Combining conditionally replicating adenovirus-mediated gene therapy with chemotherapy: a novel antitumor approach

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Despite significant improvements in diagnosis and innovations in the therapy of specific cancers, effective treatment of neoplastic diseases still presents major challenges. Recent studies have shown that conditionally replicating adenoviruses (CRAds) not only have the ability to destroy cancer cells but may also be potential vectors for the expression of therapeutic genes. Several studies in animal models have demonstrated that the combination of CRAds-mediated gene therapy and chemotherapy has greater therapeutic benefit than either treatment modality alone. In this review, an overview of specifications for a novel antitumor approach combining CRAd-gene therapy and chemotherapy is provided and recent progress in this field is discussed.

viral replication in p53 or pRb defective cancer cells5–7 (ii) by using cancer-selective promoters to control the expression of early viral genes,8,9 and (iii) by modification of viral coat proteins that function in cancer cell infection, such as the Ads53 capsid modification, to improve viral infection.10,11 Clinical trials with an E1B 55-kDa-deleted CRAd, ONYX-015 or a derivative of ONYX-015, H101 revealed some encouraging anticancer activity, when used in combination with chemotherapy.12,13

Another means of augmenting antitumor efficacy is the use of CRAd as a vehicle for the delivery of a therapeutic transgene. As an oncolytic transgene delivery system, CRAds not only selectively replicate in and lyse tumor cells but also amplify therapeutic gene expression and function in the tumor microenvironment (Fig. 1).14 In recent years, several laboratories have constructed CRAds armed with proapoptotic transgenes. Examples include the second mitochondria-derived activator of caspases (Smac),15 the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL),16 the melanoma differentiation-associated gene-7/interleukin-24 (mda-7/IL-24),17 a siRNA against the antiapoptotic factor Apollon,18 and the antioxidant enzyme manganese superoxide dismutase (MnSOD),19 which has been shown to act as an antiproliferative and apoptosis inducer in cancer cell.

Alternatively, CRAds encoding immunostimulatory cytokines, such as granulocyte-macrophage colony-stimulating factor,20 heat shock proteins21 and interleukin-12 (IL-12),22 have been used in an effort to recruit effector cells and stimulate host antitumor immunity.

Additional therapeutic genes that have received much attention are suicide genes, also known as the prodrug-activating genes, such as herpes simplex virus thymidine kinase suicide gene (HSV-TK).23 The incorporation of well-studied prodrug converting enzyme systems into highly selective CRAds may additionally enhance the therapeutic potential of
these enzymes. Moreover, preclinical studies have shown that enhanced and even synergistic antitumor activity can be achieved when CRAd-gene therapy is used in combination with chemotherapy. These studies suggest that the administration of CRAds in combination with chemotherapeutic agents could maximize the benefits of combined treatment.24,25

In this review, we provide an overview of specifications for a novel antitumor approach combining CRAd-gene therapy and chemotherapy, and discuss recent progress in their preclinical and clinical trials.

Current Status of CRAd-Gene-Chemotherapy Combination in the Treatment of Cancer: Overcoming Chemoresistance in Multiple Cancers

Several recent studies have shown that combining CRAd-gene therapy with chemotherapy holds great promise in controlling cancer cell growth. Because the therapeutic mechanisms of CRAd-gene therapy and chemotherapy are independent, cross-resistance is theoretically unlikely, and, thus, the development of treatment-resistant cancer cells would be minimized. In the following paragraphs, we summarize recent studies examining the benefit of CRAd-gene therapy in combination with chemotherapy for the treatment of human cancer (Tables 1–3).

Clinical Trials Using CRAds in Combination With Chemotherapy

ONYX-015 is an E1B 55-kDa gene-deleted adenovirus that replicate selectively in p53-deficient tumor cells.5,26 Although ONYX-015 hold promises as anticancer agents, clinical experiences show that ONYX-015 alone are not potent enough to generate sustained clinical responses or to cause complete tumor regressions. In recent clinical trials, better results of ONYX-015 have been achieved when it was combined with some chemotherapy.

Phase II trials for recurrent head and neck cancer by intratumoral injection of ONYX-015 combined with 5-fluorouracil (5-FU) and cisplatin had a 63% therapeutic rate.12 In one case, a tumor 10 cm in diameter was completely eliminated. In contrast, only a 15% remission rate was seen when using ONYX-015 alone.3,27 The combination therapy was well tolerated and did not lead to an apparent increase in toxicity.

Another Phase II trial looked at the combination of ONYX-015 with leucovorin and 5-FU in patients with gastrointestinal carcinoma metastatic to the liver.28 Although results were modest (11% with complete response and 15% with partial responses), overall safety was established. Subsequently, ONYX-015 in combination with 5-FU/leucovorin was administered in a phase II clinical trial by hepatic artery infusion to patients with metastatic colorectal cancer that have failed prior treatment with 5-FU/leucovorin.29 The phase II results showed that ONYX-015 has intrinsic and/or synergistic antitumor activity.

Additional intratumoral trials of ONYX-015 included a phase I/II clinical trial of this agent in combination with MAP (mitomycin-C, doxorubicin, cisplatin) chemotherapy in patients with advanced sarcomas.30 Intratumoral
administration of ONYX-015 in combination with MAP chemotherapy was well tolerated with no significant toxicity. Adenoviral DNA was detected by quantitative PCR in plasma samples up to 7 days after the last viral dose, thereby, verifying vector replication. In addition, there was evidence of antitumor activity in one out of six patients.

H101, an E1B-55 kDa gene and partial E3-deleted replication-selective adenovirus, was evaluated in patients with head and neck cancer in China. Clinical data show that H101 is well tolerable and has good efficacy when combined with chemotherapy in some cancer treatment modalities. In a randomized phase III clinical trial of H-101 in combination with cisplatin and 5-FU, a response rate of 39.6% was observed for chemotherapy alone, whereas chemotherapy and H-101 produced a 78.8% response rate.

Preclinical Trials of CRAds in Combination With Chemotherapy

More recently, Raki et al. obtained encouraging results with an ovarian cancer cell-specific CRAd, Ad5/3-Δ24 (a tropism-modified, adenovirus serotype 3 receptor-targeted, Rb/p16 pathway CRAd), together with gemcitabine or epirubicin. In an orthotopic murine model of peritoneally disseminated ovarian cancer, Ad5/3-Δ24 plus gemcitabine increased the survival of mice over either agent alone, and almost 60% of treated mice were cured. Liu et al. also demonstrated that a telomerase-specific CRAd, OBP-301, yielded a synergistic effect when combined with gemcitabine both in vitro and in vivo. Furthermore, they have observed that there was no reduction in viral replication or specificity for lung cancer cells when combined with gemcitabine. Synergistic efficacy and improved survival were also observed when OBP-301 was combined with cisplatin.

Another CRAd, Ad-E1B19/55, has deletions of the genes encoding both the adenoviral early gene E1B 19-kDa and E1B 55-kDa proteins. This vector exhibits marked enhancement in cytolytic and apoptotic activity in cell culture as well as in a human cervical xenograft model. A combination treatment of Ad-E1B19/55 and cisplatin demonstrated a synergistic cytotoxic effect in all the tumor cells tested.

Interleukin-24 (IL-24) is a member of the IL-10 cytokine family. Recent evidence suggests IL-24 is a promising candidate for cancer gene therapy. The expression of IL-24 from replication-defective Ads carrying IL-24 (Ad-IL-24) can suppress cancer cell growth and induce apoptosis in a variety of cancer cells without harming normal cells. This cancer cell-specific growth-inhibitory effect has been shown to occur in multiple in vivo animal models as well as in human clinical trials. To enhance therapeutic efficacy, Ad-IL-24 is administered in combination with standard chemotherapy. However, because replication-defective adenoviral vectors are used for IL-24 delivery, relatively small amounts of IL-24 can be transduced into cancer cells. Consistently, such vector systems have not yet demonstrated an advantage over standard therapy, especially for the treatment of large solid cancers.

Recent studies provided definitive evidence that ZD55, a conditionally replicating adenovirus, which mediates the expression of the IL-24 gene (ZD55-IL-24), can selectively induce apoptosis in several kinds of tumor cells, and enhance cancer-selective toxicity when combined with chemotherapy. The study by Jiang et al. investigated the combined effects of ZD55-IL-24 and dacarbazine (DTIC) on melanoma cells. The combination of ZD55-IL-24 plus DTIC enhanced apoptosis in melanoma cells by efficiently tilting the balance of Bcl-2 family proteins toward a proapoptotic pathway. Previous studies indicated that ZD55-IL-24 could improve antitumor effects, and, thereby, minimize the toxic side effects of cisplatin and adriamycin by reducing the concentrations of chemotherapeutic agents. Kaliberova et al. constructed a vascular endothelial growth factor receptor 1 (VEGFR-1/Ft-1) conditional replicating adenoviral vector encoding the IL-24 gene (CRAdRGDflt-IL24) and investigated its antitumor activity together with chemotherapy. The combination of CRAdRGDflt-IL24 and temozolomide (TMZ) significantly enhanced cytotoxicity in vitro, inhibited glioma cell growth and prolonged survival of mice harboring intracranial human glioma xenografts in comparison with CRAdRGDflt-IL24 or TMZ alone.

Several studies have shown that the inhibitors of apoptosis proteins (IAP) are a family of caspase inhibitors that bind and
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inhibit the activation of caspase-3, caspase-7 and caspase-9.\textsuperscript{45} Overexpression of IAP is associated with chemoresistance in a variety of human cancers.\textsuperscript{46} Therefore, prevention of IAP inhibition could be critical for sensitizing cancer cells to chemotherapeutic agents.\textsuperscript{47}

Smac is released from mitochondria and promotes caspase activation by eliminating IAP function during apoptosis.\textsuperscript{48} Consequently, it has been suggested that overexpression of active Smac may render resistant tumor cells sensitive to chemotherapeutic agents.\textsuperscript{48} A recent study by Pan et al.\textsuperscript{49} reported that ZD55 carrying Smac (ZD55-Smac) can augment the antitumor activity of cisplatin or 5-FU in hepatocellular carcinoma cells, whereas no growth-inhibitory or apoptotic effect was evident in normal human liver cells. Thus, this new combined strategy may prove beneficial for patients with hepatocellular carcinoma who have become resistant to chemotherapy.

Some studies have shown that overexpression of MnSOD inhibits the growth of numerous types of cancer cells.\textsuperscript{50} The inhibition of cancer cell growth can be attributed to the increase in steady-state levels of H\textsubscript{2}O\textsubscript{2} as a result of the increased dismuting activity of MnSOD.\textsuperscript{51}

Adenovirus-mediated targeting of MnSOD is an important, potential means of overexpressing MnSOD in cancer cells. However, the inhibitory effect on cancer cell growth with adenovirus-mediated MnSOD alone is not an effective treatment for cancer because of the transient nature of adenovirus expression and the immune response against adenovirus. This shortcoming was, however, overcome by combining adenovirus administration with chemotherapeutic agents.\textsuperscript{52} To this end, ZD55-MnSOD, an E1B 55 kDa-deleted CRAd that can mediate overexpression of MnSOD\textsuperscript{53} was utilized in combination with 5-FU on colorectal cancer cells.\textsuperscript{54} Remarkably, the combined treatment significantly and synergistically enhanced growth inhibition and apoptosis of colorectal cancer cells when compared with treatments with ZD55-MnSOD or 5-FU alone. This study also demonstrated that ZD55-MnSOD combined with 5-FU had an excellent anticancer effect in the nude mice model of colorectal cancer, with 60% of the nude mice becoming cancer free. Apollon, a membrane-associated inhibitor of apoptosis protein, protects cells against apoptosis and is upregulated in some chemoresistant cancer cells.\textsuperscript{54} Antisense oligonucleotides against Apollon significantly sensitize cancer cells to apoptosis induced by chemotherapeutic agents.\textsuperscript{55} These findings suggest that Apollon has an antiapoptotic role and support the notion that this protein is an attractive target for molecular cancer therapy.

RNA interference has proved to be a powerful tool for gene knockdown and holds great promise for the treatment of cancer.\textsuperscript{56} It has been shown that combining short hairpin RNA (shRNA) gene therapy with oncolytic virotherapy enhances antitumor efficacy as a result of synergism between CRAd oncolysis and shRNA antitumor responses.\textsuperscript{57} Recently, Chu et al.\textsuperscript{18} constructed ZD55 expressing shRNA against Apollon ZD55-siApollon. They showed that ZD55-siApollon can slow the rate of tumor progression \textit{in vivo} by silencing the Apollon gene, but the tumors were not eliminated. In contrast, complete tumor eradication in five out of seven xenograft mice was observed when tumors were cojected with ZD55-siApollon and 5-FU.

TRAIL, a member of the TNF receptor ligand family, can induce apoptosis in some cancer cells.\textsuperscript{57} It can trigger strong apoptosis preferentially in malignant cells, but not in normal cells, and can effectively suppress the formation and development of various xenograft cancers in nude mice via apoptosis and bystander cell death.\textsuperscript{58,59} Pan et al.\textsuperscript{60} demonstrated that a combination of ZD55 carrying TRAIL (ZD55-TRAIL) with cisplatin exhibits synergistic cytotoxicity in cancer cells while significantly abolishing toxicity in normal cells, mainly due to the reduced dosage of cisplatin that was required for the treatment. In a study by Qiu et al.\textsuperscript{57} using a nude mice model of colorectal cancer, injection of ZD55-TRAIL and 5-FU combined into subcutaneous cancers had good therapeutic effects, leaving all mice alive and one mouse completely tumor free. Furthermore, no detectable hepatotoxicity was found by serum enzyme level analysis.

Several fiber-modified CRAds are currently under investigation for their potential therapeutic effect for cancer,
including Ad5/35 chimeric CRAd. Chen et al.63 developed an Ad5/35 chimeric CRAd expressing TRAIL, SG235-TRAIL, which was used together with taxol. The combined treatment (at a low dose of 0.1 MOI SG235-TRAIL + 0.01 μM taxol) had synergistic cytotoxicity in gastric cancer cells without producing significant toxicity in normal cells.64 Similarly, Meng et al.64 demonstrated that homoharringtonine (HHT) acted synergistically with SG235-TRAIL to kill leukemia cells, without significant damage to human normal cells. Changes in mitochondrial function are key components associated with selective destruction of leukemia cells by SG235-TRAIL plus HHT.

The treatment of human pancreatic cancer cells in vitro using a CRAd with RGD-modified fibers and expressing TRAIL from the human telomerase reverse transcriptase (hTERT) promoter (Ad/TRAIL-F/RGD)65 together with gemcitabine resulted in a synergistic increase in apoptosis.66 Furthermore, suppression of the growth of pancreatic cancer in the liver by Ad/TRAIL-F/RGD in combination with gemcitabine was greater than by either agent alone.

Overall, an important issue to be mentioned here is the animal models used to examine CRAd-gene therapy for cancers. As described above, many studies have relied on human xenograft tumors established in immunodeficient mice. However, the full potential of these strategies could not be realized in the case of those vectors that express immunostimulatory molecules. The use of immunocompetent mice67,68 as well as other models such as the cotton rat,69 Syrian hamster70 and pig models71 seems reasonable for the study of the interaction between CRAds and an intact immune system. A better understanding of replicating Ads in immunocompetent hosts will lead to improvements in vectors for clinical use.

**A Novel Targeted Chemotherapeutic Strategy in the Treatment of Cancer: Suicide Transgenes Mediated by CRAds**

A group of therapeutic genes that have received much attention is suicide genes, also known as the prodrug-activating genes,72 which convert nontoxic prodrugs into toxic metabolites, thereby, avoiding the systemic toxicities associated with conventional chemotherapy. An important feature of suicide gene therapy is the “bystander effect,” in which the toxic metabolites of the prodrug diffuse away from the expressing cell and kill neighboring cells. Whereas replication-defective vectors have previously been used to deliver suicide genes, a lack of transduction efficiency limited the potency of these vectors. It was thought that the use of CRAds would offer improved tumor transduction and, thus, enhanced potency over the earlier vectors.73

The most studied prodrug-activating gene system involves the herpes simplex virus thymidine kinase/ganciclovir (HSV-TK/GCV).74 HSVTK converts GCV to a monophosphorylated form, which is then converted to a toxic triphosphate by cellular kinases. GCV triphosphate interferes with DNA synthesis, leading to cell death. Recently, Ji et al.74 demonstrated that hTERT promoter-driven CRAd in combination with HSV-TK/GCV could reduce the growth of RB cells in vitro as well as in an in vivo orthotopic nude mouse model.

Zhang et al.75 armed prodrug gene-convertiv enzyme CD with ZD55, which generates the toxic chemotherapeutic drug 5-FU from the nontoxic prodrug 5-FC.

A subsequent study showed that ZD55-CD/5-FC produces a much stronger antitumor effect than that observed with a standard replication-defective Ad-CD/ 5-FC or ONYX-015 against human colorectal carcinoma xenografts in nude mice.75 Likewise, A CRAd, Ad5-CD/TKrep, contained a bacterial cytosine deaminase (CD) /wild-type herpes simplexi virus thymidine kinase (HSV-1 TK) fusion gene under the transcriptional control of a strong viral promoter was evaluated in a phase I study in patients with prostate cancer.76 The results of this phase I study also demonstrate that intra-prostatic administration of Ad5-CD/TKrep followed by 2 weeks of 5-fluorocytosine and ganciclovir prodrug therapy can be safely applied to humans and is showing signs of biological activity.

Tumor-specific expression of the bifunctional suicide protein FCU1 is another attractive strategy for local conversion of 5-FC into the effective chemotherapeutic agent 5-FU and its active form 5-FU monophosphorlate.77 Dias et al.78 showed an increase in apoptosis in head and neck squamous cells treated with a CRAd expressing the fusion suicide gene FCU1, Ad5/3-Δ24FCU1, in combination with 5-FC comparing to Ad5/3-Δ24FCU1 or 5-FC alone. In this approach, tumor cells are killed due to virus replication and by 5-FU and 5-FUMP, and additional benefit may result from the synergy of the approaches and the direct bystander effect of passive diffusion of 5-FU that can kill untransduced neighboring tumor cells.

Carboxylesterase is another prodrug converting enzyme that looks promising for clinical trials. This enzyme converts the chemotherapeutic agent irinotecan into a more potent chemotherapeutic agent, SN-38.79 A recent study looked at ONX-015 expressing the transgene for carboxylesterase.79 This prodrug converting enzyme strategy has antitumor effects in both in vitro and in vivo studies.

**Possible Mechanisms of the Antitumor Effect of CRAd-Gene Therapy Combined With Chemotherapy**

Because cancer cells are genetically and phenotypically complex and often have multiple abnormalities, it seems reasonable to expect that a single agent, such as a chemotherapeutic agent, might not be sufficient to completely eradicate malignancies.80 Several studies have suggested enhanced and even synergistic destruction of cells and antitumor activity when CRAd-gene therapy and chemotherapy are combined. However, knowledge of the underlying molecular mechanism accounting for the synergic antitumor effects is limited. Several hypotheses have been proposed and are summarized below (Fig. 2).

E1A-expressing CRAds may augment the anticancer activity of chemotherapeutic agents. E1A is a potent inducer of p53 protein levels in infected cells and can, therefore,
increase cellular sensitivity to chemotherapeutic agents in p53-dependent apoptosis. Moreover, adenoviral-mediated E1A gene expression can sensitize chemotherapeutic agents in cancer cells by upregulating the expression of caspase proenzymes, or upregulating the activities of a proapoptotic kinase p38. Likewise, the E1A 12S and 13S proteins have been demonstrated to sensitize tumor cells to NK-cells, activated macrophages. T-cell-mediated apoptosis and effector
molecules such as TNFα, TRAIL and FasL in addition to direct sensitization to cytotoxic drugs.82–84

It has also been shown that the immune response can reduce the anticancer efficacy of CRAds, when used alone.85 Thus, the immunosuppressive effects of chemotherapeutic agents may increase CRAds efficacy by maintaining viral spread among cancer cells because of a decrease in the production of neutralizing antibodies.86,87 In keeping with this latter notion, Cheong et al.87 recently demonstrated that the poor antitumor efficacy of E3B-deleted CRAd in vivo improved when it was administered together with cisplatin or paclitaxel, and also synergistically enhanced drug-induced cell death. In addition, the immunosuppressive agent cyclophosphamide was also reported to selectively eliminate regulatory T-cells (Treg) and also synergistically enhanced drug-induced cell death. In addition, the immunosuppressive agent cyclophosphamide was also reported to selectively eliminate regulatory T-cells (Treg) prolonging viral gene expression in tumors.88 Thus, chemotherapeutic agents that overcome immune-mediated barriers, together with concomitant immune suppression, are expected to lead to more effective CRAd-gene therapy.

An additional molecular mechanism of CRAds-based combination therapy is suggested by the observation that the cellular transcription factor YB-1, involved in repair mechanisms through its interaction with repair enzymes, is one of the most highly overexpressed genes in drug-resistant cancer cells.89,90 Thus, one possible explanation for the enhanced effect of chemotherapeutic agents on viral replication is that YB-1 binds to the E2-late promoter, facilitating viral replication, while being sequestered away from its function in DNA repair.90 Consequently, the damage caused by chemotherapeutic agents cannot be repaired efficiently, leading to a potential increase in cell death. Consistent with this, a recent study showed that the CRAd dl520 in combination with the chemotherapeutic agent irinotecan could efficiently replicate in, and lyse, glioblastoma cells by augmenting nuclear localization of YB-1.91

Adenovirus replication is increased in the S/G2 phase of the cell cycle.92–94 Chemotherapeutic agents, which induce an S/G2 phase arrest, enhance adenovirus DNA replication when added after virus infection.93 Thus, S/G2 phase arrest may provide a favorable cellular environment for virus DNA replication, providing required cellular factors such as nucleotides, a mechanism, which has been confirmed by a variety of other reports.95,96

The coxsackie-adenovirus receptor (CAR) is the primary receptor of most adenovirus serotypes. Unfortunately, CAR expression is highly variable and often low on the surface of most cancer cells.96 This limitation could be detrimental to CRAd-gene therapy because no meaningful spread will occur in the absence of CAR. Interestingly, recent studies have shown that chemotherapeutic agents can upregulate expression of CAR and, consequently, cell entry of adenoviral vectors and transgene expression.97–99

As some therapeutic genes are toxic when administered systemically as a recombinant protein, the rationale for CRAd-gene therapy is to allow local production of the therapeutic genes at the cancer site. Thus, high concentrations can be achieved in and around cancer cells, but not in the serum.100 A combination of CRAd-gene therapy and chemotherapy would provide a major advantage by reducing dosages and possible overlapping toxicity while achieving a therapeutic effect.

Finally, induction of apoptosis by chemotherapeutic agents plus CRAds could augment viral distribution throughout the tumor mass, resulting in therapeutic benefit.37

These arrays of mechanisms accounting for antitumor effect reinforce the notion that CRAd-gene therapy combined with chemotherapy is a rational modality for the treatment of human cancer.

The Application of an Appropriate Regimen of CRAds and Chemotherapy

Combination of CRAds with chemotherapy presents a significant hope for the treatment of human cancer. Intensive researches are required to evaluate the potential of combination of CRAds with chemotherapy, to improve the efficacy and minimize the toxicity of the procedure. Future efforts should be directed to: (1) the development of CRAds with high transduction efficiency, high transgene capacity and acceptable toxicity profile, (2) development of CRAd vector systems allowing desired duration and regulation of the gene expression, (3) identification of the ideal CRAds in combination with chemotherapeutic agents for each therapeutic indication and (4) development of a new generation of safer and more effective strategies for CRAd-gene therapy in combination with chemotherapy.

Conclusion and Perspectives

As novel antitumor therapeutic agents, CRAds not only selectively replicate in and lyse tumor cells but can also amplify the expression and efficacy of therapeutic genes. A plethora of emerging evidence in preclinical and clinical trials concordantly supports that CRAd-gene therapy together with chemotherapy may have complementary or synergistic effects, leading to a greater antitumor effect than either treatment alone. This combination with chemotherapy could be further enhanced by the use of suicide gene therapy. This will allow for a high concentration of locally activated drugs, thus, minimizing systemic toxicity. However, the cytotoxicity of CRAds and their pharmacokinetic characteristics, make the relationship between CRAd-gene therapy and chemotherapy more complex. Further research and continuing development of molecular virology and molecular oncology will enable researchers to develop a new generation of safer and more effective strategies for CRAd-gene therapy in combination with chemotherapy. Based on these clinical results and the preclinical studies presented here, CRAd-gene therapy, in combination with chemotherapy may prove a novel and effective approach for the treatment of tumors.

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