Cauc	50	1989	1423	1.39	1950	17.6

Summary and Conclusions

- **SB-728 CD4 T-cells after Cytoxan Conditioning**
  - Cytoxan conditioning at doses up to 1 gm/m² improves adoptive transfer of total CD4 T-cells and CCR5-modified CD4 T-cells with minimal toxicity
  - Eighteen subjects have been treated with Cytoxan and SB-728-T modified CD4 T-cells and four remain on long-term treatment interruption (40-71 weeks with VL ranging from 1,230-14,300 copies/mL)
- **SB-728 CD4/CD8 T-cells after Cytoxan Conditioning**
  - Three subjects have been treated with a CCR5 modified T-cell product that contains both CD4 and CD8 T-cells
    - CD8 repletion did not affect the safety profile
    - Three subjects have shown a doubling of their CD8 T-cells (2000-4000 cells/ul)
    - CCR5 modified CD4 and CD8 T-cells were high—reflecting expansion of CD8 T-cells
    - One subject has controlled VL during TI, another subject had delayed onset of viremia
- **The large increase in CCR5 modified CD8 T-cells may mimic elite controllers and may improve HIV control through CD8 HIV cytolytic T-cells**