An image-based phenotypic screen identified the bromodomain of CBP/p300 as new cancer drug resistance target and enabled the development of the clinical candidate TT125-802

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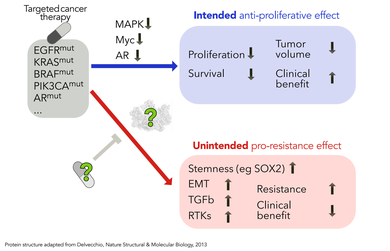
Poster #5891

A phenotypic drug screening and development platform

Problem

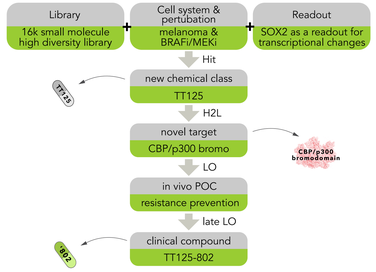
Targeted cancer therapies elicit their therapeutic effects by inhibition of proliferative and pro-survival mechanisms. At the same time, they have been reported to actively induce profound transcriptional reprogramming events that are associated with drug resistance in cancer (Boumahdi, 2020). For instance, inhibition of oncogenic MAPK signalling using a BRAF/MEK inhibitor combination in $BRAF^{V600E}$ mutated melanoma, triggers expression of the pluripotency transcription factor SOX2 along with broader expression of associated stemness and EMT genes (Benboubker, 2022). The master regulators governing transcriptional escape mechanisms to targeted cancer therapies are largely elusive.



Protein structure adapted from Delvecchio, Nature Structural & Molecular Biology, 2013

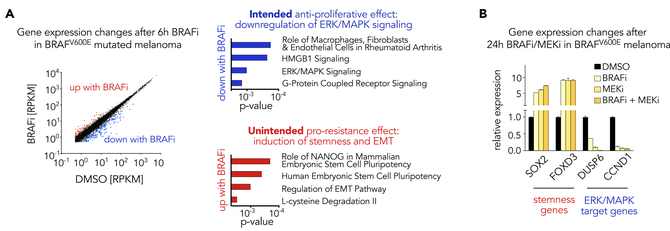
Approach and Solution

Leveraging dynamic transcriptional escape mechanisms to targeted cancer therapies, we developed a modular phenotypic screening approach that enables the identification of novel resistance modulators and the development of small molecule inhibitors against those new targets. We employed an automated high-throughput image-based screen using SOX2 as a marker for resistance-conferring transcriptional reprogramming. A battery of phenotypic assays was utilised to discover and develop the new chemical class TT125. De-orphanizing TT125 identified the bromodomain of CBP/p300 as novel target to prevent drug resistance to targeted cancer therapies.



1 Targeted cancer therapies induce transcriptional resistance mechanisms

Inhibition of oncoprotein $BRAF^{V600E}$ represses ERK/MAPK signaling target genes but at the same time induces resistance-conferring stemness and EMT

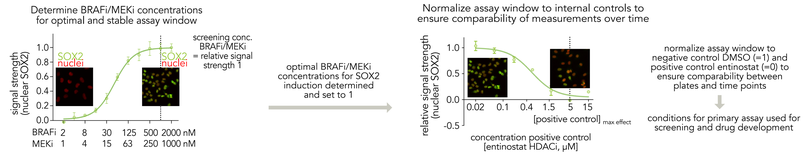


A RNA-seq of $BRAF^{V600E}$ mutated A375 melanoma cells 6h after treatment with BRAFi (1µM PLX4720). Enriched pathways ranked by p-value are shown for up- and down-regulated genes.

B Gene expression changes in $BRAF^{V600E}$ mutated A375 melanoma cells after 24h of treatment with BRAFi (1 µM PLX4720) and MEKi (0.5 µM AZD6244).

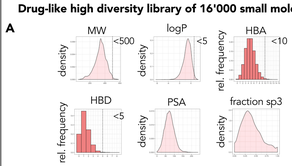
2 Phenotypic high-throughput screening assay to target transcriptional drug resistance

SOX2 immunofluorescence staining as a screening readout for broader cancer drug-induced transcriptional escape mechanism

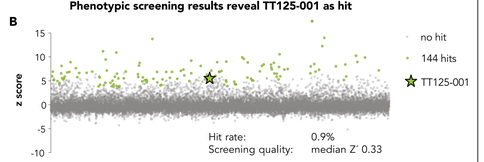


3 Small molecule library description and screening results

Drug-like high diversity library of 16'000 small molecules



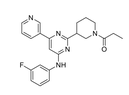
Phenotypic screening results reveal TT125-001 as hit



High-throughput screen using $BRAF^{V600E}$ mutated melanoma cells treated for 24h with BRAFi (1 µM PLX4720)/MEKi (0.5 µM AZD6244) (to induce transcriptional reprogramming) and 10 µM compound of a high diversity drug-like 16'000 small molecules library (to potentially prevent transcriptional reprogramming). **A** Characterization of the screening library **B** Z scores after applying edge and plate corrections using the median polish method Tuckey or subtracting a smooth polynomial using the loess function (Prummer; 2012).

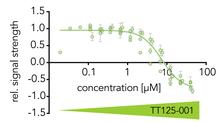
4 Screening hit TT125-001 prevents broad transcriptional reprogramming

Hit selected for H2L

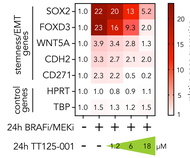


TT125-001
(racemic mixture)

TT125-001 represses drug-induced SOX2 in a concentration-dependent manner

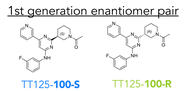


TT125-001 represses transcriptional induction of stemness/EMT genes



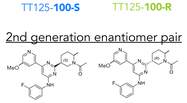
5 Battery of phenotypic assays drove stereospecific hit-to-lead phase of TT125 class

1st generation enantiomer pair



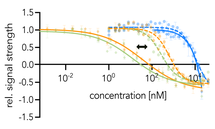
TT125-**100-S** TT125-**100-R**

2nd generation enantiomer pair



TT125-**300-S** TT125-**300-R**

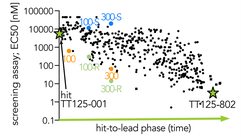
Primary phenotypic assay resolves activity of enantiomeric pairs



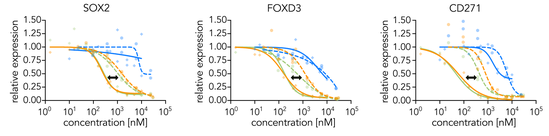
1st generation; EC50; nM	
100 (racemate)	630
100-R (active)	269
100-S (inactive)	>10000

2nd generation; EC50; nM	
300 (racemate)	13
300-R (active)	39
300-S (inactive)	>10000

Increasing potency of TT125 class during H2L phase

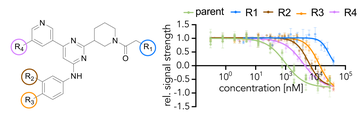


Orthogonal (qPCR) assay confirms stereoselective inhibition of stemness/EMT target genes

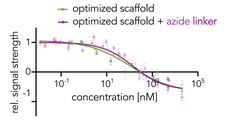


6 Target deconvolution: TT125 binds to bromodomain of transcriptional co-activators CBP/p300

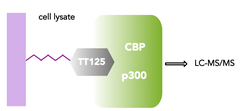
Linker position R4 delivers the most active derivatized probe



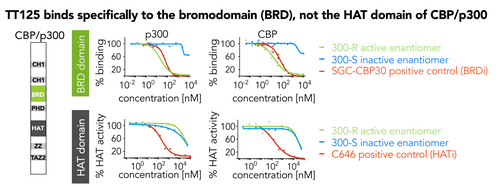
Final pulldown probe has nanomolar cellular potency



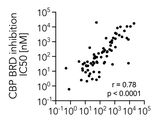
Chemoproteomics identifies CBP and p300 as targets of TT125



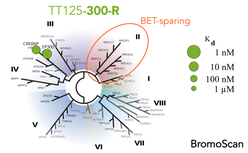
TT125 binds specifically to the bromodomain (BRD), not the HAT domain of CBP/p300



HTRF assay for CBP BRD binding correlates with primary assay

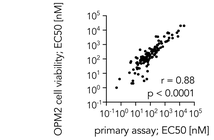


TT125 is highly selective for the bromodomains of CBP/p300 without binding to BET proteins

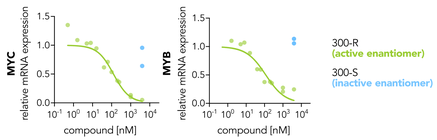


7 In vivo lead optimization was driven by a multiple myeloma tool system

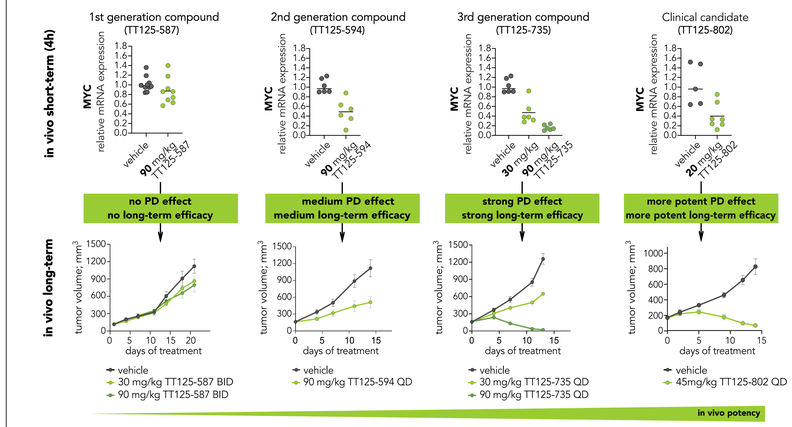
Primary assay correlates with activity on known CBP/p300 BRDi susceptible cell line



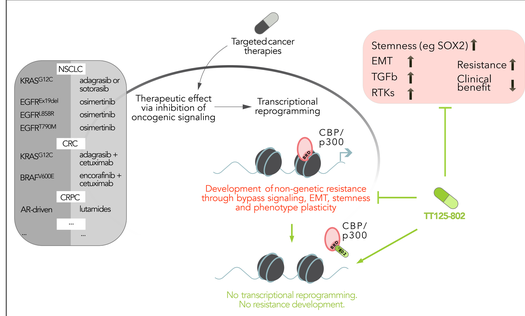
Confirmation of stereoselective inhibition of CBP/p300 target genes after 6 hours of treatment



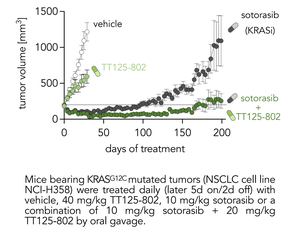
Transcriptional in vivo PD readout in multiple myeloma xenograft tumors drove lead optimization and translates into efficacy



8 TT125-802 is a versatile clinical candidate to prevent drug resistance to multiple targeted cancer therapies in solid tumor



TT125-802 prevents resistance development $KRAS^{G12C}$ lung cancer xenograft



Mice bearing $KRAS^{G12C}$ mutated tumors (NSCLC cell line NCI-H358) were treated daily (later 5d on/2d off) with vehicle, 40 mg/kg TT125-802, 10 mg/kg sotorasib or a combination of 10 mg/kg sotorasib + 20 mg/kg TT125-802 by oral gavage.

9 Conclusions: a unique strategy to drug development produces a versatile clinical molecule

Conclusions

- Transcriptional reprogramming is an important driver of drug resistance to targeted cancer therapies in the clinic.
- Inhibiting the bromodomain of CBP/p300 is a thus far unexploited strategy to address transcriptional resistance mechanisms.
- TT125-802 is currently in clinical trials. It will be developed as a combination partner for multiple targeted cancer therapies to prevent resistance development and increase clinical benefit for patients.
- We utilized the complex biology underlying transcriptional drug resistance to guide drug screening and development programs. The development of TT125-802 validates this strategy as a versatile approach to deliver new chemical scaffolds and place drug targets in a novel clinical disease context.

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