# CBX-250 is a novel Cathepsin G peptide-HLA-targeting T cell engager that exhibits high tumor antigen selectivity and potent antileukemic activity in vivo

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# AML Remains a Disease with High Unmet Need

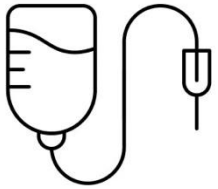
## Incidence

- Global incidence **>350k**
- Annual US incidence **~22k**
- Average age of AML patient: **68**

## Prognosis

- 5-year survival in US: **29.5%**
- >50% relapse after response
- Relapsed/refractory (R/R) setting has poor prognosis

## Available Treatments

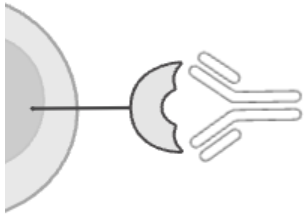


**Chemotherapy**

**Bone marrow/ stem cell transplant**

**Hypo-methylating agents** (e.g., aza)

**Small molecule inhibitors** (targeting e.g., BCL-2, FLT3, IDH1, IHD2)



**Biologics against surface proteins*** (targeting e.g., CD123, CD33, CLEC12A)

* Not yet approved, in development

**Need for potent therapies against novel tumor-selective targets in AML**

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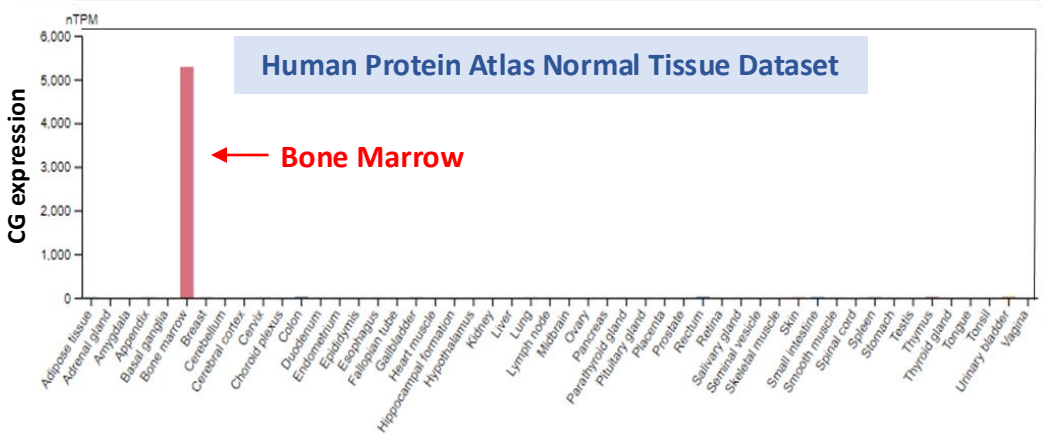
# CG1/HLA-A*02:01 is a Prevalent Tumor-Selective AML Target

## Cathepsin G (CG) Protein

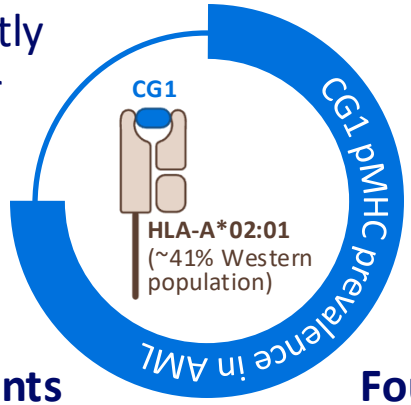



- Serine protease restricted to the myeloid lineage
- Role in inflammatory response to pathogens
- Stored in azurophilic granules in mature neutrophils
- Overexpressed in AML
  - Upregulated in primary AML blasts[1]
  - Overexpressed in LSCs vs normal HSCs[1]
  - Mis-localized in the cytoplasm within AML cells[1]

## CG1 pHLA identified in patients[1-4]

**CG1 (FLLPTGAEA)**
high affinity to HLA-A*02

**Signal peptide**
Processed efficiently
High copy number




**Graft-vs-leukemia antigen**
CG1-specific CTLs in AML patients post allo-SCT

**Identified in patients**
across FAB subtypes and cytogenetics

**Found in ~75% of HLA-A*02 AML patients**
via mass spec

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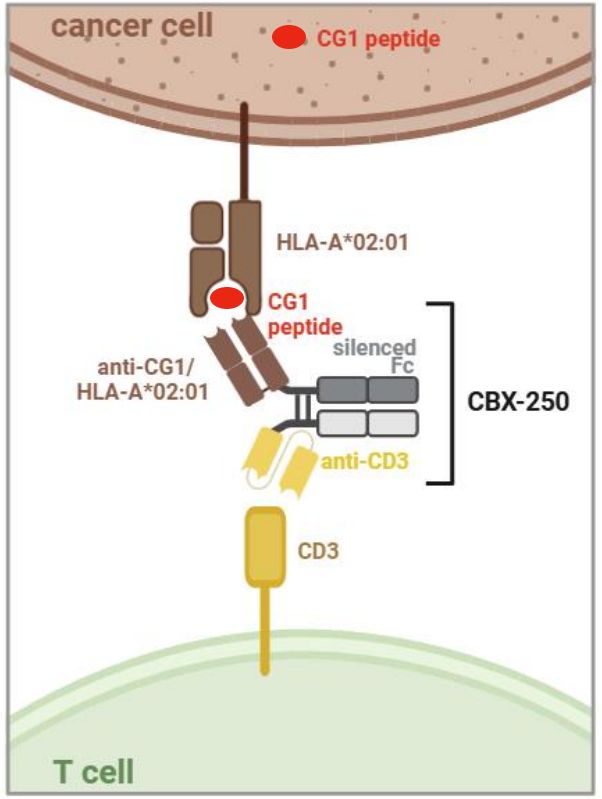
1. Zhang *et al.* CCR 2013; 2. Papadopoulos *et al.* Blood 1997; 3. Alatrash *et al.* Leukemia 2017; 4. Nelde *et al.* Blood Cancer Discov. 2023

**LSC**: leukemic stem cell; **HSC**: hematopoietic stem cell; **CTL**: cytotoxic T lymphocytes; **allo-SCT**: allogeneic stem cell transplant

# CBX-250: A Potent First-in-Class TCRm-TCE for Myeloid Malignancies

## CBX-250 Overview

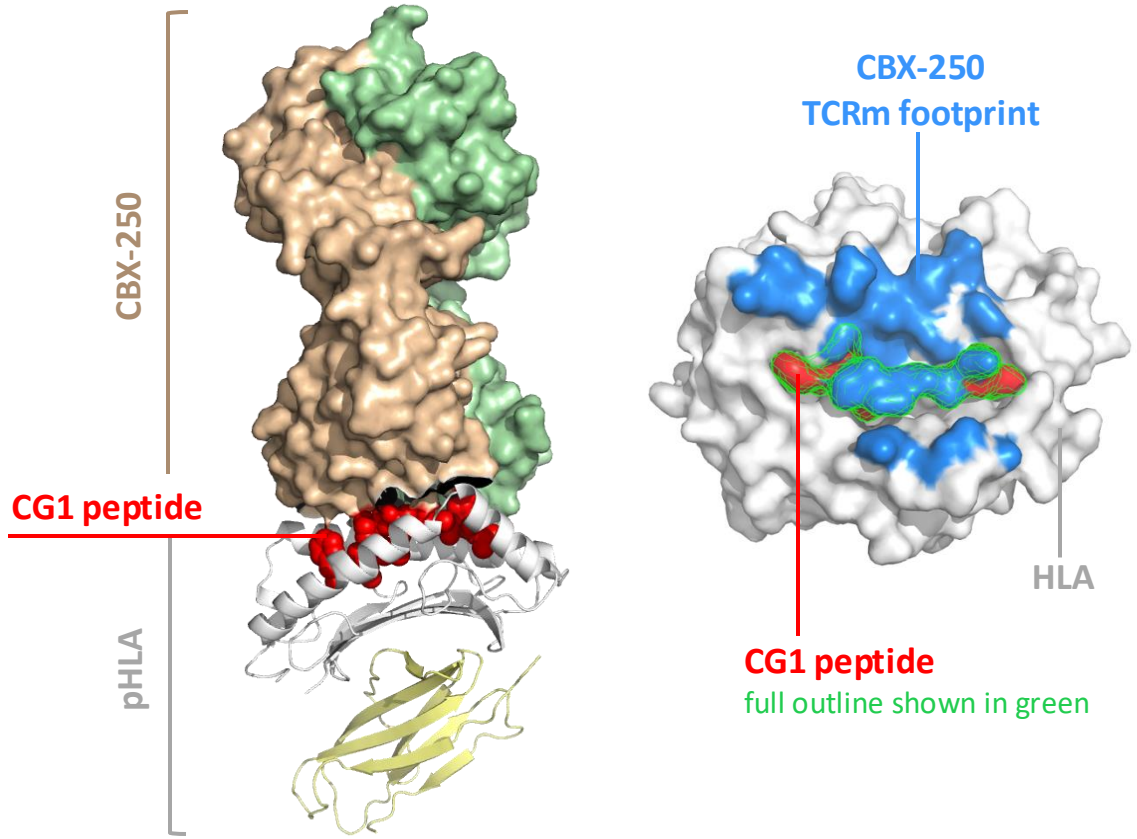
- TCR-mimetic (TCRm)-based TCE binding to CD3 and CG1/HLA-A*02:01 pMHC complex
- Sub-nM affinity for the CG1-HLA complex ~4-5x times stronger than binding to CD3




| Antigen | $k_{ON}$ ($M^{-1}s^{-1}$) | $k_{OFF}$ ($s^{-1}$) | $k_D$ (pM) |
|---|---|---|---|
| CG1-pHLA | 4.76 E+05 | 1.56 E-04 | 327 |
| CD3 | 2.65 E+05 | 3.87 E-04 | 1460 |

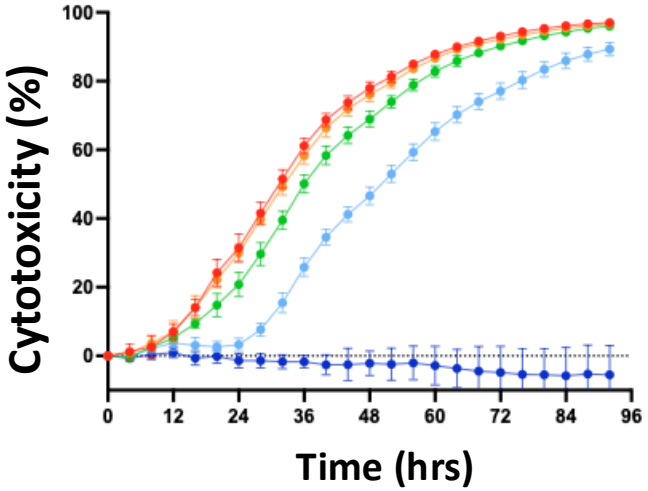
## CBX-250/pHLA Complex

**Cryo-EM structure of CBX-250/pHLA[1]**

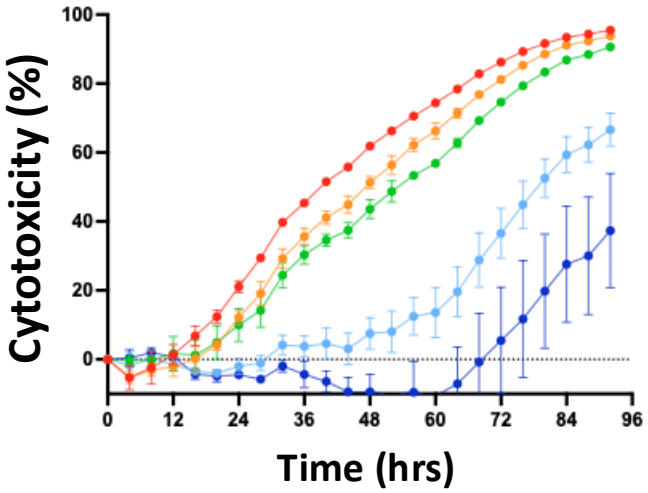



1. Structure of CBX-250 in complex with CG1 pHLA determined via cryo-EM with 4.3Å resolution (further refinement ongoing)

# CBX-250 Mediates Potent Killing of Leukemic Cells

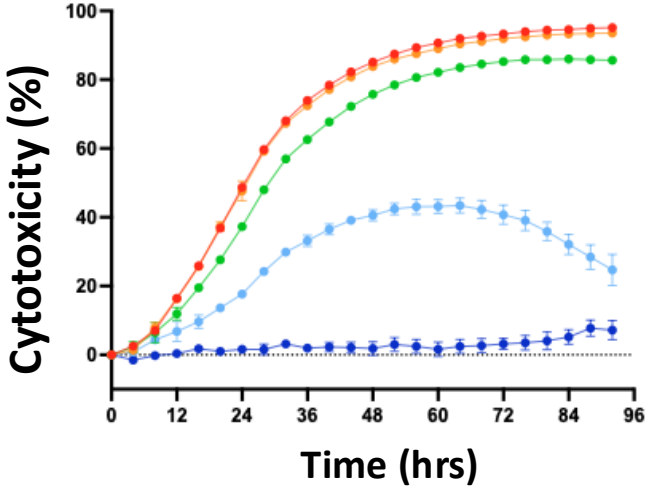



**ML2**
copy number ~3,700
E(PBMC):T=3:1
$EC50_{72hrs}$ = 0.34pM
$EC90_{72hrs}$ = 1.2pM

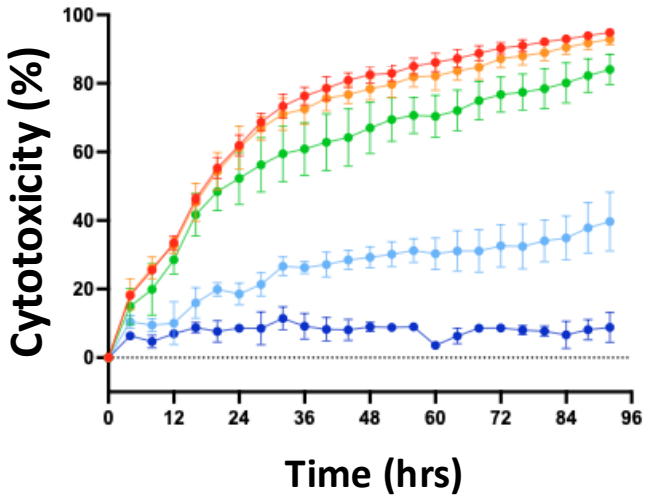



**EM2**
copy number ~950
E(PBMC):T=3:1
$EC50_{72hrs}$ = 1.9pM
$EC90_{72hrs}$ = 258pM

**CBX-250 []**
1 nM
100 pM
10 pM
1 pM
0.1 pM




**U937-A2**
copy number ~2,300
E(PBMC):T=3:1
$EC50_{72hrs}$ = 1.5pM
$EC90_{72hrs}$ = 16.5pM




**AML-14**
copy number ~750
E(PBMC):T=3:1
$EC50_{72hrs}$ = 2.4pM
$EC90_{72hrs}$ = 65.7pM

**Single digit EC50 and sub-nM EC90 across cell lines with varying CG1-HLA copy numbers**

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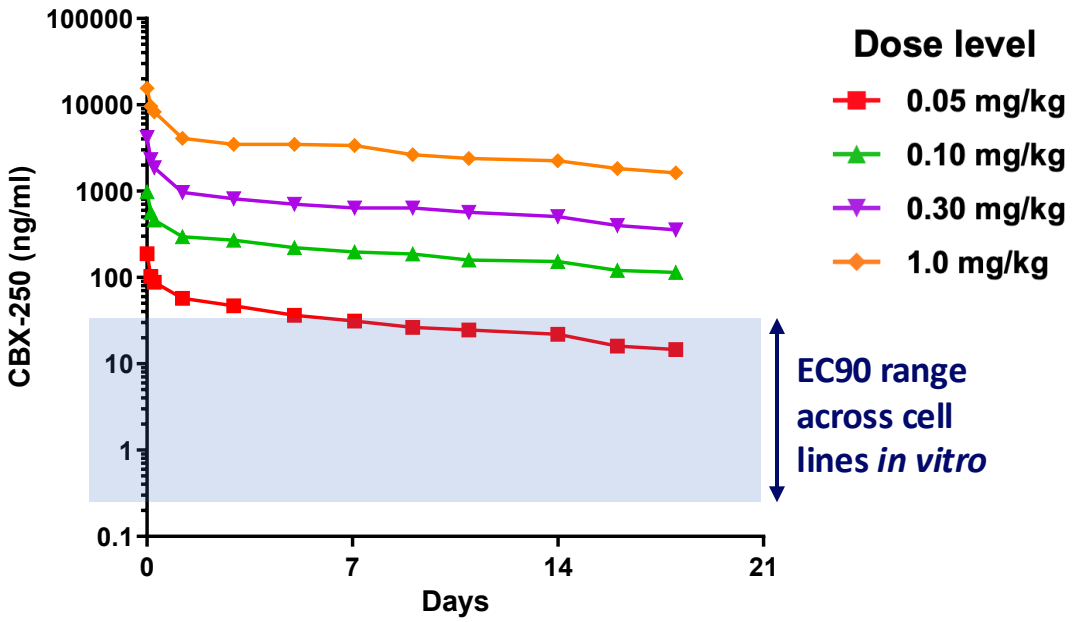
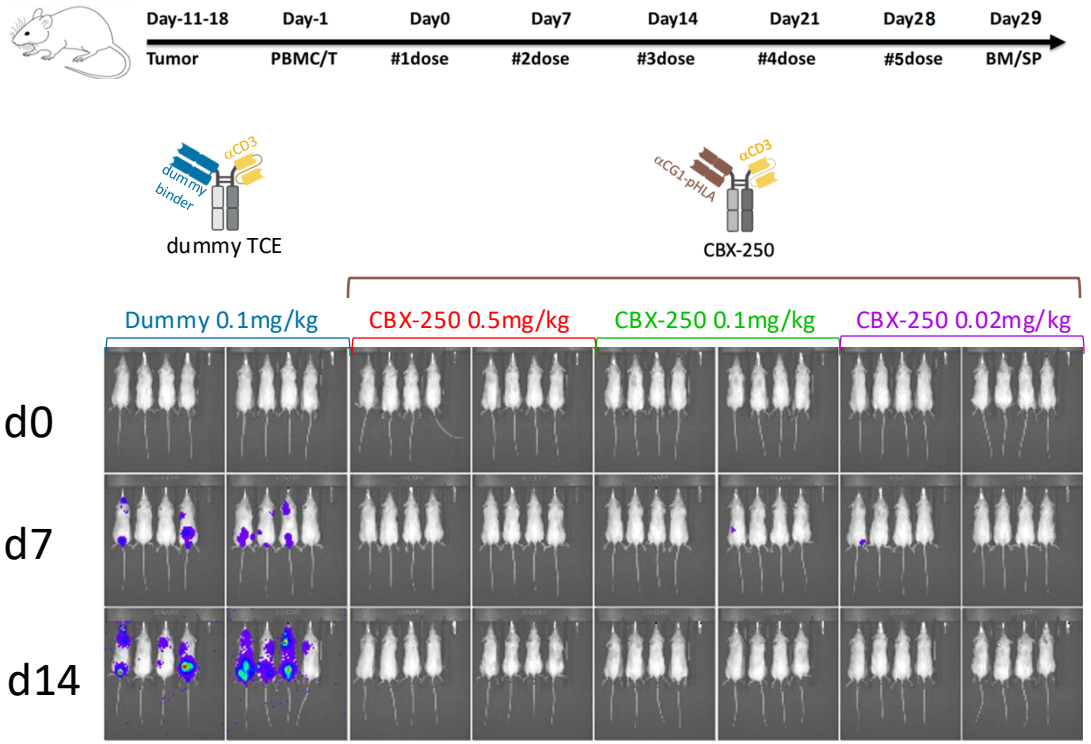
# CBX-250 Displays Long Half-Life and Potent Tumor Control *in vivo*

## Favorable PK profile in hFcRn mice




| | t½ (days) |
|---|---|
| 0.05 mg/kg | 9.04 |
| 0.1 mg/kg | 12.77 |
| 0.3 mg/kg | 12.90 |
| 1.0 mg/kg | 13.25 |

## Potent Tumor Control in AML CDX Model[1]

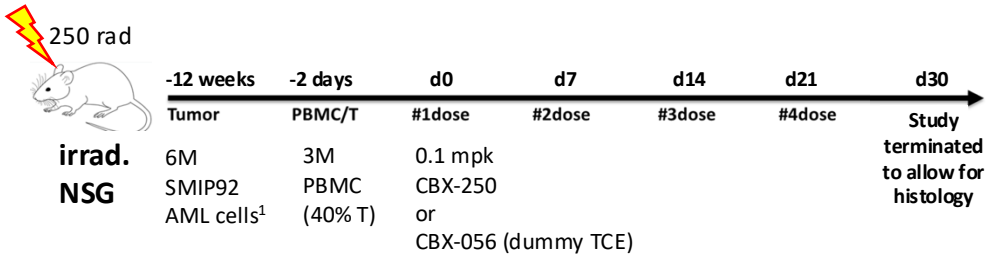


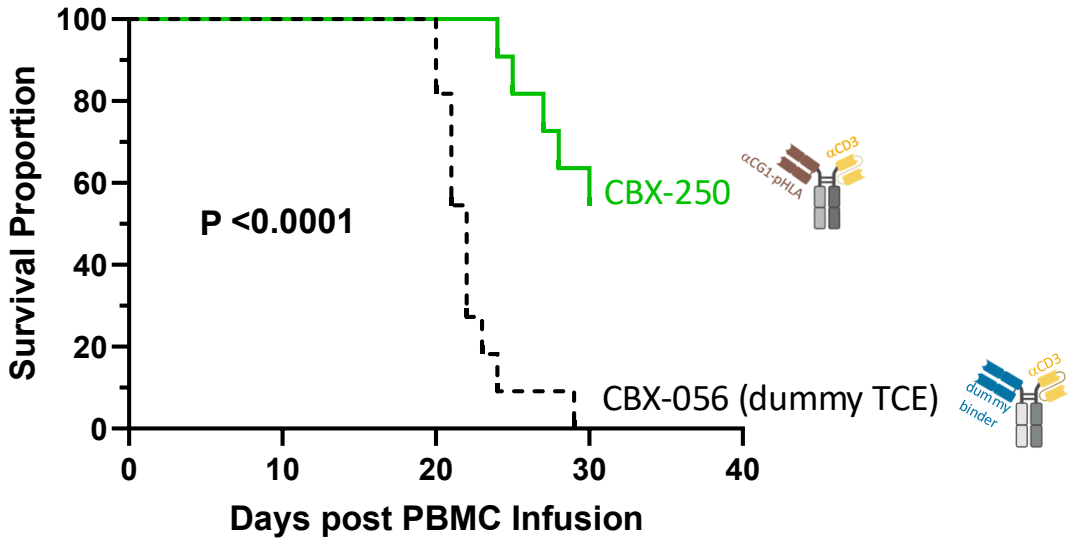

1. ML2 cell line-derived xenograft (CDX) murine model; Efficacy reproducible across 2 different PBMC donors; efficacy established with additional xenograft models

**hFcRn**: human neonatal Fc receptor; **t½**: half-life

# CBX-250 Shows Potent Efficacy in PDX *in vivo* model[1]

## Clear survival benefit

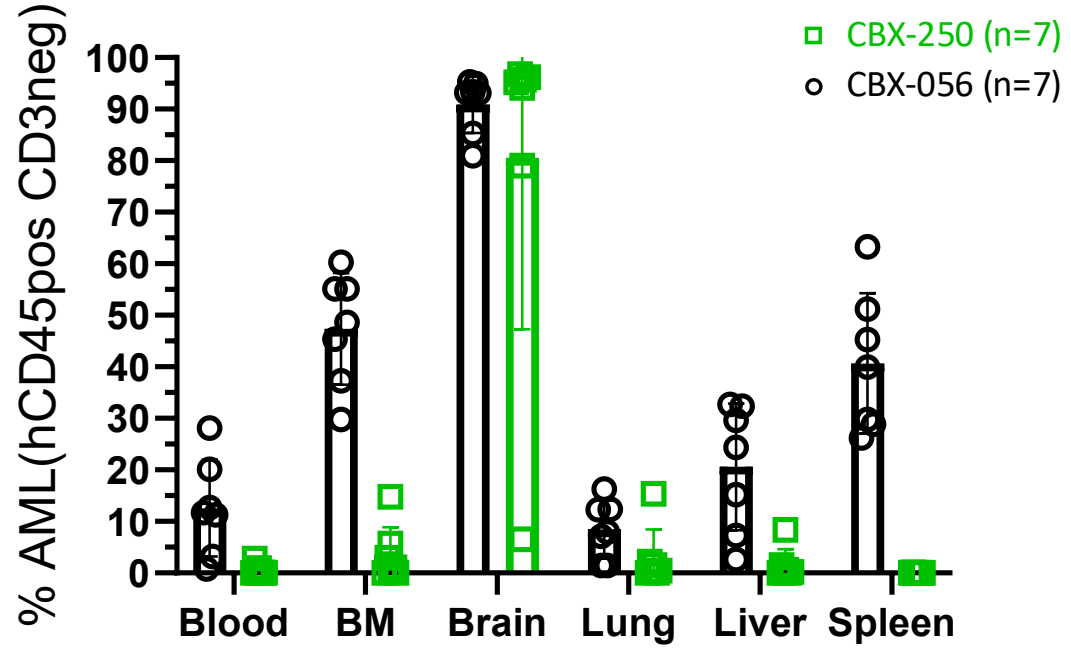



| | CBX-056 0.1mpk(n=11) | CBX-250 0.1mpk(n=11) |
|---|---|---|
| Median survival | 22 | Undefined |

## Significantly reduced leukemic burden

- CBX-250 reduces/eliminates AML in blood, bone marrow, lung, liver, spleen (not brain-penetrant[2])




1. AML cells from 63 yr old female, FLT3-ITD; DNMT3a; NPM1 mutations; >100k peripheral blasts

2. 0.6% involvement of Central Nervous System (CNS) at initial AML diagnosis (n=3,261); 2.9% CNS involvement at AML relapse (n=1,154) – Alalkel *et al.* Cancer Manag. Res. 2017

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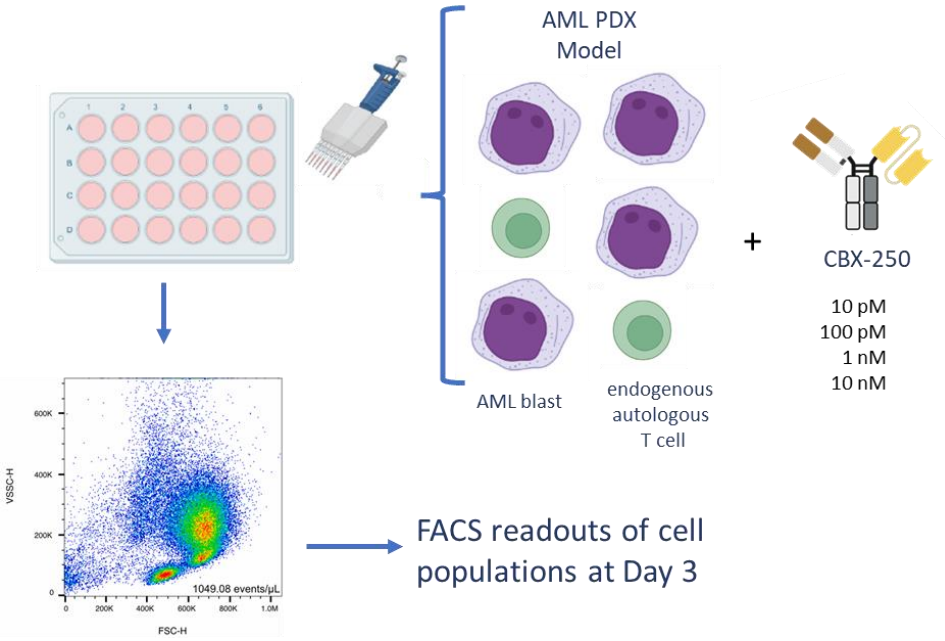
# CBX-250 *ex vivo* Efficacy Studies Using Primary AML Patient Samples

## Primary AML models

- Nine (9) HLA-A*02:01 positive AML models[1]
- Frozen primary AML samples using autologous patient T cells in each model as effector cells
- E:T ratios ranged from 1:18 to 1:165

| AML Model | Age | FAB classification/ WHO subtype | Cytogenetics | E:T ratio (CD3:total blasts) | Day 0 blast% | Noted Mutation(s) |
|---|---|---|---|---|---|---|
| CTG-2228 | 41 | M5 (monocytic; M5a and M5b) | Normal | 1:165 | 95 | FLT3-ITD, NPM |
| CTG-2235 | 66 | AML-MLD; prior MPN | 46, XY, del(20)(q11.2q13.1) [20] | 1:103 | 96 | N/A |
| CTG-2457 | 59 | NOS | 44, XY, der(3)t(3;11)(p21;q13), del(5)(q?15q?34), del(6)(q12), der(7)t(6;7)(q12;p15), -11, der(17)t(11;17)(p11.2;p11.2), -18 [11]/44, idem, -del(6)(q12), +i(6)(p10) [5]/44, idem, -Y, +i(Y)(q10), -del(6)(q12), +i(6)(p10) [4] | 1:28 | 92 | TP53 |
| CTG-2702 | 69 | M4 (myelomonocytic) | 46, X, -Y, +8 [13]/46, XY [2] | 1:113 | 89 | N/A |
| CTG-3440 | 80 | NOS | Normal | 1:140 | 96 | FLT3-ITD |
| CTG-3660 | 37 | AML with genetic abnormalities | 47, XX, inv(16)(p13.1q22),+22 [20] | 1:18 | 89 | N/A |
| CTG-3949 | 80 | NOS | Normal | 1:21 | 59 | FLT3-ITD, NPM |
| CTG-4025 | N/A | AML-MLD; prior MDS/MPN | Normal | 1:26 | 76 | FLT3-ITD, NPM |
| CTG-4051 | 69 | Not available | Not available | 1:45 | 95 | FLT3-ITD |

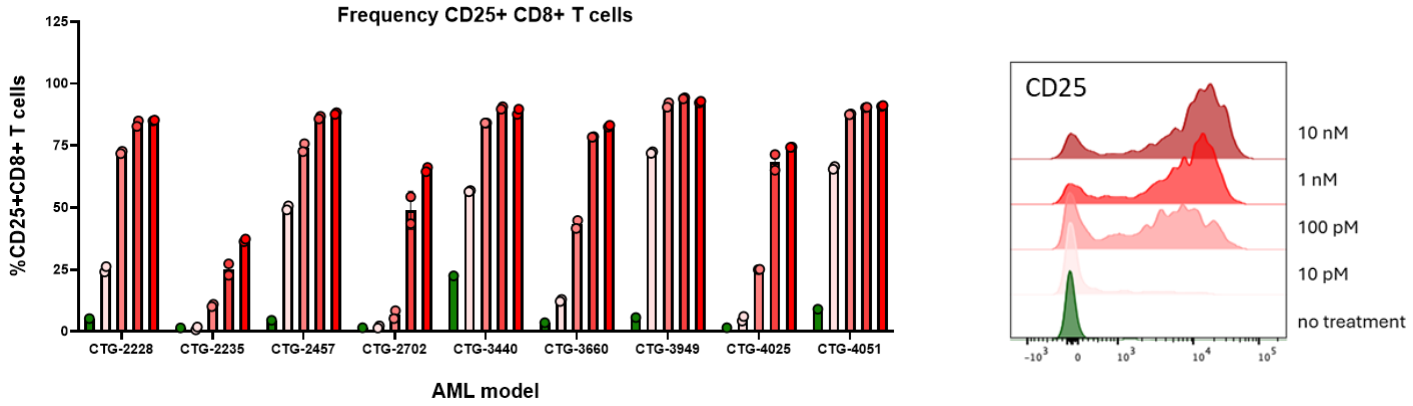
## Experimental design




1. Champions Oncology

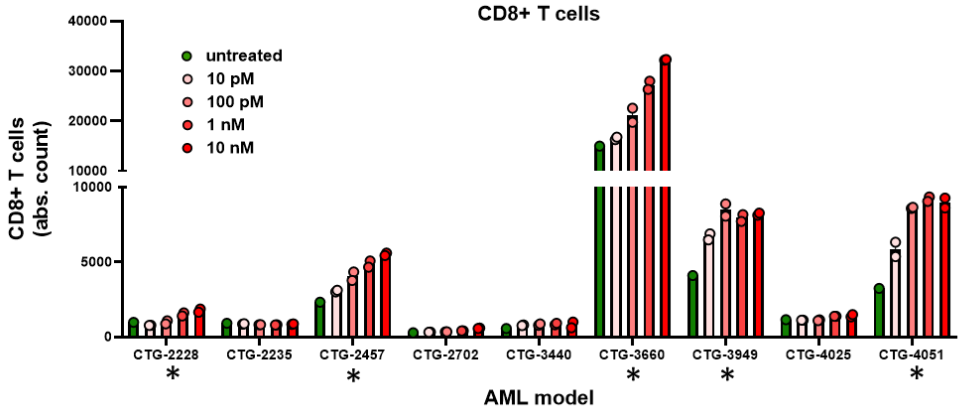
# CBX-250 Induces T cell Activation & Expansion and IFNγ Cytokine Levels

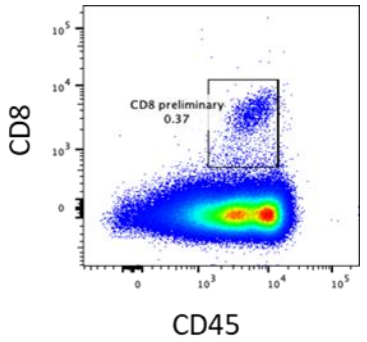
## T cell Activation & Expansion





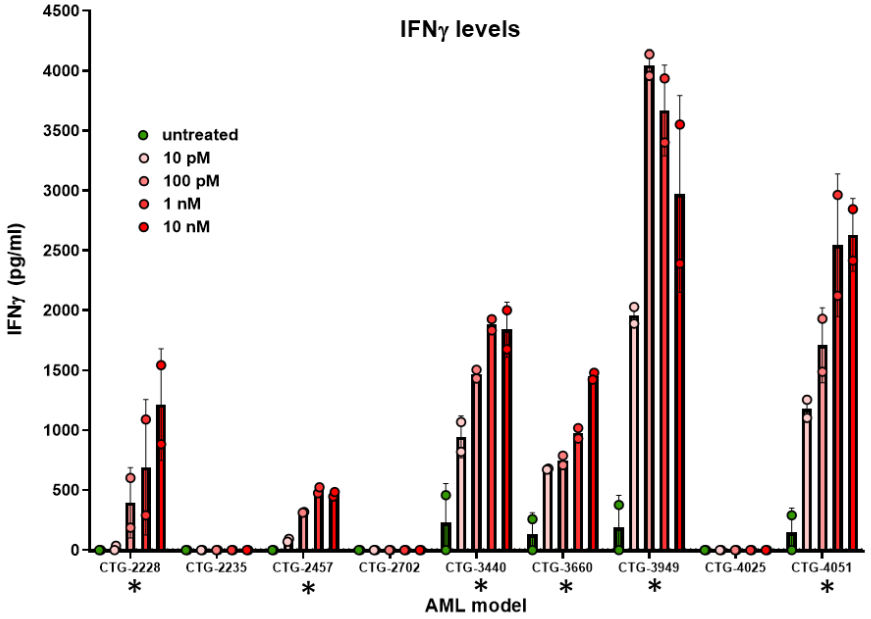





- Upregulation of CD25 on T cells observed in 9/9 models[1]
- Expansion of CD8+ T cells is observed in 5/9 models

## IFNγ



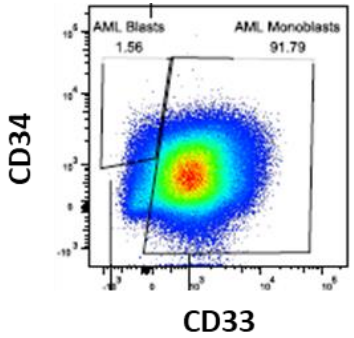

- IFNγ upregulation observed in 6/9 models in a dose-dependent manner

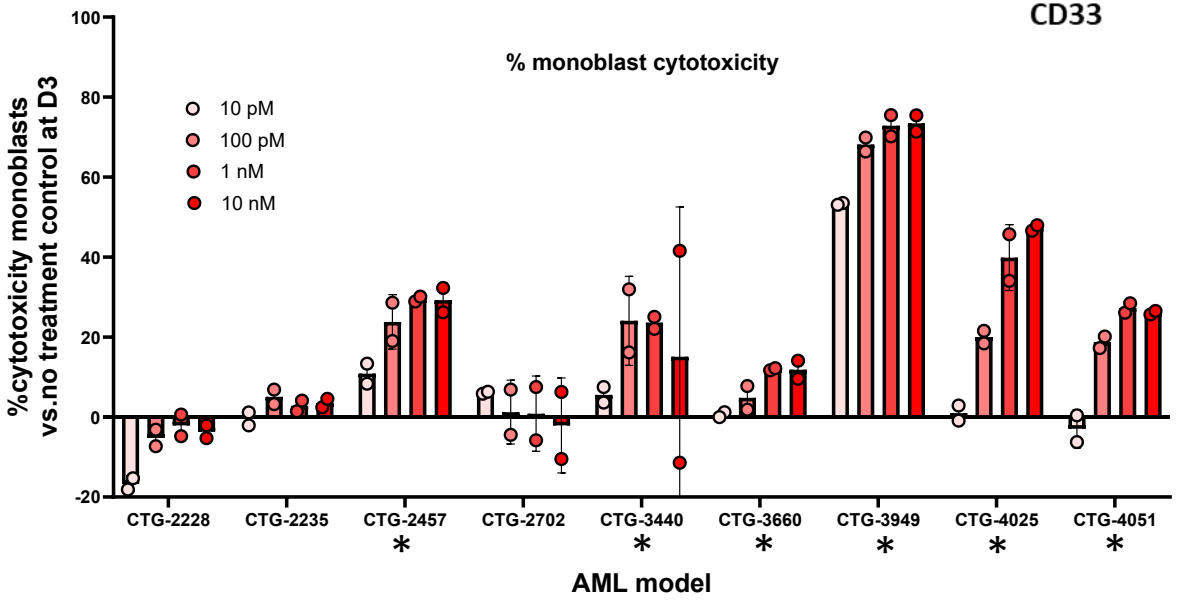
1. Upregulation of CD69 on T cells also observed in 6/9 models

# CBX-250 Dose-Dependent Cytotoxicity is Observed in AML Blasts and LSCs

## AML Blasts

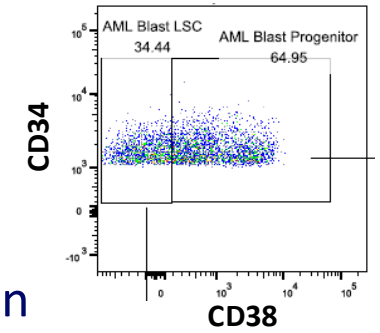
- 6/9 models exhibit CBX-250 dose-dependent cytotoxicity in AML monoblast populations

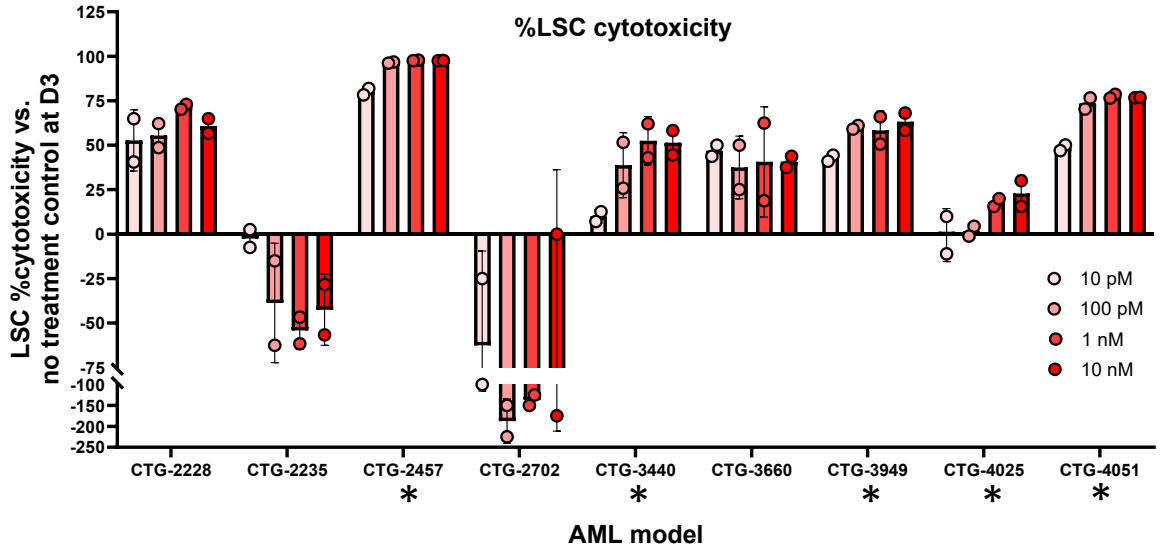



## Leukemic Stem Cells (LSCs)

- LSCs as defined by viable, CD45$^{lo}$, CD34+, CD33-, CD38- cell population
- LSC % cytotoxicity observed in 5 models which had sufficient LSC abs. cts./events for evaluation



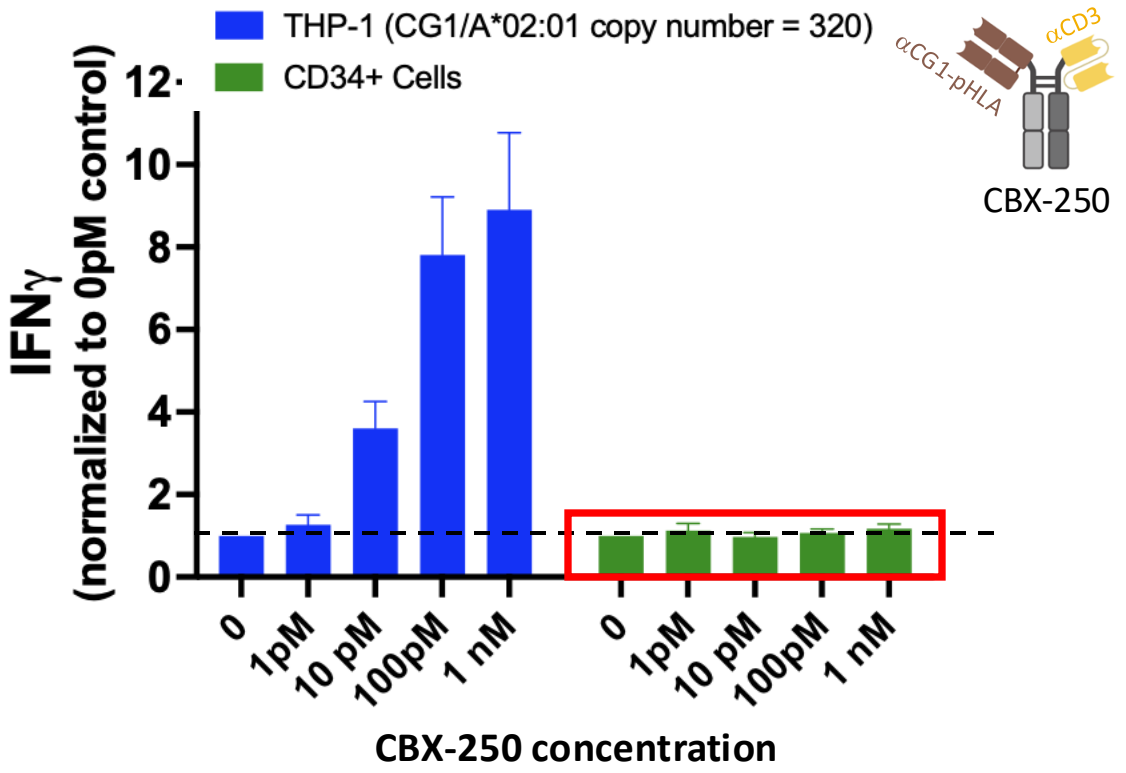




Models CTG-2228, CTG-2702, CTG-3660 with very low absolute cell counts/events (~<50 abs counts)
Models CTG-2235, CTG-3440, CTG-3949, CTG-4025 with absolute cell counts/events (~>150 abs counts)
Models CTG-2457, CTG-4051 with >~2000-80,000 and >~400-1800 absolute cell counts/events, respectively

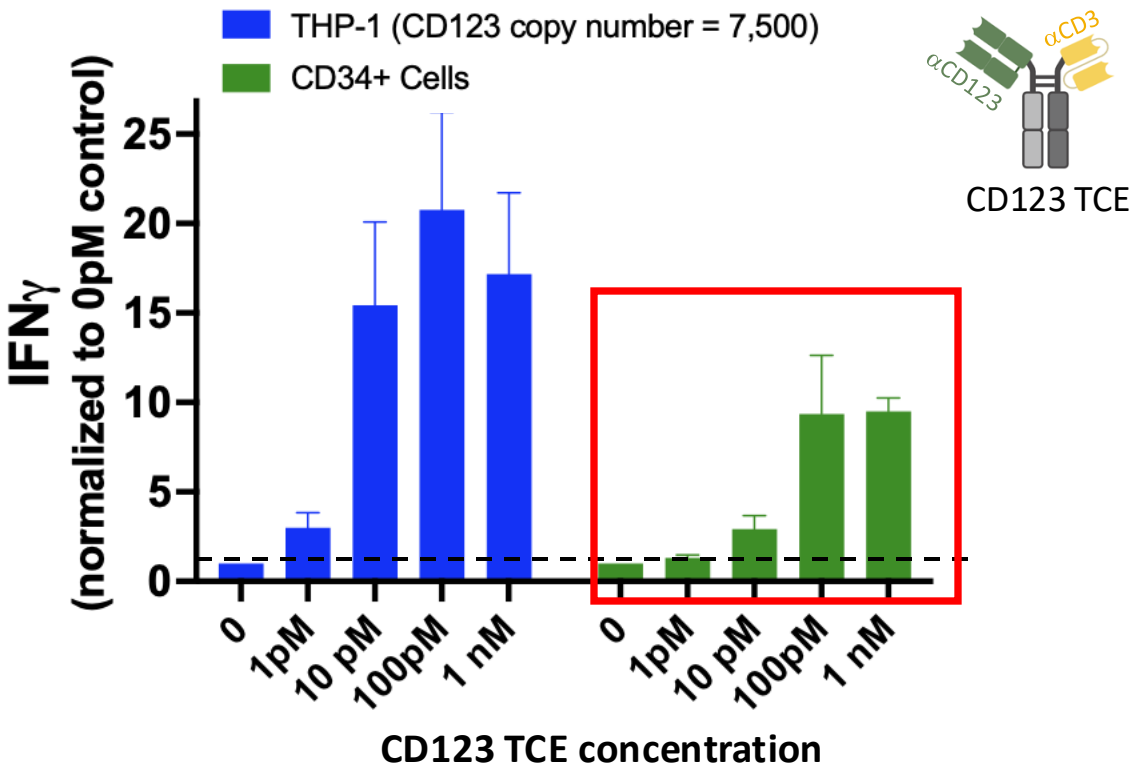
*CBX-250 responsive models

# CBX-250 Does Not Induce IFNγ Production in Normal Hematopoietic CD34+ Cells

## CBX-250




## CD123 TCE




- **CD123 TCE induces IFNγ release in normal hematopoietic CD34⁺ Cells**

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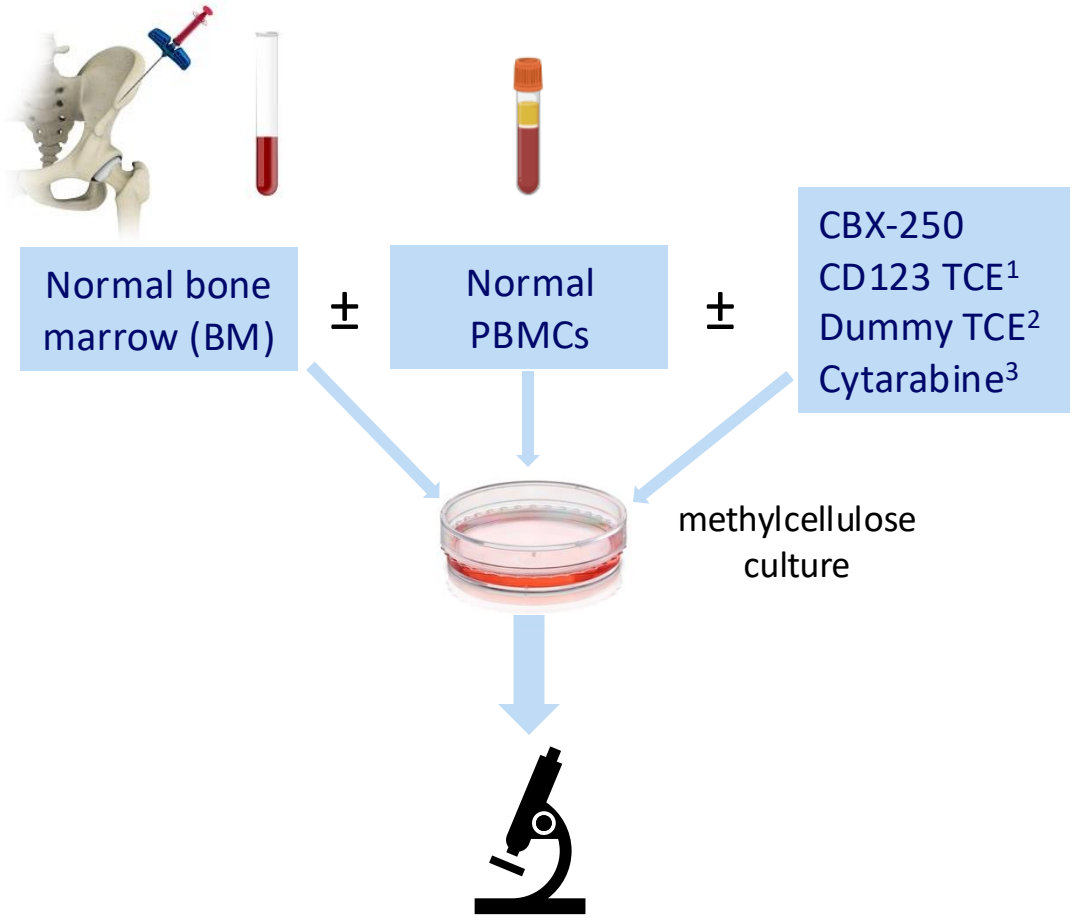
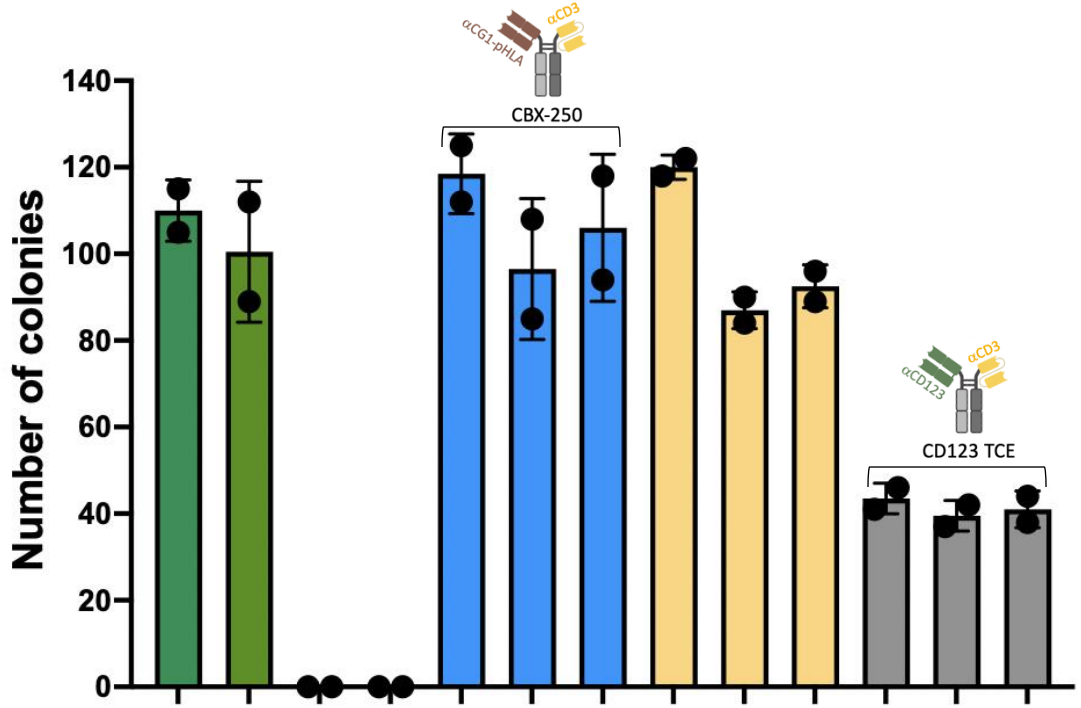
E (A02⁺ PBMC):T = 3:1

# Wide Therapeutic Index Anticipated Based on Bone Marrow CFU Assay

## Colony Formation Unit (CFU) assay design




## CBX-250 does not impair normal hematopoiesis




| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BM | + | + | + | - | + | + | + | + | + | + | + | + | + |
| PBMC | - | + | + | + | + | + | + | + | + | + | + | + | + |
| CBX-250 | - | - | - | - | 160nM | 80nM | 80pM | - | - | - | - | - | - |
| Dummy TCE[2] | - | - | - | - | - | - | - | 160nM | 80nM | 80pM | - | - | - |
| CD123 TCE[1] | - | - | - | - | - | - | - | - | - | - | 160nM | 80nM | 80pM |
| Cytarabine | - | - | 5 µg | - | - | - | - | - | - | | - | - | - |

1. CD123-TCE: tool compound targeting CD123 (same format as CBX-250)
2. Dummy TCE: negative control (same format as CBX-250)
3. Chemotherapeutic agent used as positive control

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# Conclusions

- CBX-250 is a TCRm-based T Cell Engager which targets a tumor-selective Cathepsin G (CG1) peptide HLA-A*02:01 complex
- CBX-250 displays potent killing of target-positive AML cancer cells both *in vitro* and *in vivo*
- CBX-250 induces bystander killing of neighboring target negative leukemia cells, a recognized feature of T cell engager bispecific Abs (data not shown)
- CBX-250 displays target specificity and tumor cell selectivity, suggestive of CG1/HLA's wide therapeutic index compared to other AML cell surface targets.
- IND-enabling studies are currently ongoing

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