Targeting adaptive resistance to EGFR and KRAS G12C inhibitors by TT125-802, a novel and specific CBP/p300 bromodomain inhibitor

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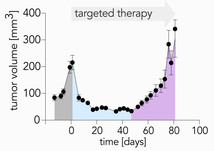
Poster #3907

Adaptive transcriptional changes lay the foundation to long-term cancer therapy failure

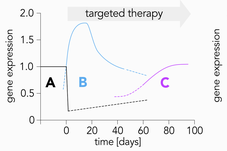
Resistance to targeted therapies is a major challenge in oncology. Disease progression is caused by multiple resistance mechanisms. Besides pre-existing and acquired *genetic* alternations, **adaptive *non-mutational* reprogramming as well as modulation of phenotypic plasticity have emerged as drivers of disease progression.** For example, in epidermal growth factor receptor (EGFR)-mutated non-small cell lung cancer (NSCLC) patients who were treated with osimertinib, ~50% of progressive disease could not be attributed to genetic mutations (Leonetti et al., *BJC* 2019). Similarly, in ~ 40% of patients suffering from KRAS G12C-mutated NSCLC and colorectal cancer (CRC), disease progressed under sotorasib treatment without identifiable acquired mutations (Zhao et al., *Nature* 2021). Thus, targeting non-genetic adaptive resistance mechanism such as drug-induced transcriptional reprogramming might be of great therapeutic benefit.

Here we identified small molecules, that interfere with cancer drug-induced transcriptional escape mechanisms using a phenotypic screen based on an SRY-Box Transcription Factor 2 (SOX2) reporter system. The screen led to the development of TT125-802, a highly specific, potent, orally available small molecule inhibitor of the bromodomain of the transcriptional regulator CBP and its paralogue p300.

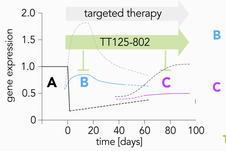
Targeted cancer therapies fail in the long-term



Adaptive resistance is driven by transcriptional changes



Combination therapy with TT125-802 represses adaptive resistance



A proliferative signatures are inhibited by targeted therapy

B early adaptive drug resistance signatures impair long-term efficacy of targeted therapy

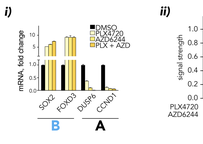
C late adaptive drug resistance signatures restore proliferation despite the targeted therapy

TT125-802 represses adaptive drug resistance signatures

TT125-802 prevented osimertinib resistance development in EGFR-mutated NSCLC cell lines, as well as sotorasib resistance development in KRAS G12C-mutated non-small cell lung cancer (NSCLC) or colorectal cancer (CRC) cell lines. Mouse xenograft studies confirmed increasing response rates and prolonged duration of response to osimertinib and sotorasib, when combined with TT125-802. In cells and tumours which were already resistant to osimertinib or sotorasib, TT125-802 still delayed cell or tumour growth. Analysis of transcriptional changes using RNA sequencing *in vitro* and *in vivo* identified several early adaptive and late acquired resistance signatures that were reversed by TT125-802. A first-in-human study of TT125-802 in cancer patients is on track to start in 2023.

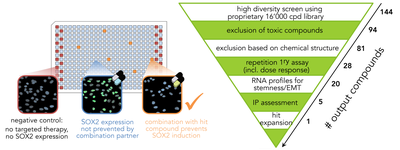
Phenotypic screen to identify compounds against adaptive cancer drug resistance

Cancer drugs inhibit oncogenic signaling and at the same time induce transcriptional changes associated with non-genetic resistance development



BRAF-V600E mutated melanoma cells (A375) treated for 24h with BRAF (PLX4720) and/or MEK (AZD6244) inhibitors. ***i)*** Targeted therapies down regulate proliferative signaling genes (A) and at the same time induce expression of early adaptive resistance (B). ***ii)*** Dose-dependent induction of stemness factor SOX2 upon treatmen with targeted therapies.

Phenotypic high-throughput screen identifies TT125 as regulator of early adaptive drug resistance

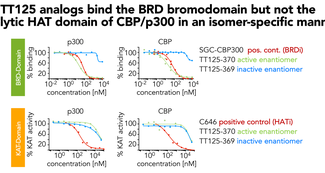


Hit compound TT125 was identified in phenotypic high throughput screen. Later a proprietary, phenotypic hit-to-lead optimization cascade was employed to increase increase the potency of the chemical series.

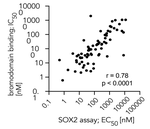
Target identification and validation: TT125 analogs bind the bromodomain of CBP and p300

TT125 analogs bind to the bromodomain (BRD) of CBP and p300. They thereby prevent binding to acetylated histones and inhibit resistance-causing transcriptional reprogramming, including gene expression of SOX2.

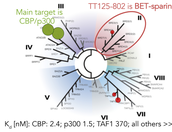
TT125 analogs bind the BRD bromodomain but not the catalytic HAT domain of CBP/p300 in an isomer-specific manner



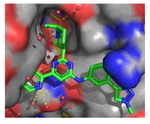
Activity of TT125 analogs in phenotypic SOX2 assay highly correlates with binding to the bromodomain of CBP



TT125-802 is highly selective to CBP/p300 with limited binding to the BET protein family

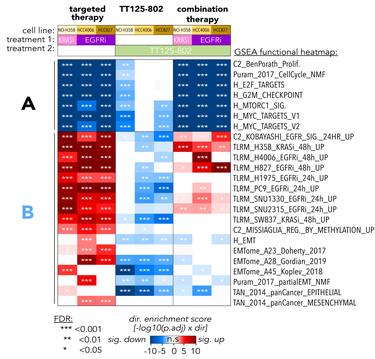


X-ray CBP-BRD with TT125 analog



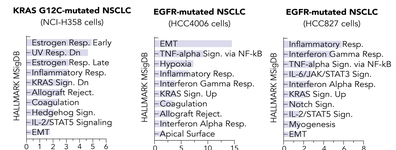
TT125-802 reverts early transcriptional changes driving adaptive resistance to KRAS and EGFR inhibitors

***i)* TT125-802 counter-regulates early adaptive response (B) across cell models driven by different oncogenic mutations and treated with different oncogene-targeting therapies**



***ii)* TT25-802 counter-regulates similar functional programs associated with adaptive resistance independent of oncogenic mutation and oncogene-targeting inhibitor**

Functional analysis of counter-regulated early adaptive gene signatures (B) by TT125-802



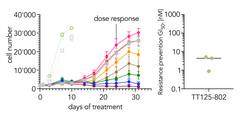
i) RNA-Seq of KRAS G12C-mutated NSCLC cells (NCI-H358) and EGFR-mutated NSCLC cells (HCC4006, HCC827) treated for 48h with DMSO, 200 nM TT125-802, the respective oncogene targeting inhibitor (100 nM KRASi sotorasib or 100 nM EGFRi osimertinib), or a combination of TT125-802 and the oncogene targeting inhibitor. Heat-map shows proliferative (A) and early adaptive response signatures (B) analysed by gene set enrichment analysis (GSEA).

ii) Functional analysis of fraction of counter-regulated genes by over representation analysis (ORA) in Enrichr (Maayan Lab).

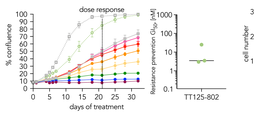
TT125-802 prevents resistance to KRASi in NSCLC and CRC and to EGFRi in NSCLC (in vitro)

In vitro life-cell tracking: TT125-802 prevents resistance development to...

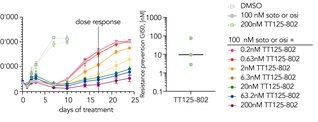
... KRASi in KRAS G12C-mutated NSCLC



... KRASi in KRAS G12C-mutated CRC



... EGFRi in EGFR-mutated NSCLC

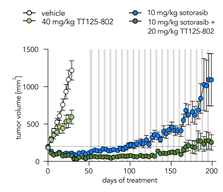


Cellular long-term resistance assays (life-cell colony tracking) in KRAS G12C-mutated NSCLC cells (NCI-H358), KRAS G12C-mutated CRC cells (SW837), and EGFR-mutated NSCLC cells (HCC4006) treated for indicated times with DMSO, 200 nM TT125-802, the respective oncogene targeting inhibitor (100 nM KRASi sotorasib or 100 nM EGFRi osimertinib), or a combination of TT125-802 and the oncogene targeting inhibitor. Half-maximal TT125-802 concentrations to prevent AMG510 or osimertinib resistance are shown as GI_{50} scatter plots. Scatter blots are from 3 experiments.

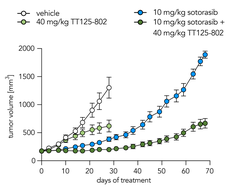
TT125-802 prevents resistance to KRASi in NSCLC and CRC and to EGFRi in NSCLC (in vivo)

In vivo xenograft studies: TT125-802 prevents resistance development to...

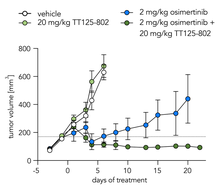
... KRASi in KRAS G12C-mutated NSCLC



... KRASi in KRAS G12C-mutated CRC



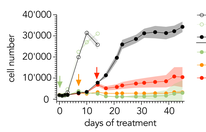
... EGFRi in EGFR-mutated NSCLC



Xenograft studies using KRAS G12C-mutated NSCLC cells (NCI-H358), KRAS G12C-mutated CRC cells (SW837), and EGFR-mutated NSCLC cells (NCI-H1975) treated for indicated times with vehicle, TT125-802, the respective oncogene targeting inhibitor, or a combination of TT125-802 and the oncogene targeting inhibitor. Drug holidays are indicated by grey bars (5 days on / 2 days off; NCI-H358 study). Mean tumor volume -/+ SEM is shown.

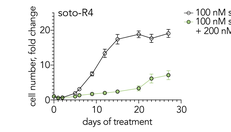
TT125-802 suppresses late adaptive resistance signatures in KRAS inhibitor-resistant cells

***i)* TT125-802 counteracts already developing resistance to KRASi**



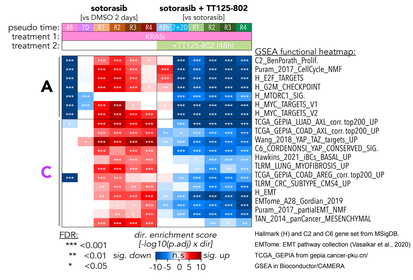
NCI-H358 cells were treated with the KRASi sotorasib over the indicated time course. TT125-802 was added from the beginning (green), after 7 days of sotorasib monotherapy (orange) or after 14 day of sotorasib monotherapy (red) once cells had already developed resistance to sotorasib and cells started regrowing in the presence of sotorasib. Even late addition of TT125-802 can counteract resistance.

***ii)* TT125-802 delays proliferation of KRASi-resistant cells**

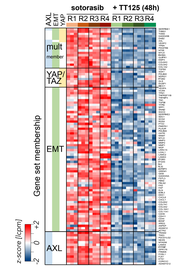


NCI-H358 cells had been made resistant to the KRASi sotorasib and were grown in the presence of sotorasib alone or in combination with TT125-802 over the indicated time course. Even in cells that are already resistant to KRASi due to established transcriptional changes, TT125-802 can delay cell growth.

***iii)* TT125-802 counteracts late adaptive resistance signatures (C)**



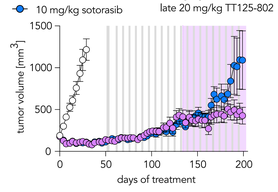
***iv)* TT125-802 suppresses well-known transcriptional resistance programs around YAP/TAZ, EMT and AXL signaling (C)**



NCI-H358 KRASi-sensitive cells were treated for 2 or 7 days with DMSO, 100 nM of the KRASi sotorasib, 200 nM TT125-802 or the combination of both for the last two days. Four sotorasib-resistant populations were maintained in sotorasib or additionally co-treated for 2 days with 200 nM TT125-802 (R1 - R4). Cells were lysed and prepared for RNA sequencing. ***iii)*** Heatmap shows proliferative (**A**) and late adaptive response signatures (**C**) analysed by by gene set enrichment analysis GSEA. Sotorasib is contrasted to DMSO, sotorasib +TT125-802 is contrasted to sotorasib. ***iv)*** Heatmap of genes contributing to significant enrichment of late adaptive response gene sets in ***iii)*** by leading edge analysis.

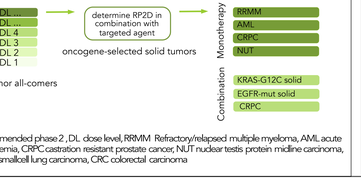
Late combination with TT125-802 delays tumor growth in KRASi-resistant NSCLC xenografts

TT125-802 breaks resistance to KRASi in KRASmut NSCLC



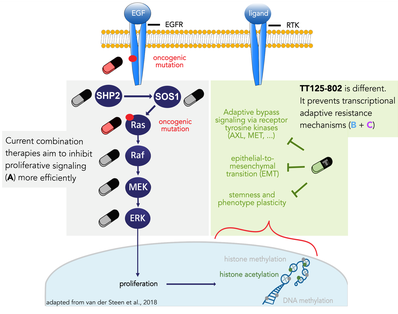
Xenograft study using KRAS G12C-mutated NSCLC cells (NCI-H358). Tumors were treated with sotorasib alone until resistance emerged. On day 136, mice were split into two groups: continued sotorasib treatment (blue group) or late addition of TT125-802 (purple group; combination treatment only in purple shaded time interval). Drug holidays are indicated by grey bars (5 days on / 2 days off). Mean tumor volume -/+ SEM is shown.

Clinical study design



RP2D recommended phase 2, DL dose level, RRMM Refractory/relapsed multiple myeloma, AML acute myeloid leukemia, CRPC castration resistant prostate cancer, NUT nuclear testis protein midline carcinoma, NSCLC non-smallcell lung carcinoma, CRC colorectal carcinoma

TT125-802 targets a novel mechanism to counteract adaptive drug resistance



- TT125-802 is a potent, highly selective and orally available small molecule inhibitor of the bromodomain of CBP/P300.
- TT125-802 reverts early and late cancer drug induced transcriptional programs that induce drug resistance, for example EMT and the expression of resistance mediating receptors.
- Early non-genetic resistance: TT125-802 *prevented* resistance development to EGFR inhibitors and KRAS G12C inhibitors in sophisticated in vitro and in vivo models
- Late non-genetic resistance: TT125-802 *counteracted* established resistance to EGFR inhibitors and KRAS G12C inhibitors in sophisticated in vitro and in vivo models
- First-in-human study in cancer patients using TT125-802 alone and in combination with standard of care is on track.

Poster #6268 on TT125-802 as monotherapy by S. Laudato:
Castration resistant prostate cancer and haematological malignancies