A combination of a CBP/p300 bromodomain inhibitor and an EGFR inhibitor for use in treating EGFR-mutant NSCLC

FIELD OF THE INVENTION

The present invention is in the field of non-small cell lung cancer (NSCLC) treatment. Thus, the present invention relates to a combination of a CBP/p300 bromodomain inhibitor and an EGFR inhibitor for use in the treatment of a patient suffering from NSCLC, wherein the NSCLC exhibits an oncogenic alteration in the EGFR.

BACKGROUND OF THE INVENTION

Non-small cell lung cancer (NCSLC) is the most prevalent malignancy and the leading cause of cancer death in the world, with a 5-years survival rate of no more than 5%.

Hou et al. recently reported that p300 promotes proliferation, migration, and invasion via inducing epithelial-mesenchymal transition in NSCLC cells (see Hou et al., *BMC Cancer* (2018) 18:641). These results were gained in NSCLC cell lines where p300 was down-regulated through RNAi. Thus, the expression of the p300 protein was down-regulated by a nucleic-acid induced mechanism.

p300 (also known as EP300 and KAT3B) is a large protein with many different domains that binds to diverse proteins including many DNA-binding transcription factors. The cyclic AMP-responsive element-binding protein (CREB) binding protein CBP (also known as CREBBP and KAT3A) is a protein that is very closely related to p300 and the two proteins are commonly referred to as paralogs in view of their extensive sequence identity and functional similarity.

CBP/p300 are lysine acetyltransferases that have been shown to catalyze the attachment of an acetyl group to a lysine side chain of histones and other proteins. CBP/p300 have been proposed to activate transcription, wherein the mechanism of action seems to reside in bridging DNA-binding transcription factors to the RNA polymerase machinery or by helping assemble the transcriptional pre-initiation complex. For this purpose, the different CBP/p300 domains are believed to interact with arrays of different transcription factors assembled at promoters and enhancers

for transcription of different genes (see Dyson and Wright, JBC Vo. 291, no. 13, pp. 6714-6722, Figure 2).

One of the multiple domains of CBP/p300 is the bromodomain. The bromodomain as such was first identified in *Drosophila* in 1992 and described to be a binding module to acetyl-lysine about 10 years later. In humans, there are many bromodomain-containing proteins that may be classified into eight groups based on sequence and structural similarities. It seems that all bromodomain-containing proteins are involved in the regulation of transcriptional programs. Oncogenic rearrangements suggest that targeting bromodomain-containing proteins and more particularly their bromodomains might be beneficial in particular in the treatment of cancer.

For this reason, several drug candidates have been developed that are presently undergoing clinical testing, which target so-called "bromodomain and extra-terminal motif" proteins, typically referred to as BET-proteins, which constitute one group of bromodomain-containing proteins. Examples of BET-protein targeting drugs are INCB054329 (Incyte Corporation), ABBV-075 (AbbVie) and I-BET762 (GlaxoSmithKline). There are also drugs that selectively target the bromodomain of CBP and p300, which are part of a separate group of bromodomain-containing proteins. Such inhibitors include e.g. CCS1477 (CellCentric) which is presently undergoing clinical studies for the treatment of metastatic castration resistant prostate cancer and haematological malignancies or FT-7051 (Forma Therapeutics Inc.) which is presently undergoing studies for the treatment of metastatic castration resistant prostate cancer.

In view of the results by Hou et al. discussed above, there is the need to further elucidate the role of p300 and in particular its different domains in NSCLC in order to provide an effective NSCLC treatment.

OBJECTS AND SUMMARY OF THE INVENTION

The inventors of the present invention have surprisingly found that a CBP/p300 bromodomain inhibitor, i.e. a bromodomain inhibitor selectively binding to the bromodomain of CBP/p300, provides an effective treatment of NSCLC exhibiting an oncogenic alteration in the EGFR if administered in combination with an EGFR inhibitor, while the CBP/p300 bromodomain inhibitor does not affect the cell proliferation of NSCLC cells if administered alone. In other words, the inventors have surprisingly found that the combination of a CBP/p300 bromodomain inhibitor and an EGFR inhibitor is more effective in treating NSCLC exhibiting an oncogenic alteration in the EGFR compared to the effect that either of the two actives exhibits on its own on the NSCLC exhibiting an oncogenic alteration in the EGFR. Thus, as noted above, the CBP/p300 bromodomain inhibitor has no effect when given alone (where "no effect" in particular means that there are no objective responses as defined by the RECIST 1.1 response criteria for target lesions or non-target lesions in a subject) while the effect of the EGFR inhibitor when given alone decreases over time, likely due to the development of resistance against the EGFR inhibitor.

In the first aspect, the present invention is directed to a combination of (i) a CBP/p300 bromodomain inhibitor and (ii) an EGFR inhibitor for use in the treatment of a patient suffering from NSCLC, wherein the NSCLC exhibits an oncogenic alteration in the EGFR. The first aspect may also be referred to as a combination of (i) a CBP/p300 bromodomain inhibitor and (ii) an EGFR inhibitor for use in the treatment of a patient suffering from NSCLC, wherein the NSCLC is characterized by the EGFR-mutational profile given in the one or more indications of the label of the EGFR inhibitor used in the combination or wherein the NSCLC is characterized by the EGFR-mutational profile targeted in the clinical trial setting by the EGFR inhibitor used in the combination.

In a preferred embodiment of the first aspect, the oncogenic alteration in the EGFR results in overactivation of the EGFR. The oncogenic alteration in the EGFR may even result in constitutively active EGFR (in the meaning that the enzymatic activity of the EGFR, namely the protein-kinase activity, is constitutively active).

In a further preferred embodiment of the first aspect, the oncogenic alteration in the EGFR is caused by a deletion and/or insertion in exon 18 or in exon 19 or in exon 20 of the EGFR gene; a kinase domain duplication in the EGFR gene; an amplification of the EGFR gene; at least one base mutation in the EGFR gene resulting in an amino acid substitution in the EGFR selected from the group consisting of L858R, G719S, G719A, G719C, V765A, T783A, S768I, S768V, L861Q, E709X, L819Q, A750P and combinations thereof; and combinations of any of the foregoing. It can be preferred that the oncogenic alteration is caused by a deletion in exon 19 of the EGFR gene; an insertion in exon 20 of the EGFR gene; at least one base mutation in the EGFR gene resulting in an amino acid substitution in the EGFR selected from the group consisting of L858R, G719S, G719A, G719C, V765A, T783A, S768I, S768V, L861Q, E709X, L819Q, A750P and combinations thereof; and combinations of any of the foregoing. It can also be preferred that the oncogenic alteration is caused by a deletion in exon 19 of the EGFR gene; at least one base mutation in the EGFR gene resulting in the amino acid substitution L858R in the EGFR; and combinations thereof. A deletion and insertion in exon 18 of the EGFR gene is in particular a deletion resulting in the deletion of E709-T710 in the EGFR and an insertion of D at this position in the EGFR. A deletion in exon 19 of the EGFR gene is in particular a deletion resulting in the deletion of E746-A750 or L747-E749 in the EGFR. A deletion and insertion in exon 19 of the EGFR is in particular a deletion resulting in the deletion of L747-A750 in the EGFR and an insertion of P at this position in the EGFR or a deletion resulting in the deletion of L747-T751 in the EGFR and an insertion of S at this position in the EGFR. An insertion in exon 20 of the EGFR gene is in particular an insertion resulting in the insertion of an amino acid (in the meaning of any amino acid or X) at a position in the EGFR between two amino acids selected from the group consisting of D761-E762, A763-Y764, Y764-V765, A767-S768, S768-V769, V769-D770, D770-N771, N771-P772, P772-H773, H773-V774, V774-C775, V765-M766, and combinations thereof. It can be most preferred that the oncogenic alteration is caused by a deletion in exon 19 of the EGFR gene (in particular a deletion resulting in the deletion of E746-A750 or L747-E749 in the EGFR); at least one base mutation in the EGFR gene resulting in the amino acid substitution L858R or A750P in the EGFR; and combinations thereof. It can also be very preferred that the oncogenic alteration is caused by a deletion in exon 19 of the EGFR gene or at least one base mutation in the EGFR gene resulting in the amino

acid substitution L858R in the EGFR. When reference is made herein to "X" as an amino acid, "X" indicates any amino acid (but of course an amino acid differing from the wild-type amino acid at the respective position, if applicable, e.g. for E709X).

In an embodiment of the first aspect, the NSCLC does not additionally exhibit a resistance alteration in the EGFR. Accordingly, the combination for use of the present invention would be used as first-line treatment, and the EGFR inhibitor in the combination may be any EGFR inhibitor that is administered (or indicated) for treating NSCLC exhibiting an oncogenic alteration in the EGFR.

In another embodiment of the first aspect, the NSCLC additionally exhibits a resistance alteration in the EGFR. The resistance alteration in the EGFR may in particular be caused by at least one base mutation in the EGFR gene resulting in an amino acid substitution in the EGFR selected from the group consisting of T790M, C797X (mainly C797S), L792X, G796X, L718Q, L718V, G724S, D761Y, V834L, T854A, and combinations thereof. It can be preferred that the resistance alteration in the EGFR is caused by at least one base mutation in the EGFR gene resulting in an amino acid substitution in the EGFR selected from the group consisting of T790M, C797X (mainly C797S), L718Q, L718V, T854A, and combinations thereof. Most preferred is that the resistance alteration in the EGFR is caused by at least one base mutation in the EGFR gene resulting in the amino acid substitution T790M in the EGFR. When reference is made herein to "X" as an amino acid, "X" indicates any amino acid (but of course an amino acid differing from the wild-type amino acid at the respective position, if applicable, e.g. for C797X).

When the NSCLC additionally exhibits a resistance alteration in the EGFR, the patient was previously treated with a (first) EGFR inhibitor that was effective initially and then became ineffective due to the development of resistance, in particular due to development of an EGFR resistance alteration. It is important to understand that in the combination for use of the present invention, the EGFR inhibitor in such a scenario is not the (first) EGFR inhibitor administered previously, but a (second or third) EGFR inhibitor that is initially therapeutically effective despite the at least one resistance alteration when administered alone. We refer to an "initial therapeutic effectiveness" here as it is a common observation that yet a further resistance towards this (second or third) EGFR inhibitor develops, rendering this (second or third) EGFR inhibitor ultimately also ineffective. In such a scenario, the combination for use of the present invention would be used as second-line or third-line treatment. To give an example, gefitinib may have been administered (alone as first-line treatment) previously to a patient suffering from NSCLC exhibiting an oncogenic alteration, with the gefitinib treatment becoming ineffective over time (typically after a period of about 10 to about 12 months) and with the finding (e.g. via a biopsy and a corresponding test in order to detect EGFR mutations) that the EGFR T790M resistance alteration developed in the tumor during the gefitinib-treatment. In such a situation, gefitinib would not be used in the combination for use of the present invention, but in particular osimertinib that has been shown (and is indicated) to be effective in the treatment of patients with EGFR T790M mutation-positive NSCLC, whose disease has progressed on or after EGFR tyrosine kinase inhibitor (TKI) therapy.

Taking the above into account, the present invention in an embodiment relates to the combination of (i) a CBP/p300 bromodomain inhibitor and (ii) an EGFR inhibitor for use in the treatment of a patient suffering from NSCLC, wherein the NSCLC exhibits an oncogenic alteration in the EGFR, with the proviso that, if the NSCLC additionally exhibits a resistance alteration in the EGFR due to previous administration of an EGFR inhibitor, the EGFR inhibitor of the combination is not the EGFR inhibitor previously administered but in particular an EGFR inhibitor, which is therapeutically effective despite the resistance alteration in the EGFR (namely the resistance alteration that rendered the previously administered EGFR inhibitor therapeutically ineffective). One may also refer to the combination for use according to the first aspect of the present invention with the proviso that, if the NSCLC additionally exhibits a resistance alteration in the EGFR due to previous administration of an EGFR inhibitor, the EGFR inhibitor of the combination is not the EGFR inhibitor previously administered but an EGFR inhibitor which is therapeutically effective during the first treatment cycles if administered alone despite the resistance alteration or with the proviso that, if the NSCLC additionally exhibits a resistance alteration in the EGFR due to previous administration of an EGFR inhibitor, the EGFR inhibitor of the combination is not the EGFR inhibitor previously administered but an EGFR inhibitor that is indicated for treatment of NSCLC additionally exhibiting the resistance alteration in the EGFR.

To give examples when considering two specific EGFR inhibitors (namely "X" and "the EGFR inhibitor of the combination"), the above paragraph is understood to refer in an embodiment to the combination of (i) a CBP/p300 bromodomain inhibitor and (ii) an EGFR inhibitor for use in the treatment of a patient suffering from NSCLC, wherein the NSCLC exhibits an oncogenic alteration in the EGFR, with the proviso that, if the NSCLC additionally exhibits a resistance alteration in the EGFR due to previous administration of EGFR inhibitor X, the EGFR inhibitor of the combination is not EGFR inhibitor X. It is noted that the EGFR inhibitor of the combination is therapeutically effective despite the resistance alteration in the EGFR (namely the resistance alteration that rendered the previously administered EGFR inhibitor X therapeutically ineffective).

In another embodiment of the first aspect, the CBP/p300 bromodomain inhibitor is a small molecule inhibitor. Thus, in such an embodiment, the CBP/p300 bromodomain inhibitor is not a nucleic acid-based inhibitor, such as e.g. a shRNA or RNAi directed to CBP and/or p300.

In another embodiment of the first aspect, the EGFR inhibitor is a small molecule inhibitor or an antibody. Thus, in such an embodiment, the EGFR inhibitor is not a nucleic acid-based inhibitor, such as e.g. a shRNA or RNAi directed to EGFR. In yet another embodiment of the first aspect, the EGFR inhibitor is a small molecule inhibitor. In a further embodiment of the first aspect, the EGFR inhibitor inhibits the tyrosine kinase activity of the EGFR.

The CBP/p300 bromodomain inhibitor may be selected from the group consisting of Compound A, Compound C, Compound 00030, Compound 00071, CCS1477, GNE-781, GNE-049, SGC-CBP30, CPI-637, FT-6876, Compound 462, Compound 424 and Compound 515. These compounds are either commercially available or publicly disclosed as outlined further below, or their synthesis and structures are shown in the examples of the present application. It can be preferred

that the CBP/p300 bromodomain inhibitor is selected from the group consisting of Compound A, Compound C, CCS1477, GNE-781, GNE-049, CPI-637, Compound 462, Compound 424 and Compound 515.

The EGFR inhibitor may be selected from the group consisting of ABBV-321, abivertinib, afatinib, alflutinib, almonertinib, apatinib, AZD3759, brigatinib, D 0316, D 0317, D 0318, dacomitinib, DZD9008, erlotinib, FCN-411, gefitinib, icotinib, lapatinib, lazertinib, mobocertinib, nazartinib, neratinib, olafertinib, osimertinib, poziotinib, pyrotinib, rezivertinib, TAS6417, vandetanib, varlitinib, XZP-5809, amivantamab, CDP1, cetuximab, GC1118, HLX07, JMT101, M1231, necitumumab, nimotuzumab, matuzumab, panitumumab, SCT200, SI-B001, SYN004, zalutumumab, and combinations thereof. The EGFR inhibitor may also be selected from the group consisting of cetuximab, panitumumab, zalutumumab, nimotuzumab, matuzumab, gefitinib, erlotinib, lapatinib, neratinib, vandetanib, necitumumab, osimertinib, afatinib, dacomitinib, brigatinib, poziotinib, and combinations thereof. In a preferred embodiment, the EGFR inhibitor is selected from the group consisting of abivertinib, afatinib, alflutinib, almonertinib, apatinib, AZD3759, brigatinib, D 0316, D 0317, D 0318, dacomitinib, DZD9008, erlotinib, FCN-411, gefitinib, icotinib, lapatinib, lazertinib, mobocertinib, nazartinib, neratinib, olafertinib, osimertinib, poziotinib, pyrotinib, rezivertinib, TAS6417, vandetanib, varlitinib, XZP-5809, and combinations thereof. In a more preferred embodiment, the EGFR inhibitor is gefitinib or osimertinib. It can be most preferred that the EGFR inhibitor is osimertinib.

In a preferred embodiment of the first aspect, the combination is administered to the patient during each treatment cycle.

In still another embodiment of the first aspect, the EGFR inhibitor is administered as sole active agent during the first treatment cycle, followed by the additional administration of the CBP/p300 bromodomain inhibitor during the later treatment cycle, wherein a resistance alteration in the EGFR has not yet developed in response to the administration of the EGFR inhibitor alone during the first treatment cycle (i.e. prior to the administration of the combination of the present invention). As noted above, the development of a resistance alteration can be assessed e.g. via a biopsy and a corresponding test in order to detect EGFR mutations. Since the development of a resistance may be prevented when using the combination for use according to the present invention, the administration of the combination during each treatment cycle is preferred.

In another embodiment of the first aspect, the CBP/p300 bromodomain inhibitor and the EGFR inhibitor are administered as separate dosage forms or comprised in a single dosage form. If the CBP/p300 bromodomain inhibitor and the EGFR inhibitor are administered as separate dosage forms, the administration during each treatment cycle may be concomitantly or sequentially. This includes the option that the CBP/p300 bromodomain inhibitor is administered first, followed by the administration of the EGFR inhibitor.

In yet another embodiment of the first aspect, the treatment results in an extended duration of the therapeutic effect of the EGFR inhibitor compared to the duration of the therapeutic effect of

the EGFR inhibitor when administered as the sole active agent. In still another embodiment, the treatment results in an increased therapeutic efficacy of the EGFR inhibitor compared to the therapeutic efficacy of the EGFR inhibitor when administered as the sole active agent. In another embodiment, the treatment results in the prevention of resistance to the EGFR inhibitor.

In another embodiment of the first aspect, the CBP/p300 bromodomain inhibitor is administered at a daily amount of between about 1 mg and about 3000 mg, preferably of between about 10 mg and about 2000 mg, more preferably of between about 15 mg and about 1000 mg. It can be preferred to administer the CBP/p300 bromodomain inhibitor at a daily amount of about 10 mg, about 15 mg, about 20 mg, about 50 mg, about 100 mg, about 250 mg, about 500 mg, about 1000 mg, about 1500 mg, about 2000 mg, about 2500 mg, or about 3000 mg. The administration may take place intermittently, i.e. not every day, but on a day the administration takes place, the afore-mentioned daily amount may be administered. If CCS1477, Compound 462, Compound 424 or Compound 515 is used as CBP/p300 bromodomain inhibitor, the respective compound may be administered at a daily amount of between about 10 mg and about 600 mg.

In another embodiment of the first aspect, the EGFR inhibitor is administered at a daily amount that is in the range of a typical daily amount (in particular the daily amount mentioned for the EGFR inhibitor in the label, if available) if the EGFR inhibitor is administered as the sole active agent. The typical daily amount (or the indicated daily amount, if available) depends on the specific EGFR inhibitor that will be used. Thus, gefitinib may e.g. be administered in the combination for use of the present invention at a daily amount of between about 50 and about 300 mg, preferably of between about 100 mg and about 250 mg, and most preferably of between about 150 mg and about 250 mg. Osimertinib may e.g. be administered in the combination for use of the present invention at a daily amount of between about 5 and about 1500 mg, preferably of between about 10 mg and about 100 mg, and most preferably of between about 50 mg and about 80 mg. Erlotinib may e.g. be administered in the combination for use of the present invention at a daily amount of between about 10 mg and about 300 mg, preferably of between about 25 mg and about 200 mg, and most preferably of between about 100 mg and about 150 mg. Afatinib may e.g. be administered in the combination for use of the present invention at a daily amount of between about 5 mg and about 100 mg, preferably of between about 10 mg and about 80 mg, and most preferably of between about 20 mg and about 40 mg. Dacomitinib may e.g. be administered in the combination for use of the present invention at a daily amount of between about 5 mg and about 100 mg, preferably of between about 10 mg and about 80 mg, and most preferably of between about 15 mg and about 50 mg.

In another embodiment of the first aspect, the EGFR inhibitor is administered at a daily amount that is lower than the above-mentioned typical daily amount if the EGFR inhibitor is administered as the sole active agent. In other words, if an EGFR inhibitor is not administered as the sole active agent but in the combination for use according to the present invention, the EGFR inhibitor may be administered at a lower amount than the amount used when the EGFR inhibitor is administered as the sole active agent. This e.g. means for the examples given above that the daily amount would be at the lower ends of the ranges given or even below these ranges.

In yet a further embodiment of the first aspect, the present invention is directed to a combination of (i) Compound A and (ii) an EGFR inhibitor for use in the treatment of a patient suffering from non-small cell lung cancer (NSCLC), wherein the NSCLC exhibits an oncogenic alteration in the EGFR. It can be preferred in this embodiment that the EGFR inhibitor is osimertinib and that the oncogenic alteration is caused by a deletion in exon 19 of the EGFR gene (in particular a deletion resulting in the deletion of E746-A750 or L747-E749 in the EGFR); at least one base mutation in the EGFR gene resulting in the amino acid substitution L858R or A750P in the EGFR; and combinations thereof. The at least one base mutation in the EGFR gene resulting in the amino acid substitution T790M in the EGFR corresponding to a resistance alteration in the EGFR may or may not be present in the embodiment where the EGFR inhibitor is osimertinib.

In yet a further embodiment of the first aspect, the present invention is directed to a combination of (i) Compound A or Compound C and (ii) an EGFR inhibitor for use in the treatment of a patient suffering from non-small cell lung cancer (NSCLC), wherein the NSCLC exhibits an oncogenic alteration in the EGFR. It can be preferred in this embodiment that the EGFR inhibitor is osimertinib and that the oncogenic alteration is caused by a deletion in exon 19 of the EGFR gene (in particular a deletion resulting in the deletion of E746-A750 or L747-E749 in the EGFR); at least one base mutation in the EGFR gene resulting in the amino acid substitution L858R or A750P in the EGFR; and combinations thereof. The at least one base mutation in the EGFR gene resulting in the amino acid substitution T790M in the EGFR corresponding to a resistance alteration in the EGFR may or may not be present in the embodiment where the EGFR inhibitor is osimertinib.

In yet a further embodiment of the first aspect, the present invention is directed to a combination of (i) CCS1477 and (ii) an EGFR inhibitor for use in the treatment of a patient suffering from non-small cell lung cancer (NSCLC), wherein the NSCLC exhibits an oncogenic alteration in the EGFR. It can be preferred in this embodiment that the EGFR inhibitor is osimertinib and that the oncogenic alteration is caused by a deletion in exon 19 of the EGFR gene (in particular a deletion resulting in the deletion of E746-A750 or L747-E749 in the EGFR); at least one base mutation in the EGFR gene resulting in the amino acid substitution L858R or A750P in the EGFR; and combinations thereof. The at least one base mutation in the EGFR gene resulting in the amino acid substitution T790M in the EGFR corresponding to a resistance alteration in the EGFR may or may not be present in the embodiment where the EGFR inhibitor is osimertinib.

In yet a further embodiment of the first aspect, the present invention is directed to a combination of (i) GNE-781 or GNE-049 and (ii) an EGFR inhibitor for use in the treatment of a patient suffering from non-small cell lung cancer (NSCLC), wherein the NSCLC exhibits an oncogenic alteration in the EGFR. It can be preferred in this embodiment that the EGFR inhibitor is osimertinib and that the oncogenic alteration is caused by a deletion in exon 19 of the EGFR gene (in particular a deletion resulting in the deletion of E746-A750 or L747-E749 in the EGFR); at least one base mutation in the EGFR gene resulting in the amino acid substitution L858R or A750P in the EGFR; and combinations thereof. The at least one base mutation in the EGFR gene resulting in the amino

acid substitution T790M in the EGFR corresponding to a resistance alteration in the EGFR may or may not be present in the embodiment where the EGFR inhibitor is osimertinib.

In yet a further embodiment of the first aspect, the present invention is directed to a combination of (i) CPI-637 and (ii) an EGFR inhibitor for use in the treatment of a patient suffering from non-small cell lung cancer (NSCLC), wherein the NSCLC exhibits an oncogenic alteration in the EGFR. It can be preferred in this embodiment that the EGFR inhibitor is osimertinib and that the oncogenic alteration is caused by a deletion in exon 19 of the EGFR gene (in particular a deletion resulting in the deletion of E746-A750 or L747-E749 in the EGFR); at least one base mutation in the EGFR gene resulting in the amino acid substitution L858R or A750P in the EGFR; and combinations thereof. The at least one base mutation in the EGFR gene resulting in the amino acid substitution T790M in the EGFR corresponding to a resistance alteration in the EGFR may or may not be present in the embodiment where the EGFR inhibitor is osimertinib.

In yet a further embodiment of the first aspect, the present invention is directed to a combination of (i) Compound 462 or Compound 424 or Compound 515 and (ii) an EGFR inhibitor for use in the treatment of a patient suffering from non-small cell lung cancer (NSCLC), wherein the NSCLC exhibits an oncogenic alteration in the EGFR. It can be preferred in this embodiment that the EGFR inhibitor is osimertinib and that the oncogenic alteration is caused by a deletion in exon 19 of the EGFR gene (in particular a deletion resulting in the deletion of E746-A750 or L747-E749 in the EGFR); at least one base mutation in the EGFR gene resulting in the amino acid substitution L858R or A750P in the EGFR; and combinations thereof. The at least one base mutation in the EGFR gene resulting in the amino acid substitution T790M in the EGFR corresponding to a resistance alteration in the EGFR may or may not be present in the embodiment where the EGFR inhibitor is osimertinib.

In a second aspect, the present invention is directed to a method of treating NSCLC in a patient in need thereof, said method comprising administering to the patient an effective amount of (i) a CBP/p300 bromodomain inhibitor and an effective amount of (ii) an EGFR inhibitor, wherein the NSCLC exhibits an oncogenic alteration in the EGFR.

In a third aspect, the present invention is directed to a method of extending the duration of the therapeutic effect of an EGFR inhibitor in a patient in need thereof, said method comprising administering to the patient an effective amount of (i) a CBP/p300 bromodomain inhibitor and an effective amount of (ii) the EGFR inhibitor, wherein the NSCLC exhibits an oncogenic alteration in the EGFR. In other words, the duration of the therapeutic effect of the EGFR inhibitor (when administered in the combination) is extended compared to the duration of the therapeutic effect of the EGFR inhibitior when administered as the sole active agent in NSCLC treatment.

In a fourth aspect, the present invention is directed to a method of increasing the therapeutic efficacy of an EGFR inhibitor in a patient in need thereof, said method comprising administering to the patient an effective amount of (i) a CBP/p300 bromodomain inhibitor and an effective amount of (ii) the EGFR inhibitor, wherein the NSCLC exhibits an oncogenic alteration in the

EGFR. In other words, the therapeutic efficacy of the EGFR inhibitor (when administered in the combination) is increased compared to the therapeutic efficacy of the EGFR inhibitor when administered as the sole active agent in NSCLC treatment.

In a fifth aspect, the present invention is directed to a method of blocking proliferation of a NSCLC cell, said method comprising administering to the cell an effective amount of (i) a CBP/p300 bromodomain inhibitor and an effective amount of (ii) an EGFR inhibitor, wherein the NSCLC cell exhibits an oncogenic alteration in the EGFR.

In a sixth aspect, the present invention is directed to a method of retarding the proliferation of a NSCLC cell, said method comprising administering to the cell an effective amount of (i) a CBP/p300 bromodomain inhibitor and an effective amount of (ii) an EGFR inhibitor, wherein the NSCLC cell exhibits an oncogenic alteration in the EGFR.

The embodiments outlined above for the first aspect equally apply for the methods of the second to sixth aspects.

DESCRIPTION OF THE FIGURES

FIG. 1: Only CBP/p300 inhibitors that bind to the bromodomain (Compound A, CCS1477) or the HAT domain (A485) blunt EGFR inhibitor-induced gene expression in EGFR-mutated non-small cell lung cancer cells (NSCLC), but not inhibitors that prevent the interaction of CBP with β-catenin (ICG001). Two different EGFR inhibitors are used in cell lines that carry the resistance-causing gatekeeper mutation T790M or not. Examples of regulated genes shown are ALPP (Alkaline phosphatase, placental type; A and C) and HOPX (Homeodomain-only protein; B and D). Data from 2 independent experiments with qPCRs in duplicate (mean ± SD).

FIG. 2: Only the enantiomer that binds the bromodomain (BRD) of CBP/p300 (Compound A) but not the enantiomer that does not bind the bromodomain of CBP/p300 (Compound B) potentiates EGFR inhibitor-mediated NSCLC cell proliferation inhibition in a concentration-dependent manner. Cell numbers of EGFR-mutated HCC827 cells were monitored over time. (A) Cells were treated with DMSO alone (filled circles), with 20 nM EGFR inhibitor alone (Gefitinib; 1st generation EGFR inhibitor, open circles) or in combination with the bromodomain-binding enantiomer of the CBP/p300 BRD inhibitor (Compound A). (B) HCC827 cells were exposed to Compound A & B in absence of EGFR inhibitor. In the absence of an EGFR inhibitor Compound A loses its effect on proliferation of EGFR-mutated NSCLC cells and behaves like Compound B that does not bind the bromodomain of CBP/p300. Presented graphs are from one experiment with triplicates for each time-point and condition (mean ± SD).

FIG. 3: Only inhibitors that bind to the bromodomain of CBP/p300 (Compound A, CCS1477) potentiate the effect of an EGFR inhibitor without affecting cell growth in the absence of the EGFR inhibitor. CBP/p300 inhibitors that inhibit the histone acetyl transferase (HAT) domain of CBP/p300 inhibitors (A485) affect cell proliferation of EGFR-mutated NSCLC cells even in the ab-

sence of EGFR inhibitors. (A), (B), (C), (D) and (E) Cell numbers of the EGFR-mutated NSCLC cell line HCC827 are plotted as function of drug treatments (symbols in graph legends) over time [days] measured in 96-well plates using nuclear fluorescent staining. Figure 3 (A) and (B) and (C) Left side: Single agent treatment of cells with Compound A (Fig 3A), CCS1477 (Fig 3B), SGC-CBP30 (Fig 3C) or A485. Compound A CCS1477, SGC-CBP30 that targets the bromodomain of CBP/p300 do not affect cell proliferation of EGFR-mutated NSCLC cells in the absence of an EGFR inhibitor. Figure 3 (A) and (B) and (C) Right side and figure 3 (D) and (E): Anti-proliferative activity of Compound A (A) and CCS1477 (CBP/p300 BRD-I) (B) and SGC-CBP30 (C) and compound 00071 (D) and compound 00030 (E) and A485 (CBP/p300 HAT-I) in presence of 300 nM Gefitinib (EGFR inhibitor). Compound A and CCS1477 and SGC-CBP30 and compound 00071 and compound 00030 that target the bromodomain of CBP/p300 potentiate the effect of the EGFR inhibitor in EGFR-mutated NSCLC cells despite the absence of an anti-proliferative effect when the compounds are used without the EGFR inhibitor (left). Represented curves are from one experiment in triplicate (mean ± SD).

FIG 4: (A) Assessment of HCC827 cell number over time in [h]. Compound A does not affect cell proliferation of EGFR-mutated NSCLC cells in the absence of an EGFR inhibitor but prevents the development of drug resistance when combined with an EGFR inhibitor. Treatments: DMSO, 1 µM Compound A, 300 nM Gefitinib or a combination of 300 nM Gefitinib and 1 µM Compound A according to legend. Example graphs show the cell number (mean ± SD) of 6 wells for DMSO and Compound A or of 24 wells for Gefitinib or Gefitinib + Compound A treatments. (B) Cell numbers per well, as dot plots, treated with Gefitinib or Gefitinib + Compound A for 0 or 22 days (from 2 experiment plates as in A, 48 wells per condition). **** $p<0.0001$, Kruksal-Wallis Test, with Dunn's multiple comparison. (C) Waterfall plot of wells treated with 300 nM Gefitinib or 300 nM Gefitinib + 1µM Compound A from 2 plates (48 wells/per condition) analyzed as in (A) showing the response to the treatment after 22 days as log fold change of the initial cell number on the particular well. Wells were sorted from highest to lowest log fold changes. Empty bars are Gefitinib-treated wells, filled bars represent wells treated with Gefitinib + Compound A. Even though cells do not respond to Compound A alone, Compound A significantly increases the response to the EGFR inhibitor when combined with such.

FIG 5: Inhibitors that bind to the bromodomain of CBP/p300 potentiate the effect of 3rd generation EGFR inhibitors without affecting cell growth in the absence of the EGFR inhibitor in EGFR-mutated NSCLC cells that carry a T790M-gatekeeper mutation. (A) Assessment of NCI-H1975 cell numbers as function of time [h] in presence of DMSO, 50 nM osimertinib, 2 µM Compound A or combinations of 50 nM osimertinib with 2.0, 0.5 or 0.125 µM Compound A. Compound A does not affect cell proliferation of EGFR-mutated NSCLC cells that carry a T790M-gatekeeper mutation in the absence of a 3rd generation EGFR inhibitor but prevents the development of drug resistance when combined with the EGFR inhibitor. (B) Assessment of NCI-H1975 cell growth in presence of DMSO, 50 nM osimertinib, 2 µM CCS1477 or combinations of 50 nM osimertinib with 2.0, 0.5 or 0.125 µM CCS1477. CCS1477 does not affect cell proliferation of EGFR-mutated NSCLC cells that carry a T790M-gatekeeper mutation in the absence of a 3rd generation EGFR inhibitor but prevents the development of drug resistance when combined with the EGFR inhibitor. (Exam-

ple graphs show duplicates of each data and timepoint, with a logistic growth curve fit calculated in GraphPad Prism).

FIG 6: Inhibitors that bind to the bromodomain of CBP/p300 have no effect in vivo when used in the absence of EGFR inhibitors but they potentiate the effect EGFR inhibitors to provide better tumor-control over time and better response rates to the therapy when combined with such. (A) The mean tumor volumes (+SEM) of EGFR-mutated NCI-H1975 xenografts are plotted over time. Four different treatment groups are depicted: vehicle (30% PEG300/H2O; crossed circle; n=4), 20 mg/kg CCS1477 (open circles; n=4), 2 mg/kg osimertinib (filled circle; n=9) or with 2 mg/kg osimertinib in combination with 20 mg/kg CCS1477 (half-filled circles; n=10). When CCS1477 is used in the absence of the EGFR inhibitor it has no effect on tumor growth. However, when it is combined with an EGFR inhibitor, response to therapy is increased and tumor size is better controlled over the course of the therapy. (B) The best average response for all 4 treatment groups is shown in a waterfall plot (vehicle in grey, 20 mg/kg CCS1477 in white, 2 mg/kg osimertinib in black and 2 mg/kg osimertinib in combination with 20 mg/kg CCS1477 in squared). The dashed line indicates the reduction of 30% of initial tumor volume. There is an increased response rate to the therapy when the bromodomain-binding inhibitor (CCS1477) is combined with the EGFR inhibitor (osimertinib), despite the absence of response to the bromodomain-binding inhibitor (CCS1477) alone.

FIG 7: The initial Fo-Fc difference electron density map of the model (contoured at 4.0 σ) resulting from refinement of the initial model prior to modelling of the compound with REFMAC5, in the determination of the crystal structure of the bromodomain of human CREBBP in complex with compound 00004.

FIG 8: Compound A in combination with the EGFR inhibitor Gefitinib mediates inhibition of HCC4006 long-term cell proliferation - the details are provided in example 9.

FIG 9: Compound A, Compound C and structurally unrelated selective CBP/p300 bromodomain inhibitors (CCS1477, FT-6876 and GNE-781) in combination with the EGFR inhibitor osimertinib mediate inhibition of HCC827 long-term cell proliferation – the details are provided in example 10.

FIG 10: Compound A, Compound C and structurally unrelated selective CBP/p300 bromodomain inhibitors (CCS1477, FT-6876 and GNE-781) in combination with the EGFR inhibitor osimertinib mediate inhibition of HCC4006 long-term cell proliferation – the details are provided in example 11.

FIG 11: Inhibitors that bind to the bromodomain of CBP/p300 have no effect in vivo when used in the absence of EGFR inhibitors but they potentiate the effect EGFR inhibitors to provide better tumor-control over time and better response rates to the therapy when combined with EGFR inhibitors. (A) The mean tumor volumes (+SEM) of EGFR-mutated NCI-H1975 xenografts are plotted over time. Four different treatment groups are depicted: vehicle (crossed circle), 90 mg/kg

Compound C (open circles), 2 mg/kg osimertinib (filled circle) and 2 mg/kg osimertinib in combination with 90 mg/kg Compound C (half-filled circles). (B) The best average response for all 4 treatment groups is shown in a waterfall plot (vehicle in grey, 90 mg/kg Compound C in white, 2 mg/kg osimertinib in black and 2 mg/kg osimertinib in combination with 90 mg/kg Compound C in squared). The dashed line indicates the reduction of 30% of initial tumor volume – the details are provided in example 12.

DETAILED DESCRIPTION OF THE INVENTION

Before the present invention is described in more detail, the following definitions are introduced.

1. Definitions

As used in the specification and the claims, the singular forms of "a" and "an" also include the corresponding plurals unless the context clearly dictates otherwise.

The term "about" in the context of the present invention denotes an interval of accuracy that a person skilled in the art will understand to still ensure the technical effect of the feature in question. The term typically indicates a deviation from the indicated numerical value of ±10% and preferably ±5%.

It needs to be understood that the term "comprising" is not limiting. For the purposes of the present invention, the term "consisting of" is considered to be a preferred embodiment of the term "comprising". If hereinafter a group is defined to comprise at least a certain number of embodiments, this is also meant to encompass a group which preferably consists of these embodiments only.

The term "a CBP/p300 bromodomain inhibitor" as used herein means a small molecule that strongly and selectively binds to the bromodomain of CBP and to the bromodomain of p300. This term is synonymous with the terms "a bromodomain inhibitor selectively binding to the bromodomain of CBP/p300" and "a bromodomain inhibitor selective for the inhibition of CBP/p300". "Strong binding" in this respect means a Kd of less than about 300 nM, preferably less than about 100 nM when binding to the bromodomain of CBP and the bromodomain of p300. "Selective binding" in this respect means that the small molecule binds to the bromodomain of CBP and the bromodomain of p300 with a Kd that is at least about 20 fold lower, preferably at least about 30 fold lower, more preferably at least about 50 fold lower and most preferably at least about 70 fold lower than the Kd for binding of any other bromodomain-containing protein or bromodomain of the BROMOscan™, preferably when compared to the further bromodomain-containing proteins or bromodomains indicated by the DiscoveRx Gene Symbols in the Table of example 4 of the present application when carrying out the BROMOscan™ as indicated in example 4. For the comparison, the lowest Kd of any bromodomain-containing protein or bromodomain of the BROMOscan™ except for CBP and p300 is compared to the highest Kd of CBP and p300. Thus, if e.g. the Kd for BRD4 (full-length, short-iso.) is the lowest Kd of all bromodomain-containing pro-

teins or bromodomains except for CBP and p300, and is 7100 nM, this is compared to the Kd for CBP, which is 29 nM (and not to the Kd for p300, which is 12 nM and thus lower than the Kd for CBP). The afore-mentioned example is made for Compound A in the Table of example 4 below.

By the strong and selective binding as outlined above, interactions with interaction partners in the cell that usually take place via the bromodomain of CBP/p300 are inhibited such that the molecule is referred to as "inhibitor". The term "inhibiting interactions" means that preferably no interaction at all (at least not to a detectable level) between the bromodomain of CBP/p300 and an interaction partner takes place anymore. However, when a given interaction between the bromodomain of CBP/p300 and an interaction partner (set to 100%) is greatly reduced, e.g. to a level of about 50%, about 40%, about 30%, preferably about 20%, more preferably about 10% or most preferably about 5% or less, such a reduced interaction is still encompassed by the term "inhibiting interactions". In terms of the medical use of a compound inhibiting an interaction, a complete inhibition of an interaction may not be required to achieve a sufficient therapeutic effect. Thus, it needs to be understood that the term "inhibiting" as used herein also refers to a reduction of an interaction, which is sufficient to achieve a desired effect.

The term "EGFR" as used herein refers to the protein "epidermal growth factor receptor". EGFR is a transmembrane protein that is activated by binding of its specific ligands, including epidermal growth factor. Upon activation by its growth factor ligands, EGFR undergoes a transition from an inactive monomeric form to an active homodimer. In addition to forming homodimers after ligand binding, EGFR may pair with another member of the ErbB receptor family, such as ErbB2/Her2/neu, to create an activated heterodimer. EGFR dimerization stimulates its intrinsic intracellular protein-tyrosine kinase activity. As a result, autophosphorylation of several tyrosine residues in the C-terminal domain of EGFR occurs, which elicits downstream activation and signaling by several other proteins that associate with the phosphorylated tyrosines through their own phosphotyrosine-binding SH2 domains. These downstream signaling proteins initiate several signal transduction cascades, principally the MAPK, Akt and JNK pathways, leading to DNA synthesis and cell proliferation. Mutations that lead to EGFR overactivation have been associated with a number of cancers, including lung cancer, and may *inter alia* result in its constant activation, which results in uncontrolled cell division.

The term "EGFR inhibitor" as used herein refers to molecules capable of acting on EGFR such that intracellular downstream signaling, which ultimately results in cell proliferation, is inhibited. The term "inhibited" in this context means that preferably no downstream signaling takes place any more. However, when a given downstream signaling (set to 100%) is greatly reduced, e.g. to a level of about 70%, about 60%, about 50%, about 40%, about 30%, preferably about 20%, more preferably about 10% or most preferably about 5% or less, such a reduced downstream signaling is still encompassed by the term "inhibiting intracellular downstream signaling". In terms of the medical use of a compound inhibiting downstream signaling, a complete inhibition of the signaling may not be required to achieve a sufficient therapeutic effect. Thus, it needs to be understood that the term "inhibiting" as used herein in this context also refers to a reduction of a downstream signaling, which is sufficient to achieve a desired effect. An EGFR inhibitor may bind

to and thus block the extracellular ligand binding domain of the EGFR. Such an EGFR inhibitor is typically an antibody, in particular a monoclonal antibody selected from the group consisting of amivantamab, CDP1, cetuximab, GC1118, HLX07, JMT101, M1231, necitumumab, nimotuzumab, matuzumab, panitumumab, SCT200, SI-B001, SYN004, zalutuzumab, and combinations thereof. An EGFR inhibitor may also bind to the cytoplasmic side of the receptor and thereby inhibit the EGFR tyrosine kinase activity. Such an EGFR inhibitor is typically a small molecule, in particular a small molecule selected from the group consisting of abivertinib, afatinib, alflutinib, almonertinib, apatinib, AZD3759, brigatinib, D 0316, D 0317, D 0318, dacomitinib, DZD9008, erlotinib, FCN-411, gefitinib, icotinib, lapatinib, lazertinib, mobocertinib, nazartinib, neratinib, olafertinib, osimertinib, poziotinib, pyrotinib, rezivertinib, TAS6417, vandetanib, varlitinib, XZP-5809, and combinations thereof.

The term "wherein the NSCLC exhibits an oncogenic alteration in the EGFR" as used herein means that the NSCLC tumors have a mutated version of the EGFR, wherein this mutated version of the EGFR is implicated in the development of the NSCLC. In other words, the mutated version of the EGFR can be regarded as being linked to or causative of the development of the NSCLC, optionally amongst other factors. The mutated version of the EGFR is present in the NSCLC tumors because of an alteration in the EGFR gene, wherein such an alteration is in particular a deletion in the EGFR gene, an insertion in the EGFR gene, a deletion and insertion in the EGFR gene, a duplication in the EGFR gene, an amplification of the EGFR gene, and/or at least one base mutation in the EGFR gene resulting in an amino acid substitution in the EGFR. Corresponding specific alterations are outlined above. Frequently, combinations of such alterations in the EGFR gene are found. The "oncogenic alteration in the EGFR" is not a "resistance alteration in the EGFR" as defined below.

The term "resistance alteration in the EGFR" as used herein means that, upon treatment with an EGFR inhibitor, the NSCLC tumors have acquired (in addition to the oncogenic alteration) a further alteration in the EGFR, wherein this further alteration in the EGFR renders the NSCLC resistant to a treatment by said EGFR inhibitor (i.e. the EGFR inhibitor that was used for the treatment and to which the NSCLC was initially sensitive). The resistance is mediated by an alteration in the EGFR gene, which can in particular be at least one base mutation in the EGFR gene resulting in an amino acid substitution in the EGFR. Thus, in contrast to the "oncogenic alteration in the EGFR" as defined above, the "resistance alteration" is not regarded as being linked to or causative of the initial development of the NSCLC. Rather, it provides a further growth advantage to the NSCLC, namely in that it confers resistance to the NSCLC to the treatment by a specific EGFR inhibitor that was previously administered (and that was effective in treating the NSCLC before the resistance alteration developed as response of the tumor to this treatment). A prominent "resistance alteration in the EGFR" is the amino acid substitution T790M in the EGFR, which is also referred to as gate-keeper mutation. The "resistance alteration in the EGFR" is not an "oncogenic alteration in the EGFR" as defined above. However, both types of alterations can of course be present in the EGFR of a NSCLC tumor and are frequently detected in patients and corresponding cell lines exist as model systems (see e.g. the cell line NCI-H1975).

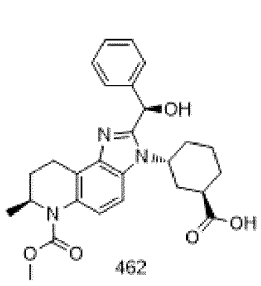
The term "overactivation" of the EGFR as used herein means that the EGFR is more active compared to the wild-type situation, in particular more active with respect to downstream activation and signaling, thus resulting in cancerous cell growth.

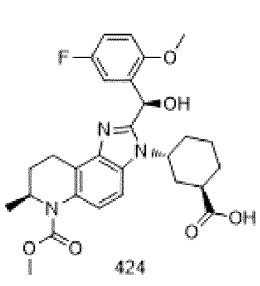
The term "small molecule" as used herein refers to a small organic compound having a low molecular weight. A small molecule in the context of the present invention preferably has a molecular weight of less than 5000 Dalton, more preferably of less than 4000 Dalton, more preferably less than 3000 Dalton, more preferably less than 2000 Dalton or even more preferably less than 1000 Dalton. In a particularly preferred embodiment a small molecule in the context of the present invention has a molecular weight of less than 800 Dalton. In another preferred embodiment, a small molecule in the context of the present invention has a molecular weight of 50 to 3000 Dalton, preferably of 100 to 2000 Dalton, more preferably of 100 to 1500 Dalton and even more preferably of 100 to 1000 Dalton.

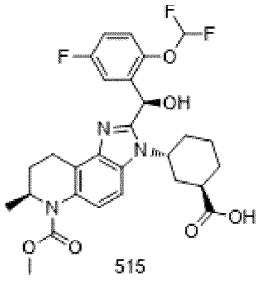
The term "treatment" as used herein refers to clinical intervention in order to cure or ameliorate a disease, prevent recurrence of a disease, alleviate symptoms of a disease, diminish any direct or indirect pathological consequences of a disease, achieve a stabilized (i.e., not worsening) state of disease, prevent metastasis, decrease the rate of disease progression, and/or prolong survival as compared to expected survival if not receiving treatment.

The term "treatment cycle" as used herein means that a medicament is administered for a period of time after an initial assessment of the patient's condition, wherein the patient's condition is then typically reassessed before starting another treatment cycle.

The details of the CBP/p300 bromodomain inhibitors referred to herein are as follows: The structures of Compound A, Compound C, Compound 00030 and Compound 00071 are shown in the example section of the present application. Further, the synthesis routes for these compounds are shown in the example section of the present application. CCS1477 is commercially available e.g. at Aobious and its CAS-no. is 2222941-37-7. GNE-781 is commercially available e.g. at MCE (MedChemExpress) and its CAS-no. is 1936422-33-1. GNE-049 is commercially available e.g. at MCE (MedChemExpress) and its CAS-no. is 1936421-41-8. SGC-CBP30 is commercially available e.g. at MCE (MedChemExpress) and its CAS-no. is 1613695-14-9. CPI-637 is commercially available e.g. at MCE (MedChemExpress) and its CAS-no. is 1884712-47-3. FT-6876 is commercially available e.g. at MCE (MedChemExpress) and its CAS-no. is 2304416-91-7 (FT-6876 is also referred to as "CBP/p300-IN-8"). The structures of Compounds 462, 424 and 515 are depicted below, wherein these structures and the synthesis routes are given in WO 2020/006483 (see in particular pages 33 and 34 for Compound 424, pages 42 and 43 for Compound 462, and pages 47 and 48 for Compound 515):


462


424


515

2. The surprising findings by the inventors

The present inventors identified novel compounds that strongly bind to the bromodomain of CBP/p300 and showed that the binding to the bromodomain of CBP/p300 is also selective, as it is well known that there are many proteins that comprise bromodomains.

CBP/p300 have been identified as central nodes in eukaryotic transcriptional regulatory networks and as interacting with more than 400 transcription factors and other regulatory proteins. CBP/p300 regulate crosstalk and interference between numerous cellular signaling pathways and are targeted by tumor viruses to hijack the cellular regulatory machinery (see Dyson and Wright, *supra*, page 6714, right column). CBP/p300 are large proteins that contain several domains, as can be derived from Figure 1 of Dyson and Wright, *supra*. These domains are the NRID, TAZ1, TAZ2, KIX, CRD1, BRD, CH2 (with a PHD domain and a RING finger domain), HAT, ZZ and NCBD domains. It is already evident from the size of these proteins and their different domains that their cellular functions are very diverse, e.g. by interacting with many different interaction partners due to the variety of interactions that CBP/p300 are capable of. CBP/p300's enzymatic activity as a histone acetyltransferase is located in the HAT domain. As noted above, this enzymatic function is mainly implicated in transcriptional activation. CBP/p300 is also subject to posttranslational modifications, in particular phosphorylation. Their own enzymatic activity as well as the proteins being subject to posttranslational modifications introduces yet another level of complexity to the various functions and effects of CBP/p300. That these functions and effects can even be opposed is nicely summarized in the introductory section of Goodman and Smolk, *Genes & Development* 2000, *14*:1553-1577, where it is stated that one of the major paradoxes in CBP/p300 function is that these proteins appear to be capable of contributing to diametrically opposed cellular processes, and that it appears to be highly context dependent whether CBP/p300 promote apoptosis or cell proliferation. For the implication in diseases and in particular in cancer, this means that the context of the specific disease and specific cancer type will be decisive on how CBP/p300 are involved, if they are involved at all.

In view of the above, it is not surprising that it is not possible to assign a single function to CBP/p300 in a cellular process, which could be influenced e.g. by a general "CBP/p300 inhibitor". Rather, due to the enormous level of complexity, the dissection of the various functions of CBP/p300 appears only to be possible when investigating the specific domains of CBP/p300, i.e. by analyzing the effects achieved when e.g. inhibiting the enzymatic activity of CBP/p300 in their HAT domains or when rendering specific interactions to interaction partners impossible by block-

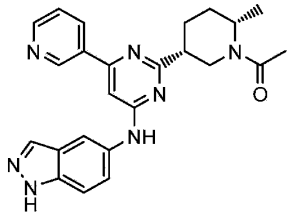
ing (or "inhibiting") certain domains. Furthermore, this must be seen in the respective context, e.g. a specific disease or cancer type, as outlined above.

Thus, the inventors moved on to study their effects in specific contexts, where their inhibitors render interactions with interaction partners via the bromodomain of CBP/p300 impossible. At present, it is known that CBP/p300's bromodomain recognizes acetyl-lysine residues in histone tails and in transcription factor IDRs (intrinsically disordered regions) including those of p53 and CREB (see Dyson and Wright, *supra*, page 6717, right column). The inventors set out to investigate the effect of their inhibitors in non-small cell lung cancer (NSCLC) cells in view of a recent publication by Hou et al. (Hou et al., *supra*). In this publication, it is concluded on the basis of shRNA-mediated down-regulation of p300 that p300 promotes cell proliferation, migration and invasion in NSCLC cells as a crucial tumor promoter. However, the inventors failed to see an effect on the proliferation of the tested NSCLC cell lines when applying the inhibitor alone. Thus, contrary to other cancer types such as e.g. prostate cancer, a CBP/p300 bromodomain inhibitor fails to have an effect on the proliferation of NSCLC cells, and it remains to be seen whether inhibitors targeting different domains of CBP/p300 might show the effect that is observed if the complete p300 protein is downregulated by RNAi in NSCLC cells.

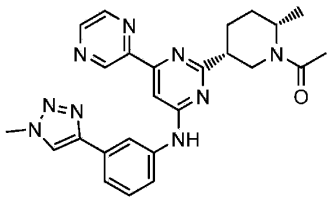
The inventors moved on to test the CBP/p300 bromodomain inhibitors and surprisingly found that their CBP/p300 bromodomain inhibitors prolonged the effect of an EGFR inhibitor in NSCLC cells exhibiting an oncogenic alteration in the EGFR compared to the administration of the EGFR inhibitor alone. In other words, while failing to have an effect on its own on the proliferation of NSCLC exhibiting an oncogenic alteration in the EGFR, the CBP/p300 bromodomain inhibitors of the inventors exhibited an effect with an EGFR inhibitor. Still in other words, the combination of the CBP/p300 bromodomain inhibitors of the inventors and the EGFR inhibitor resulted in a remarkable proliferation inhibition of the tested EGFR-mutant NSCLC cells over time.

For their experiments, the inventors used NSCLC cell lines with deletions in exon 19 of EGFR gene as oncogenic alteration (HCC827 with the deletion in exon 19 resulting in the deletion of E746 to A750 in the EGFR and HCC4006 with the deletion in exon 19 resulting in the deletion of L747 to E749 in the EGFR) but without a resistance alteration in the EGFR. These cell lines may thus be regarded as model system for first-line treatment of patients with NSCLC whose tumors have "EGFR exon 19 deletions. Gefitinib and osimertinib were used as respective EGFR inhibitor in combination with the CBP/p300 bromodomain inhibitors (see the examples below). The inventors also used a NSCLC cell line with EGFR exhibiting the oncogenic alteration L858R as well as the resistance alteration T790M (NCI-H1975). EGFR T790M is known to develop as resistance alteration in response to e.g. gefitinib treatment, rendering gefitinib treatment ineffective. This cell line may thus be regarded as model system for second-line treatment of patients with NSCLC whose tumors have developed resistance to an initial EGFR inhibitor treatment. In this cell line, the inventors used only osimertinib in combination with the CBP/p300 bromodomain inhibitor (as osimertinib has been shown to be effective despite the resistance alteration T790M in EGFR, which renders gefitinib ineffective). Testing of the combination of gefitinib with the CBP/p300 bromodomain inhibitor was moot in view of the T790M mutation.

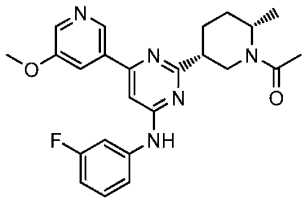
The observed remarkable proliferation inhibition over long-term incubation for the combination in all tested cell lines is in particular noteworthy since – due to the development of resistance - the proliferation inhibition over time will not remain complete when using an EGFR inhibitor alone. As the data in the experimental section below show, this is not only the case for gefitinib when applied alone, but also for osimertinib when applied alone. Thus, while osimertinib is initially capable of overcoming the resistance provided by the mutation EGFR T790M and thus initially effective (contrary to gefitinib, to which the NSCLC is already resistant), also resistance towards osimertinib develops over time, which ultimately results in osimertinib becoming ineffective. Given the results for their CBP/p300 inhibitors, the inventors went on to investigate whether the observed effect can be generalized to CBP/p300 inhibitors as such. To this aim, further CBP/p300 bromodomain inhibitors were tested, namely CCS1477, SGC-CBP30, FT-6876 and GNE-781. It is noted that the structures of the different sets of CBP/p300 inhibitors that were tested by the inventors are not related such that their feature in common exclusively relates to the effect that is achieved by these inhibitors, namely the selective inhibition of the CBP/p300 bromodomain. The structures of all tested CBP/p300 inhibitors are as follows:



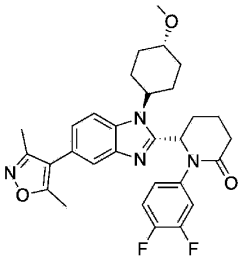
Compound 00030



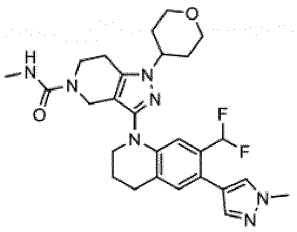
Compound C



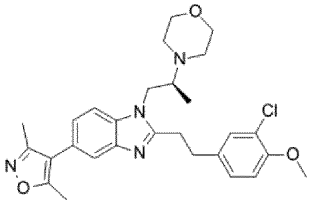
Compound A



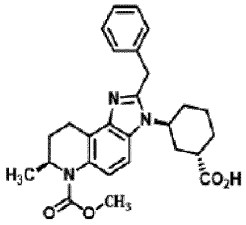
CCS1477



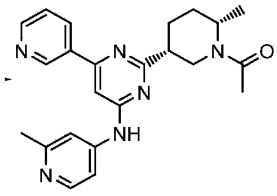
GNE-781



SGC-CBP30



FT-6876



Compound 00071

It should also be mentioned that the tested EGFR inhibitors gefitinib and osimertinib are quite different in their structure and action (gefitinib is a "non-covalent inhibitor", whereas osimertinib is a "covalent inhibitor") but have in common their inhibitory function against the kinase activity of the EGFR. Furthermore, they are quite different in terms of their development, namely a drug of the first generation for treating NSCLC (i.e. gefitinib) and a drug of the third generation for treating NSCLC (i.e. osimertinib).

Furthermore, not only a single NSCLC cell line was tested by the inventors, but three different NSCLC cell lines (HCC827, HCC4006 and NCI-H1975) were used. This is in particular important as *in vitro* experiments with a single cell line of a given disease might provide unreliable results, whereas obtaining identical results in at least two different cell lines of a given disease is a much stronger indicator that the obtained results are reliable. Furthermore, it appears to be preferred to use NSCLC cell lines in such assays that are initially fully growth-inhibited by EGFR inhibitors to be in a position to more reliably analyze the effect of the CBP/p300 bromodomain inhibitor, in particular after a few days of treatment. Of course, results that are based on xenograft models and that are consistent with the initial findings when using NSCLC cell lines even better confirm the overall conclusion that can be drawn from the experiments. Such xenograft data were gained by the inventors as well, as shown in the example section herein below.

3. Pharmaceutical composition of the compound of the present invention

The "CBP/p300 bromodomain inhibitor" and the "EGFR inhibitor" are "pharmaceutically active agents" for the use as claimed herein. As noted above, they may either be present in separate dosage forms or comprised in a single dosage form.

"Pharmaceutically active agents" as used herein means that the compounds are potent of modulating a response in a patient, i.e. a human or animal being *in vivo*. The term "pharmaceutically acceptable excipient" as used herein refers to excipients commonly comprised in pharmaceutical compositions, which are known to the skilled person. Such excipients are exemplary listed below. In view of the definition "pharmaceutically active agents" as given above, a pharmaceutically acceptable excipient can be defined as being pharmaceutically inactive.

If a marketed EGFR inhibitor is used in combination with the CBP/p300 bromodomain inhibitor, it is preferred that the administration occurs via separate dosage forms and that the EGFR inhibitor is administered in the dosage form and via the administration route that is authorized. The CBP/p300 bromodomain inhibitor may be administered in a dosage form as set out in the following or in a dosage form in which it is currently undergoing clinical testing.

A dosage form for use according to the present invention may be formulated for oral, buccal, nasal, rectal, topical, transdermal or parenteral application. Oral application can be preferred. Parenteral application can also be preferred and includes intravenous, intramuscular or subcutaneous administration. A dosage form of the present invention may also be designated as formulation or pharmaceutical composition.

In general, a pharmaceutical composition according to the present invention can comprise various pharmaceutically acceptable excipients which will be selected depending on which functionality is to be achieved for the composition. A "pharmaceutically acceptable excipient" in the meaning of the present invention can be any substance used for the preparation of pharmaceutical dosage forms, including coating materials, film-forming materials, fillers, disintegrating agents, release-modifying materials, carrier materials, diluents, binding agents and other adjuvants. Typical pharmaceutically acceptable excipients include substances like sucrose, mannitol, sorbitol, starch and starch derivatives, lactose, and lubricating agents such as magnesium stearate, disintegrants and buffering agents.

The term "carrier" denotes pharmaceutically acceptable organic or inorganic carrier substances with which the active ingredient is combined to facilitate the application. Suitable pharmaceutically acceptable carriers include, for instance, water, salt solutions, alcohols, oils, preferably vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, surfactants, perfume oil, fatty acid monoglycerides and diglycerides, petroethral fatty acid esters, hydroxymethylcellulose, polyvinylpyrrolidone and the like. The pharmaceutical compositions can be sterilized and if desired, mixed with auxiliary agents, like lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compound.

If liquid dosage forms are considered for the present invention, these can include pharmaceutically acceptable emulsions, solutions, suspensions and syrups containing inert diluents commonly used in the art such as water. These dosage forms may contain e.g. microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer and sweeteners/flavouring agents.

For parenteral application, particularly suitable vehicles consist of solutions, preferably oily or aqueous solutions, as well as suspensions, emulsions, or implants. Pharmaceutical formulations for parenteral administration are particularly preferred and include aqueous solutions in water-soluble form. Additionally, suspensions may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran.

Particularly preferred dosage forms are injectable preparations of a pharmaceutical composition of the present invention. Thus, sterile injectable aqueous or oleaginous suspensions can for example be formulated according to the known art using suitable dispersing agents, wetting agents and/or suspending agents. A sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that can be used are water and isotonic sodium chloride solution. Sterile oils are also conventionally used as solvent or suspending medium.

Suppositories for rectal administration of a pharmaceutical composition of the present invention can be prepared by e.g. mixing the compound with a suitable non-irritating excipient such as cocoa butter, synthetic triglycerides and polyethylene glycols which are solid at room temperature but liquid at rectal temperature such that they will melt in the rectum and release the active agent from said suppositories.

For administration by inhalation, the pharmaceutical composition comprising a compound according to the present invention may be conveniently delivered in the form of an aerosol spray from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

Oral dosage forms may be liquid or solid and include e.g. tablets, troches, pills, capsules, powders, effervescent formulations, dragees and granules. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. The oral dosage forms may be formulated to ensure an immediate release of the active agent or a sustained release of the active agent.

4. Further disclosure and embodiments

The clinical anti-tumor effect of receptor tyrosine kinase (RTK) inhibitors and other kinase inhibitors is not durable. Resistance to these inhibitors usually develops. More specifically the clinical anti-tumor effect of EGFR inhibitors (EGFRi) is not durable. Resistance to EGFR inhibitors usually develops within 9 to 19 months depending on the therapeutic agent and clinical setting. Therefore it is desirable to develop a mode of cancer treatment that would prevent drug resistance in cancer patients. Historically, most approaches to tackle drug resistance have focused on the genetic drivers of relapsing tumors. In an effort to overcome already established drug resistance, a newly mutated protein that drives tumor regrowth would be therapeutically targeted alone or in combination with the primary cancer drug. One resistance mechanism to EGFRi treatment is the development of a gatekeeper mutation in the EGFR protein – a mutation that renders the EGFRi ineffective. Most commonly this gatekeeper mutation is a T790M mutation. Mutation-specific inhibitors such as Osimertinib are used to overcome established drug resistance to first generation EGFR inhibitors that are not inhibiting mutated EGFR T790M. Another resistance

mechanism to EGFRi treatment is bypass signalling which is activated via other receptor tyrosine kinases, for example through the amplification, overexpression or activation of MET, ErbB2, HGF, ErbB3, IGF1R, AXL, NTRK1, BRAF, FGFR3, or FGFR1. Therapeutic interventions to inhibit bypass signalling have been tested in the clinic with mixed results.

Previous disclosures such as patent application WO2018022637, describe the use of CBP/p300 inhibitors as novel cancer therapies, particularly for the treatment of cancers harbouring p300 mutations. WO2011085039 describes methods for treating cancer comprising inhibiting the activity of CBP/p300 histone acetyltransferase (HAT) and the use of CBP/p300 HAT inhibitors for treating a subject having cancer, in particular in combination with DNA damaging chemotherapeutic anti-cancer agents.

There is a need for new effective methods and compositions to prevent the development of cancer drug resistance. This is *inter alia* addressed by the embodiments of the present section 4.

Embodiment 1: A CBP/p300 bromodomain inhibitor for use in a method of treating cancer in an animal comprising administering to the animal in need thereof, a CBP/p300 bromodomain inhibitor and a receptor tyrosine kinase inhibitor selected from the group consisting of EGFR, ALK, MET, HER2, ROS1, RET, NTRK1 and AXL inhibitor, or a KRas (Kirsten Rat Sarcoma) or BRAF (proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B) inhibitor, wherein the cancer comprises an alteration in the corresponding receptor tyrosine kinase or in KRas or BRAF and wherein the CBP/p300 bromodomain inhibitor alone does not slow the progression of the cancer.

Embodiment 2: A CBP/p300 bromodomain inhibitor for use in a method of extending the duration of response to a receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor cancer therapy in an animal, comprising administering to an animal with cancer a CBP/p300 bromodomain inhibitor or a pharmaceutically acceptable salt thereof, wherein the duration of response to the cancer therapy when the CBP/p300 bromodomain inhibitor or a pharmaceutically acceptable salt thereof is administered is extended compared to the duration of response to the cancer therapy in the absence of the administration of the CBP/p300 bromodomain inhibitor or a pharmaceutically acceptable salt thereof, and wherein the receptor tyrosine kinase inhibitor is selected from the group consisting of EGFR, ALK, MET, HER2, ROS1, RET, NTRK1 and AXL inhibitor.

Embodiment 3: A composition for use in the treatment of cancer, said composition comprising a synergistic combination of a CBP/p300 bromodomain inhibitor or a pharmaceutically acceptable salt thereof, and a receptor tyrosine kinase inhibitor selected from the group consisting of an inhibitor of EGFR, ALK, MET, HER2, ROS1, RET, NTRK1 and AXL, or a KRas or BRAF inhibitor, wherein the cancer comprises an alteration in the corresponding receptor tyrosine kinase or KRas or BRAF and wherein the CBP/p300 bromodomain inhibitor alone does not slow the progression of the cancer.

Embodiment 4: A method of inhibiting the growth of a cancer cell comprising administering a CBP/p300 bromodomain inhibitor and a receptor tyrosine kinase inhibitor selected from the group consisting of EGFR, ALK, MET, HER2, ROS1, RET, NTRK1 and AXL inhibitor, or a KRas or BRAF inhibitor and wherein the cancer cell comprises an alteration in the corresponding receptor tyrosine kinase or KRas or BRAF and wherein the CBP/p300 bromodomain inhibitor alone does not inhibit the growth of the cancer cell.

Embodiment 5: The CBP/p300 bromodomain inhibitor or composition for use or method according to any preceding embodiment, wherein the alteration to the receptor tyrosine kinase or to KRas or BRAF is an oncogenic alteration.

Embodiment 6: The CBP/p300 bromodomain inhibitor or composition for use or method according to any preceding embodiment, wherein the receptor tyrosine kinase inhibitor is an EGFR inhibitor.

Embodiment 7: The CBP/p300 bromodomain inhibitor or composition for use or method according to embodiment 6, wherein the alteration to the receptor tyrosine kinase is a mutation in EGFR.

Embodiment 8: The CBP/p300 bromodomain inhibitor or composition for use or method according to any preceding embodiment, wherein the composition or combination of a CBP/p300 bromodomain inhibitor or a pharmaceutically acceptable salt thereof, and receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor, is synergistic in treating cancer, compared to the CBP/p300 inhibitor alone or the receptor tyrosine kinase or KRas or BRAF inhibitor alone.

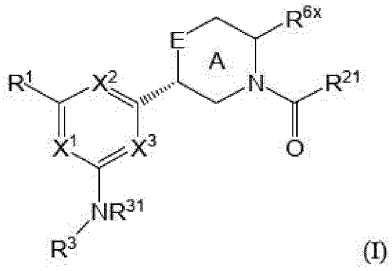
Embodiment 9: The CBP/p300 bromodomain inhibitor or composition for use or method according to any preceding embodiment, wherein the composition or combination of a CBP/p300 bromodomain inhibitor or a pharmaceutically acceptable salt thereof, and receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor, delays or reduces the risk of resistance of the cancer to the receptor tyrosine kinase inhibitor or Kras or BRAF inhibitor.

Embodiment 10: The CBP/p300 bromodomain inhibitor or composition for use or method according to any preceding embodiment, wherein the CBP/p300 bromodomain inhibitor is administered in an effective amount to prevent resistance of the cancer cell to the receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor.

Embodiment 11: The CBP/p300 bromodomain inhibitor or composition for use or method according to any preceding embodiment, wherein the EGFR inhibitor is selected from the group comprising cetuximab, panitumumab, zalutumumab, nimotuzumab, matuzumab, gefitinib, erlotinib, dacomitinib, lapatinib, neratinib, vandetanib, necitumumab, osimertinib, afatinib, AP26113, EGFR inhibitor (CAS No. 879127-07-8), EGFR/ErbB2/ErbB-4 Inhibitor (CAS No. 881001-19-0), EGFR/ErbB-2 Inhibitor (CAS No. 17924861-4), EGFR inhibitor II (BIBX 1382,CAS No. 196612-93-8),

EGFR inhibitor III (CAS No. 733009-42-2), EGFR/ErbB-2/ErbB-4 Inhibitor II (CAS No. 944341-54-2) or PKCβII/EGFR Inhibitor (CAS No. 145915-60-2).

Embodiment 12: The CBP/p300 bromodomain inhibitor or composition for use or method according to any preceding embodiment, wherein the CBP/p300 inhibitor is a compound of formula (I)



(I)

wherein

R^1 is selected from halogen and −(optionally substituted hydrocarbon group which contains from 1 to 20 carbon atoms and optionally 1 to 15 heteroatoms selected from O, N and S);

R^{21} is selected from hydrogen, −(optionally substituted C_{1-6} alkyl) which may contain one to three oxygen atoms between carbon atoms, and −(optionally substituted C_{3-6} cycloalkyl);

R^3 is selected from −(optionally substituted heterocyclyl), −(optionally substituted carbocyclyl), −(optionally substituted C_{1-6} alkylene)−(optionally substituted heterocyclyl) and −(optionally substituted C_{1-6} alkylene)−(optionally substituted carbocyclyl);

each of X^1, X^2 and X^3 is independently selected from N, CH and CR^x, wherein at least one of said X^1, X^2 and X^3 is N;

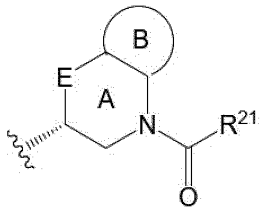
R^{31} is selected from –hydrogen, –C_{1-6}-alkyl, and –(C_{1-6}-alkyl substituted with one or more F);

wherein R^3 and any R^{31} can be optionally linked; and

E is either absent or is selected from –CH_2–, –CHR^x–, –CR^x_2–, –NH–, –NR^x–, –O–, –L^1–L^2– and –L^2–L^1–, wherein L^1 is selected from –CH_2–, –CHR^x–, –CR^x_2–, –NH–, –NR^x– and –O– and L^2 is selected from –CH_2–, –CHR^x– and –CR^x_2–;

R^{6x} is –halogen, –OH, =O, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkyl substituted with one or more OH, monocyclic aryl optionally substituted with one or more R^{xb}, monocyclic heteroaryl optionally substituted with one or more R^{xb}, monocyclic cycloalkyl optionally substituted with one or more R^{xb}, monocyclic heterocycloalkyl optionally substituted with one or more R^{xb}, monocyclic cycloalkenyl optionally substituted with one or more R^{xb}, monocyclic heterocycloalkenyl optionally substituted with one or more R^{xb}, wherein said R^{xb} is independently selected from –halogen, –OH, =O, C_{1-4} alkyl, C_{1-2} haloalkyl, C_{1-2} alkyl substituted with one or two OH;

wherein Ring A may further be substituted with one or more groups R^x, wherein any two R^x groups at ring A can be optionally linked and/or any R^x group at ring A can be optionally linked with R^{21}; and/or wherein Ring A may be further substituted with one group R^x so as to form together with R^{6x} a bicyclic moiety having the following partial structure:

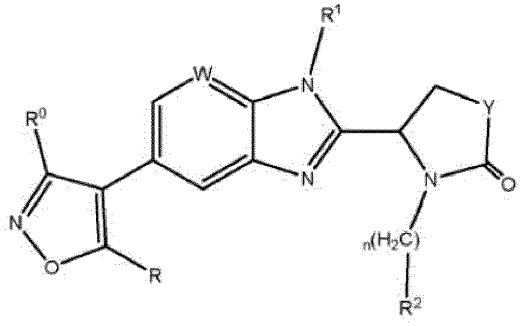


wherein Ring B is an −(optionally substituted heterocycle) or −(optionally substituted carbocycle); each R^x is independently selected from –halogen, –OH, −O–(optionally substituted C_{1-6} alkyl), −NH–(optionally substituted C_{1-6} alkyl), −N(optionally substituted C_{1-6} alkyl)$_2$, =O, −(optionally substituted C_{1-6} alkyl), –(optionally substituted carbocyclyl), –(optionally substituted heterocyclyl), −(optionally substituted C_{1-6} alkylene)–(optionally substituted carbocyclyl), −(optionally substituted C_{1-6} alkylene)–(optionally substituted heterocyclyl), –O−(optionally substituted C_{1-6} alkylene)–(optionally substituted carbocyclyl), and –O–(optionally substituted C_{1-6} alkylene)–(optionally substituted heterocyclyl), and

wherein the optional substituent of the optionally substituted hydrocarbon group, optionally substituted C_{3-6} cycloalkyl, optionally substituted heterocyclyl, optionally substituted heterocycle, optionally substituted carbocyclyl, optionally substituted carbocycle and optionally substituted C_{1-6} alkylene is independently selected from –(C_{1-6} alkyl which is optionally substituted with one or more halogen), −halogen, −CN, −NO_2, oxo, −C(O)R*, −COOR*, −C(O)NR*R*, −NR*R*, −N(R*)−C(O)R*, −N(R*)−C(O)−OR*, −N(R*)−C(O)−NR*R*, −N(R*)−$S(O)_2$R*, −OR*, −O−C(O)R*, −O−C(O)−NR*R*, −SR*, −S(O)R*, −$S(O)_2$R*, −$S(O)_2$−NR*R*, −N(R*)−$S(O)_2$−NR*R*, heterocyclyl which is optionally substituted with halogen or C_{1-6} alkyl, and carbocyclyl which is optionally substituted with halogen or C_{1-6} alkyl; wherein each R* is independently selected from H, C_{1-6} alkyl which is optionally substituted with halogen, heterocyclyl which is optionally substituted with halogen or C_{1-6} alkyl, and carbocyclyl which is optionally substituted with halogen or C_{1-6} alkyl; wherein any two R* connected to the same nitrogen atom can be optionally linked, and

wherein the optional substituent of the optionally substituted C_{1-6} alkyl and of the optionally substituted C_{1-6} alkylene is independently selected from −halogen, −CN, −NO_2, oxo, −C(O)R**, −COOR**, −C(O)NR**R**, −NR**R**, −N(R**)−C(O)R**, −N(R**)−C(O)−OR**, −N(R**)−C(O)−NR**R**, −N(R**)−$S(O)_2$R**, −OR**, −O−C(O)R**, −O−C(O)−NR**R**, −SR**, −S(O)R**, −$S(O)_2$R**, −$S(O)_2$−NR**R**, and −N(R**)−$S(O)_2$−NR**R**; wherein R** is independently selected from H, C_{1-6} alkyl which is optionally substituted with halogen, heterocyclyl which is optionally substituted with halogen or C_{1-6} alkyl, and carbocyclyl which is optionally substituted with halogen or C_{1-6} alkyl; wherein any two R** connected to the same nitrogen atom can be optionally linked.

Embodiment 13: The CBP/p300 bromodomain inhibitor or composition for use or method according to any preceding embodiment, wherein the CBP/p300 inhibitor is an arylimidazolyl isoxazole of formula (A)



(A), wherein

R° and R, which are the same or different, are each H or C_1-C_6alkyl which is unsubstituted or substituted by OH, -OC(O)R' or OR' wherein R' is unsubstituted C_1-C_6alkyl;

W is N or CH;

R^1 is a group which is unsubstituted or substituted and is selected from C-linked 4- to 6-membered heterocyclyl; C_3-C_6 cycloalkyl; C_1-C_6 alkyl which is unsubstituted or substituted by C_6-C_{10} aryl, 5- to 12-membered N-containing heteroaryl, C_3-C_6 cycloalkyl, OH, -OC(O)R' or OR' wherein R' is as defined above; and a spiro group of the following formula:



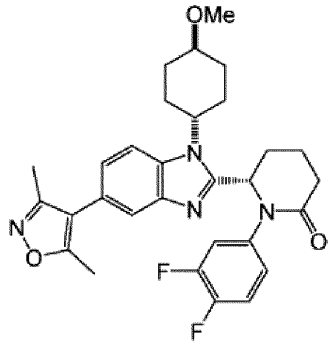
;

Y is -CH_2-, -CH_2CH_2- or -$CH_2CH_2CH_2$-;

n is 0 or 1;

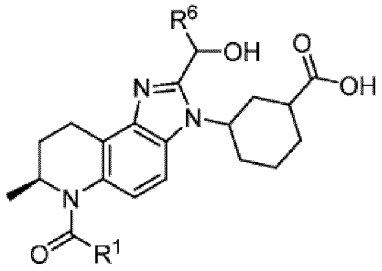
R^2 is a group selected from C_6-C_{10} aryl, 5- to 12-membered N-containing heteroaryl, C_3-C_6 cycloalkyl and C_5-C_6 cycloalkenyl, wherein the group is unsubstituted or substituted and wherein C_6-C_{10} aryl is optionally fused to a 5- or 6-membered heterocyclic ring;

or a pharmaceutically acceptable salt thereof, and wherein preferably said arylimidazolyl isoxazole has the formula (Aa*):



(Aa*; CCS1477 [CAS 2222941-37-7]).

Embodiment 14: The CBP/p300 bromodomain inhibitor or composition for use or method according to any preceding embodiment, wherein the CBP/p300 inhibitor is a compound of formula (Ba)

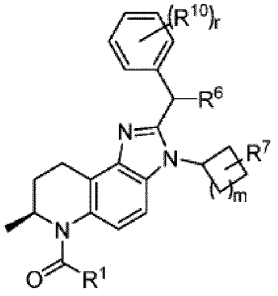


(Ba), wherein

R^1 is -O(C_1-C_3alkyl);

R^6 is phenyl optionally substituted independently with one or more R^B, wherein R^B is selected from –O-C_{1-6}alkyl, -O-C_{3-6}cycloalkyl, -O-aryl, or –O-heteroaryl, wherein each alkyl, cycloalkyl, aryl or heteroaryl is optionally substituted independently with one or more halogen;

or wherein the CBP/p300 inhibitor is a compound of formula (Bc)



(Bc), wherein

R^1 is -OR^5;

R^5 is –C_{1-6}alkyl, -C_{3-8}cycloalkyl, heterocyclyl, aryl, or heteroaryl;

R^6 is -OH, halogen, oxo, -NO_2, -CN, -NH2, -C_{1-6}alkyl, -C_{3-8}cycloalkyl, -C_{4-8}cycloalkenyl, heterocyclyl, aryl, spirocycloalkyl, spiroheterocyclyl, heteroaryl, -OC_{3-6}cycloalkyl, -Oaryl, -Oheteroaryl, -(CH_2)n-OR^8, -C(O)$R^{8'}$, -C(O)OR^8, or -C(O)NR^8R^9, - NHC_{1-6}alkyl, -N(C_{1-6}alkyl)2, -$S(O)_2NH(C_{1-6}$alkyl), -$S(O)_2N(C_{1-6}$alkyl)$_2$, -$S(O)_2C_{1-6}$alkyl, -N(C_{1-6}alkyl)SO_2C_{1-6}alkyl, -S(O)(C_{1-6}alkyl), -S(O)N(C_{1-6}alkyl)$_2$, or -N(C_{1-6}alkyl)S(O)(C_{1-6}alkyl), wherein each alkyl, cycloalkyl, cycloalkenyl, heterocyclyl, spirocycloalkyl, spiroheterocyclyl, heteroaryl, or aryl is optionally substituted with one or more R^{10};

R^7 is independently, at each occurrence, -H, halogen, -OH, -CN, -OC_{1-6}alkyl, -NH_2, - NH(C_{1-6}alkyl), -N(C_{1-6}alkyl)$_2$, -$S(O)_2H(C_{1-6}$alkyl), -$S(O)_2N(C_{1-6}$alkyl)$_2$, -$S(O)_2(C_{1-6}$alkyl, -$S(O)_2$OH, -C(O)C_{1-6}alky, -C(O)NH_2, -C(O)NH(C_{1-6}alkyl), -C(O)N(C_{1-6}alkyl)$_2$, -C(O)OH, -C(O)OC_{1-6}alkyl, -N(C_{1-6}alkyl)SO_2C_{1-6}alkyl, -S(O)(C_{1-6}alkyl), -S(O)N(C_{1-6}alkyl)$_2$, -$S(O)_2NH_2$, -N(C_{1-6}alkyl)S(O)(C_{1-6}alkyl) or tetrazole;

R^{10} is independently, at each occurrence, -C_{1-6}alkyl, -C_{2-6}alkenyl, -C_{2-6}alkynyl, -C_{3-8}cycloalkyl, -C_{4-8}cycloalkenyl, heterocyclyl, heteroaryl, aryl, -OH, halogen, oxo, -NO_2, - CN, -NH_2, -OC_{1-6}alkyl, -OC_{3-6}cycloalkyl, -Oaryl, -Oheteroaryl, -NHC_{1-6}alkyl, -N(C_{1-6}alkyl)$_2$, -$S(O)_2NH(C_{1-6}$alkyl), -$S(O)_2N(C_{1-6}$alkyl)$_2$, -$S(O)_2C_{1-6}$alkyl, -C(O)C_{1-6}alkyl, -C(O)NH_2, -C(O)NH(-C_{1-6}alkyl), -NHC(O)C_{1-6}alkyl -C(O)N(C_{1-6}alkyl)$_2$, -C(O)OC_{1-6}alkyl, -N(C_{1-6}alkyl)SO2-C_{1-6}alkyl, -S(O)(C_{1-6}alkyl), -S(O)N(C_{1-6}alkyl)$_2$, or -N(C_{1-6}alkyl)S(O)(C_{1-6}alkyl), wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclyl, heteroaryl, or aryl is optionally substituted with one or more -R^{12};

R^{12} is independently, at each occurrence, halogen;

m is an integer from 0 to 5;
r is an integer from 0 to 5.

Embodiment 15: The CBP/p300 bromodomain inhibitor or composition for use or method according to the preceding embodiment, wherein a slow progression of the cancer is measured using the RECIST 1.1. Response Criteria for target lesions or non-target lesions in the animal.

Embodiment 16: The CBP/p300 bromodomain inhibitor or composition for use or method according to any preceding embodiment, wherein the cancer is non-small cell lung cancer (NSCLC).

Embodiment 17: The CBP/p300 bromodomain inhibitor or composition for use or method according to the preceding embodiment, wherein the CBP/p300 bromodomain inhibitor is a compound of formula (I) of embodiment 12, the receptor tyrosine kinase inhibitor is an EGFR inhibitor, the receptor tyrosine kinase is EGFR and the cancer is NSCLC, more preferably the NSCLC comprises an EGFR T790M mutation, more preferably wherein the receptor tyrosine kinase inhibitor is Osimertinib.

Embodiment 18: The CBP/p300 bromodomain inhibitor or composition for use or method according to the preceding embodiment, wherein the CBP/p300 bromodomain inhibitor is a compound of formula (A) of embodiment 13, preferably CCS1477 (CAS 2222941-37-7), the receptor tyrosine kinase inhibitor is an EGFR inhibitor, the receptor tyrosine kinase is EGFR and the cancer is NSCLC, more preferably the NSCLC comprises an EGFR T790M mutation, more preferably wherein the receptor tyrosine kinase inhibitor is Osimertinib.

As regards the above embodiment 13, it is noted that the compounds of formula (A) has been described in WO2016170324, WO2018073586 and WO2019202332, all applications and their disclosures are incorporated herein by reference in its entirety, in particular with respect to the synthesis of the compounds of formula (A).

In another embodiment, there is provided a method of treating cancer in an animal comprising administering to the animal in need thereof, a CBP/p300 bromodomain inhibitor and a receptor tyrosine kinase inhibitor selected from the group consisting of EGFR, ALK, MET, HER2, ROS1, RET, NTRK1 and AXL inhibitor, or a KRas or BRAF inhibitor, wherein the cancer comprises an alteration in the corresponding receptor tyrosine kinase or KRas or BRAF and wherein the CBP/p300 bromodomain inhibitor alone does not slow the progression of the cancer.

In another embodiment, there is provided a method of treating cancer with a composition, said composition comprising a synergistic combination of a CBP/p300 bromodomain inhibitor or a pharmaceutically acceptable salt thereof, and a receptor tyrosine kinase inhibitor selected from the group consisting of EGFR, ALK, MET, HER2, ROS1, RET, NTRK1 and AXL inhibitor, or a Kras or BRAF inhibitor, wherein the cancer comprises an alteration in the corresponding receptor tyrosine kinase or Kras or BRAF and wherein the CBP/p300 bromodomain inhibitor alone does not slow the progression of the cancer.

In another embodiment, there is provided a method of extending the duration of response to a receptor tyrosine kinase inhibitor or Kras or BRAF inhibitor cancer therapy in an animal, comprising administering to an animal with cancer a CBP/p300 bromodomain inhibitor or a pharmaceutically acceptable salt thereof, wherein the duration of response to the cancer therapy when the CBP/p300 inhibitor or a pharmaceutically acceptable salt thereof is administered is extended compared to the duration of response to the cancer therapy in the absence of the administration of the CBP/p300 inhibitor or a pharmaceutically acceptable salt thereof, and wherein the receptor tyrosine kinase inhibitor is selected from the group consisting of EGFR, ALK, MET, HER2, ROS1, RET, NTRK1 and AXL or is a KRas or BRAF inhibitor.

In another embodiment, there is provided a method for inhibiting growth of a cancer cell which comprises administering to the cancer cell a CBP/p300 bromodomain inhibitor and a receptor tyrosine kinase inhibitor selected from the group consisting of EGFR, ALK, MET, HER2, ROS1, RET, NTRK1 and AXL inhibitor, or a KRas or BRAF inhibitor, wherein the cancer cell comprises an alteration in the corresponding receptor tyrosine kinase or KRas or BRAF and wherein the CBP/p300 bromodomain inhibitor alone does not inhibit the growth of the cancer cell and wherein the CBP/p300 bromodomain inhibitor is administered in an effective amount to prevent resistance of the cancer cell to the kinase inhibitor.

In another embodiment, there is provided a method for inducing cell death in a cancer cell comprising administering to the cancer cell a CBP/p300 bromodomain inhibitor and a receptor tyrosine kinase inhibitor selected from the group consisting of EGFR, ALK, MET, HER2, ROS1, RET, NTRK1 and AXL inhibitor, or a KRas or BRAF inhibitor wherein the cancer cell comprises an alteration in the corresponding receptor tyrosine kinase or KRas or BRAF and wherein the CBP/p300 bromodomain inhibitor alone does not induce cell death in a cancer cell.

In one embodiment, the alteration to the receptor tyrosine kinase may be an oncogenic alteration, wherein the term "oncogenic alteration" in this embodiment of section 4 may refer to the genetic changes to cellular proto-oncogenes. The consequence of these genetic changes / alterations may be to confer a growth advantage to the cell. In one embodiment the genetic mechanisms of mutation, gene amplification, gene fusions and/or chromosome rearrangements may activate oncogenes in human neoplasms.

In another embodiment, the oncogenic alteration is an EGFR gene mutation selected from the group comprising EGFR-Exon 19 deletion, EGFR-L858R, EGFR-T790M, EGFR-T854A, EGFR-D761Y, EGFR-L747S, EGFR-G796S/R, EGFR-L792F/H, EGFR-L718Q, EGFR-exon 20 insertion, EGFR-G719X (where X is any other amino acid), EGFR-L861X, EGFR-S768I, or EGFR amplification. In a preferred embodiment the alteration is EGFR-T790M. In another embodiment the cancer is NSCLC and the alteration is a mutation comprising EGFR Exon 19 deletion, L858R or T790M.

In another embodiment, the oncogenic alteration is a RET gene mutation or rearrangement selected from the group comprising KIF5B-RET, CCDC6-RET, NCOA4-RET, TRIM33-RET, RET-V804L, RET-L730, RET-E732, RET-V738, RET-G810A, RET-Y806, RET-A807 or RET-S904F.

In another embodiment, the oncogenic alteration is a HER2 gene mutation selected from the group comprising HER2 exon 20 insertion or mutation and HER2-C805S, HER2 T798M, HER2 L869R, HER2 G309E, HER2 S310F or HER2 amplification.

In another embodiment, the oncogenic alteration is a ROS1 gene fusion or rearrangement selected from the group comprising CD74-ROS1, GOPC-ROS1, EZR-ROS1, CEP85L-ROS1, SLC34A2-ROS1, SDC4-ROS1, FIG-ROS1, TPM3-ROS1, LRIG3-ROS1, KDELR2-ROS1, CCDC6-ROS1, TMEM106B-ROS1, TPD52L1-ROS1, CLTC-ROS1 and LIMA1-ROS1 or a mutation comprising ROS1 G2032R, D2033N, S1986Y/F, L2026M and/or L1951R.

In another embodiment, the oncogenic alteration is a MET gene amplification, a MET gene mutation such as MET Y1230C, D1227N, D1228V, Y1248H as well as MET exon 14 skipping, or gene fusion or rearrangements selected from the group comprising TPR-MET, CLIP2-MET, TFG-MET Fusion, KIF5B-MET fusion.

In another embodiment, the oncogenic alteration is a KRas gene mutation selected from the group comprising G12C, G12V, G12D, G13D, Q61H or L or R, K117N.

In another embodiment, the oncogenic alteration is an ALK gene mutation or gene fusion or rearrangement selected from the group comprising EML4-ALK, TFG-ALK, KIF5B-ALK, KLC1-ALK, STRN-ALK in NSCLC, EML4-ALK, C2orf44-ALK, EML4-ALK, TPM-ALK, VCL-ALK, TPM3-ALK, EML4-ALK, or VCL-ALK.

In another embodiment, the oncogenic alteration is a BRAF gene mutation selected from the group comprising V600E or V600K.

In another embodiment, the oncogenic alteration is an NTKR gene fusion or rearrangement selected from the group comprising TPM3-NTRK1, ETV6-NTRK3, TPM3-NTRK1, TPR-NTRK1, TFG-NTRK1, PPL-NTRK1, ETV6-NTRK3, TPR-NTRK1, MPRIP-NTRK1, CD74-NTRK1, SQSTM1-NTRK1, TRIM24-NTRK2, LMNA-NTRK, ETV6-NTRK3, BCAN-NTRK1, ETV6-NTRK3, AML, GIST, NFASC-NTRK1, BCAN-NTRK1, AGBL4-NTRK2, VCL-NTRK2, ETV6-NTRK3, BTBD1-NTRK3, RFWD2-NTRK1, RABGAP1L-NTRK1, TP53-NTRK1, AFAP1-NTRK2, NACC2-NTRK2, OKI-NTRK2, PAN3-NTRK2, or an NTKR1 gene mutation selected from the group comprising F589L, G595R, G667C/S, A608D, or an NTRK3 gene mutation selected from the group comprising G623R, G696A.

In another embodiment, the receptor tyrosine kinase inhibitor is an EGFR inhibitor. In another embodiment, the EGFR inhibitor is selected from the group cetuximab, panitumumab, zalutumumab, nimotuzumab, matuzumab, gefitinib, erlotinib, lapatinib, neratinib, vandetanib, necitumumab, osimertinib, afatinib, dacomitinib, AP26113, poziotinib, EGFR inhibitor (CAS No. 879127-

07-8), EGFR/ErbB2/ErbB-4 Inhibitor (CAS No. 881001-19-0), EGFR/ErbB-2 Inhibitor (CAS No. 17924861-4), EGFR inhibitor II (BIBX 1382,CAS No. 196612-93-8), EGFR inhibitor III (CAS No. 733009-42-2), EGFR/ErbB-2/ErbB-4 Inhibitor II (CAS No. 944341-54-2) or PKCβII/EGFR Inhibitor (CAS No. 145915-60-2).

In another embodiment, the alteration to the receptor tyrosine kinase is a mutation in an EGFR gene.

In another embodiment, the receptor tyrosine kinase inhibitor is an RET inhibitor. In another embodiment, the RET inhibitor is selected from the group comprising Cabozantinib, Vandetanib, Lenvatinib, Alectinib, Apatinib, Ponatinib, LOXO-292, BLU-667, or RXDX-105.

In another embodiment, the receptor tyrosine kinase inhibitor is an HER2 inhibitor. In another embodiment, the HER2 inhibitor is selected from the group comprising trastuzumab, hyaluronidase/trastuzumab fam-trastumzumab deruxtecan, ado-trastuzumab emtansine, lapatinib, neratinib, pertuzumab, tucatinib, poziotinib, or dacomitinib.

In another embodiment, the receptor tyrosine kinase inhibitor is an ROS1 inhibitor. In another embodiment, the ROS1 inhibitor is selected from the group comprising Crizotinib, Ceritinib, Brigatinib, Lorlatinib, Etrectinib, Cabozantinib, DS-6051b, TPX-0005.

In another embodiment, the receptor tyrosine kinase inhibitor is an MET inhibitor. In another embodiment, the MET inhibitor is selected from the group comprising crizotinib, cabozantinib, MGCD265, AMG208, altiratinib, golvatinib, glesantinib, foretinib, avumatinib, tivatinib, savolitinib, AMG337, capmatinib and tepotinib, OMO-1 [JNJ38877618] or anti-MET antibodies onartuzumab and emibetuzumab [LY2875358] or anti-HGF antibodies ficlatuzumab [AV-299] and rilotumumab [AMG102].

In another embodiment, the inhibitor is a KRas inhibitor. In another embodiment, the KRas inhibitor is selected from the group comprising AMG510, MRTX849, JNJ-74699157/ARS-3248, BI1701963, BAY-293, or "RAS(ON)" inhibitors.

In another embodiment, the receptor tyrosine kinase inhibitor is an ALK inhibitor. In another embodiment, the ALK inhibitor is selected from the group comprising Crizotinib, Ceritinib, Alectinib, Loratinib or Brigatinib.

In another embodiment, the inhibitor is a BRAF inhibitor. In another embodiment, the BRAF inhibitor is selected from the group comprising Vemurafenib, dabrafenib, encorafenib or any unspecific RAF inhibitor.

In another embodiment, the receptor tyrosine kinase inhibitor is an NTRK inhibitor. In another embodiment, the NTRK inhibitor is selected from the group comprising Entrectinib, larotrectinib (LOXO-101), LOCO-195, DS-6051b, cabozantinib, merestinib, TSR-011, PLX7486, MGCD516, crizo-

tinib, regorafenib, dovitinib, lestaurtinib, BMS-754807, danusertib, ENMD-2076, midostaurin, PHA-848125 AC, BMS-777607, altriratinib, AZD7451, MK5108, PF-03814735, SNS-314, foretinib, nintedanib, ponatinib, ONO-5390556 or TPX-0005.

In another embodiment, the composition or combination of a CBP/p300 bromodomain inhibitor or a pharmaceutically acceptable salt thereof, and receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor, is synergistic in treating cancer, compared to the CBP/p300 inhibitor alone or the receptor tyrosine kinase or KRas or BRAF inhibitor alone. As used in the context of the embodiments of section 4, the term "synergistic" refers to an interaction between two or more drugs that causes the total effect of the drugs to be greater than the sum of the individual effects of each drug. In a preferred embodiment the synergistic effect is an increase in response rate of the animal to the combination of the CBP/p300 bromodomain inhibitor and the receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor. In another embodiment the increase in response rate is measured as an increase in efficacy in the treatment of the cancer.

In another embodiment, the anti-cancer effect provided by the composition or combination of a CBP/p300 bromodomain inhibitor or a pharmaceutically acceptable salt thereof, and receptor tyrosine kinase or Kras or BRAF inhibitor, is greater than the anti-cancer effect provided by a monotherapy with the same dose of the CBP/p300 inhibitor or the receptor tyrosine kinase inhibitor or the KRas or BRAF inhibitor. As used in the context of the embodiments of section 4, the term "anti-cancer" refers to the treatment of malignant or cancerous disease. In another embodiment, the present invention provides a composition for use or method, wherein the anti-cancer effect provided by the composition or combination of a CBP/p300 bromodomain inhibitor or a pharmaceutically acceptable salt thereof, and receptor tyrosine kinase inhibitor or Kras or BRAF inhibitor, is at least 2 fold greater, at least 3 fold greater, at least 5 fold greater, or at least 10 fold greater than the monotherapy alone.

In another embodiment, the composition or combination of a CBP/p300 bromodomain inhibitor or a pharmaceutically acceptable salt thereof, and receptor tyrosine kinase inhibitor or Kras or BRAF inhibitor, delays or reduces the risk of resistance of the cancer to the receptor tyrosine kinase inhibitor or Kras or BRAF inhibitor. As used in the context of the embodiments of section 4, the term "resistance of the cancer" refers to the reduction in effectiveness of a medication; more specifically the term may refer to the development of drug resistance by the cancer cells. In another embodiment, the cancer does not become resistant to the receptor tyrosine kinase inhibitor or Kras or BRAF inhibitor for at least 3 months, 6 months, 9 months, 12 months, 24 months, 48 months, or 60 months. In another embodiment, the CBP/p300 bromodomain inhibitor is administered in an effective amount to prevent resistance of the cancer cell to the receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor.

In another embodiment, the CBP/p300 bromodomain inhibitor inhibits a bromodomain of CBP and/or p300. p300 (also called Histone acetyltransferase p300, E1A binding protein p300, E1A-associated protein p300) and CBP (also known as CREB-binding protein or CREBBP) are two structurally very similar transcriptional co-activating proteins.

As used in the context of the embodiments of section 4, the term "CBP/p300 bromodomain inhibitor" may be regarded as referring to a compound that binds to the CBP bromodomain and/or p300 bromodomain and inhibits and/or reduces a biological activity or function of CBP and/or p300. In some embodiments, CBP/p300 bromodomain inhibitor may bind to the CBP and/or p300 primarily (e.g., solely) through contacts and/or interactions with the CBP bromodomain and/or p300 bromodomain. In some embodiments, CBP/p300 bromodomain inhibitor may bind to the CBP and/or p300 through contacts and/or interactions with the CBP bromodomain and/or p300 bromodomain as well as additional CBP and/or p300 residues and/or domains. In some embodiments, CBP/p300 bromodomain inhibitor may substantially or completely inhibit the biological activity of the CBP and/or p300. In some embodiments, the biological activity may be binding of the bromodomain of CBP and/or p300 to chromatin (e.g., histones associated with DNA) and/or another acetylated protein. In certain embodiments in the context of the embodiments of section 4, an inhibitor may have an IC50 or binding constant of less about 50 μM, less than about 1 μM, less than about 500 nM, less than about 100 nM, less than about 10 nM, or less than about 1nM. In some embodiments, the CBP/p300 bromodomain inhibitor may bind to and inhibit CBP bromodomain. In some embodiments, the CBP/p300 bromodomain inhibitor may bind to and inhibit p300 bromodomain. In some embodiments the CBP/p300 bromodomain inhibitor may not inhibit histone acetyl transferase activity of CBP/p300.

In one embodiment, the CBP/p300 bromodomain inhibitor is a compound of formula (I). In one embodiment, the CBP/p300 bromodomain inhibitor is a compound of formula (A), preferably CCS1477 (CAS 2222941-37-7). In another embodiment, the CBP/p300 bromodomain inhibitor is FT-7051. In another embodiment the compound of formula (I), the compound of formula (A), preferably CCS1477, or FT-7051 is a daily dose of the drug at a concentration selected from the list comprising 10mg, 15mg, 25mg, 50mg, 100mg, 150mg, or 200mg. In another embodiment the CCS1477 is administered 2, 3, 4, 5, 6, or 7 days a week. In another embodiment the CCS1477 is administered twice a day. In another embodiment, the administering to the cancer cell comprises contacting the cancer cell with the CBP/p300 inhibitor and the receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor.

In another embodiment, the dosage depends on a variety of factors including the age, weight and condition of the patient and the route of administration. Daily dosages can vary within wide limits and will be adjusted to the individual requirements in each particular case. Typically, however, the dosage adopted for each route of administration when a compound is administered alone to adult humans may be in the range of 0.0001 to 50 mg/kg, most commonly in the range of 0.001 to 10 mg/kg, body weight, for instance 0.01 to 1 mg/kg. Such a dosage may be given, for example, from 1 to 5 times daily. For intravenous injection a suitable daily dose can be from 0.0001 to 1 mg/kg body weight, preferably from 0.0001 to 0.1 mg/kg body weight. A daily dosage can be administered as a single dosage or according to a divided dose schedule.

In another embodiment, a progression of the cancer or duration of response to the cancer therapy may be measured using the RECIST 1.1. response criteria for target lesions or non-target lesions in a subject / animal.

In another embodiment, the term "does not slow progression of the cancer" may be defined in the embodiments of section 4 as the subjects not achieving any RECIST 1.1 clinical response. In another embodiment, the term "does not slow progression of the cancer" may be defined in the embodiments of section 4 as the subjects / animals not achieving a partial RECIST 1.1 clinical response. In another embodiment, the term "does not slow the progression of the cancer" is measured as no objective response rate and/or no increased progression free survival according to RECIST 1.1. In another embodiment, the term "does not slow the progression of the cancer" is measured as a decrease of less than 30% in the sum of the longest diameters of target lesions, taking as reference the baseline sum of the longest diameters of target lesions.

In certain embodiments, the cancer is selected from acoustic neuroma, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute t-cell leukemia, basal cell carcinoma, bile duct carcinoma, bladder cancer, brain cancer, breast cancer, bronchogenic carcinoma, cervical cancer, chondrosarcoma, chordoma, choriocarcinoma, chronic leukemia, chronic lymphocytic leukemia, chronic myelocytic leukemia, chronic myelogenous leukemia, colon cancer, colorectal cancer, craniopharyngioma, cystadenocarcinoma, diffuse large B-cell lymphoma, dysproliferative changes, embryonal carcinoma, endometrial cancer, endotheliosarcoma, ependymoma, epithelial carcinoma, erythroleukemia, esophageal cancer, estrogen-receptor positive breast cancer, essential thrombocythemia, Ewing's tumor, fibrosarcoma, follicular lymphoma, germ cell testicular cancer, glioma, glioblastoma, gliosarcoma, heavy chain disease, head and neck cancer, hemangioblastoma, hepatoma, hepatocellular cancer, hormone insensitive prostate cancer, leiomyosarcoma, leukemia, liposarcoma, lung cancer, lymphagioendotheliosarcoma, lymphangiosarcoma, lymphoblastic leukemia, lymphoma, lymphoid malignancies of T-cell or B-cell origin, medullary carcinoma, medulloblastoma, melanoma, meningioma, mesothelioma, multiple myeloma, myelogenous leukemia, myeloma, myxosarcoma, neuroblastoma, NUT midline carcinoma (NMC), non-small cell lung cancer (NSCLC), oligodendroglioma, oral cancer, osteogenic sarcoma, ovarian cancer, pancreatic cancer, papillary adenocarcinomas, papillary carcinoma, pinealoma, polycythemia vera, prostate cancer, rectal cancer, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, sarcoma, sebaceous gland carcinoma, seminoma, skin cancer, small cell lung carcinoma, solid tumors (carcinomas and sarcomas), small cell lung cancer, stomach cancer, squamous cell carcinoma, synovioma, sweat gland carcinoma, thyroid cancer, Waldenstrom's macroglobulinemia, testicular tumors, uterine cancer, and Wilms' tumor. In certain embodiments, the cancer is melanoma, NSCLC, renal, ovarian, colon, pancreatic, hepatocellular, or breast cancer. In certain embodiments of any of the methods, the cancer is lung cancer, breast cancer, pancreatic cancer, colorectal cancer, and/or melanoma. In certain embodiments, the cancer is lung. In certain embodiments, the lung cancer is non-small cell lung cancer NSCLC. In certain embodiments, the cancer is breast cancer. In certain embodiments, the cancer is melanoma. In certain embodiments, the cancer is colorectal cancer.

In another embodiment, the CBP/p300 bromodomain inhibitor and receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor are administered to the animal simultaneously as a single composition. In another embodiment, the CBP/p300 bromodomain inhibitor and receptor tyrosine ki-

nase inhibitor or KRas or BRAF inhibitor are administered to the animal separately. In another embodiment, the CBP/p300 bromodomain inhibitor and receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor are administered to the animal concurrently. In another embodiment, the CBP/p300 bromodomain inhibitor is administered to the animal prior to the receptor tyrosine kinase inhibitor or the KRas or BRAF inhibitor. In another embodiment, the animal is a human.

In an embodiment, the term "effective amount" of an agent, e.g., a pharmaceutical formulation, may refer to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. In some embodiments, the effective amount refers to an amount of a CBP/p300 bromodomain inhibitor and receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor that (i) treats the particular disease, condition or disorder, (ii) attenuates, ameliorates or eliminates one or more symptoms of the particular disease, condition, or disorder, or (iii) prevents or delays the onset of one or more symptoms of the particular disease, condition or disorder described herein. In some embodiments, the effective amount of the CBP/p300 bromodomain inhibitor and receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor may reduce the number of cancer cells; may reduce the tumor size; may inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; may inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; may inhibit, to some extent, tumor growth; and/or may relieve to some extent one or more of the symptoms associated with the cancer. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR). In some embodiments, an effective amount is an amount of a CBP/p300 bromodomain inhibitor and receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor entity described herein sufficient to significantly decrease the activity or number of drug tolerant or drug tolerant persisting cancer cells.

In an embodiment, a compound of the disclosure may be administered to a human or animal patient in conjunction with radiotherapy or another chemotherapeutic agent for the treatment of cancer. In another embodiment, a combination therapy may be provided, where the CBP/P300 inhibitor or RTK inhibitor or KRas or BRAF inhibitor is administered concurrently or sequentially with radiotherapy; or is administered concurrently sequentially or as a combined preparation with another chemotherapeutic agent or agents, for the treatment of cancer. The or each other chemotherapeutic agent will typically be an agent conventionally used for the type of cancer being treated. Classes of chemotherapeutic agents for combination may in an embodiment be e.g. for the treatment of prostate cancer androgen receptor antagonists, for instance Enzalutamide, and inhibitors of CYP17A1 (17a-hydroxylase/C 17,20 lyase), for instance Abiraterone. In other embodiments, other chemotherapeutic agents in combination therapy can include Docetaxel.

In one embodiment, the term "combination" may in the section 4 refer to simultaneous, separate or sequential administration. Where the administration is sequential or separate, the delay in administering the second component should not be such as to lose the beneficial effect of the combination

In another embodiment, the response to the CBP/p300 bromodomain inhibitor and receptor tyrosine kinase inhibitor or KRas or BRAF inhibitor is a sustained response. In one embodiment, "sustained response" may refer to the sustained effect on reducing tumor growth after cessation of a treatment. For example, the tumor size may remain to be the same or smaller as compared to the size at the beginning of the administration phase.

In another embodiment, the term "treatment" (and variations such as "treat" or "treating") may refer to clinical intervention in an attempt to alter the natural course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment might include one or more of preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, stabilized (i.e., not worsening) state of disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, prolonging survival as compared to expected survival if not receiving treatment and remission or improved prognosis. In certain embodiments, a CBP/p300 bromodomain inhibitor and receptor tyrosine kinase or a KRas or BRAF inhibitor might be used to delay development of a disease or disorder or to slow the progression of a disease or disorder. In an embodiment, those individuals in need of treatment may include those already with the condition or disorder as well as those prone to have the condition or disorder, (for example, through a genetic mutation or aberrant expression of a gene or protein) or those in which the condition or disorder is to be prevented.

In an embodiment, the term "delay" might refer to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease (such as cancer) or resistance of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

5. Examples

The following Examples are merely illustrative and shall describe the present invention in a further way. These Examples shall not be construed to limit the present invention thereto.

The preparation of compounds 00003 (Compound B), 00004 (Compound A), 00030, 00071 and Compound C is described in the following. If deemed helpful, the synthesis route for an intermediate compound and/or a compound close to the afore-mentioned compounds are given.

General experimental methods

LCMS methods:
Method A: Apparatus: Agilent 1260 Bin. Pump: G1312B, degasser; autosampler, ColCom, DAD: Agilent G1315D, 220-320 nm, MSD: Agilent LC/MSD G6130B ESI, pos/neg 100-800, ELSD Alltech

3300 gas flow 1.5 mL/min, gas temp: 40°C; column: Waters XSelect™ C18, 30x2.1 mm, 3.5µ, Temp: 35ºC, Flow: 1 mL/min, Gradient: t_0 = 5% A, $t_{1.6min}$ = 98% A, t_{3min} = 98% A, Posttime: 1.3 min, Eluent A: 0.1% formic acid in acetonitrile, Eluent B: 0.1% formic acid in water).

Method B: Apparatus: Agilent 1260 Bin. Pump: G1312B, degasser; autosampler, ColCom, DAD: Agilent G1315D, 220-320 nm, MSD: Agilent LC/MSD G6130B ESI, pos/neg 100-800, ELSD Alltech 3300 gas flow 1.5 mL/min, gas temp: 40°C; column: Waters XSelect™ C18, 50x2.1 mm, 3.5µ,Temp: 35ºC, Flow: 0.8 mL/min, Gradient: t_0 = 5% A, $t_{3.5min}$ = 98% A, t_{6min} = 98% A, Posttime: 2 min; Eluent A: 0.1% formic acid in acetonitrile, Eluent B: 0.1% formic acid in water).

Method C: Apparatus: Agilent 1260 Bin. Pump: G1312B, degasser; autosampler, ColCom, DAD: Agilent G1315C, 220-320 nm, MSD: Agilent LC/MSD G6130B ESI, pos/neg 100-800; column: Waters XSelect™ CSH C18, 30x2.1 mm, 3.5µ,Temp: 25 ºC, Flow: 1 mL/min, Gradient: t_0 = 5% A, $t_{1.6min}$ = 98% A, t_{3min} = 98% A, Posttime: 1.3 min, Eluent A: 95% acetonitrile + 5% 10 mM ammoniumbicarbonate in water in acetonitrile, Eluent B: 10 mM ammoniumbicarbonate in water (pH=9.5).

Method D: Apparatus: Agilent 1260 Bin. Pump: G1312B, degasser; autosampler, ColCom, DAD: Agilent G1315C, 220-320 nm, MSD: Agilent LC/MSD G6130B ESI, pos/neg 100-800; column: Waters XSelect™ CSH C18, 50x2.1 mm, 3.5µ, Temp: 25 ºC, Flow: 0.8 mL/min, Gradient: t_0 = 5% A, $t_{3.5min}$ = 98% A, t_{6min} = 98% A, Posttime: 2 min, Eluent A: 95% acetonitrile + 5% 10 mM ammoniumbicarbonate in water in acetonitrile, Eluent B: 10 mM ammoniumbicarbonate in water (pH=9.5).

UPLC methods:

Method A: Apparatus: Agilent Infinity II; Bin. Pump: G7120A, Multisampler, VTC, DAD: Agilent G7117B, 220-320 nm, PDA: 210-320 nm, MSD: Agilent G6135B ESI, pos/neg 100-1000, ELSD G7102A: Evap 40°C, Neb 50°C, gasflow 1.6 mL/min, Column: Waters XSelect CSH C18, 50x2.1 mm, 2.5 µm Temp: 25ºC, Flow: 0.6 mL/min, Gradient: t_0 = 5% B, t_{2min} = 98% B, $t_{2.7min}$ = 98% B, Post time: 0.3 min, Eluent A: 10 mM ammonium bicarbonate in water (pH=9.5), Eluent B: acetonitrile.

Method B: Apparatus: Agilent Infinity II; Bin. Pump: G7120A, Multisampler, VTC, DAD: Agilent G7117B, 220-320 nm, PDA: 210-320 nm, MSD: Agilent G6135B ESI, pos/neg 100-1000, ELSD G7102A: Evap 40°C, Neb 40°C, gasflow 1.6 mL/min, Column: Waters XSelect™ CSH C18, 50x2.1 mm, 2.5 µm Temp: 40ºC, Flow: 0.6 mL/min, Gradient: t_0 = 5% B, t_{2min} = 98% B, $t_{2.7min}$ = 98% B, Post time: 0.3 min, Eluent A: 0.1% formic acid in water, Eluent B: 0.1% formic acid in acetonitrile.

GCMS methods:

Method A: Instrument: GC: Agilent 6890N G1530N and MS: MSD 5973 G2577A, EI-positive, Det.temp.: 280 °C Mass range: 50-550; Column: RXi-5MS 20 m, ID 180 µm, df 0.18 µm; Average velocity: 50 cm/s; Injection vol: 1 µl; Injector temp: 250°C; Split ratio: 100/1; Carrier gas: He; Initial temp: 100°C; Initial time: 1.5 min; Solvent delay: 1.0 min; Rate 75°C/min; Final temp 250°C; Hold time 4.3 min.

Method B: Instrument: GC: Agilent 6890N G1530N, FID: Det. temp: 300 °C and MS: MSD 5973 G2577A, EI-positive, Det.temp.: 280°C Mass range: 50-550; Column: Restek RXi-5MS 20 m, ID 180 µm, df 0.18 µm; Average velocity: 50 cm/s; Injection vol: 1 µl; Injector temp: 250°C; Split ratio: 20/1; Carrier gas: He; Initial temp: 60°C; Initial time: 1.5 min; Solvent delay: 1.3 min; Rate 50°C/min; Final temp 250°C; Hold time 3.5 min.

Method C: Instrument: GC: Agilent 6890N G1530N, FID: Det. temp: 300 °C and MS: MSD 5973 G2577A, EI-positive, Det.temp.: 280°C Mass range: 50-550; Column: Restek RXi-5MS 20 m, ID 180 µm, df 0.18 µm; Average velocity: 50 cm/s; Injection vol: 1 µl; Injector temp: 250°C; Split ratio: 20/1; Carrier gas: He; Initial temp: 100°C; Initial time: 1.5 min; Solvent delay: 1.3 min; Rate 75°C/min; Final temp 250°C; Hold time 4.5 min.

Chiral LC:
Method A: (apparatus: Agilent 1260 Quart. Pump: G1311C, autosampler, ColCom, DAD: Agilent G4212B, 220-320 nm, column: Chiralcel® OD-H 250x4.6 mm, Temp: 25ºC, Flow: 1 mL/min, Isocratic: 90/10, time: 30 min, Eluent A: heptane, Eluent B: ethanol).

Preparative reversed phase chromatography:
Method A: Instrument type: Reveleris™ prep MPLC; Column: Phenomenex LUNA C18 (150x25 mm, 10µ); Flow: 40 mL/min; Column temp: room temperature; Eluent A: 0.1% (v/v) formic acid in water, Eluent B: 0.1% (v/v) formic acid in acetonitrile; Gradient: t=0 min 5% B, t=1 min 5% B, t=2 min 30% B, t=17 min 70% B, t=18 min 100% B, t=23 min 100% B; Detection UV: 220/254 nm. Appropriate fractions combined and lyophilized.
Method B: Instrument type: Reveleris™ prep MPLC; Column: Waters XSelect™ CSH C18 (145x25 mm, 10µ); Flow: 40 mL/min; Column temp: room temperature; Eluent A: 10 mM ammoniumbicarbonate in water pH = 9.0); Eluent B: 99% acetonitrile + 1% 10 mM ammoniumbicarbonate in water; Gradient: t=0 min 5% B, t=1 min 5% B, t=2 min 30% B, t=17 min 70% B, t=18 min 100% B, t=23 min 100% B; Detection UV: 220/254 nm. Appropriate fractions combined and lyophilized.

Chiral (preparative) SFC
Method A: (Column: SFC instrument modules: Waters Prep100q SFC System, PDA: Waters 2998, Fraction Collector: Waters 2767; Column: Phenomenex Lux Amylose-1 (250x20 mm, 5 µm), column temp: 35°C; flow: 100 mL/min; ABPR: 170 bar; Eluent A: CO_2, Eluent B: 20 mM ammonia in methanol; isocratic 10% B, time: 30 min, detection: PDA (210-320 nm); fraction collection based on PDA).
Method B: (Column: SFC instrument modules: Waters Prep100q SFC System, PDA: Waters 2998, Fraction Collector: Waters 2767; Column: Phenomenex Lux Celulose-1 (250x20 mm, 5 µm), column temp: 35°C; flow: 100 mL/min; ABPR: 170 bar; Eluent A: CO_2, Eluent B: 20 mM ammonia in methanol; isocratic 10% B, time: 30 min, detection: PDA (210-320 nm); fraction collection based on PDA).
Method C: (Column: SFC instrument modules: Waters Prep100q SFC System, PDA: Waters 2998; Column: Chiralpak IC (100x4.6 mm, 5 µm), column temp: 35°C; flow: 2.5 mL/min; ABPR: 170 bar; Eluent A: CO_2, Eluent B: methanol with 20 mM ammonia; t=0 min 5% B, t=5 min 50% B, t=6 min 50% B, detection: PDA (210-320 nm); fraction collection based on PDA).
Method D: (Column: SFC instrument modules: Waters Prep 100 SFC UV/MS directed system; Waters 2998 Photodiode Array (PDA) Detector; Waters Acquity QDa MS detector; Waters 2767 Sample Manager; Column: Waters Torus 2-PIC 130A OBD (250x19 mm, 5 µm); Column temp: 35°C; Flow: 70 mL/min; ABPR: 120 bar; Eluent A: CO2, Eluent B: 20 mM Ammonia in Methanol; Linear

gradient: t=0 min 10% B, t=4 min 50% B, t=6 min 50% B; Detection: PDA (210-400 nm); Fraction collection based on PDA TIC).

Starting materials

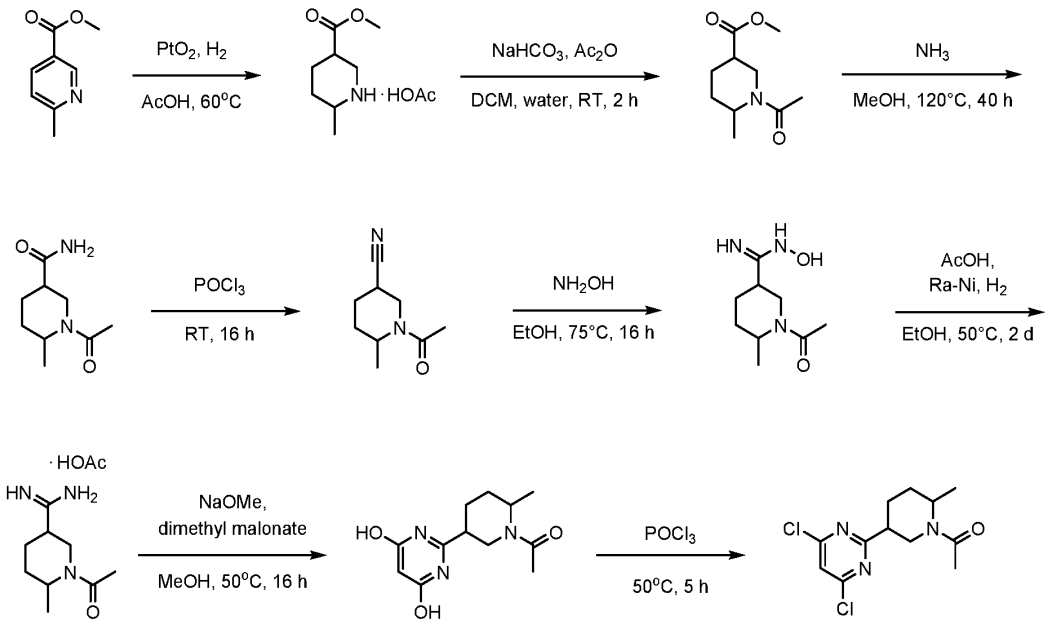
Standard reagents and solvents were obtained at highest commercial purity and used as such, specific reagents purchased are described below.

Compound name	Supplier	CAS
tetrakis(triphenylphosphine)palladium(0)	Sigma-Aldrich	14221-01-3
1,1'-bis(diphenylphosphino)ferrocenepalladium(II) dichloride	Sigma-Aldrich	72287-26-4
2-dicyclohexylphosphino-2',4',6'-triisopropyl-biphenyl	Sigma-Aldrich	564483-18-7
bis(triphenylphosphine)palladium(II) dichloride	Fluorochem	13965-03-2
2-tributylstannylpyrazine	Combi-Blocks	205371-27-3
N-acetyl-D-leucine	Accela Chembio	19764-30-8
methyl 6-methylpiperidine-3-carboxylate	Combi-Blocks	908245-03-4
3-bromo-5-fluoroaniline	Combi-Blocks	134168-97-1
1-methyl-4-(tributylstannyl)-1H-imidazole	Synthonix	446285-73-0
3-fluoro-5-iodoaniline	Combi-Blocks	660-49-1
4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazol	Combi-Blocks	269410-08-4
3-bromoaniline	Combi-Blocks	591-19-5
1,3,5-trimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole	Combi-Blocks	844891-04-9
3-fluoro-5-nitrobenzoic acid	Combi-Blocks	14027-75-9
acetohydrazide	Combi-Blocks	1068-57-1
N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride	Fluorochem	25952-53-8
1-hydroxy-7-azabenzotriazole	Enamine	39968-33-7
(methoxycarbonylsulfamoyl)triethylammonium hydroxide (Burgess reagent)	Combi-Blocks	29684-56-8
3-nitrophenylacetylene	Combi-Blocks	3034-94-4
L-ascorbic acid sodium salt	Sigma-Aldrich	134-03-2
2-azidopropane, 2.5M in DMF	Enamine	691-57-6
azidooxetane, 0.5M in MTBE	Enamine	81764-67-2
azidotrimethylsilane	Acros	4648-54-8
1-fluoro-3-iodo-5-nitrobenzene	Combi-Blocks	3819-88-3
1-bromo-3-chloro-5-nitrobenzene	Combi-Blocks	219817-43-3
2-Iodo-1-methyl-4-nitrobenzene	Fluorochem	7745-92-8
3-bromo-5-nitrotoluene	Combi-Blocks	52488-28-5
4-bromo-1-methyl-1,2,3-triazole	Combi-Blocks	13273-53-5

3-nitrobenzaldehyde	Acros	99-61-6
3-nitrophenylacetylene	Combi-Blocks	3034-94-4
chloro(pentamethylcyclopentadienyl)bis(triph-enylphosphine)ruthenium(II)	STREM chemicals	92361-49-4
tetrabutylammonium fluoride 1.0M solution in THF	Fluorochem	429-41-4
3-ethynyl-4-fluoroaniline	Synthonix	77123-60-5
tert-butyl 3-cyanopiperidine-1-carboxylate	Combi-Blocks	91419-53-3
Raney®-Nickel, 50% slurry in water	Acros Organics	7440-02-0
tris(dibenzylideneacetone)dipalladium(0)	Sigma-Aldrich	51364-51-3
Xphos	Sigma-Aldrich	564483-18-7
2-(tributylstannyl)-pyrimidine	Sigma-Aldrich	153435-63-3
10% palladium on activated carbon	ACROS	7440-05-3

Synthetic procedures for key intermediates

Intermediate 1: 1-(5-(4,6-dichloropyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one

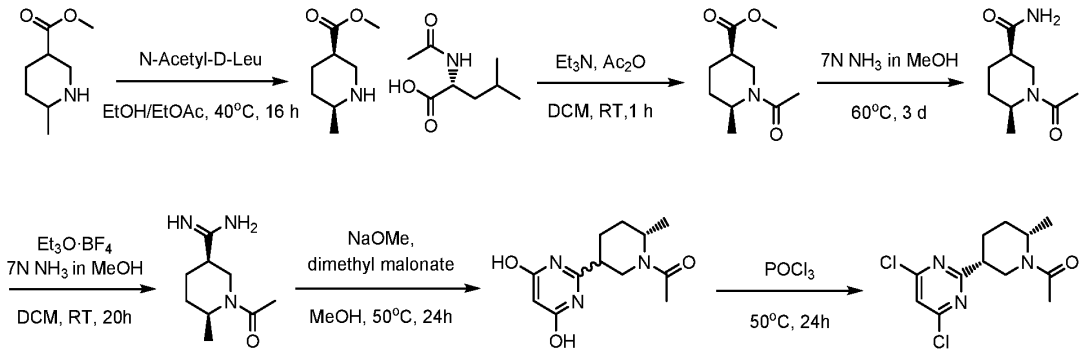


To a solution of methyl 6-methylnicotinate (100 g, 662 mmol) in acetic acid (250 mL) in a 1L steel autoclave, platinum(IV) oxide (0.5 g, 2.202 mmol) was added after which the reaction mixture was stirred under 10 bar hydrogen atmosphere at 60°C. Rapid hydrogen consumption was observed and the autoclave was refilled several times until hydrogen consumption stopped and the reduction was complete. The mixture was cooled to room temperature and filtrated over Celite. The filtrate was concentrated to afford methyl 6-methylpiperidine-3-carboxylate acetate as a mixture of diastereoisomers (143.8 g, 100%) that was used as such in the next step. GCMS (Method A): tR 2.40 (80%) and 2.48 min (20%), 100%, MS (EI) 157.1 (M)+, 142.1 (M-Me)+. To a solution of methyl 6-methylpiperidine-3-carboxylate acetate (53 g, 244 mmol) in a mixture of water (500 mL) and dichloromethane (500 mL), sodium bicarbonate (82 g, 976 mmol) was added carefully (effervescence!!) after which acetic anhydride (29.9 g, 293 mmol) was added slowly. The reaction mixture

was stirred at room temperature for 2 hours. The organic layer was separated, dried over sodium sulfate, filtered and concentrated in vacuo to afford methyl 1-acetyl-6-methylpiperidine-3-carboxylate (49 g, 100%) as a yellow oil. A solution of methyl 1-acetyl-6-methylpiperidine-3-carboxylate (49 g, 246 mmol) in ammonia in methanol (7N, 500 mL, 3.5 mol) was stirred in a pressure vessel at 120 °C for 40 hours. The mixture was cooled to room temperature and concentrated to afford a light yellow solid. This solid was dissolved in dichloromethane and filtered over a plug of silica. The filtrate was concentrated to afford 1-acetyl-6-methylpiperidine-3-carboxamide as an off white solid that was used as such in the next step. A solution of 1-acetyl-6-methylpiperidine-3-carboxamide (266 mmol) from the previous step in phosphorus oxychloride (500 mL, 5.37 mol) was stirred at room temperature for 16 hours. The reaction mixture was evaporated in vacuo affording a thick oil. This oil was co-evaporated twice with toluene and carefully partitioned between cold saturated sodium carbonate (effervescence!) and ethyl acetate. The organic layer was separated from the basic water layer, dried on sodium sulfate, filtered and concentrated in vacuo to afford the product as a thick oil that solidified upon standing. The crude was dissolved in dichloromethane and filtered over a plug of silica (eluted with 10% methanol in dichloromethane). This afforded 1-acetyl-6-methylpiperidine-3-carbonitrile (28 g, 63%) as an oil that solidified upon standing. GCMS (Method A): tR 3.78 (63%) and 3.89 min (378%), 100%, MS (EI) 166.1 (M)+. To a solution of 1-acetyl-6-methylpiperidine-3-carbonitrile (23 g, 138 mmol) in ethanol (300 ml), hydroxylamine solution (50 % in water, 25.4 mL, 415 mmol) was added after which the reaction mixture was stirred at reflux for 16 hours. The reaction mixture was concentrated and co-evaporated with ethyl acetate three times to dryness to afford 1-acetyl-N-hydroxy-6-methylpiperidine-3-carboximidamide as a sticky solid. LCMS (Method A): tR 0.13 min, 100%, MS (ESI) 200.2 (M+H)+. Assuming quantitative yield, the product was used as such in the next step. To a solution of 1-acetyl-N-hydroxy-6-methylpiperidine-3-carboximidamide (23 g, 138 mmol) from the previous step in ethanol (500 mL), acetic acid (23.79 mL, 416 mmol) and 50% Raney®-Nickel slurry in water (5 mL) were added after which the reaction mixture was stirred under hydrogen atmosphere for 2 days at 50 °C. The mixture was filtered over Celite, washed with some ethanol and concentrated to afford 70 g of a thick oil. This was co-evaporated twice with ethyl acetate and extensively dried in vacuo to afford 1-acetyl-6-methylpiperidine-3-carboximidamide acetate (33 g, 98%) as a greenish yellow oil that was used as such in the next step. LCMS (Method A): tR 0.14 min, 90%, MS (ESI) 184.1 (M+H)+. To a solution of sodium (18.14 g, 789 mmol) in dry methanol under nitrogen atmosphere (60 mL) 1-acetyl-6-methylpiperidine-3-carboximidamide acetate (32 g, 132 mmol) and dimethyl malonate (26.1 g, 197 mmol) were added, after which the reaction mixture was stirred at 50 °C for 16 hours. The reaction mixture was concentrated, taken up in water (300 mL), acidified to pH 4 using 6N hydrochloric acid and allowed to precipitate. The precipitate was filtered off to afford 1-(5-(4,6-dihydroxypyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one as a yellow solid (10.4 g, 31%) that was used as such in the next step. A suspension of 1-(5-(4,6-dihydroxypyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (10.4 g, 41.4 mmol) in phosphorus oxychloride (200 mL, 2146 mmol) was stirred at 50 °C. The solids slowly dissolved after approximately 3 hours. After 5 hours, the reaction mixture was concentrated in vacuo and co-evaporated with toluene twice. The remaining oil was carefully quenched with ice and neutralised with saturated aqueous sodium bicarbonate and extracted with ethyl acetate (2 x 100 mL). The combined organic layers were dried over sodium sulfate and concentrated in vacuo to afford 1-

(5-(4,6-dichloropyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (Intermediate 1, 6.8 g, 57%) as a yellow oil that solidified upon standing. LCMS (Method A): tR 1.88 min, 100%, MS (ESI) 288.1 (M+H)+.

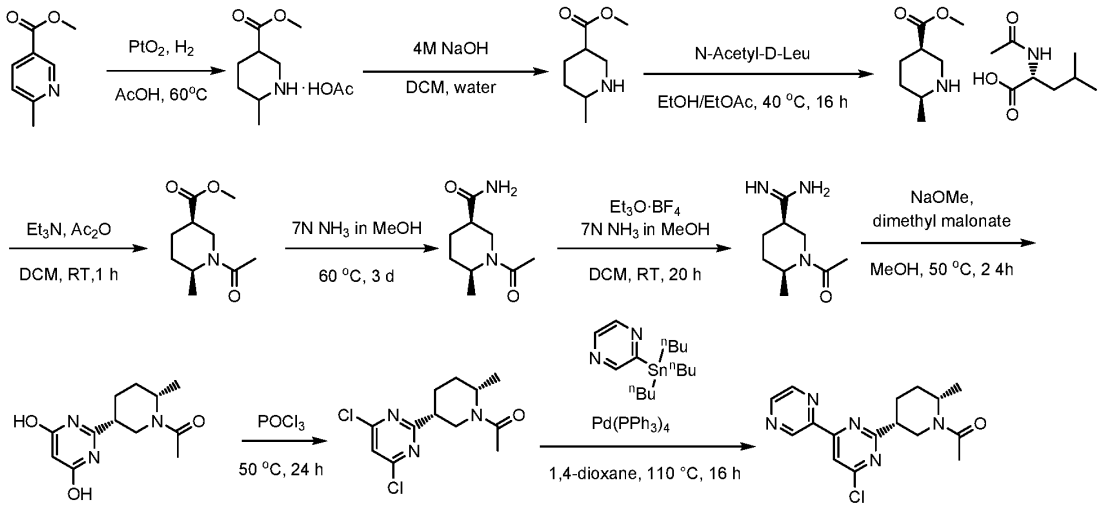
Intermediate 2: 1-((2S,5R)-5-(4,6-dichloropyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one



To a solution of N-acetyl-D-leucine (1 kg, 5.77 mol) in ethanol (1.5 L) was added a solution of methyl 6-methylpiperidine-3-carboxylate (934 g, 2.38 mol, prepared under Intermediate 1) in ethyl acetate (3 L) and the mixture was heated to 40°C. The resulting solution was allowed to reach room temperature over 16 hours during which precipitation occurred. The precipitate was filtered off, washed with diethyl ether (500 mL) and air dried to afford crude methyl (3R,6S)-6-methylpiperidine-3-carboxylate acetyl-D-leucinate (287 g, 34%) as a white solid. The crude methyl (3R,6S)-6-methylpiperidine-3-carboxylate acetyl-D-leucinate (287 g, 869 mmol) was crystallised from a hot mixture of ethanol and ethyl acetate 1:2 (1 L). The precipitate was filtered off and the filtercake was triturated in a mixture of diethyl ether and n-pentane 1:1 (500 mL). The precipitate was filtered off and air dried to afford methyl (3R,6S)-6-methylpiperidine-3-carboxylate acetyl-D-leucinate (128 g, 44%) as a white solid. To a solution of methyl (3R,6S)-6-methylpiperidine-3-carboxylate acetyl-D-leucinate (128 g, 387 mmol) in dichloromethane (1 L) was added a saturated sodium carbonate solution (1 L). The biphasic system was stirred vigorous for 10 minutes and the layers were separated. The organic layer was dried with sodium sulfate and filtered to afford a clear solution. Next, triethylamine (65 mL, 465 mmol) and acetic anhydride (44 mL, 465 mmol) were added and the mixture was stirred at room temperature for 1 hour. The mixture was washed with saturated sodium bicarbonate solution, dried over sodium sulfate and concentrated to afford methyl (3R,6S)-1-acetyl-6-methylpiperidine-3-carboxylate (93 g) as a light yellow solid. An autoclave was charged with methyl (3R,6S)-1-acetyl-6-methylpiperidine-3-carboxylate (93 g, 387 mmol) in 7N ammonia in methanol (600 mL, 4200 mmol) and was heated to 60˚C for 3 days. The mixture was concentrated to afford (3R,6S)-1-acetyl-6-methylpiperidine-3-carboxamide (102 g) as a pale yellow oil. Assuming quantitative yield, the product was used as such in the next step. Chiral LC (Method A) tR= 12.35 min, >98% ee. To a solution of (3R,6S)-1-acetyl-6-methylpiperidine-3-carboxamide (50 g, 271 mmol) in dichloromethane (500 mL) was added triethyloxonium tetrafluoroborate (77 g, 407 mmol) portion wise and the mixture was stirred at room temperature for 4 hours. Slowly, 7N ammonia in methanol (200 ml, 9.15 mol) was added and the mixture was stirred at room temperature for 16 hours. The mixture was concentrated to afford (3R,6S)-1-acetyl-6-methylpiperidine-3-carboximidamide (50 g) as

a pink solid which was used as such in the next step. To a solution of 5.4M sodium methoxide in methanol (99 mL, 535 mmol) in methanol (200 mL) was added, (3R,6S)-1-acetyl-6-methylpiperidine-3-carboximidamide (49 g, 267 mmol) in methanol (400 mL) and dimethyl malonate (61.4 mL, 535 mmol). The mixture was heated to 50°C and stirred for 24 hours. The mixture was acidified (pH ~3) with concentrated hydrochloric acid and was concentrated to a smaller volume. The residue was filtered through silica (20% methanol in dichloromethane) and concentrated to afford an orange oil. The crude product was purified with silica column chromatography (0% to 20% methanol in dichloromethane) to afford 1-((2S,5R)-5-(4,6-dihydroxypyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (12 g, 17%) as a colorless gum. LCMS (Method C): tR 0.17 min, 100%, MS (ESI) 252.1 (M+H)+. A solution of 1-((2S,5R)-5-(4,6-dihydroxypyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (12 g, 47.8 mmol) in phosphorus oxychloride (80 mL, 858 mmol) was stirred at 60°C for 24 hours. The reaction mixture was concentrated and co-evaporated with toluene twice to afford a yellow oil. The oil was dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution. The aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried over sodium sulfate and concentrated to afford a yellow oil. The oil was purified with silica column chromatography (0% to 20% tetrahydrofuran in toluene) to afford 1-((2S,5R)-5-(4,6-dichloropyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (Intermediate 2, 1.5 g, 11%) as a colorless gum. LCMS (Method B): tR 3.34 min, 100%, MS (ESI) 288.0 (M+H)+; Chiral UPLC (Method: A) tR 2.54 min, >95% ee and de.

Intermediate 3: synthesis of 1-((2S,5R)-5-(4-chloro-6-(pyrazin-2-yl)pyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one



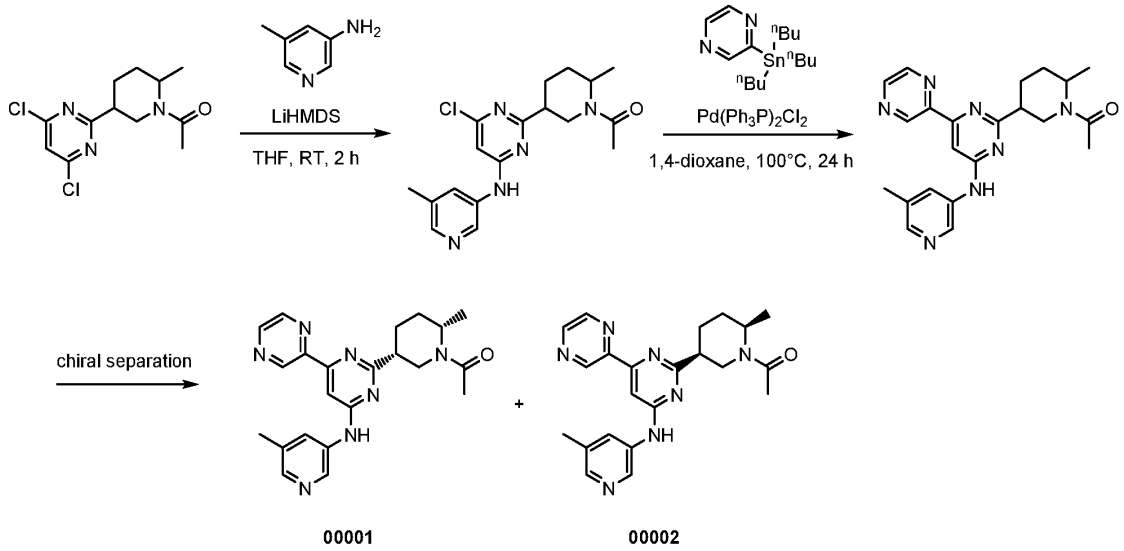
To a solution of methyl 6-methylnicotinate (100 g, 662 mmol) in acetic acid (250 mL) in a 1L steel autoclave, platinum(IV) oxide (0.5 g, 2.202 mmol) was added after which the reaction mixture was stirred under 10 bar hydrogen atmosphere at 60 °C. Rapid hydrogen consumption was observed and the autoclave was refilled several times until hydrogen consumption stopped. The mixture was cooled to room temperature and filtered over Celite. The filtrate was carefully concentrated

to afford methyl 6-methylpiperidine-3-carboxylate acetate as a mixture of diastereoisomers (143.8 g, 100%) that was used as such in the next step. GCMS (Method A): t_R 2.40 (80%) and 2.48 min (20%), 100%, MS (EI) 157.1 $(M)^+$. Methyl 6-methylpiperidine-3-carboxylate acetate as a mixture of diastereoisomers (2.1 kg, 9924 mmol) was diluted with dichloromethane (4 L) and 4M sodium hydroxide solution was added slowly until pH ~ 9. The layers were separated and the aqueous layer was extracted with dichloromethane twice (the aqueous layer was re-basified with 4M sodium hydroxide solution to pH~9 after each extraction). The combined organic layers were dried with sodium sulfate and concentrated (35°C, 450 mbar) to a smaller volume (~2 L) to afford methyl 6-methylpiperidine-3-carboxylate (2.8 kg, 8905 mmol) as a ~50% yellow solution in dichloromethane. ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 5.10 (s,.3H), 3.63 (s, 1H), 3.49 – 3.42 (m, 2.2H), 3.41 – 3.34 (m, 0.8H), 3.18 – 3.10 (m, 0.8H), 3.09 – 3.03 (m, 0.2H), 2.64 – 2.54 (m, 0.8H), 2.53 – 2.34 (m, 1.2H), 2.30 – 2.20 (m, 1H), 1.95 – 1.76 (m, 1H), 1.53 – 1.36 (m, 1H), 1.35 – 1.21 (m, 1H), 1.04 – 0.90 (m, 1H), 0.89 – 0.84 (m, 0.8H), 0.83 – 0.76 (m, 2.2H). To a solution of *N*-acetyl-D-leucine (1 kg, 5.77 mol) in ethanol (1.5 L) was added a solution of methyl 6-methylpiperidine-3-carboxylate (934 g, 2.38 mol) in ethyl acetate (3 L) and the mixture was heated to 40 °C. The resulting solution was allowed to reach room temperature over 16 hours during which precipitation occurred. The precipitate was filtered off, washed with diethyl ether (500 mL) and air dried to afford crude methyl (3R,6S)-6-methylpiperidine-3-carboxylate acetyl-D-leucinate (287 g, 34%) as a white solid. The crude methyl (3R,6S)-6-methylpiperidine-3-carboxylate acetyl-D-leucinate (287 g, 869 mmol) was crystallized from a hot mixture of ethanol and ethyl acetate 1:2 (1 L). The precipitate was filtered off and the filter cake was triturated in a mixture of diethyl ether and n-pentane 1:1 (500 mL). The precipitate was filtered off and air dried to afford methyl (3R,6S)-6-methylpiperidine-3-carboxylate acetyl-D-leucinate (128 g, 44%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-*d*6) δ 7.80 (d, *J* = 8.2 Hz, 1H), 5.80 – 5.00 (s, 2H), 4.20 – 4.04 (m, 1H), 3.63 (s, 3H), 3.32 – 3.21 (m, 1H), 2.93 – 2.80 (m, 2H), 2.73 – 2.65 (m, 1H), 2.04 – 1.94 (m, 1H), 1.82 (s, 3H), 1.68 – 1.49 (m, 3H), 1.49 – 1.37 (m, 2H), 1.30 – 1.15 (m, 1H), 1.02 (d, *J* = 6.4 Hz, 3H), 0.85 (m, 6H). To a solution of methyl (3R,6S)-6-methylpiperidine-3-carboxylate acetyl-D-leucinate (128 g, 387 mmol) in dichloromethane (1 L) was added a saturated sodium carbonate solution (1 L). The biphasic system was stirred vigorous for 10 minutes and the layers were separated. The organic layer was dried with sodium sulfate and filtered to afford a clear solution. Next, triethylamine (65 mL, 465 mmol) and acetic anhydride (44 mL, 465 mmol) were added and the mixture was stirred at room temperature for 1 hour. The mixture was washed with saturated aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated to afford methyl (3R,6S)-1-acetyl-6-methylpiperidine-3-carboxylate (93 g) as a light yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 5.02 – 4.87 (m, 0.5H), 4.84 – 4.68 (m, 0.5H), 4.18 – 4.05 (m, 0.5H), 3.89 – 3.77 (m, 0.5H), 3.71 (d, *J* = 11.6 Hz, 3H), 3.31 – 3.18 (m, 0.5H), 2.79 – 2.67 (m, 0.5H), 2.51 – 2.31 (m, 1H), 2.11 (d, *J* = 6.7 Hz, 3H), 2.01 – 1.90 (m, 1H), 1.88 – 1.55 (m, 3H), 1.33 – 1.21 (m, 1.5H), 1.20 – 1.06 (m, 1.5H). An autoclave was charged with methyl (3R,6S)-1-acetyl-6-methylpiperidine-3-carboxylate (93 g, 387 mmol) in 7N ammonia in methanol (600 mL, 4200 mmol) and was heated to 60 °C for 3 days. The mixture was concentrated to afford (3R,6S)-1-acetyl-6-methylpiperidine-3-carboxamide (102 g) as a pale yellow oil. Assuming quantitative yield, the product was used as such in the next step. ^{1}H-NMR (400 MHz, DMSO-*d*6, mixture of rotamers) δ 7.38 (s, 1H), 6.89 (d, *J* = 24.7 Hz, 1H), 4.76 – 4.59 (m, 0.5H), 4.39 – 4.24 (m, 0.5H), 4.16 – 4.01 (m, 0.5H), 3.72 – 3.51 (m, 0.5H), 3.14 –

2.99 (m, 0.5H), 2.68 – 2.51 (m, 0.5H), 2.30 – 2.12 (m, 0.5H), 2.11 – 1.92 (m, 3.5H), 1.78 – 1.38 (m, 4H), 1.23 – 1.11 (m, 1.5H), 1.09 – 0.94 (m, 1.5H); Chiral LC (Method A) t_R= 12.35 min, >98% ee. To a solution of (3R,6S)-1-acetyl-6-methylpiperidine-3-carboxamide (50 g, 271 mmol) in dichloromethane (500 mL) was added triethyloxonium tetrafluoroborate (77 g, 407 mmol) portion wise and the mixture was stirred at room temperature for 4 hours. Slowly, 7N ammonia in methanol (200 mL, 9.15 mol) was added and the mixture was stirred at room temperature for 16 hours. The mixture was concentrated to afford (3R,6S)-1-acetyl-6-methylpiperidine-3-carboximidamide (50 g) as a pink solid which was used as such in the next step. To a solution of 5.4M sodium methoxide in methanol (99 mL, 535 mmol) in methanol (200 mL) was added, (3R,6S)-1-acetyl-6-methylpiperidine-3-carboximidamide (49 g, 267 mmol) in methanol (400 mL) and dimethyl malonate (61.4 mL, 535 mmol). The mixture was heated to 50 °C and stirred for 24 hours. The mixture was acidified (pH ~3) with concentrated hydrochloric acid and was concentrated to a smaller volume. The residue was filtered through silica (20% methanol in dichloromethane) and concentrated to afford an orange oil. The crude product was purified with silica column chromatography (0% to 20% methanol in dichloromethane) to afford 1-((2S,5R)-5-(4,6-dihydroxypyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (12 g, 17%) as a colorless gum. LCMS (Method C): t_R 0.17 min, 100%, MS (ESI) 252.1 $(M+H)^+$. A solution of 1-((2S,5R)-5-(4,6-dihydroxypyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (12 g, 47.8 mmol) in phosphorus oxychloride (80 mL, 858 mmol) was stirred at 60 °C for 24 hours. The reaction mixture was concentrated and co-evaporated with toluene twice to afford a yellow oil. The oil was dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution. The aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried over sodium sulfate and concentrated to afford a yellow oil. The oil was purified with silica column chromatography (0% to 20% tetrahydrofuran in toluene) to afford 1-((2S,5R)-5-(4,6-dichloropyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (1.5 g, 11%) as a colorless gum. ^{1}H-NMR (400 MHz, DMSO-*d*6, mixture of rotamers) δ 7.95 (d, *J* = 7.3 Hz, 1H), 4.85 – 4.72 (m, 1H), 4.69 – 4.62 (m, 1H), 4.23 – 4.13 (m, 1H), 4.07 – 3.98 (m, 1H), 3.97 – 3.88 (m, 1H), 3.00 – 2.89 (m, 1H), 2.81 – 2.67 (m, 1H), 2.09 – 1.72 (m, 7H), 1.71 – 1.58 (m, 2H), 1.25 – 1.14 (m, 3H), 1.12 – 1.05 (m, 2H); LCMS (Method B): t_R 3.34 min, MS (ESI) 288.0 $(M+H)^+$; Chiral UPLC (Method: A) t_R 2.54 min, >95% ee and de. Under argon, 2-tributylstannylpyrazine (607 mg, 1.65 mmol), 1-((2S,5R)-5-(4,6-dichloropyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (500 mg, 1.74 mmol) and bis(triphenylphosphine)palladium(II) chloride (244 mg, 0.34 mmol) in 1,4-dioxane (20 mL) were heated to 100 °C and stirred for 32 hours. The mixture was diluted with dichloromethane containing 1% triethylamine and coated onto silica. This was purified with silica column chromatography (0% to 40% acetonitrile in dichloromethane containing 1% triethylamine) to afford 1-((2S,5R)-5-(4-chloro-6-(pyrazin-2-yl)pyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (Intermediate 3**,** 134 mg, 18%) as an orange gum. ^{1}H-NMR (400 MHz, DMSO-*d*6, mixture of rotamers) δ 9.46 – 9.41 (m, 1H), 8.80 – 8.76 (m, 1H), 8.65 – 8.59 (m, 1H), 8.33 – 8.29 (m, 1H), 7.66 – 7.59 (m, 1H), 4.86 – 4.70 (m, 0.5H), 4.27 – 4.17 (m, 0.5H), 4.09 – 3.97 (m, 0.5H), 3.55 – 3.41 (m, 0.5H), 3.06 – 2.98 (m, 0.5H), 2.88 – 2.82 (m, 0.5H), 2.10 – 1.90 (m, 6H), 1.89 – 1.76 (m, 0.5H), 1.75 – 1.61 (m, 1.5H), 1.29 – 1.20 (m, 1.5H), 1.17 – 1.10 (m, 1.5H); LCMS (Method C): t_R 1.81 min, MS (ESI) 331.1 $(M+H)^+$.

Synthetic procedures for final products

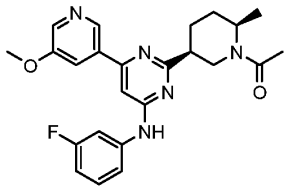
Example 1: synthesis of 1-((2S,5R)-2-methyl-5-(4-((5-methylpyridin-3-yl)amino)-6-(pyrazin-2-yl)pyrimidin-2-yl)piperidin-1-yl)ethan-1-one (00001) and 1-((2R,5S)-2-methyl-5-(4-((5-methylpyridin-3-yl)amino)-6-(pyrazin-2-yl)pyrimidin-2-yl)piperidin-1-yl)ethan-1-one (00002)

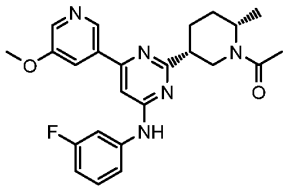


00001 00002

To a solution of 3-amino-5-methylpyridine (0.751 g, 6.94 mmol) in tetrahydrofuran (20 mL) was added 1M lithium bis(trimethylsilyl)amide in tetrahydrofuran (6.94 mL, 6.94 mmol) and the mixture was stirred at room temperature for 10 minutes. Next, 1-(5-(4,6-dichloropyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (Intermediate 1, 1 g, 3.47 mmol) in tetrahydrofuran (20 ml) was added and the mixture was stirred at room temperature for 2 hours. The mixture was poured into saturated ammonium chloride solution and was extracted with ethyl acetate twice. The combined organic layers were washed with brine once, dried over sodium sulfate and concentrated to afford a yellow solid. The solid was purified with silica column chromatography (0% to 5% methanol in dichloromethane) to afford 1-(5-(4-chloro-6-((5-methylpyridin-3-yl)amino)pyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (788 mg, 60%) as a yellow foam. LCMS (Method B): tR 1.81 min, 100%, MS (ESI) 360.1 (M+H)+. Under nitrogen, 2-(tributylstannyl)pyrazine (103 mg, 0.28 mmol), 1-(5-(4-chloro-6-((5-methylpyridin-3-yl)amino)pyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (50 mg, 0.14 mmol) and bis(triphenylphosphine)palladium(II) dichloride (9.75 mg, 0.01 mmol) were dissolved in N,N-dimethylformamide (3 mL). The mixture was heated to 80˚C for 24 hours and cooled to room temperature. The mixture was eluted through a C18 plug using acetonitrile, the filtrate was purified with reversed phase chromatography (method B) and lyophilized to afford 1-(2-methyl-5-(4-((5-methylpyridin-3-yl)amino)-6-(pyrazin-2-yl)pyrimidin-2-yl)piperidin-1-yl)ethan-1-one (22 mg, 37%) as a white solid. The obtained mixture of cis enantiomers was submitted for chiral preparative SFC (Method A) and lyophilized to afford both stereoisomers. 1-((2S,5R)-2-methyl-5-(4-((5-methylpyridin-3-yl)amino)-6-(pyrazin-2-yl)pyrimidin-2-yl)piperidin-1-yl)ethan-1-one (5 mg, 22%) LCMS (Method D): tR 3.17 min, 100%, MS (ESI) 404.1 (M+H)+; Chiral UPLC (Method: A):tR 3.17 min, >95% ee and de. 1-((2R,5S)-2-methyl-5-(4-((5-methylpyridin-3-yl)amino)-6-(pyrazin-2-yl)pyrimidin-2-yl)piperidin-1-yl)ethan-1-one (6 mg, 27%) LCMS (Method D): tR 3.17 min, 100%, MS (ESI) 404.2 (M+H)+; Chiral UPLC (Method A): tR 4.60 min, >95% ee and de.

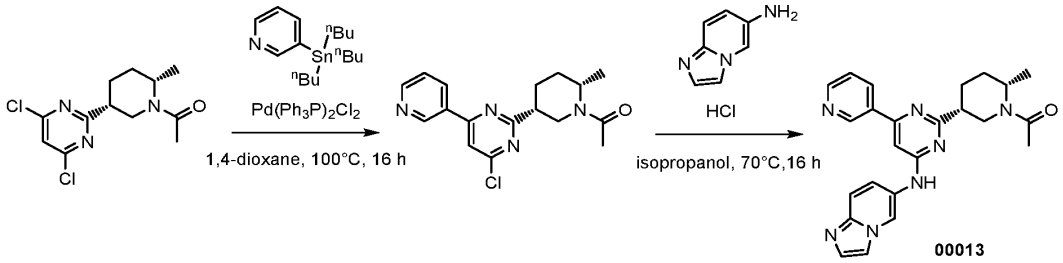
Compounds 00003 (which is also referred to herein as Compound B) and 00004 (which is also referred to herein as Compound A) were prepared using procedures analogous to Example 1, using the appropriate starting materials.





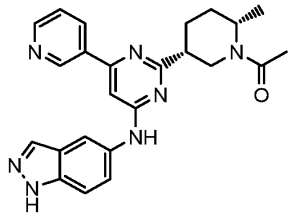
00003 00004

Example 2: synthesis of 1-((2S,5R)-5-(4-(imidazo[1,2-a]pyridin-6-ylamino)-6-(pyridin-3-yl)pyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (00013)



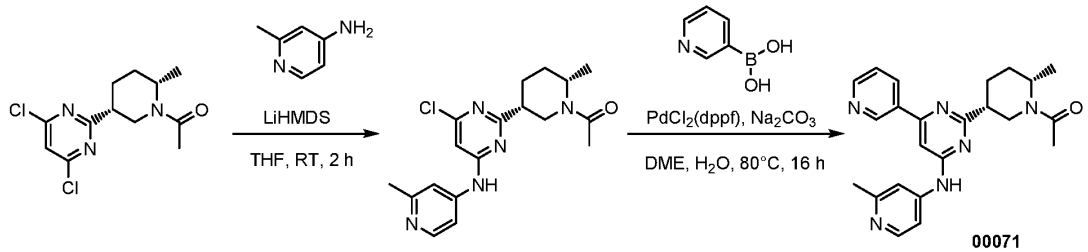
Under argon, 3-(tributylstannyl)pyridine (607 mg, 1.65 mmol), 1-((2S,5R)-5-(4,6-dichloropyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (Intermediate 2, 500 mg, 1.74 mmol) and bis(triphenylphosphine)palladium(II) chloride (244 mg, 0.34 mmol) in 1,4-dioxane (20 mL) were heated to 100°C and stirred for 32 hours. The mixture was diluted with dichloromethane containing 1% triethylamine and coated onto silica. This was purified with silica column chromatography (0% to 40% acetonitrile in dichloromethane containing 1% triethylamine) to afford 1-((2S,5R)-5-(4-chloro-6-(pyridin-3-yl)pyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (134 mg, 18%) as an orange gum. LCMS (Method C): tR 1.81 min, 100%, MS (ESI) 331.1 (M+H)+. To a solution of 1-((2S,5R)-5-(4-chloro-6-(pyridin-3-yl)pyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (30 mg, 0.09 mmol) in 2-propanol (2 mL), was added imidazo[1,2-a]pyridin-6-amine (36.2 mg, 0.27 mmol) and hydrochloric acid (0.02 mL, 0.27 mmol). The mixture was stirred at 60oC for 16 hours, poured into saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate twice. The combined organic layers were dried over sodium sulfate and concentrated to afford a yellow oil. The oil was purified with reversed phase chromatography (method B) and lyophilized to afford 1-((2S,5R)-5-(4-(imidazo[1,2-a]pyridin-6-ylamino)-6-(pyridin-3-yl)pyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan1-one as a blue-ish solid. LCMS (Method B): tR 2.19 min, 100%, MS (ESI) 428.1 (M+H)+.

Compound 00030 was prepared following procedures analogous to Example 2, using the appropriate starting materials.



00030

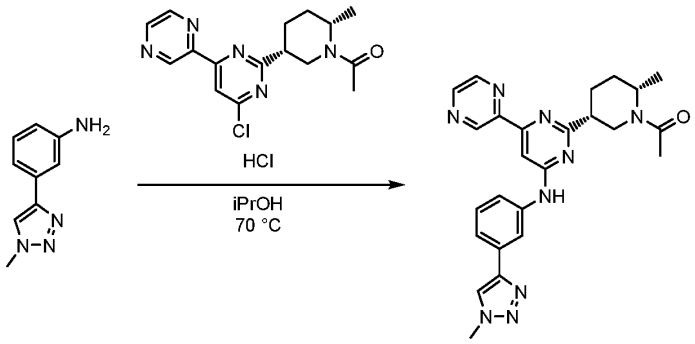
Example 3A: synthesis of 1-((2S,5R)-2-methyl-5-(4-((2-methylpyridin-4-yl)amino)-6-(pyridin-3-yl)pyrimidin-2-yl)piperidin-1-yl)ethan-1-one (00071)



00071

To a solution of 2-methylpyridin-4-amine (3.19 g, 29.5 mmol) in dry tetrahydrofuran (100 mL) was added 1M lithium bis(trimethylsilyl)amide in tetrahydrofuran (29.5 mL, 29.5 mmol) and the mixture was stirred for 10 minutes. Next, 1-((2S,5R)-5-(4,6-dichloropyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (Intermediate 2, 850 mg, 2.95 mmol) in dry tetrahydrofuran (100 mL) was added over 10 minutes and the mixture was stirred at room temperature for 2 hours. The mixture was poured into saturated ammonium chloride solution and was extracted with ethyl acetate twice. The combined organic layers were washed with brine once, dried over sodium sulfate and concentrated to afford a brown oil. The oil was purified with silica column chromatography (80% to 100% ethyl acetate in n-heptane followed by 0% to 10% methanol in dichloromethane) to afford 1-((2S,5R)-5-(4-chloro-6-((2-methylpyridin-4-yl)amino)pyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (275 mg 25%) as a yellow oil. LCMS (Method A): tR 1.49 min, 100%, MS (ESI) 360.1 (M+H)+. Under nitrogen, 1-((2S,5R)-5-(4-chloro-6-((2-methylpyridin-4-yl)amino)pyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (275 mg, 0.76 mmol), sodium carbonate (162 mg, 1.53 mmol), pyridine-3-boronic acid (188 mg, 1.53 mmol) and PdCl2(dppf)-CH2Cl2 adduct (62.4 mg, 0.08 mmol) were dissolved in a mixture of 1,2-dimethoxyethane (6 mL) and water (2 mL). The mixture was heated to 80°C for 1 hour, filtered through a C18-plug and concentrated to afford a dark residue. The residue was purified with reversed phase chromatography (method B) and lyophilized to afford a light yellow solid. The product was further purified by chiral preparative SFC (Method B) and lyophilized to afford 1-((2S,5R)-2-methyl-5-(4-((2-methylpyridin-4-yl)amino)-6-(pyridin-3-yl)pyrimidin-2-yl)piperidin-1-yl)ethan-1-one (135 mg, 41%) as beige solid. LCMS (Method D): tR 3.06 min, 100%, MS (ESI) 403.2 (M+H)+; Chiral SFC (Method B): tR 3.60 min, >95% ee and de.

Example 3B: synthesis of 1-((2S,5R)-2-methyl-5-(4-((3-(1-methyl-1H-1,2,3-triazol-4-yl)phenyl)amino)-6-(pyrazin-2-yl)pyrimidin-2-yl)piperidin-1-yl)ethan-1-one (Compound C)



To a solution of 1-((2S,5R)-5-(4-chloro-6-(pyrazin-2-yl)pyrimidin-2-yl)-2-methylpiperidin-1-yl)ethan-1-one (Intermediate 3, 120 mg, 0.36 mmol) in 2-propanol (2 mL), was added 3-(1-methyl-1H-1,2,3-triazol-4-yl)aniline (188 mg, 1.08 mmol) and hydrochloric acid (0.08 mL, 1.08 mmol). The mixture was stirred at 70 °C for 16 hours, poured into saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate twice. The combined organic layers were dried over sodium sulfate and concentrated to afford a yellow oil. The oil was purified with reversed phase chromatography (method B) and lyophilized to afford 1-((2S,5R)-2-methyl-5-(4-((3-(1-methyl-1H-1,2,3-triazol-4-yl)phenyl)amino)-6-(pyrazin-2-yl)pyrimidin-2-yl)piperidin-1-yl)ethan-1-one (Compound C, 102 mg, 60%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-*d6*, mixture of rotamers)δ 10.01 (d, *J* = 5.6 Hz, 1H), 9.56 (dd, *J* = 11.0, 1.1 Hz, 1H), 8.80 (d, *J* = 1.5 Hz, 2H), 8.54 – 8.42 (m, 2H), 7.72 – 7.54 (m, 2H), 7.53 – 7.39 (m, 2H), 4.86 – 4.76 (m, 1H), 4.27 – 4.16 (m, 0.5H), 4.15 – 4.03 (m, 3.5H), 3.58 – 3.42 (m, 0.5H), 3.00 – 2.86 (m, 1H), 2.86 – 2.68 (m, 0.5H), 2.17 – 1.96 (m, 5H), 1.93 – 1.77 (m, 0.5H), 1.76 – 1.64 (m, 1.5H), 1.27 (d, *J* = 6.8 Hz, 1.5H), 1.13 (d, *J* = 7.0 Hz, 1.5H); LCMS (Method D): t_R 3.31 min, MS (ESI) 470.2 $(M+H)^+$.

Example 4: Crystal Structure of the Bromodomain of Human CREBBP in Complex with Compound 00004 and BROMOscan™ results for Compound A, Compound C, and CCS1477

CRYSTALLIZATION

Experimental setup: The construct used for crystallization comprised residues 1081 to 1197. Crystals of CREBBP in complex with compound 00004 were obtained using hanging-drop vapour-diffusion set-ups. CREBBP at a concentration of 20.3 mg/ml (10mM Hepes, 500mM NaCl, 5% Glycerol, 0.5mM TCEP, pH 7.4) was pre-incubated with 4.3 mM (3.0-fold molar excess) of 00004 (150 mM in DMSO) for 1 h. 1 µl of the protein solution was then mixed with 1 µl of reservoir solution (0.1 M MgCl2, 0.1 M MES/NaOH pH 6.3, 18% (w/v) PEG 6000 and 10% (v/v) ethylene glycol) and equilibrated at 4°C over 0.4 ml of reservoir solution. Well diffracting crystals appeared and grew to full size over 4 days.

DATA COLLECTION

Crystals were cryo-protected by addition of 10% glycerol (final concentration) to the crystallization drop before mounting. A complete 1.6 Å data set of a CREBBP/00004crystal was collected at Diamond Light Source (Didcot, UK, beamline i03) and the data were integrated, analyzed and scaled by XDS, Pointless and Aimless within the autoPROC pipeline (Table 1).

Table 1: Data collection statistics

Space group	$P2_1$
Unit cell parameters [Å]	a=70.4, b=58.6, c=73.2 α=90.0, β=115.4, γ=90.0
Resolution [Å]	66.14-1.60 (1.63-1.60)
# Unique reflections	68872 (2664)
I/σ(I)	14.9 (2.2)
Completeness [%]	97.2 (75.5)
Multiplicity	3.3 (2.1)
Rmeas	0.050 (0.460)

STRUCTURE DETERMINATION AND REFINEMENT

Molecular replacement was done using a previously determined structure of CREBBP as a starting model. Several rounds of alternating manual re-building and refinement with REFMAC5 resulted in the final model (Table 2). Atomic displacement factors were modelled with a single isotropic B-factor per atom.

Table 2: Refinement statistics

Resolution	35.00-1.60 (1.64-1.60)
R_{work}	0.151 (0.305)
R_{free}	0.190 (0.351)
Completeness [%]	97.2 (77.6)

Results: We have produced crystals of CREBBP/00004 that diffracted to 1.6 Å resolution and determined the 3-dimensional structure of the protein-ligand complex. Clear electron density in the Fo-Fc omit map of the initial model at the compound binding site in each chain of CREBBP revealed the binding of the entire compound (Figure 7) and allowed its unambiguous placement. Additionally, the structure also confirms the absolute stereochemistry of compound 00004 (2S, 5R on the piperidine moiety).

BromoKdMAX-Assay

A BromoKdMAX was performed at DiscoverX. This assay may be used for determining whether compounds bind to the bromodomain of p300 and/or the bromodomain of CBP with a particular Kd (e.g. 100 nM or less).

The assay principle is the following: BROMOscan™ is a novel industry leading platform for identifying small molecule bromodomain inhibitors. Based on proven KINOMEscan™ technology, BROMOscan™ employs a proprietary ligand binding site-directed competition assay to quantitatively measure interactions between test compounds and bromodomains. This robust and reliable assay panel is suitable for high throughput screening and delivers quantitative ligand binding data to facilitate the identification and optimization of potent and selective small molecule bromodomain inhibitors. BROMOscan™ assays include trace bromodomain concentrations (<0.1 nM) and

thereby report true thermodynamic inhibitor Kd values over a broad range of affinities (<0.1 nM to >10 uM).

The assay was conducted as follows: For the Bromodomain assays, T7 phage strains displaying bromodomains were grown in parallel in 24-well blocks in an E. coli host derived from the BL21 strain. E. coli were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32°C until lysis (90-150 minutes). The lysates were centrifuged (5,000 x g) and filtered (0.2 µm) to remove cell debris. Streptavidin-coated magnetic beads were treated with biotinylated small molecule or acetylated peptide ligands for 30 minutes at room temperature to generate affinity resins for bromodomain assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1 % BSA, 0.05 % Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific phage binding. Binding reactions were assembled by combining bromodomains, liganded affinity beads, and test compounds (i.e. either Compound A, Compound C or CCS1477) in 1x binding buffer (17% SeaBlock, 0.33x PBS, 0.04% Tween 20, 0.02% BSA, 0.004% Sodium azide, 7.4 mM DTT). Test compounds were prepared as 1000X stocks in 100% DMSO. Kds were determined using an 11-point 3-fold compound dilution series with one DMSO control point. All compounds for Kd measurements are distributed by acoustic transfer (non-contact dispensing) in 100% DMSO. The compounds were then diluted directly into the assays such that the final concentration of DMSO was 0.09%. All reactions performed in polypropylene 384-well plates. Each was a final volume of 0.02 ml. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05% Tween 20, 2 µM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The bromodomain concentration in the eluates was measured by qPCR.

The results were as follows:

	compound A	compound C	CCS1477
DiscoveRx Gene Symbol	Kd [nM]	Kd [nM]	Kd [nM]
ATAD2A	>10000	>10000	>10000
ATAD2B	>10000	>10000	>10000
BAZ2A	>10000	>10000	>10000
BAZ2B	>10000	>10000	>10000
BRD1	>10000	>10000	>10000
BRD2(1)	>10000	3700	230
BRD2(1,2)	7600	4500	610
BRD2(2)	>10000	>10000	2100
BRD3(1)	>10000	3700	320
BRD3(1,2)	>10000	7300	1400
BRD3(2)	>10000	>10000	3900
BRD4(1)	>10000	2500	250

BRD4(1,2)	>10000	>10000	6900
BRD4(2)	>10000	>10000	5200
BRD4(full-length,short-iso.)	7100	1200	440
BRD7	>10000	8000	5100
BRD8(1)	>10000	8400	>10000
BRD8(2)	>10000	>10000	>10000
BRD9	>10000	6300	790
BRDT(1)	>10000	3600	390
BRDT(1,2)	>10000	8500	2400
BRDT(2)	>10000	>10000	8900
BRPF1	>10000	7800	1400
BRPF3	>10000	>10000	>10000
CECR2	>10000	9800	>10000
CREBBP	29	3,2	0,47
EP300	12	2,1	0,26
FALZ	>10000	>10000	5500
GCN5L2	>10000	>10000	>10000
PBRM1(2)	>10000	>10000	>10000
PBRM1(5)	>10000	>10000	3100
PCAF	>10000	9400	>10000
SMARCA2	>10000	>10000	>10000
SMARCA4	>10000	>10000	>10000
TAF1(2)	>10000	230	7900
TAF1L(2)	>10000	1600	>10000
TRIM24(Bromo.)	>10000	7900	680
TRIM24(PHD,Bromo.)	>10000	>10000	1900
TRIM33(PHD,Bromo.)	>10000	>10000	>10000
WDR9(2)	>10000	4100	>10000

Corresponding data is publicly available i) for SGC-CBP30 e.g. in the supplementary information of Wu et al., NATURE COMMUNICATIONS (2019)10:1915 https://doi.org/10.1038/s41467-09672-2; ii) for GNE-781 e.g. in Romero et al., *J. Med. Chem.* 2017, 60, 9162-9183; and iii) for FT-6876 e.g. in Poster #3079 of the AACR Annual Meeting 2020, Virtual Meeting II, June 22-24, 2020 (entitled "FT-6876, a potent and selective inhibitor of CBP/p300 with antitumor activity in AR-positive breast cancer").

Example 5

Material and Methods

Gene expression analysis:

250000 HCC827 (ATCC; CRL 2868; with EGFR exon 19 deletion E746-A750) cells/well or 200000 NCI-H1975 (ATCC; CRL 5908; with EGFR L858R and T790M) cells/well were seeded in 6-well dishes (Greiner Bio-One, 7657160) the day before drug treatment in RPMI medium containing

10%FCS and 2 mM L-Glutamine. Cells were then treated for 24 h either with DMSO, EGFR inhibitor (for HCC827 Gefitinib [100 nM final, LC Laboratories; G-4408]; for NCI-H1975 osimertinib [20 nM final, LC Laboratories; O-7200]) with or without 1 µM Compound A (Compound A is also referred to herein as compound 00004), CCS1477 (Chemgood; C1505), A-485 (Lucerna-Chem; HY-107455) or ICG-001 (Selleckchem, S2662). Subsequently cells were washed 3 times with 2 ml PBS and lysed in 300 µl lysis buffer (RA1 + 1% TCEP [Sigma 646547]). RNA was extracted according to Macherey-Nagel NucleoSpin 8 RNA Kit protocol for vacuum (740698.5) for RNA extraction and RNA was eluted in H2O. 0.5 -2 µg RNA were reversed transcribed using the Thermo Scientific High-capacity cDNA Reverse Transcription Kit (4368813). Equal amount of cDNA was then subjected to qPCR analysis using the Kapa SYBR fast kit (KK4611) in a 384 well format in a Roche Light Cycler 480. Gene expression of genes was evaluated by subtraction of housekeeping gene CT-values (b-Actin) from CT of the genes of interest, and by calculating the ΔΔCT by subtracting the DMSO control value from sample of interest to finally calculate fold change differences of treated versus control treated sample.

Primers:
ACTB (b-Actin): fwd 5` GCC CCAGCTCACCATGGAT 3` (SEQ ID NO: 1), rev 5` TGGGCCTCGTCGCCCACATA 3` (SEQ ID NO: 2);
ALPP: fwd 5` AGAAAGCAGGGAAGTCAGTGG 3` (SEQ ID NO: 3), rev 5` CGAGTACCAGTTGCGGTTCA 3` (SEQ ID NO: 4);
HOPX: fwd 5` GACCATGTCGGCGGAGACC 3` (SEQ ID NO: 5), rev 5` GCGCTGCTTAAACCATTTCTGGG 3` (SEQ ID NO: 6).

Bromodomain- and HAT domain-binding CBP/p300 inhibitors but not inhibitors that prevent the interaction between CBP and β-Catenin blunt EGFR inhibitor-induced gene expression in EGFR-mutated non-small cell lung cancer cells (NSCLC).

Gene expression was assessed for Compound A in parallel with other CBP/p300-Inhibitors (CBP-I) with different modes of action (i.e. binding to different protein domains of CBP/p300). Compound A and CCS1477 bind to the bromodomain (BRD-I) of CBP/p300, A-485 targets the catalytic histone acetyl transferase (HAT) activity of CBP/p300 (HAT-I), ICG-001 disrupts the interaction of CBP with β-Catenin (CBP/β-Cat-I).

Figure 1 shows the results, the regulated genes shown are ALPP (Alkaline phosphatase, placental type; Figure 1A and Figure 1C) and HOPX (Homeodomain-only protein; Figure 1B and Figure 1D). Gene expression in HCC827 exposed for 24h to 20 nM Gefitinib (EGFR inhibitor) or with 1 µM of different CBP-Inhibitors in the presence of 20 nM Gefitinib (Figure 1 A-B). Gene expression in NCI-H1975 (carrying EGFR T790M mutation resulting in Gefitinib-resistance) were exposed for 24h to 20 nM osimertinib (3rd generation EGFR inhibitor) or with 1 µM of different CBP/p300 inhibitors in the presence of 20 nM osimertinib. Data is from 2 independent experiments with qPCRs in duplicate (mean ± SEM) (Figure 1 C-D).

Results: Both EGFR-mutated NSCLC cell lines respond with upregulation of example genes ALPP and HOPX to the treatment with two different EGFR inhibitors (1st generation Gefitinib and 3rd generation osimertinib). Compound A and CCS1477 (both CBP/p300 bromodomain binders) and A-485 (catalytic inhibition of CBP/p300) reverse EGFR inhibitor induced gene expression. Different mode of action compound (ICG001) that prevents the interaction of CBP with β-Catenin does not blunt EGFR-induced gene expression.

Example 6

Material and Methods

Cell counting:

2000 HCC827 (ATCC; CRL 2868) cells/well were seeded into 96 well plates (Greiner BioOne 655090) one day prior drug treatment in RPMI medium containing 10% FCS and 2 mM L-Glutamine. Several plates were seeded, as for each time point of cell counting one plate was fixed and stained for analysis. The next day cells were treated with indicated compounds and concentrations [Figure 2 shows Compound A and B; Figure 3 shows Compound A, CCS1477 (Chemgood; C1505), SGC-CBP30 (Selleckchem; S7256; CAS No. 1613695-14-9), A-485 (Lucerna-Chem; HY-107455), compound 00071 and compound 00030] in combination with DMSO or Gefitinib (20 nM in Figure 2 or 300 nM in Figure 3). Cell cultivation was continued until time of fixation and analysis for each plate. For extended time-points, medium and drugs were replenished twice weekly.

Fixation and Imaging:

At the given time plates were washed 3 times with PBS and cells fixed with 80µl 4% PFA for 10 min, RT. After 3 wash steps with PBS cells were stained with 10 µg/ml Hoechst33342 (Thermo Scientific H12492) in 100 µl PBS for 2 h, RT, dark. After 3x PBS washing-steps, Hoechst33342 signals were acquired on in an automated imaging mode using a Zeiss Apotome with motorized X/Y stage and a 5x objective. Image analysis and determination of Hoechst33342 spots (nuclei) was done using ImageJ. Number of nuclei was plotted as function of time using GraphPad Prism.

Label-free determination of cell proliferation:

2000 HCC827 (ATCC; CRL 2868) cells/well or 2000 NCI-H1975 (ATCC; CRL 5908) were seeded into 96 well plates (Greiner BioOne 655090) one day prior to drug treatment in RPMI medium containing 10% FCS and 2 mM L-Glutamine. The next day wells were imaged label-free using brightfield imaging on a CELIGO Imaging Cytometer to determine the initial cell number. Subsequently cells were treated with either DMSO, with single drugs or drug combinations and regularly imaged over weeks using brightfield mode (CELIGO Imaging Cytometer) to track cell proliferation in each well over time. Growth medium and treatments were replenished twice weekly. Drugs and concentrations for HCC827: 300 nM Gefitinib, 1 µM Compound A and 300 nM Gefitinib + 1 µM Compound A. Drugs and concentrations NCI-H1975: 50 nM osimertinib (LC Laboratories; O-7200), 0.125, 0.5 or 2 µM CCS1477 (Chemgood; C1505) and 0.125, 0.5 or 2 µM Compound A or combinations as indicated in the figures. Cell numbers were determined using CELIGO software's built-in "direct cell counting" analysis tool in the brightfield mode.

Only the enantiomer that binds the bromodomain of CBP/p300 (Compound A) but not the enantiomer that does not bind the bromodomain of CBP/p300 (Compound B) potentiates EGFR inhibitor-mediated NSCLC inhibition of cell proliferation in a concentration-dependent manner.

Cell numbers of EGFR-mutated HCC827 cells were monitored over time. Figure 2A shows cells which were treated with DMSO alone (filled circles), with 20 nM EGFR inhibitor alone (Gefitinib; 1st generation EGFR inhibitor, open circles) or in combination with the active enantiomer of the CBP/p300 BRD inhibitor Compound A (top) or its enantiomer Compound B (Compound B is also referred to herein as compound 00003) that does not bind to the bromodomain of CBP/p300 (bottom), at indicated compound concentrations. Figure 2B HCC827 cells were exposed to Compound A & B in absence of EGFR inhibitor. Presented graphs are from one experiment with triplicates for each time-point and condition (mean ± SD).

Results: HCC827 cell numbers initially decrease under treatment with 20 nM Gefitinib but start to re-grow under continuous Gefitinib exposure. Re-growth is inhibited over the investigated period by the inhibition of CBP/p300 using BRD-I Compound A but not with the corresponding non-bromodomain-binding enantiomer Compound B. Interestingly, the BRD-I effects in combination therapy to prevent re-growth, occurs despite its inactivity as a single agent.

Compound A and benchmark CBP/p300 inhibitors in combination with EGFR inhibitor mediated HCC827 cell proliferation inhibition.

Figure 3 (A) and (B) and (C) and (D) and (E) shows cell numbers of the EGFR-mutated NSCLC cell line HCC827 as a function of drug treatments (symbols in graph legends) over time [days] measured in 96-well plates using nuclear fluorescent staining. Figure 3(A) and (B) and (C) Left graph: Single agent treatment of cells with Compound A (Fig 3A), CCS1477 (Fig 3B), SGC-CBP30 (Fig 3C) or A485. Figure 3 (A) and (B) and (C) Right graph and Figure 3(C) and (D): Anti-proliferative activity of Compound A (Fig 3A) and CCS1477 (CBP/p300 BRD-I) (Fig 3B) and compound SGC-CBP30 (Fig 3C) and compound 00071 (Fig 3D) and compound 00030 (Fig 3E) and A485 (CBP/p300 HAT-I) in the presence of 300 nM Gefitinib. Represented curves are from one experiment in triplicate (mean ± SD), similar results were repeatedly obtained in similar experiments.

Figure 4 A shows the assessment of HCC827 cell number over time in [h]. Compound A does not affect cell proliferation of EGFR-mutated NSCLC cells in the absence of an EGFR inhibitor but prevents the development of drug resistance when combined with an EGFR inhibitor (1 example plate is shown n= 24 wells for Gefitinib and n=24 wells for Gefitinib + Compound A treatment, DMSO: n=6, Compound A: n=6, mean ± SD). Fig 4B depicts cell numbers per well, as dot plots, treated with Gefitinib or Gefitinib + Compound A for 0 or 22 days (from 2 experiment plates as in A, n=48 wells per condition). Figure 4 C shows a waterfall plot of wells treated with 300 nM Gefitinib or 300 nM Gefitinib + 1µM Compound A from 2 plates (n= 48 wells/per condition) analyzed as in Figure 4 A. The increase in cell numbers at Day 22 in each well was calculated as log fold change from the initial cell number of each well before drug treatment (Day 0).

Results:
Figure 3A and 3B and 3C and 3D and 3E: HCC827 cell numbers initially decrease post-treatment with 300 nM Gefitinib but start to re-grow under continuous Gefitinib exposure. Re-growth is inhibited over the investigated period by inhibition of CBP/p300 using five independent BRD-I or HAT-I. Interestingly the BRD-I effects in combination therapy to prevent re-growth, occurs despite its inactivity as a single agent.
Figure 4A 4B and 4C: Compound A has weak/no effect on cell numbers on its own, whereas 300 nM Gefitinib initially completely blocks cell proliferation. In the long-term cultures however cells re-grow if treated with Gefitinib only, while co-treatment with Compound A significantly delays or completely prevents re-grow for the investigated time (>22 days).

Compound A and benchmark CBP/p300 inhibitors in combination with EGFR inhibitor-mediated NSCLC cell proliferation inhibition - NCI-H1975.

Figure 5 (A) shows the assessment of NCI-H1975 cell numbers as a function of time [h] in the presence of DMSO, 50 nM osimertinib, 2 µM Compound A or combinations of 50 nM osimertinib with 2.0, 0.5 or 0.125 µM Compound A. Figure 5 (B) shows the assessment of NCI-H1975 cell growth in the presence of DMSO, 50 nM osimertinib, 2 µM CCS1477 or combinations of 50 nM osimertinib with 2.0, 0.5 or 0.125 µM CCS1477. (Example graphs show duplicates of each data and timepoint, with a logistic growth curve fit calculated in GraphPad Prism).

Results: Figure 5: The combination effect of CBP/p300 BRD-I and EGFR-I is true for further EGFR-mutated NSCLC cell lines and for different EGFR-I compounds. Compound A and CCS1477 have no/weak effect on cell numbers on their own, whereas 50 nM osimertinib initially blocks cell proliferation. Long-term however, cells continue growing with a slow rate even in the presence of 50 nM osimertinib, which is delayed by co-treatment with Compound A or CCS1477 dose-dependently.

Example 7

Xenograft: 2 million NCI-H1975 cells (ATCC; CRL 5908) were injected into both flanks of NMRI-Nude mice (Janvier). Mice were distributed into treatment groups when the bigger tumor had reached the volume of about 200 mm^3. Mice were treated daily, orally with vehicle (30% PEG300/H2O; 202371 Sigma-Aldrich), 20 mg/kg CCS1477 (ChemieTek; CT-CCS1477), 2 mg/kg osimertinib (O-7200 LC Laboratories) or with 2 mg/kg osimertinib in combination with 20 mg/kg CCS1477 (pre-mixed DMSO stocks). Tumor volume was measured using a manual caliper two to three times a week. The tumor volume was calculated using the formula: lager tumor diameter x square of the smaller tumor diameter divided by 2. Based on a linear fit of the mean tumor volumes the slopes of the curves were compared by regression analysis (two-tailed). A significant difference between the osimertinib and the osimertinib/CCS1477 group was detected. No significant difference between the vehicle and CCS1477 single agent treatment could be observed.

The response was determined by comparing tumor volume change at time t to its baseline: % tumor volume change = ΔVolt = 100% × ((Vt – Vinitial) / Vinitial). The Best Response was the mini-

mum value of ΔVolt for t ≥ 10 d. For each time t, the average of ΔVolt from t = 0 to t was also calculated. The BestAvgResponse is defined as the minimum value of this average for t ≥ 10 d. This metric captures a combination of speed, strength and durability of response into a single value. The criteria for response (mRECIST) were adapted from RECIST criteria21 and defined as follows (applied in this order): mCR, BestResponse < −95% and BestAvgResponse < −40%; mPR, BestResponse < −50% and BestAvgResponse < −20%; mSD, BestResponse < 35% and BestAvgResponse < 30%; mPD, not otherwise categorized. Mice that were sacrificed because of an adverse event before they had completed 14 d on trial were removed from the data set.

In vivo efficacy of the combination of EGFRi and CBP/p300i

Figure 6 (A) shows the mean tumor volumes (+SEM) of EGFR-mutated NCI-H1975 xenografts are plotted over time. Four different treatment groups are depicted: vehicle (30% PEG300/H2O; crossed circle; n=4), 20 mg/kg CCS1477 (open circles; n=4), 2 mg/kg osimertinib (filled circle; n=9) or with 2 mg/kg osimertinib in combination with 20 mg/kg CCS1477 (half-filled circles; n=10). Figure 6 (B) shows the best average response for all 4 treatment groups is shown in a waterfall plot (vehicle in grey, 20 mg/kg CCS1477 in white, 2 mg/kg osimertinib in black and 2 mg/kg osimertinib in combination with 20 mg/kg CCS1477 in squared). The dashed line indicates the reduction of 30% of initial tumor volume.

Results: Figure 6: When bromodomain inhibitors of CBP/p300 are used in absence of the EGFR inhibitor, they have no effect on EGFR-mutated NSCLC xenograft tumor growth. However, when they are combined with an EGFR inhibitor, response to therapy is increased and tumor size is better controlled over the course of the therapy. Surprisingly, when the bromodomain-binding inhibitor (CCS1477) is combined with the EGFR inhibitor (osimertinib) there is an increased response rate to the therapy despite the absence of response to the bromodomain-binding inhibitor (CCS1477) alone.

Example 8

Materials and Methods:

CBP bromodomain binding assay (TR-FRET):

Compounds solutions of 10mM in DMSO were pre-diluted in DSMO to 25x stock solutions in DMSO. These were then diluted down to 4x in Assay buffer. A dilution series in Assay buffer was performed keeping the DMSO concentration stable. 5µl compound in assay buffer was transferred into the assay plate (provided by assay kit) and the TR-FRET assay Cayman chemicals; 600850) was performed using the manufactor's instructions. After 1 hour incubation at room temperature in the dark, assay plates were read in a Tecan M1000 plate reader using the TR-FRET mode (top read; excitation 340nM bandwidth 20nM; emission 620nM bandwidth 7nM; gain optimal determined for the first well, number of flashes: 5; flash frequency 100Hz; integration time: 500µs, lag time: 100µs, room temperature). The TR-FRET ratio was calculated by dividing 670nm emission by 620nm emission. Calculation of EC50 was done on normalized values (DMSO =1) and positive control (0). Values were log transformed and non-linear regression with variable

slope (4 parameters) was used to fit values to a dose-response curve to evaluate EC50 values (see table 3 below).

Table 3:

Legend EC50: A* < 0.2 µM < A < 1µM < B < 10µM < C

Compound #	EC50
00003	C
00004	A*
00030	A
00071	A*

It is evident from the TR-FRET data that Compound 00003 with an EC50 of > 10 µM does not correspond to a CBP/p300 bromodomain inhibitor as defined herein.

Example 9

Material and Methods

Label-free determination of cell proliferation:

2000 HCC4006 (ATCC; CRL2871; with EGFR exon 19 deletion L747 to E749) were seeded into 96 well plates (Greiner BioOne 655090) one day prior to drug treatment in RPMI medium containing 10% FCS and 2 mM L-Glutamine. The next day wells were imaged label-free using brightfield imaging on a CELIGO ImageCytometer to determine the initial cell number. Subsequently cells were treated with either DMSO, with single drugs or drug combinations and regularly imaged over weeks using brightfield mode (CELIGO Imaging Cytometer) to track cell proliferation in each well over time. Growth medium and treatments were replenished twice weekly. Drugs and concentrations for HCC4006: 300 nM Gefitinib (LC Laboratories; G-4408), 1 µM Compound A and 300 nM Gefitinib + 1 µM Compound A. Cell numbers were determined using CELIGO software's built-in "direct cell counting" analysis tool in the brightfield mode.

Figure 8A shows the assessment of HCC4006 cell number over time [in days]. Compound A does not affect cell proliferation of EGFR-mutated NSCLC cells in the absence of an EGFR inhibitor but prevents the development of drug resistance when combined with an EGFR inhibitor (DMSO: n=6, Compound A: n=6, Gefitinib: n=24, Gefitinib + Compound A: n= 24, mean ± SD). Fig 8B depicts cell numbers per well, as dot plots, treated with Gefitinib or Gefitinib + Compound A for 0 or 20 days (from experiment in A, 24 wells per condition, "+" in x-axis label indicates Gefitinib + Compound A). Figure 8C shows a waterfall plot of wells treated with 300 nM Gefitinib or 300 nM Gefitinib + 1µM Compound A (24 wells/per condition) analyzed as in Figure 4A. The increase in cell numbers at Day 20 in each well was calculated as log fold change from the initial cell number of each well before drug treatment (Day 0).

Results: Figure 8A, 8B and 8C: Compound A has no/at best a very weak effect on HCC4006 cell numbers on its own, whereas 300 nM Gefitinib initially completely blocks cell proliferation. In the long-term cultures, HCC4006 cells re-grow if treated with Gefitinib only, whereas co-treatment with Compound A completely prevents re-growth for the investigated time period (>20 days).

Example 10

Material and Methods

Label-free determination of cell proliferation:

2000 HCC827 (ATCC; CRL 2868) were seeded into 96 well plates (Greiner BioOne 655090) one day prior to drug treatment in RPMI medium containing 10% FCS and 2 mM L-Glutamine. The next day wells were imaged label-free using brightfield imaging on a CELIGO Image Cytometer to determine the initial cell number. Subsequently cells were treated with either DMSO, with single drugs (osimertinib and each of the CBP/p300 bromodomain inhibitors) or drug combinations of 100 nM osimertinib and one CBP/p300 bromodomain inhibitor with drug concentrations given below. Plates were regularly imaged over weeks using brightfield mode (CELIGO Imaging Cytometer) to track cell proliferation in each well over time. Growth medium and treatments were replenished twice weekly. Drugs and concentrations for HCC827: 100 nM osimertinib (EGFR-inhibitor, LC Laboratories; O-7200) and for CBP/p300 bromodomain inhibitors: 1 µM Compound A, 0.2 µM Compound C, 0.2 µM CCS1477 (ChemiTek; CT-CCS1477), 1 µM FT-6876 ("CBP/P300-IN-8", MedChemExpress; HY-136920) and 0.2 µM GNE-781 (MedChemExpress; HY-108696). Cell numbers were determined using CELIGO software's built-in "direct cell counting" analysis tool in the brightfield mode.

Figure 9A-E show the assessment of HCC827 cell numbers over 21 days. CBP/p300 bromodomain inhibitors [(A)Compound A, (B) Compound C, (C) CCS1477, (D) FT-6876 and (E) GNE-781)] do not affect cell proliferation of EGFR-mutated NSCLC cells in the absence of an EGFR inhibitor but prevent the development of drug resistance towards 100 nM osimertinib when combined with osimertinib. Note that DMSO curves and time courses for 100 nM osimertinib treatment are identical in panels 9A-E, as all conditions were run in parallel (DMSO: 18 wells, CBP/p300 bromodomain inhibitor: 6 wells each, osimertinib: 12 wells and all combinations of osimertinib + CBP/p300 bromodomain inhibitor: 12 wells, mean ± SD).

Results: Figure 9A-E: CBP/p300 bromodomain inhibitors no/at best a weak effect on HCC827 cell numbers on their own, whereas 100 nM osimertinib initially blocks cell proliferation. In the long-term cultures HCC827 cells re-grow if treated with osimertinib only, whereas co-treatment with the different CBP/p300 bromodomain inhibitors completely prevents re-growth for the investigated time of 21 days.

Example 11

Material and Methods

Label-free determination of cell proliferation:

2000 HCC4006 (ATCC; CRL2871) were seeded into 96 well plates (Greiner BioOne 655090) one day prior to drug treatment in RPMI medium containing 10% FCS and 2 mM L-Glutamine. The next day wells were imaged label-free using brightfield imaging on a CELIGO Image Cytometer to determine the initial cell number. Subsequently cells were treated with either DMSO, with single drugs (osimertinib and each of the CBP/p300 bromodomain inhibitors) or drug combinations of 100 nM osimertinib and one CBP/p300 bromodomain inhibitor with drug concentrations given

below. Plates were regularly imaged over weeks using brightfield mode (CELIGO Imaging Cytometer) to track cell proliferation in each well over time. Growth medium and treatments were replenished twice weekly. Drugs and concentrations for HCC827: 100 nM osimertinib (EGFR-inhibitor, LC Laboratories; O-7200) and for CBP/p300 bromodomain inhibitors: 1 µM Compound A, 0.2 µM Compound C, 0.2 µM CCS1477 (ChemiTek; CT-CCS1477), 1 µM FT-6876 ("CBP/P300-IN-8", MedChemExpress; HY-136920) and 0.2 µM GNE-781 (MedChemExpress; HY-108696). Cell numbers were determined using CELIGO software's built-in "direct cell counting" analysis tool in the brightfield mode.

Figure 10A-E shows the assessment of HCC4006 cell number over 21 days. CBP/p300 bromodomain inhibitors [(A)Compound A, (B) Compound C, (C) CCS1477, (D) FT-6876 and (E) GNE-781)] do not affect cell proliferation of EGFR-mutated NSCLC cells in the absence of an EGFR inhibitor but prevent the development of drug resistance towards 100 nM osimertinib when combined with osimertinib. Note that DMSO curves and time courses for 100 nM osimertinib treatment are identical in panels (A-E), as all conditions were run in parallel (DMSO: 18 wells, CBP/p300 bromodomain inhibitor: 6 wells each, osimertinib: 12 wells and all combinations of osimertinib + CBP/p300 bromodomain inhibitor: 12 wells, mean ± SD).

Results: Figure 10A-E: CBP/p300 bromodomain inhibitors have no/at best a weak effect on HCC4006 cell numbers on their own, whereas 100 nM osimertinib initially blocks cell proliferation. In the long-term cultures HCC4006 cells re-grow if treated with osimertinib only, whereas co-treatment with the different CBP/p300 bromodomain inhibitors significantly delays or completely prevents re-growth for the investigated time of 21 days.

Example 12

This xenograft example was carried out along the lines as example 7 above. Thus, 2 million NCI-H1975 cells (ATCC; CRL 5908) were injected into both flanks of NMRI-Nude mice (Janvier). Mice were distributed into treatment groups when the bigger tumor had reached the volume of about 200 mm^3. Mice were treated daily, orally with vehicle (0.8% (vol) DMSO (CAS [67-68-5]; 5% (vol) NMP (CAS [872-50-4]), 4.2% (vol) DMA (CAS [127-19-5]), 90% (vol) of 40% (wt/vol) Captisol (CAS [182410-00-0]) in pH4 acetate buffer 0.1M), 90 mg/kg Compound C, 2 mg/kg osimertinib (O-7200 LC Laboratories) or with 2 mg/kg osimertinib in combination with 90 mg/kg Compound C (premixed). Tumor volume was measured using a caliper two to three times a week. The tumor volume was calculated using the formula: lager tumor diameter x square of the smaller tumor diameter divided by 2. Based on a linear fit of the mean tumor volumes the slopes of the curves were compared by regression analysis (two-tailed). A significant difference between the osimertinib and the osimertinib/Compound C group was detected. No significant difference between the vehicle and Compound C single agent treatment could be observed.

The response was determined by comparing tumor volume change at time t to its baseline: % tumor volume change = ΔVolt = 100% × ((Vt – Vinitial) / Vinitial). The Best Response was the minimum value of ΔVolt for t ≥ 6 d. For each time t, the average of ΔVolt from t = 0 to t was also calculated. The BestAvgResponse is defined as the minimum value of this average for t ≥ 10 d. This

metric captures a combination of speed, strength and durability of response into a single value. The criteria for response (mRECIST) were adapted from RECIST criteria21 and defined as follows (applied in this order): mCR, BestResponse < −95% and BestAvgResponse < −40%; mPR, BestResponse < −50% and BestAvgResponse < −20%; mSD, BestResponse < 35% and BestAvgResponse < 30%; mPD, not otherwise categorized. Mice that were sacrificed because of an adverse event before they had completed 14 d on trial were removed from the data set.

Figure 11 (A) shows the mean tumor volumes (+SEM) of EGFR-mutated NCI-H1975 xenografts plotted over time. Four different treatment groups are depicted: vehicle; crossed circle; n=8, 90 mg/kg Compound C (open circles; n=6), 2 mg/kg osimertinib (filled circle; n=9) or with 2 mg/kg osimertinib in combination with 90 mg/kg Compound C (half-filled circles; n=12). A linear regression fit of mean tumor volumes over time of treatment was performed, and the slopes of the osimertinib and osimertinib in combination with compound C were compared. A significant difference in the slopes between the two groups could be detected, which was positive in the osimertinib group (+6.4) and negative in the combination group (-7.4), respectively. Figure 11 (B) shows the best average response for all 4 treatment groups in a waterfall plot (vehicle in grey, 90 mg/kg Compound C in white, 2 mg/kg osimertinib in black and 2 mg/kg osimertinib in combination with 90 mg/kg Compound C in squared). The dashed line indicates the reduction of 30% of initial tumor volume.

Results: The results confirm the results obtained in example 7 above, this time for Compound C instead of CCS1477 as the CBP/p300 bromodomain inhibitor, wherein the two inhibitors are structurally not related but have the same function. Thus, when a CBP/p300 bromodomain inhibitor is used in absence of an EGFR inhibitor, there is no effect on EGFR-mutated NSCLC xenograft tumor growth. However, when a CBP/p300 bromodomain inhibitor (CCS1477 in example 7, Compound C in the present example) is combined with an EGFR inhibitor (here osimertinib), response to therapy is significantly increased and tumor size is much better controlled over the course of the therapy.