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Characterization of CBX-250, a first-in-class TCR-mimetic-based T-cell engager targeting a Cathepsin G peptide-HLA complex for the treatment of myeloid leukemia

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Disclosure Information

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I have the following relevant financial relationships to disclose:

Employee of: Crossbow Therapeutics, Inc.

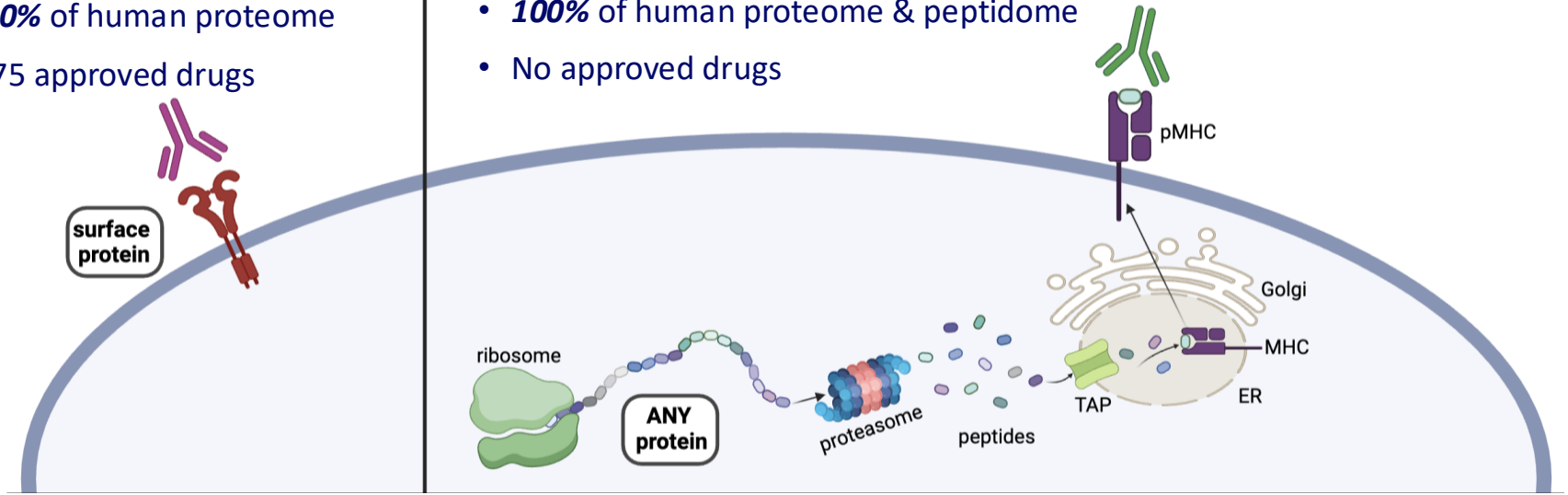
TCR-mimetics Expand the Reach of Antibodies to Intracellular Targets

Traditional antibody-based tx

- ***< 30%*** of human proteome
- ~175 approved drugs

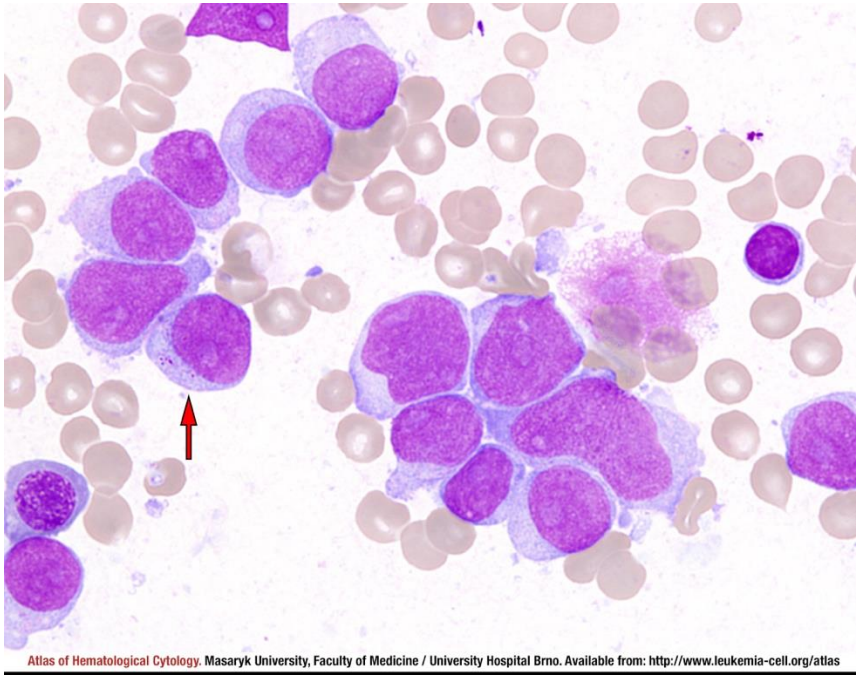
TCRm antibody-based therapeutics

- ***100%*** of human proteome & peptidome
- No approved drugs



Acute Myeloid Leukemia (AML) remains a Disease with High Unmet Need

- ~ 20K new cases and 11K deaths per year in the US; Average age at diagnosis: 68
- With conventional chemotherapy ~2/3 go into remission, 50% of those may be cured
- Allogeneic hematopoietic stem cell transplant increases cure rate through **graft-versus-leukemia** effect
- Targeted agents (e.g. FLT-3, IDH 1, IDH2) and BCL-2 inhibitor regimes increase survival but are not curative
- Antibody-based therapies target cell surface proteins not unique to cancer



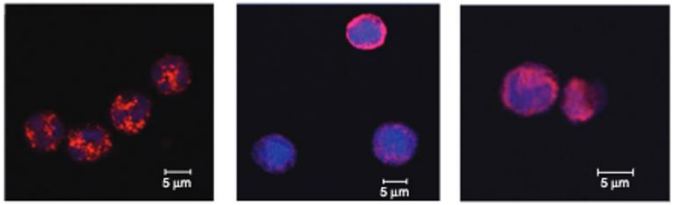
New immune based approaches against tumor selective targets are needed

Cathepsin G peptide-HLA (CG1-HLA) is a Tumor-Selective AML Target

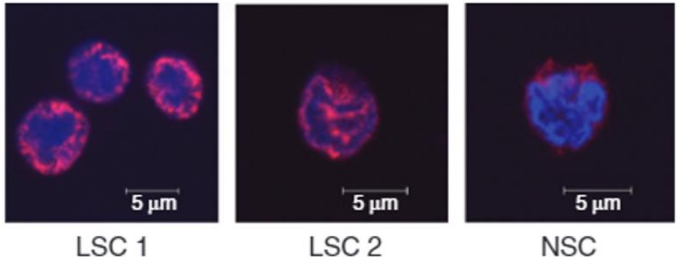
Protein is overexpressed and mis-localized

- In AML and other heme malignancies, Cathepsin G is
  - over-expressed in LSC, vs. in normal HSCs[1]
  - present in the cytoplasm vs. in normal granulocytes

Focal, granular vs. diffuse Cathepsin G localization in AML vs. normal granulocytes



Higher Cathepsin G expression in LSC vs. normal HSC



High copy number peptide identified

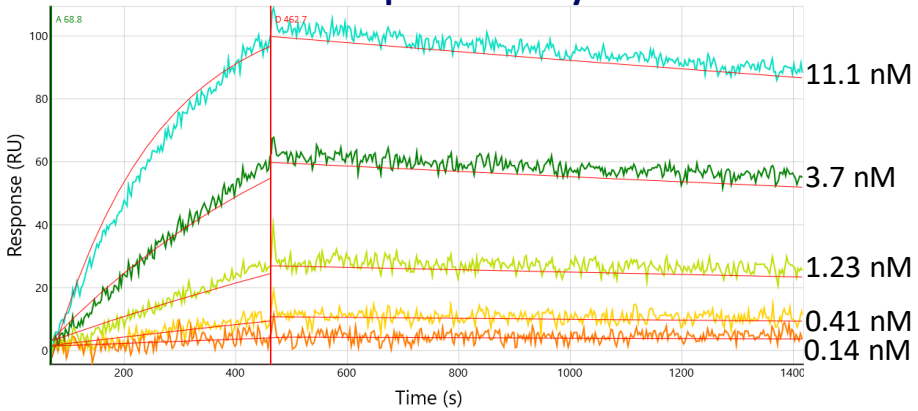
- CG1 = HLA-A*02:01-restricted Cathepsin G ***peptide***
  - CG1 binds HLA-A*02:01 with high affinity
  - Signal peptide – efficiently processed and presented
  - First identified with high copy number on the surface of CD34^{+} blasts of a CML patient[2]
  - Identified via mass spectrometry in AML patients with various cytogenetics and FAB subtypes[3,4]
  - Recognized by cytotoxic T lymphocytes (CTLs) in AML patients post allo-SCT[1]

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

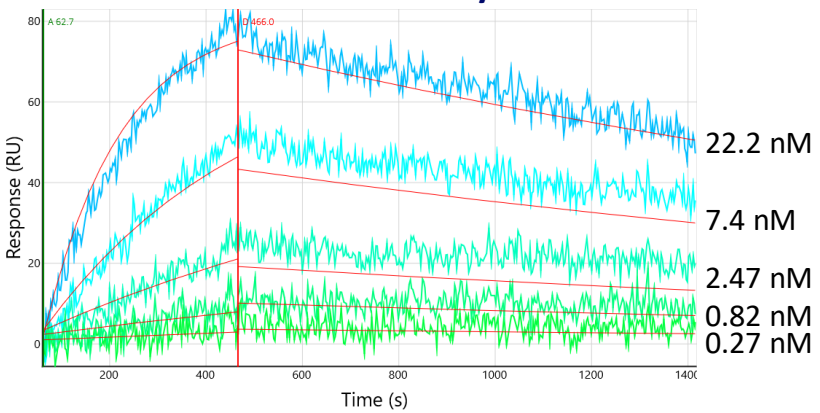
CBX-250: A Potent First-in-Class TCRm-TCE for Myeloid Malignancies

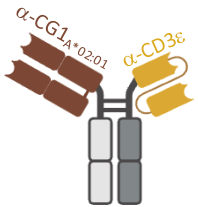
- CBX-250 is a TCR-mimetic (TCRm)-based T Cell Engager (TCE) binding to CD3 and $CG1_{A*02:01}$
- CBX-250 has sub-nM affinity for CG1-pHLA, and binds to the pHLA 4-5x more potently than to CD3

CG1-pHLA affinity



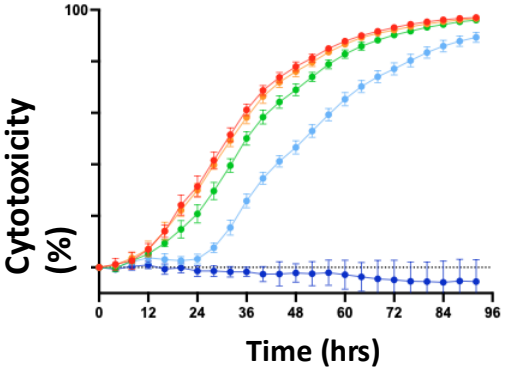
CD3 affinity



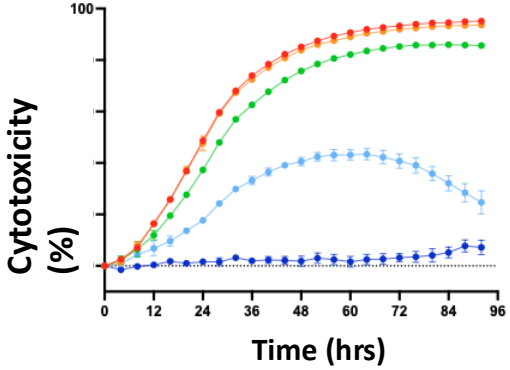


TCE	Antigen	pH	$k_{ON}(M^{-1}s^{-1})$	$k_{OFF}(s^{-1})$	k_D (pM)
CBX-250	CG1-pHLA	7.3	4.76 E+05	1.56 E-04	327
CBX-250	CD3	7.3	2.65 E+05	3.87 E-04	1460

CBX-250 Mediates Potent Killing of Leukemic Cells

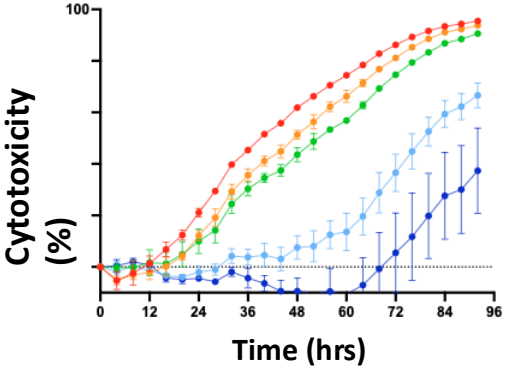


ML2
copy number ~3,700
E(PBMC):T=3:1
$EC50_{72hrs}$ = 0.34pM
$EC90_{72hrs}$ = 1.2pM

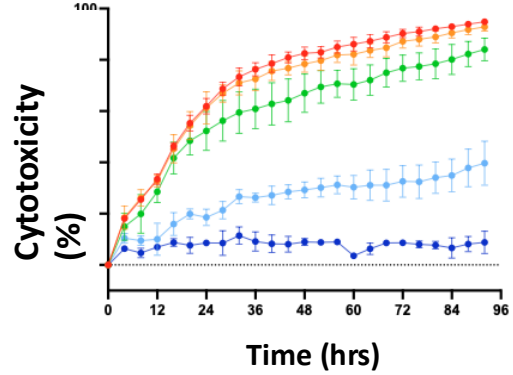


U937-A2
copy number ~2,300
E(PBMC):T=3:1
$EC50_{72hrs}$ = 1.5pM
$EC90_{72hrs}$ = 16.5pM

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



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

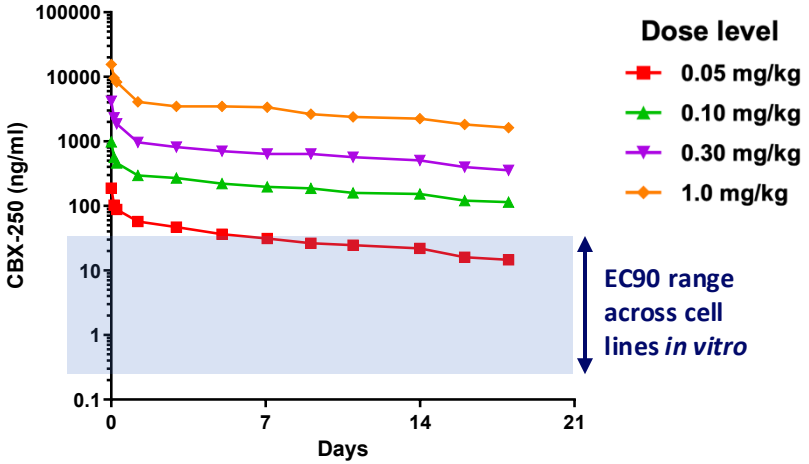


AML-14
copy number ~750
E(PBMC):T=3:1
$EC50_{72hrs}$ = 2.4pM
$EC90_{72hrs}$ = 65.7pM

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

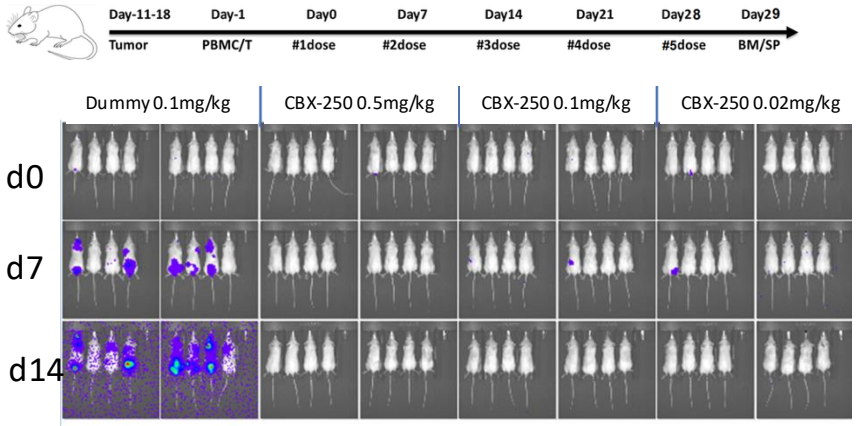
CBX-250 Displays Long Half-Life and Potent Tumor Control *in vivo*

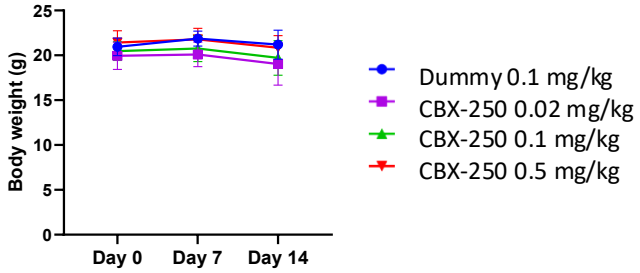
Favorable PK profile in hFcRn mice



	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]



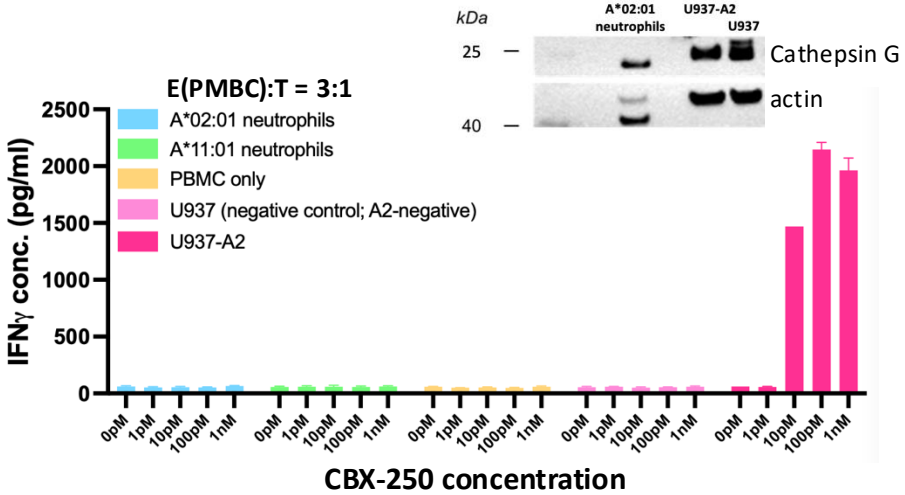


1. Efficacy reproducible across 2 different PBMC donors

CBX-250 Displays No Toxicity towards Normal Hematopoietic Cells

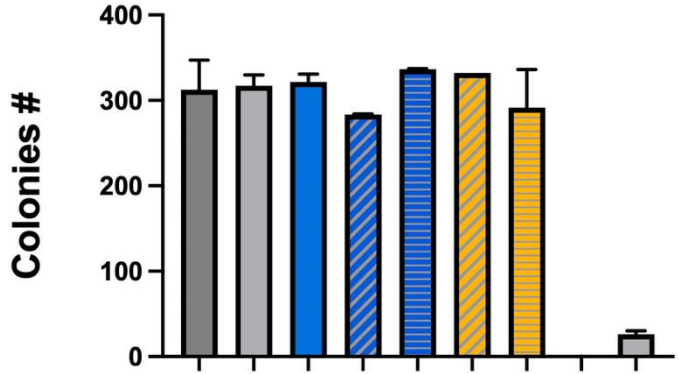
Neutrophils

- Cathepsin G ***protein*** is present in neutrophils, specifically stored in azurophilic granules[1]
- CBX-250 does not activate T Cells in the presence of neutrophils



Hematopoietic Progenitor Cells

- Cathepsin G is expressed in HSCs (less than in LSCs)[2]
- CBX-250 does not negatively impact normal hematopoiesis (CFU assay[3])

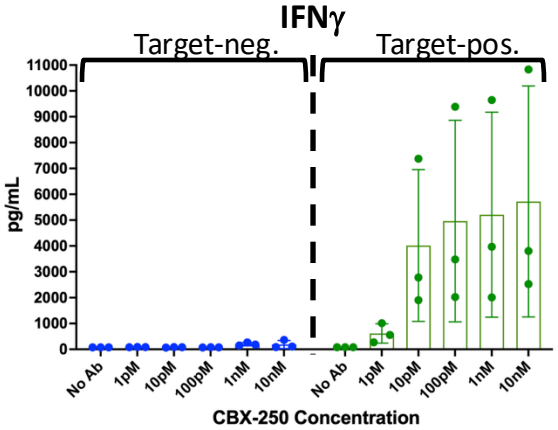


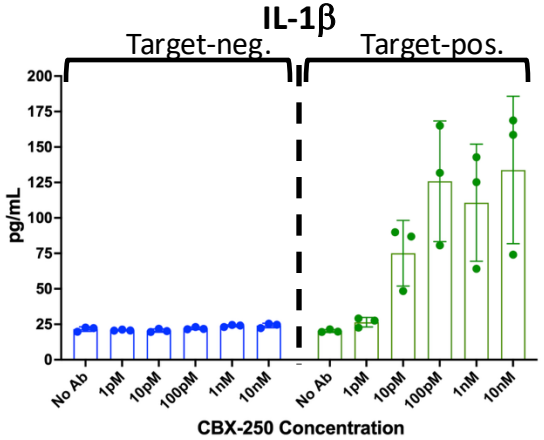
	1	2	3	4	5	6	7	8	9
BM	+	+	+	+	+	+	+	+	-
PBMC	-	+	+	+	+	+	+	-	+
CBX-250 (µg)	-	-	1	5	10	-	-	-	-
Isotype Ab (µg)	-	-	-	-	-	5	10	-	-
Cytarabine (µg)	-	-	-	-	-	-	-	5	-

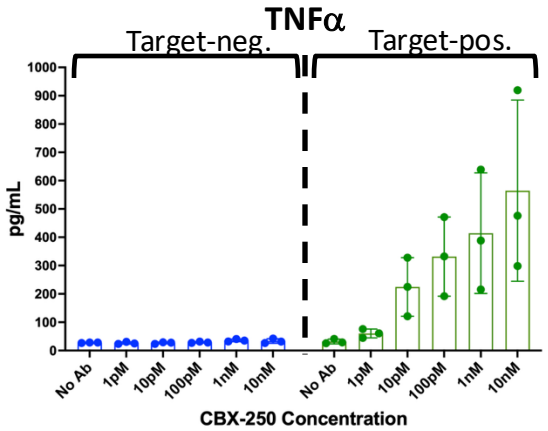
1. Gao *et al.* Arch Rheumatol. 2018; Aghdassi *et al.* Scientific Reports 2019; in-house data; 2. Zhang *et al.* CCR 2013; 3. Protocol described in *Alatrash et al.* Leukemia 2017, Shi *et al.* 2024 (in preparation)

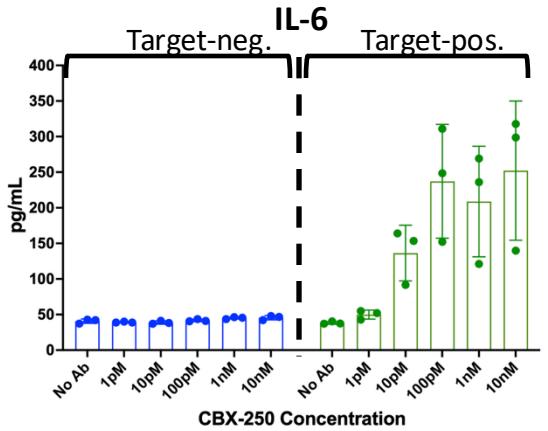
CBX-250 Does Not Induce Cytokine Release in Absence of Target-Positive Cells

- Tested dose-range of CBX-250 (1pm-10nM)
- E:T = 3:1
  - Effector cells: tested w/ 3 different HLA-A*02:01 PBMC donors
  - Target-negative cells: U937: positive for Cathepsin G, negative for HLA-A*02:01 (blue)
  - Target-positive cells: U937-A2: positive for Cathepsin G, positive for HLA-A*02:01 (green)
- Tested 8 different cytokines: IFNγ, IL-1β, IL-6, TNF-α, GM-CSF, IL-2, IL-8, IL-10 (shown 4)





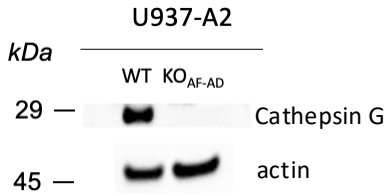


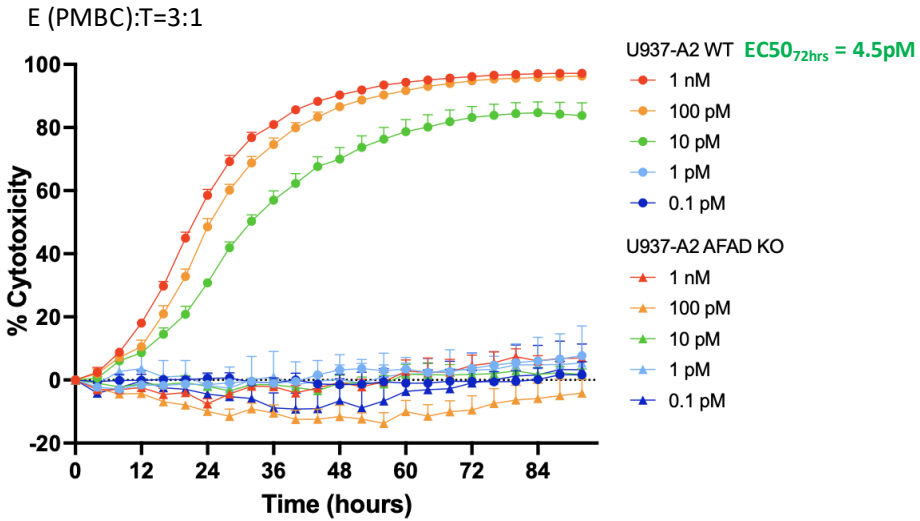


CBX-250 Does Not Kill Target-Negative Cancer Cell Lines

Cathepsin G KO cells

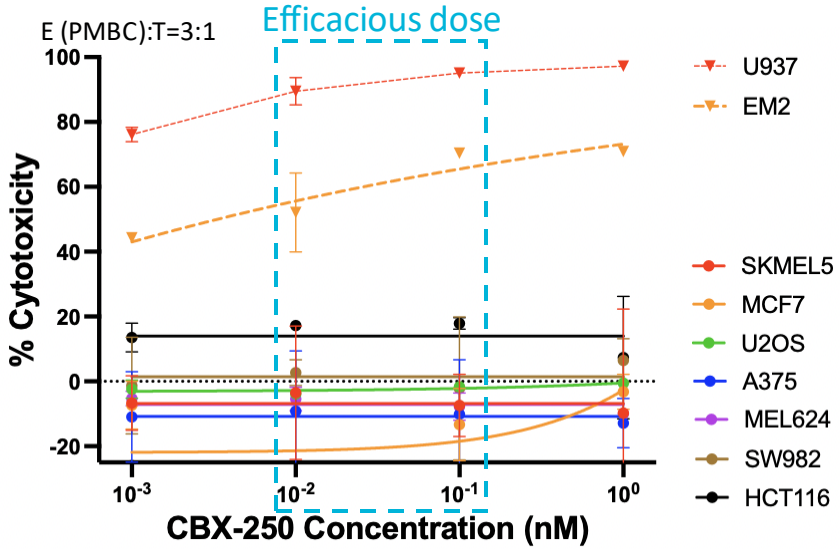
- No killing in Cathepsin G KO cells generated via CRISPR-Cas9





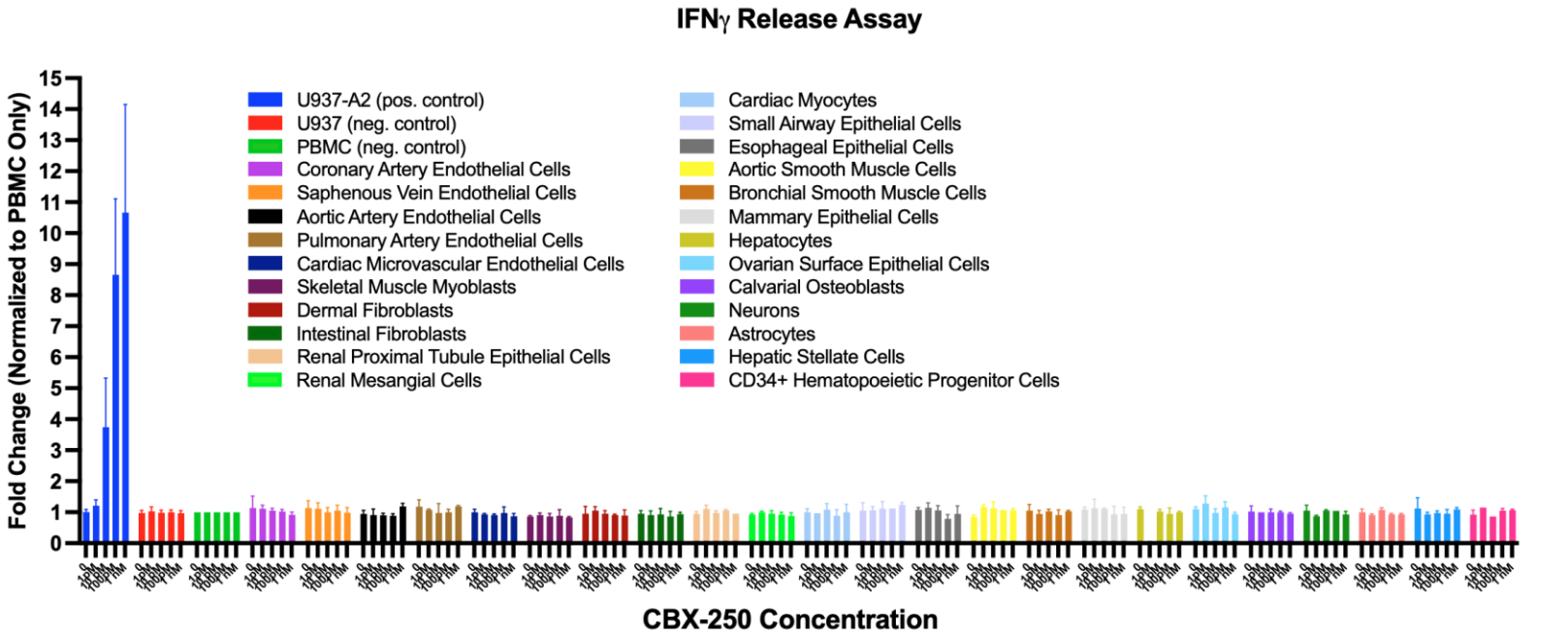
Solid tumor cell lines

- No killing of solid tumor A*02:01-positive solid tumor cell lines presumed to be Cathepsin G-negative



CBX-250 Has No Detectable Interactions with Normal Primary Tissues

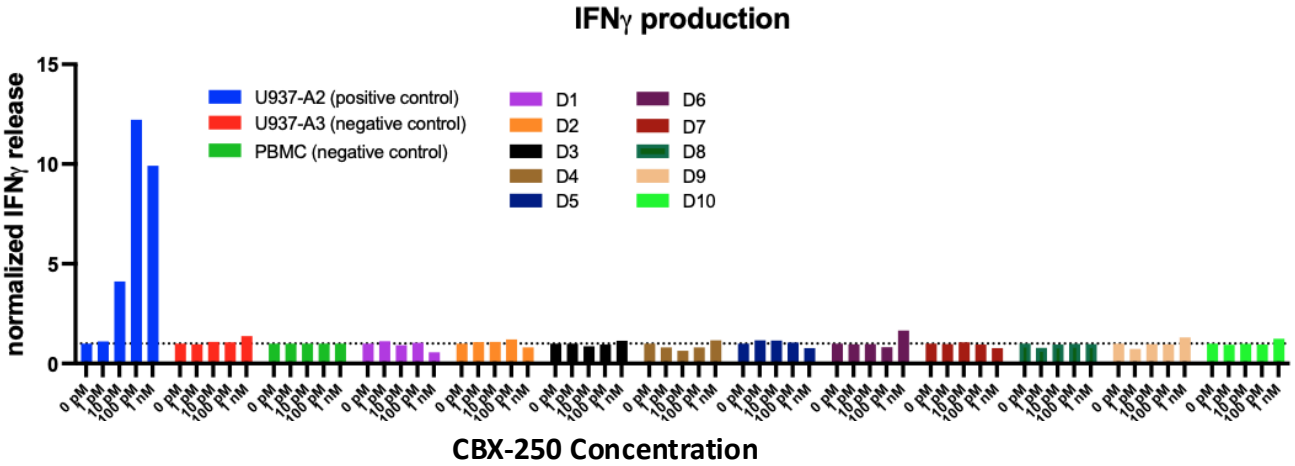
- Panel of 23 A02$^+$ ***normal primary*** cell types across vital organs + human PBMCs
  - T-cell activation (NFAT reporter, %CD69, %CD25) and IFNγ response in the presence of dose response of CBX-250



No T-cell activation or IFNγ secretion when CBX-250 is exposed to normal cells

CBX-250 Has No Detectable Cross-HLA Interactions

- Panel of 10 ***B-LCL*** cells selected covering 37 unique HLA haplotypes
  - All HLA-types which occur in over 5% of the US population included
  - All selected B-LCL cell lines are HLA-A*02:01 negative
  - T-cell activation and IFNγ response tested
  - CBX-250 dose response (1pM, 10pM, 100pM, 1nM)



No T-cell activation or IFNγ secretion when CBX-250 is exposed to non-HLA-A*02:01 cells

Conclusions

- Current immunotherapies in myeloid leukemia are focused on cancer cell surface antigens rarely unique to cancer cells
- CBX-250 is a TCRm-based T Cell Engager which targets a tumor-selective Cathepsin G peptide on HLA-A*02:01
- CBX-250 displays potent killing of target-positive cancer cells *in vitro* and potent tumor control *in vivo*
- CBX-250 displays target specificity, suggestive of a wide therapeutic index
- IND-enabling studies ongoing

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