Potentiation of Immunotherapy by LSD1 Modulation

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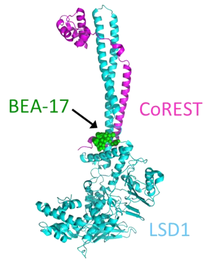
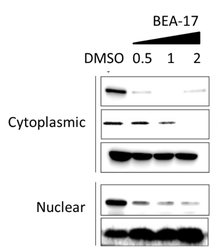
Abstract

LSD1 has emerged as a potential therapeutic target that increases the effectiveness of immunotherapy. We have developed a series of novel small molecules, exemplified by the lead substance BEA-17, that modulates LSD1 via binding to an allosteric site, without directly inhibiting its enzymatic activity. In cells, BEA-17 induces a reduction of LSD1 and its partner protein CoREST. In addition, BEA-17 upregulates the expression of endogenous retroviral genes and T cell-attractant chemokines and does so in an LSD1-dependent manner. In a co-culture of HeLa and PBMCs, BEA-17 potentiates the cell kill of cancer cells by cytotoxic T cells, also in an LSD1-dependent manner. In a CT26 syngeneic animal model of colon cancer, BEA-17 potentiates the activity of anti-PD1 inhibitors. Finally, in a syngeneic animal GL261 model of glioblastoma, BEA-17 increases the effectiveness of standard-of-care temozolomide + radiation.

BEA-17 Induced LSD1/CoREST Degradation



Allosteric binding site of BEA-17 near the LSD1/CoREST interface



Western blot analysis of LSD1 and CoREST in THP-1 cells exposed to BEA-17

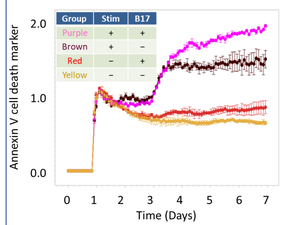
Degradation of LSD1 and CoREST is blocked by proteasome inhibitor bortezomib.

Gene Expression Profiling in HeLa Cells

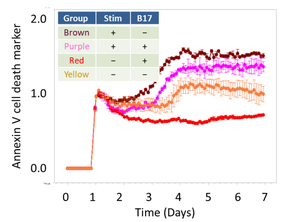
Pathway/Gene	WT		LSD1 KO		Conclusion
	Vehicle	BEA-17	Vehicle	BEA-17	
ERVV-2 (endogenous retrovirus gene)	—	↑	—	—	LSD1-**dependent** upregulation of ERVV-2 by BEA-17
Cholesterol metabolism	—	↑	—	↑	LSD1-**independent** upregulation of cholesterol biosynthesis genes by BEA-17
TGFβ pathway	—	—	↑	↑	LSD1 KO upregulates TGFβ pathway
SLC2A3 (GLUT3)	—	—	—	↑	Upregulation of SLC2A3 by BEA-17 in LSD1 KO (higher glucose demand in KO upon treatment)

Cell Kill Assay

- Co-culture of HeLa cells + human PBMCs (ratio 10:1)
- Monitor confluence of tumour cells (cell viability)
- Four conditions:
  - +/- stimulation (IL2/CD3)
  - +/- BEA-17



BEA-17 induces cell kill in wt cells

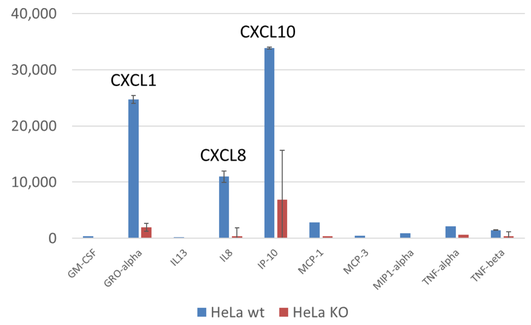


No induction in LSD1 KO cells

- Cell kill assay:
  - Dose-response viability/proliferation studies were performed on PBMCs from three different donors, using BEA-17.
  - One PBMC batch was chosen, based on the capacity to proliferate upon stimulation. The same batch was used throughout the study.
  - PBMCs were cultured in 24-well plates.
  - The tumor cells were seeded the day before addition of PBMC and drug.
  - Stimulation = treatment with anti-CD3 and IL2 (called Stim).
  - Supernatants were harvested at different timepoints (Day1 –Day6)

Cytokine Profiling of Cell Kill Supernatant

Quantification of cytokines and chemokines in supernatants from either HeLa wt or LSD1 KO cells and PBMCS in co-culture. Stimulated with the combination of IL-2 + anti-CD3 and BEA-17.



CT26 (Colon Cancer) Syngeneic Animal Study

Female BALB/c, mice 10 weeks old, 10 animals per group, received a subcutaneous injection of 3 x 10^5 CT26 cells in the right flank. The animals were randomized into six different treatment groups.

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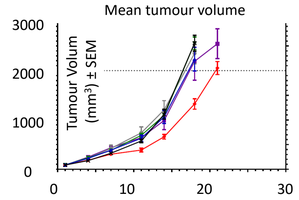
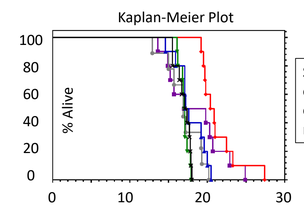


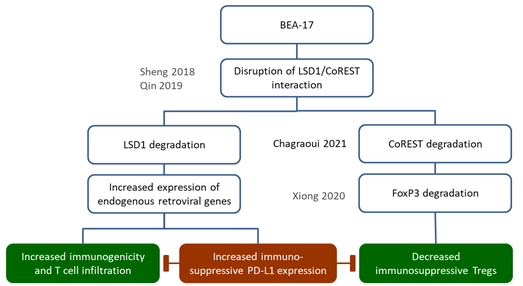
Only the combination of BEA-17 and anti-PD-1 achieved statistically significant (20%, $P < 0.001$) tumour growth delay compared to the control



Survival was significantly improved by combination of BEA-17 and anti-PD-1 compared to control ($P < 0.001$) and monotherapy anti-PD-1 ($P < 0.01$)

Gr.		Agent	Formulation dose	Route	Schedule
1		IgG2a//vehicle	20 mg/kg//N/A	ip//ip	biwk x 3//biwk x 3
2		anti-PD-1//vehicle	20 mg/kg//N/A	ip//ip	biwk x 3//biwk x 3
3		Rat IgG2a//BEA-17	20 mg/kg//25 mg/kg	ip//ip	biwk x 3//qd x 40
4		anti-PD-1//BEA-17	20 mg/kg//25 mg/kg	ip//ip	biwk x 3//qd x 40
5		IgG2a//GSK2879552	20 mg/kg//1.5 mg/kg	ip//po	biwk x 3//qd x 40
6		anti-PD-1//GSK2879552	20 mg/kg//1.5 mg/kg	ip//po	biwk x 3//qd x 40

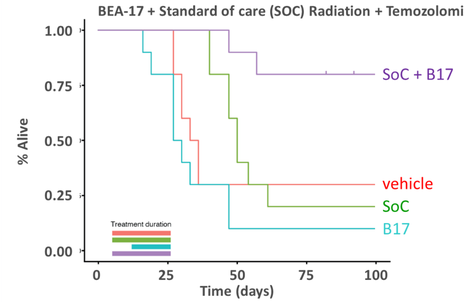
Proposed Mechanism of Action



- PD-L1 elevation explains why there is no stand-alone activity
- Must neutralize with checkpoint inhibitor to see an immunotherapeutic effect

GL261 (Glioblastoma) Syngeneic Animal Study

Female C57BL6, 6-12 weeks old (All mice of same age, age depends on treatment start), 10 animals per group, injected with 20,000 GL261 cells in the striatum. The treatment schedule started 5 days after injection of tumour cells. During the first week all mice were treated the same. The animals were then randomized to four different treatment groups.



	TMZ-25 RT	TMZ-25 RT	TMZ-25 RT	TMZ-25 RT	TMZ-25 RT	TMZ-25 RT	TMZ-25 RT
Days	1	2	3	4	5	6	7
	BEA-17 TMZ-50	BEA-17 TMZ-50	BEA-17 TMZ-50	BEA-17 TMZ-50	BEA-17 TMZ-50	BEA-17 TMZ-50	BEA-17 TMZ-50
Days	8	9	10	11	12	13	14
	BEA-17 TMZ-50	BEA-17 TMZ-50	BEA-17 TMZ-50	BEA-17 TMZ-50	BEA-17 TMZ-50	BEA-17 TMZ-50	BEA-17 TMZ-50
Days	15	16	17	18	19	20	21

RT External beam irradiation, 2 Gy
TMZ-25 I.P. Temozolomide, 25 mgs/kg b.w.
TMZ-50 I.P. Temozolomide, 50 mgs/kg b.w.
BEA-17 I.P. BEA-17, 20 mgs/kg b.w.

P-values from pairwise comparisons using Log-Rank (adj. p-value method: BH)

Group	vehicle	SoC
SoC	0.0698	-
BEA-17	0.1918	0.0053
Soc + BEA-17	0.0063	**0.0083**

Conclusions

- We have developed a novel small molecule BEA-17 that modulates LSD1 via an allosteric site inducing a reduction of cellular LSD1 and CoREST levels
- In an LSD1-dependent manner, BEA-17
  - Upregulates ERVV-2, an endogenous retroviral gene
  - In a co-culture of HeLa cells and PBMCs, induces cell kill
  - Increases the expression of T-cell attractant chemokines
- In syngeneic animals, BEA-17 potentiates the activity of
  - PD1 checkpoint inhibitors in a CT28 model of colon cancer
  - Standard of care (radiation + temozolomide) in a GL261 model of glioblastoma
- BEA-17 was well tolerated at 25 mg/kg daily IP dosing for 8 weeks

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