Histone deacetylase inhibitors for epigenetic therapy of cancer

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Histone acetylation and histone deacetylation play key roles in the epigenetic regulation. Thus, inhibition of deacetylation controlled by histone deacetylases may result in chromatin remodeling, upregulation of key tumor repressor genes, differentiation or apoptosis. Therefore many naturally occurring and synthetic histone deacetylase inhibitors have been shown to display potent anticancer activities in preclinical studies. The exact mechanism by which histone deacetylases exert their effect, however, is still obscure; in any case it is more complicated than originally understood. Although several representatives of this novel class of therapeutic agents are currently at early stages of clinical development, rational design leading to highly selective histone deacetylase inhibitors against histone deacetylase isoforms will not only probably offer more potent anticancer drugs, but also critical insights into their mechanism of action. ***Anti-Cancer Drugs*** **18:363–370**

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Introduction

Histones are small basic protein that, by complexing with DNA, form nucleosomes leading to the compact structure of chromatin. They can be in one of the two antagonist forms, acetylated or deacetylated, and enzymes responsible for this conversion are histone acetyl transferases (HAT) producing the acetylation and histone deacetylases (HDACs) that reverse this process. Deacetylation led to the removal of an acetyl group from the ε-amino groups near the N-termini of histones. Histones can be also methylated on their lysine (K4 and 27 of H-3 and K-20 of H-4) and also on their arginine residues, but so far, this modification seems to be less dynamic than those caused by the acetylation process.

This reversible acetylation of histones has a critical role in transcriptional regulation of genes [1–3]. Whereas deacetylation of histone tails induces transcriptional repression through chromatin condensation, acetylation correlates with nucleosome remodeling and transcriptional activation. This may be explained by the fact that neutralization of the positive charge of lysine residues in the N-terminal tail by this acetylation process leads to loosening histone–DNA contacts. This relaxation of the chromatin structure facilitates the accessibility of a variety of factors to DNA.

Besides this change at the nucleosome scale, acetylation or deacetylation of histones could also interfere [4] with the formation of the chromatin itself by modulating the interactions between the nucleosomes. Moreover, N-terminal amino acids may also act as signals for interactions with other proteins, directly involved in transcription, or via the modification of the chromatin environment. The opposite functions of HAT and HDAC, in both activating and repressing transcription, show the intricate regulatory processes that are involved in turning genes on or off.

Recruitment of HDAC and mutations of HAT are associated with transcriptional repression of a set of genes, which may result in cell growth and tumor cell proliferation [5,6].

Deregulation of HDAC recruitment to promoters appears to be one of the mechanisms by which these enzymes contribute to tumorigenesis. Several reports [7–9] show that inappropriate transcriptional repression mediated by HDAC is a common molecular mechanism that is used by oncoproteins. For all these reasons, HDAC inhibition has been regarded as a promising anticancer drug target [9–12]. Increased interest in HDAC inhibition results from the recent advances in understanding the role of epigenetic mechanisms, which involve DNA and histone modifications, by which occurs the heritable silencing of genes without a change in their coding sequence [13–16].

It is now well established that HDAC inhibitors (HDACIs) cause transforming cells to undergo growth arrest, terminal differentiation and/or apoptosis, activate transcription of the cyclin-dependent kinase inhibitor WAF1, and downregulate cyclins A and D. In addition,

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HDACIs might lead [17,18] to activation of the host immune response and inhibition of tumor angiogenesis by multifactorial processes.

So far, 17 human genes that encode proven or putative HDACs have been identified which belong to three major classes on the basis of their homologies to yeast proteins [19,20].

Class I HDACs (size 42–45 kDa) are nuclear proteins homologous to yeast protein Rpd3, and includes HDAC 1, 2, 3 and 8 human enzymes. Contrary to HDACs of class I which are exclusively expressed in the nucleus, HDAC 4, 5, 6, 7, 9 and 10 belonging to the class II (size 120–130 kDa) shuttle between the cytoplasm and the nucleus. They are homologous to the yeast protein HDA1.

The third class of HDACs has been identified on the basis of sequence similarity with Sir2, a yeast transcriptional repressor that requires the cofactor nicotinamide adenine dinucleotide for its deacetylase activity [21,22].

Short-chain fatty acid histone deacetylase inhibitors

The first encountered HDACIs belong to the class of short-chain fatty acid. The first ones were sodium *N*-butyrate [23], phenylacetate and phenylbutyrate [24], but limited efficacy has been observed during the clinical trials [25] with some toxicities including the central nervous system and fatigue. Phase II clinical trials are going.

The antiepileptic agent, valproic acid, has been reported as an HDACI [26] delaying the growth of primary breast cancers, activating the Notch signaling cascade in human neuroblastoma [27], and inducing growth arrest, apoptosis and senescence in medulloblastomas [28].

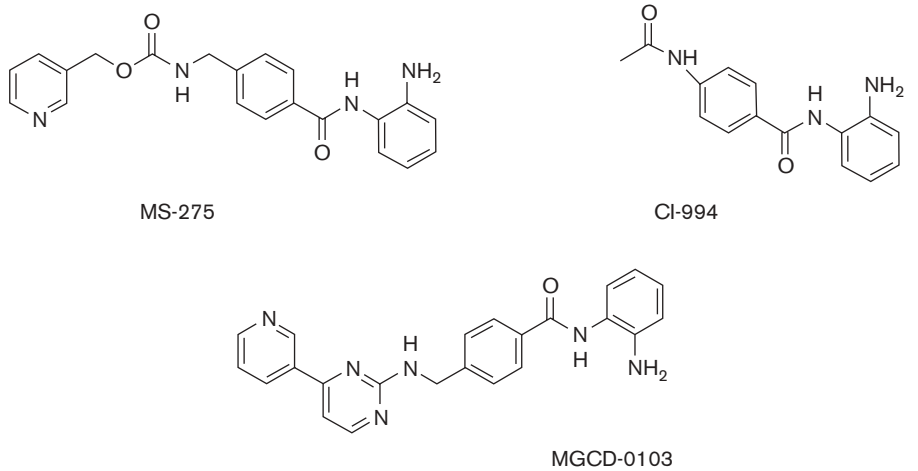
All these compounds were weak inhibitors, however, in the high micromolar or low millimolar range, and nonselective. Among several disadvantages was their low biodisponibility. Some progress have been made with the use of prodrugs such as AN-9, the pivaloyloxymethyl butyrate, or pivanex [29,30], which is in phase II studies in chronic lymphocytic leukemia and has been completely evaluated in a phase II trial with patients with refractory non-small-cell lung cancer [31].

Natural histone deacetylase inhibitors

Beside these fatty acids, the following HDACIs were natural products which have been isolated from fungus strains: trichostatin A and C (from *Streptomyces hydroscopicus*) [32], depudecin (from *Alternaria brassicicola*) [33], trapoxins A and B (from *Helicoma ambiens*) [34], apicidin or OSI-2040 (from *Fusarium* species ATCC 74289 and ATCC 74322) [35]. Closely related cyclic hydroxamic-acid-containing peptides (CHAPs) are hybrids of trichostatin and trapoxin, which have been designed and synthesized [36]. Among them, CHAP31 was the most potent HDACI of the series with an $IC_{50} = 3.32$ nmol/l (against HDAC prepared form B16/BL6 cells). On the other hand, based on the natural Apicidin, a novel series of potent and selective HDACIs has been recently reported [37]. Azumamides A–E, which belong to the same class of cyclotetrapeptides as trapoxins, were recently isolated from the marine sponge *Mycale izuensis* as new HDCAIs [38] (Fig. 1).

So far the most studied compound belonging to this class is undoubtedly, depsipeptide FK228 (or NSC630176), a

Fig. 1



Benzamide-containing histone deacetylase inhibitors.

naturally occurring polypeptide, which has been isolated from *Chromobacterium violaceum*. Studies carried out by Yoshida *et al.* [39] have shown that FK228 is in fact a stable prodrug which is activated by reduction by glutathione, after uptake into the cells. The reduced form, namely redFK, would be the active form, as supported by several experiments. RedK having a four-carbon-long chain between one of the sulfhydryl and the cyclic depsipeptide core, this SH would react with the only one conserved cysteine residue present in the pocket to form a covalent disulfide bond. The synthesis of FK228 has been achieved by Li *et al.* [40].

FK228, which inhibits selectively class I HDACs, is currently progressing through phase II clinical trials in renal cell carcinoma, hormone-refractory prostate cancer as well as in cutaneous T-cell lymphoma in which it has led to complete or partial response at the end of the phase I study [41]. More recent results suggest that FK228 might be a promising drug for use against Ewing's family tumors [42]. As electrocardiogram abnormalities were observed in preclinical studies and focal necrosis and hemorrhage in cardiac tissues at lethal doses, two recent studies have been conducted in patients: one in patients treated in a phase II trial for T-cell lymphoma [43], another in patients with metastatic neuroendocrine tumor [44] during a National Cancer Institute sponsored phase II clinical trial. The first study concluded that no evidence of cardiotoxicity was detected, the only electrocardiogram changes being reversible and generally of short duration. On the contrary, the second study was terminated prematurely because of an unexpected high number of serious cardiac adverse events. Following this main message, some controversy appeared in the literature with an editorial and two letters to the editor, around the assertion of 'serious adverse events'. Using the same arguments as Peikartz *et al.*, in their editorial who considered that HADCs have documented efficacy, including durable complete response [101], Molife *et al.* [102], conclude that they should not lead them to slow the development of an agent that has shown promising activity in castration refractory prostate cancer and cutaneous T-cell lymphoma. In response to both remarks, Shah *et al.* held their own [103], choosing to focus on alternative histone deacetylase. It remains unclear if this cardiotoxicity is mediated through HDAC inhibition, and further investigations are needed.

First generation of synthetic analogues from library screening

Suberoylanilide hydroxamic acid (SAHA; Vorinostat was selected by a group at the Memorial Sloan-Kettering Cancer Institute [45] among a library of 600 synthesized hybrid molar compounds as an active inducer of differentiation of murine erythroleukemia cells, such cells having proved of value in inducing the differentiation of other transformed cell lines. In a following article, the same group [46] reported that SAHA inhibits HDACs 1 and 3. Further experiments demonstrated that SAHA inhibits prostate cancer cell growth *in vitro* and *in vivo* [47] when administered to nude mice bearing human androgen-dependent CWR22 prostate tumors. A dose of 50 mg/kg/day caused a reduction of 97% of the tumors versus untreated animals. Phase I and II clinical trials of SAHA are currently going on in patients with various hematologic tumors (acute and chronic myeloid leukemia, chronic lymphocytic leukemia, etc.), but also with refractory solid tumors. Adverse events include anorexia, anemia and thrombocytopenia, all reversible [48].

Oxamflatin was originally identified [49] as a compound inducing the morphological reversion of v-K-*ras*-transformed NIH3T3 cells from a chemical library and it was shown [50] that oxamflatin induces a morphological change of HeLa cells similar to that induced by HDACIs like = trichostatin A. Oxamflatin inhibits HDAC *in vitro* and *in vivo*, improving the expression of gelsolin, cyclin E and cyclin-dependent kinase inhibitors including $p21^{WAF1/Cip1}$.

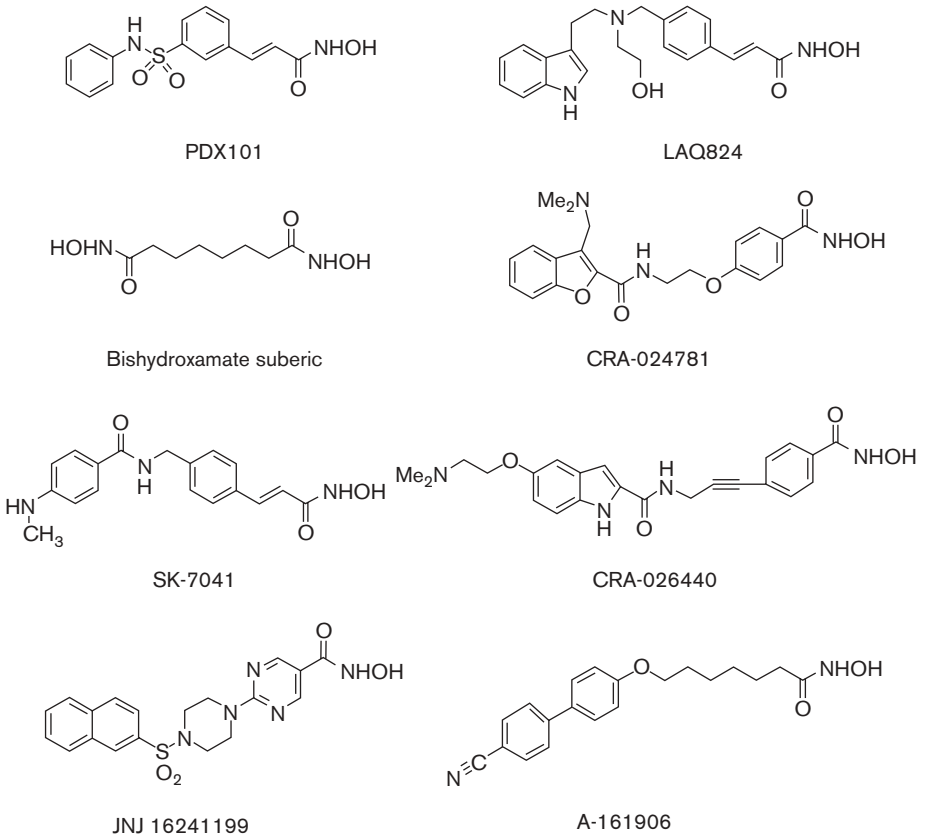
Using high-throughput systems, Su *et al.* [51] and Abbott Laboratories [52,53] identified novel HDACIs, termed scriptaid and A-161906, respectively. Scriptaid and A-161906, discovered serendipitously in a transforming growth factor-β mimetic screen, were soon speculated to be novel HDACIs because of their structural similarity to the class of hydroxamic acid-containing inhibitors.

Synthetic hydroxamic acid-containing histone deacetylase inhibitors

From the X-ray crystal structure of = trichostatin A bound to high-density lipoprotein (HDLP), an archebacterial homologue of human HDAC isolated from *Aquifex aeolicus*, Finnin *et al.* [54] pointed out that the hydroxamic acid coordinates the zinc ion through its CO and OH groups resulting in a penta-coordinate Zn^{2+}. Three additional hydrogen-bonds exist between the CO, the NH and the OH groups of SAHA and Tyr 297, His 132 and His 131 of HDLP, respectively. Therefore, by comparing the structures of known HDACIs like = trichostatin A, SAHA and trapoxins, it clearly appeared at this stage that all these HDACIs possess a metal-binding functionality, linked by an hydrocarbon chain to a cap substructure that interacts with amino acids at the entrance of the *N*-acetyl lysine-binding channel (Fig. 2).

On the other hand, the first crystal structures of the human HDC-8 complexed with = trichostatin A, SAHA, CRA-A and MS344, four hydroxamic acid-containing HDACIs, have been reported [55,56]. The catalytic mechanism is similar to the mechanism proposed for HDLP despite some structural differences.

Fig. 2



Hydroxamate-containing histone deacetylase inhibitors.

Based upon the molecular structure of known HDACIs such as = trichostatin A, oxamflatin and SAHA, several groups have designed and synthesized new inhibitors consisting of a zinc-binding group, a 5- or 6-methylene hydrophobic spacer attached to a hydrophobic group via a connection unit. This has been outlined in recent review articles [57–59]. Few of them have, however, reached the preclinical level and less, the clinical level. Among HDACIs having been the subject of preclinical evaluation, one can cite the following: CRA-026440 [60], (*S*)-HDAC-42, an orally bioavailable inhibitor endowed with antitumor effect in prostate cancer xenografts [61] and potent antiproliferative activity in ovarian cancer cells [62], ITF2357 [63], SK7041, a specific HDAC1 and HDAC2 inhibitor [64], and suberic bishydroxamate [65].

Several hydroxamic acid-containing HDACIs are currently under clinical evaluation: R306465 (or JNJ16241199) which is undergoing phase I clinical trials [66], CRA-024781 (Celera) [67], which is under evaluation in phase I clinical trials for cancer and LBH589 (Novartis) [68] in a phase I study in patients with refractory hematologic tumors (acute myeloid leukemia, acute lymphocytic leukemia, myelodysplastic syndrome) [69], but the most advanced are LAQ824 (Novartis) and PDX-101 (Topotarget Prolifix).

From a synthetic program based on the general HDACI structure combined with a high-throughput screen of the Novartis Pharmaceutical compound library, Remiszewski *et al.* [70] identified NVP-LAK974 as a hit. Undertaking a systematic structural exploration of *N*-hydroxy-3-phenyl-2-propen-hydroxyamides, the same group further identified NVP-LAQ824 (formerly LAQ824) exhibiting high cytotoxicity against HDAC enzyme, HT1299 and HT116 tumors cell lines. Efficacy of LAQ824 in mice bearing HCT116 xenografts at 100 mg/kg combined with the least gross toxicity and with a maximum tolerated dose above 200 mg/kg was confirmed in the A549 human lung carcinoma model. On the basis, in part, of these properties, LAQ824 had entered clinical trials. Significant activity of LAQ824 against multiple myeloma has been reported by Catley *et al.* [71] On the other hand, LAQ downregulates Her-2 and thus sensitizes human

breast cancer cells to monoclonal antibodies like trastuzumab, but also to drugs like taxotere, gemcitabine and epothilone B by enhancing their induced apoptosis [72].

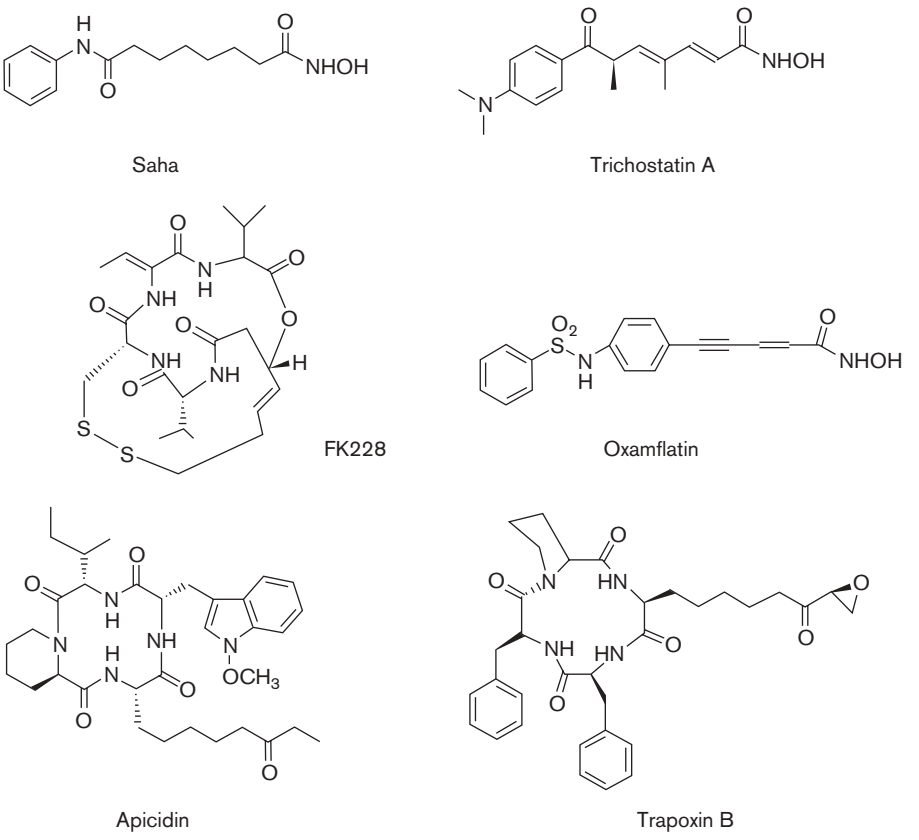
Once the inhibition of growth of ovarian and colon tumor xenografts in mice with no apparent of toxicity has been demonstrated by using PDX-101 [73], this compound entered in clinical trials. Thus PDX-101 is currently in phase II clinical trial evaluation, as a single agent or in combination with dexamethasone for the treatment of advanced multiple myeloma. On the other hand, phase I clinical trials in colorectal, ovarian [74] and multiple myeloma versus velcade seem to be achieved. It has also undergoing phase II study in patients with T-cell lymphomas.

Synthetic benzamide-containing histone deacetylase inhibitors

Although sharing no structural similarity with the previous HDACIs, benzamide derivatives which inhibit HDAC *in vitro* and *in vivo* have been reported in 1999 by Suzuki *et al.* [75] from Mitsui Pharmaceuticals. One of the most active derivatives is MS-275 which is developed by Schering (Berlin) in collaboration with the National Cancer Institute. In a phase I trial conducted in patients with refractory/relapsed acute myeloid leukemia [76], no dose-limiting toxicity was observed and further trials were planned with increased dosage. It is now currently in phase I/II clinical evaluation in patients with lung or colorectal cancer [77]. A recent investigation has shown that MS-275 may be efficient in malignant brain tumor such as experimental glioblastomas as a single injection of MS-275 7 days after orthotopic implantation of glioma cells led to significant reduction of tumor growth [78] (Fig. 3).

p-N-acetyl dinaline (or CI-994 or GOE 5549), the 4-acetylamino-*N*-(2′-aminophenyl)-benzamide, is a bioavailable cytostatic oral drug. It has been introduced in clinical trials for a number of tumor diseases, mainly in colorectal cancer [79], but it displayed only little activity as a single agent. It is, however, only after many years of study on its mechanism that it was identified [80] as a HDAC. A phase I study was started 4 years ago with chronic oral administration by Prakash *et al.* [81], and more recently phase I studies of oral CI-0994 in

Fig. 3



Natural histone deacetylase inhibitors.

association with capecitabine [82] and carboplatin and paclitaxel [83] were reported. In all these clinical trials, thrombocytenia was dose-limiting for CI-994 at the maximum tolerated dose of 6–8 mg/m^2/day.

MGCD-0103 currently developed by Methylgene, Pharmion and Taiho belongs to the class of substituted *N*-(2-aminophenyl)-benzamides [59,84]. It has been claimed, as well as some structural analogues [85], as inhibiting a specific subset of HDAC isoforms with IC_{50} values in the submicromolar range. Three phase I trials have been completed in patients with solid tumors or hematologic malignancies refractory to current available therapies. Phase II trials are expecting to start during second half of 2006 in elderly patients with acute myeloid leukemia or high-risk myelodysplastic syndrome.

Miscellaneous

Design and synthesis of SAHA-based nonhydroxamates such as semicarbazide and bromoacetamide were reported [86]. Arising from Abbott researchers, three series have been designed which contain trifluoromethyl ketones [87], heterocyclic ketones [88] and α-keto-esters or α-keto-amides [89], as zinc linkers.

The pioneering work of Schreiber *et al.* [90] provides evidence that class II HDAC6 (a histone with two catalytic domains) is the intracellular target of tubacin, a small molecule which inhibits α-tubulin deacetylation in mammalian cells [91]. Most hydroxamic acid HDACIs inhibit all the HDAC isoforms, whereas cyclic peptides and benzamide derivatives do not inhibit HDAC6. Owing to the fact that selective HDAC6 inhibitors may represent a promising target class for applications as antimetastatic and antiangiogenic agents alone or in association with hsp90 or bortezomib [92], recent reports have focused on synthesis of such selective HDAC6 inhibitors [93,94].

Screening of a sample collection looking for L-2-amino-8-oxodecanoic acid (L-AODA), an unusual amino acid present in Apicidin, the group of IRBM/Merck reported a new series of potent and selective nonhydroxamate HDACIs [95]. Optimization led to IRBM-1, which selectively inhibits HDAC1, 2, 3 and 6, and was active against a wide range of cancer cell lines [96].

Conclusion

Owing to the fact that HDACs play critical roles in a large number of pathological pathways and that their inhibitors are now undoubtedly implicated in the treatment of cancer, increased numbers of new inhibitors have been identified or designed. Their exact mechanism of action, especially in the case of benzamide derivatives, however, is still not completely elucidated and class-specific inhibitors should offer greater clinical usefulness. So there is a need for designing more selective HDACIs in the next future, based on the better knowledge of the biochemical role of different HDAC subtypes [97,98], along with an increased knowledge of the structural elements responsible for these selectivities [99] that have fuelled this need.

On the other hand, optimal evaluation of HDACIs remains a question to be solved as most studies have shown that the relationship between histone acetylation *per se* and biological activity is not clear. Noninvasive imaging methods for quantifying the biological activity of HDACIs, which are currently going on, may be one way to solve this problem [100].

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