Multicellular life relies on epigenetic processes to specialize the function of groups of cells for optimal physiology. Be it for development, differentiation, stemness, or sex chromosome dosage compensation, stable, cell specific regulation of gene expression is essential for normal function (1). Multiple mechanisms have evolved to perform these essential functions: DNA methylation, a bewildering array of histone modifications, RNA-based regulation, etc. It took a while to recognize that these same processes are intimately involved in the pathophysiology of disease states, but research into epigenetic deregulation is now as commonplace as research into genetic etiology in various medical conditions.

It is easy to understand how cancer can be an epigenetic disease. Cells have built machinery to turn off and on gene expression permanently. Usurping these processes to deregulate functional pathways is likely simpler than generating mutations or chromosomal aberrations (unless DNA repair is deficient and genetic instability is present). Indeed, early cancer research focused on the fact that neoplastic cells have aberrant gene expression and differentiation states, pointing to shared mechanisms between normal development and cancer (2). In retrospect, the fact that cancer was viewed as a genetic disease for so long was likely an artifact of focus and technology: Chromosomal changes began to be detectable before we understood epigenetics at a molecular level, and the early focus on familial cancer and rapidly induced viral tumors favored the discovery of mutational carcinogenesis, which has largely colored our views over the past three decades (3, 4). But it became clear in the past decade that genetic changes cannot fully explain cancer and, indeed, whole genome analyses are revealing surprisingly few shared mutational events in cases that lack genetic instability (5, 6). In parallel, deciphering the mediators of cellular epigenetic inheritance has led to an explosion of information on epigenetic alterations in cancer, and it is now accepted that these play an important part in defining the transformed phenotype (2, 7).

In mammals, as in most (but not all) complex organisms, DNA methylation is an essential component of the epigenetic machinery of the cells. By stably regulating gene expression and providing a mechanism for sustaining these expression patterns through mitosis, DNA methylation fulfills the basic requirements of an epigenetic code (8). Thus, DNA methylation is necessary and sufficient to explain some of the peculiar patterns of gene expression observed in mature organisms, from imprinting to X-inactivation to germ cell restricted gene expression. Not surprisingly then, altered DNA methylation is also a central component of the molecular nature of neoplasia, and there is ample evidence documenting the central role this process plays in deregulating key pathways important to the transformed phenotype. Much remains to be learned about the causes of DNA methylation abnormalities in cancer; for the most part, methylation seems to be gene specific. In some cases, a rare methylation event appears in cancer because of selection (2), while in others methylation anomalies are downstream of an oncogenic event (9). Methylation is discussed in more detail in this issue (10). Current knowledge indicates that all malignancies have a mixture of genetic and epigenetic defects,

Abstract Two nucleoside inhibitors of DNA methylation, azacitidine and decitabine, are now standard of care for the treatment of the myelodysplastic syndrome, a deadly form of leukemia. These old drugs, developed as cytotoxic agents and nearly abandoned decades ago were resurrected by the renewed interest in DNA methylation. They have now provided proof of principle for epigenetic therapy, the final chapter in the long saga to provide legitimacy to the field of epigenetics in cancer. But challenges remain; we don’t understand precisely how or why the drugs work or stop working after an initial response. Extending these promising findings to solid tumors faces substantial hurdles from drug uptake to clinical trial design. We do not know yet how to select patients for this therapy and how to move it from life extension to cure. The epigenetic potential of DNA methylation inhibitors may be limited by other epigenetic mechanisms that are also worth exploring as therapeutic targets. But the idea of stably changing gene expression in vivo has transformative potential in cancer therapy and beyond.
and no purely genetic or epigenetic neoplasms have been identified. Most remarkably, interfering with maintenance of DNA methylation reactivates silenced gene expression and reverses much of this pathway deregulation, leading to therapeutically desirable effects such as differentiation, apoptosis, enhanced recognition by the immune system, etc. (11, 12). DNA methylation inhibitors have made it to the clinic, and are now part of the standard of care in certain forms of leukemias.

**Inhibiting DNA Methylation**

We all start life thanks to inhibition of DNA methylation. As soon as embryogenesis begins, a massive decrease in DNA methylation reprograms the epigenome and creates a nearly blank slate on which development and differentiation can be written (13). Thus, a decrease in DNA methylation is compatible with life, at least in embryogenesis. Nuclear transplantation-induced reprogramming can also erase (if incompletely) DNA methylation in adult cells (14) and, when applied to cancer, seems to reverse the malignant phenotype, even in the face of genetic alterations (15). Outside of epigenetic reprogramming, inhibition of DNA methylation can only be achieved by genetic or pharmacologic targeting of DNA methyltransferase enzymes. Given that DNA methylation is a post-DNA synthesis event that needs to be sustained by the presence of methylating enzymes, cellular replication in the face of reduced levels of these enzymes results in significant demethylation in daughter cells, accompanied by gene reactivation (12). When applied to cancer cells, this approach does have a therapeutic ratio; normal cells tend to survive hypomethylation whereas cancer cells tend to be killed (or at least stop proliferating) when this happens, perhaps because cancer cells are dependent on critical gene silencing for survival (whereas normal cells are not).

Drugs that inhibit DNA methylation were discovered by pure serendipity (12). Cytosine analogs developed as cytotoxic anticancer agents in the 1960s and tested in the clinic in the 1970s were found to induce peculiar differentiation phenotypes in vitro (16). This DNA hypomethylating property is limited to cytosine analogs with 5′ modifications of the ring (Fig. 1). Other cytosine or nucleoside analogs do not affect DNA methylation directly. Eventually, this property of the two main analogs, 5-azaacytidine (AZA) and 5-aza-2′-deoxycytidine (DAC), was traced to their ability to incorporate into DNA, trap DNA methyltransferases (DNMTs), and target these enzymes for degradation (refs. 11, 17; Fig. 2). DNA synthesis in the absence of these enzymes then results in hypomethylation in the daughter cells and eventually to reactivation of silenced gene expression (Fig. 3). Several other 5′ modified nucleoside analogs have been described (Fig. 1) either in preclinical studies or in early stage clinical trials (18).

Various nonnucleoside analog drugs were also reported to inhibit DNA methylation, including procainamide, hydralazine, epigallocatechin gallate (EGCG), and others. However,
their ability to induce DNA hypomethylation in vitro has been questioned (19, 20), and they are “weak” hypomethylators at best. Some confusion may have arisen through equating inhibition of DNA methyltransferase activity and actual hypomethylation induction. DNA methyltransferase activity is proliferation dependent and cell cycle regulated (21, 22). Thus, any insult that leads to proliferation arrest also leads to measurable decreases in DNA methyltransferase activity, but in this setting inhibited activity has not been conclusively shown to affect DNA methylation per se. Any clinical effect of these weak “demethylating” drugs may require prolonged exposure to high doses of the compounds and the uncertainties over their potency have limited enthusiasm for clinical trials in neoplasia.

Other than nucleoside analogs and “weak” hypomethylators, there has been interest in the development of small molecules targeting DNA methyltransferases (20). These small molecules have theoretical advantages in that the DNA incorporation requirement of nucleoside analogs makes these drugs exquisitely S-phase specific, a considerable barrier in some malignancies (see further). However, the three known DNA methyltransferases have redundant functions to a certain extent, and it is clear that inhibiting more than one is required to optimally activate tumor-suppressor genes (23). This may explain why, despite considerable effort, no small molecule inhibitor of DNA methyltransferases has made it to the clinic yet. MG98, an antisense compound directed at DNMT1 is effective in vitro, but human clinical trials have shown disappointing results so far (24).

It is important to recognize that while inhibiting DNA methylation is a molecularly precise targeted therapy approach, the downstream effects on neoplastic behavior are quite nonspecific (Figs. 2 and 3). The trapping of DNMTs onto DNA creates bulky adducts that can inhibit DNA synthesis and eventually result in cell death by cytotoxicity (25). In fact, in vitro experiments showed that at high doses of the compounds, inhibition of DNA synthesis masks effects on DNA methylation, resulting in a U shaped dose-response for hypomethylation induction (26). Even at optimal (low) doses for hypomethylation induction, if one considers reactivated genes, a case could be made (and data be found) for effects of the inhibitors on multiple pathways (2, 7) including senescence (via P16 activation, for example), apoptosis (via activation of proapoptotic genes), differentiation (e.g., responsiveness to retinoic acid), stem cell renewal (by abrogating self-renewal signals), invasion (by upregulating inhibitors of motility), angiogenesis (through angiogenesis inhibitors such as THBS1), immune recognition (by activation of cancer testis antigens), etc. With the recent discovery of many microRNAs (miRNA) silenced by DNA methylation in cancer (27), one needs to add to the list effects of the drugs on downregulation of oncogenes such as BCL6, CDK6, and other growth promoters. Finally, given the promiscuity of hypermethylation in cancer, one also needs to keep in mind the possibility that hypomethylation can activate those rare oncogenes known to be silenced in cancer (e.g., COX2, EGFR, etc.; ref. 2). This nonspecificity makes predictions of clinical outcomes in vivo quite difficult, and...
indeed likely tumor and patient specific. In other words, in some patients responses may be predominantly apoptosis mediated and rapid, while in others a differentiation effect is seen. However, it is likely that a mixture of effects reflecting the sum total of the pathways activated is in fact the reality in most cases.

Fig. 3. Pleiotropic therapeutic effects of DNA methylation inhibition and gene reactivation in cancer. DNA methylation is maintained postreplication by the action of DNA methyltransferases. DAC and AZA lead to degradation of the main DNA methyltransferases, and continued replication results in passive demethylation that eventually results in reactivated gene expression. Activated gene expression, in turn, has effects on multiple different pathways, each of which could contribute to a clinical response.
Table 1. Selected recent clinical results with DNA methylation inhibitors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Indication</th>
<th>Results</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azacitidine</td>
<td>75 mg/m² SQ, daily × 7, q4 wk</td>
<td>MDS (symptomatic)</td>
<td>CR 7%, PR 16%, HI 37%</td>
<td>Improved progression-free survival compared with supportive care</td>
<td>(30)</td>
</tr>
<tr>
<td>Azacitidine</td>
<td>75 mg/m² SQ, daily × 7, q4 wk</td>
<td>MDS (advanced)</td>
<td>CR 17%, PR 12%, HI 20%</td>
<td>Improved survival compared with conventional therapy</td>
<td>(33)</td>
</tr>
<tr>
<td>Azacitidine + phenylbutyrate</td>
<td>AZA 25-75 mg/m² SQ, daily for 5-14 d, q4 wk + phenylbutyrate 375 mg/kg/d IV, CI × 7 d beginning on the final day of AZA</td>
<td>MDS and AML</td>
<td>CR 14%, PR 3%, HI 21%</td>
<td>Tumor suppressor gene hypomethylation more pronounced in responders</td>
<td>(44)</td>
</tr>
<tr>
<td>Azacitidine + valproic acid + ATRA</td>
<td>AZA 75 mg/m² SQ, daily × 7, q4 wk + valproic acid 50-75 mg/kg PO days 1-7 + ATRA 45 mg/m² PO days 3-7</td>
<td>Poor prognosis or relapsed or refractory leukemias</td>
<td>CR 22%, PR 5%, Other responses 13%</td>
<td>Higher response rate in previously untreated patients. Correlation between Valproic acid levels and response</td>
<td>(41)</td>
</tr>
<tr>
<td>Azacitidine + VPA</td>
<td>AZA 20-95 mg/m² SQ, daily × 10, q4 wk + VPA titrated to plasma levels of 75-100 μg/mL</td>
<td>Relapsed refractory solid tumors</td>
<td>SD 25%</td>
<td></td>
<td>(55)</td>
</tr>
<tr>
<td>Decitabine</td>
<td>15 mg/m² IV over 3 h q8 h for 9 doses, q6 wk</td>
<td>MDS (advanced)</td>
<td>CR 24%, PR 10%, HI 14%</td>
<td>Improved progression-free survival compared with supportive care</td>
<td>(56)</td>
</tr>
<tr>
<td>Decitabine</td>
<td>15 mg/m² IV over 3 h q8 h for 9 doses, q6 wk</td>
<td>MDS (advanced)</td>
<td>CR 9%, PR 8%, HI 13%</td>
<td></td>
<td>(57)</td>
</tr>
<tr>
<td>Decitabine</td>
<td>5-20 mg/m² IV over 1 h daily × 10</td>
<td>Poor prognosis or relapsed or refractory leukemias</td>
<td>CR 18%, PR 6%, HI 8%</td>
<td>Greater response rate at lower doses. Flat hypomethylation dose-response past 150 mg/m² total dose</td>
<td>(32, 42)</td>
</tr>
<tr>
<td>Decitabine</td>
<td>3 dose schedules; most patients treated at 20 mg/m² IV over 1 h daily × 5, q4 wk</td>
<td>MDS (advanced)</td>
<td>CR 34%, PR 1%, CRi + HI 37%</td>
<td>Improved survival compared with chemotherapy in a case-control study. Association between P15 reactivation and response. Addition of valproic acid did not impact responses.</td>
<td>(36, 37)</td>
</tr>
<tr>
<td>Decitabine</td>
<td>15-20 mg/m² IV over 1 h daily × 10 (some patients also received valproic acid)</td>
<td>AML</td>
<td>CR 16%, CRi 16%, PR 12%</td>
<td>Correlation between ER reactivation and response. Addition of valproic acid did not impact responses.</td>
<td>(45)</td>
</tr>
<tr>
<td>Decitabine</td>
<td>2.5-20 mg/m² IV over 1 h daily × 5-10 d</td>
<td>Relapsed or refractory solid tumors</td>
<td>PR 4%, minor response 11%, SD 46% (at 4 weeks)</td>
<td>No correlation between tumor and peripheral blood mononuclear cell hypomethylation after therapy</td>
<td>Stewart, et al., in press¹</td>
</tr>
<tr>
<td>Decitabine + valproic acid</td>
<td>DAC 15 mg/m² IV over 1 h daily × 10 + valproic acid 25-50 mg/kg PO days 1-10</td>
<td>Poor prognosis or relapsed or refractory leukemias</td>
<td>CR 19%, CRi 4%</td>
<td>Higher response rate in previously untreated patients. Correlation between valproic acid levels and response.</td>
<td>(59)</td>
</tr>
<tr>
<td>Decitabine + interleukin 2</td>
<td>DAC 0.1-0.3 mg/kg SQ d days/week for 2 wk + IL2 600,000 IU/kg IV q8 h × 14 doses on weeks 3 and 6</td>
<td>Metastatic melanoma and renal tumors</td>
<td>CR 6%, PR 12%, minor response 12%, SD 18%</td>
<td></td>
<td>(60)</td>
</tr>
<tr>
<td>Decitabine + carboplatin</td>
<td>DAC 45-135 mg/m² IV × 1, q4 wk + carboplatin AUC 5-6 IV d8</td>
<td>Relapsed or refractory solid tumors</td>
<td>PR 3%, SD 9%</td>
<td>Hypomethylation of MAGE1 documented in tumors</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete remission; PR, partial response; HI, hematologic improvement; MDS, myelodysplasia; AML, acute myeloid leukemia; CRi, complete remission incomplete; ATRA, all-trans retinoid acid; SD, stable disease; VPA, valproic acid; carbo, carboplatin; AUC, area under the curve; CI, continuous infusion.
Clinical Results

AZA and DAC have had two lives as antineoplastic agents. Early clinical trials in the 1970s and 1980s were based on the presumed cytotoxic effects of the drugs. Tested mostly at very high doses for relatively short exposure times, the drugs were found to be sporadically effective, more so in hematologic malignancies than in solid tumors, but also quite toxic (28). The ratio of efficacy to toxicity was deemed unfavorable, and their usage was largely abandoned after the U.S. Food and Drug Administration (FDA) rejected an application for registration of AZA. This was not particularly surprising in hindsight. The drugs only work as epigenetic modifiers when given at low doses, given that high doses will inhibit DNA synthesis, which will short-circuit their DNA hypomethylating effect. In vitro, differentiation after AZA is only observed at low doses (17), and hypomethylation after DAC (in vitro) is U shaped, with no effects on DNA methylation at all at high doses (which are achievable in vivo; ref. 29). Thus, the traditional phase I/II sequence of drug testing failed in this case because of the unusual dose-response properties of the drugs. Sadly, this failure may not be limited to this class of agents, a sobering fact considering that even today, most drugs including targeted agents still follow the classical phase I/II paradigm of clinical testing at maximally tolerated dose.

The hypomethylating nucleoside analogs came back to life over the past decade through the persistence of a few investigators (30, 31) and the renewed interest in DNA methylation as a therapeutic target brought about by basic investigations (7). Through a convergence of serendipity (the desire to treat older patients who cannot tolerate high doses) and mechanism of action-based dose finding trials (32), both AZA and DAC were tested in relatively large studies at low to moderate doses and over multiple cycles of administration, thus optimizing their epigenetic modulation potential. Following promising phase II studies, AZA was tested in two separate phase III studies (30, 33) in the myelodysplastic syndrome (MDS). Response rates ranging from 30% to 60% were observed, with documented improved survival compared with either supportive care or cytotoxic chemotherapy. Those trials used an open-ended treatment approach where patients were encouraged to continue receiving the drug until progression or death. DAC also had promising early studies in MDS, and phase III studies confirmed responses and modulation of disease (for example delay in acute myeloid leukemia [AML] progression) but failed to show substantial effects on survival (34, 35). As opposed to the AZA studies, the DAC phase III studies limited the number of cycles patients received. When using an open ended approach similar to that of AZA trials (36, 37), DAC resulted in a very high response rate (40% complete response, over 70% total response), survival comparable to that seen in the AZA trials and superior to that observed with cytotoxic chemotherapy. It remains to be seen whether AZA (which incorporates into both RNA and DNA) is truly clinically different from DAC (which incorporates only in DNA) as the two drugs have not been directly compared. The results of recent clinical trials in leukemia and solid tumors are summarized in Table 1.

Translational Results

Much effort has been devoted to molecular evaluation, prediction, and understanding of clinical responses to hypomethylating agents. In various leukemias where this therapy has been tested [MDS, AML, chronic myelogenous leukemia (CML)], pretreatment molecular characteristics (clinical factors, chromosomal changes, mutations, gene methylation) have not been able to accurately predict the chances of response (40). This inability could be due to the limited set of markers/genes examined so far. It is also possible that critical factors to response do not lie in the intrinsic molecular nature of the neoplasm but in the patient and tumor-specific pharmacologic disposition of the drugs. In any case, this remains an open area of investigation.

An interesting and complex issue is whether drug-induced epigenetic modulation of the neoplastic cells in vivo happens,
and whether it is important to clinical responses. The complexity lies in the dynamic nature of the changes, in the clonal shifts that occur while on therapy, and in the variability of current technology to measure changes in gene methylation and expression. An ideal study would sample sorted/purified neoplastic cells everyday (or nearly so) throughout a whole treatment course to capture the entire ranges of observed effects. For the most part, this is neither practical nor really feasible as clonal shifts within a neoplasm can be impossible to detect. Indeed, if therapy is really effective acutely, the most hypomethylated cells may have died and would therefore not be available for sampling and analysis. With these acknowledged limitations, what can be gleaned from various studies is that (a) global DNA methylation measured directly (5-methylcytosine content) or indirectly (repetitive element methylation) decreases shortly after treatment is started, nadirs around 10 days, and recovers back to baseline 28 to 35 days after treatment starts (38, 40, 41); (b) tumor-suppressor gene methylation decreases in some patients but not in all after therapy (42–44); recovery to baseline is also variable; and (c) gene expression of silenced genes (e.g., P15, ER, etc.) is induced shortly after treatment in some patients (37, 45) with, again, variable drift back to baseline.

In terms of correlations between methylation/epigenetic modulation and response, several patterns emerge. Global methylation changes are not consistently associated with responses (40). In CML, there is even an inverse relation (46), which may be related to the clonal shifts described earlier. Tumor-suppressor gene methylation change at early time points was associated with response in some studies but not all (40, 44, 45), likely because of the complexity of this analysis described earlier. Gene expression induction generally correlates with response better than gene demethylation (37, 45). Sustained changes in methylation/gene expression do correlate with response (43), but they are confounded by clonal elimination. Of interest, simultaneous tracking of DNA methylation and gene mutation confirmed that epigenetic modulation precedes clonal elimination in some cases (38, 43).

The lack of consistent association between epigenetic patterns at baseline, DNA hypomethylation induction, and response to therapy has raised the issue of the precise in vivo mechanism of action of the drugs. As mentioned earlier, AZA and DAC do induce dose-dependent cytotoxicity and it is possible that some (or all) of the responses seen are related to this effect. In our opinion, this is unlikely because observed clinical responses are not consistent with a cytotoxic effect in that (a) increasing doses of the active agents has not resulted in increased responses as one would expect with a pure cytotoxic drug (32); (b) the kinetics of responses (early hypomethylation, delayed clonal elimination) are also inconsistent with direct cytotoxicity (38); (c) responses have clearly been seen in patients highly resistant to the classical cytotoxic drug cytarabine (32), which shares pharmacologic disposition with DAC; and (d) survival with DAC or AZA is clearly superior to survival achieved with combination cytotoxic therapy (33, 36). Of course, it is likely that cytotoxicity plays a role in the responses seen in some patients, but the uniqueness of the clinical outcomes after DAC or AZA strongly suggest a contribution by epigenetic effects of the drugs.

Barriers to Effective Epigenetic Therapy

With molecular and clinical proof of principle at hand, it is clear that hypomethylation therapy is here to stay. In order for it to achieve the broad impact that the extent of DNA methylation abnormalities in cancer suggest, several barriers need to be overcome: drug delivery, appropriate clinical testing, understanding resistance, and understanding sensitivity.

Drug delivery is perhaps the single most daunting barrier to effective translation of the leukemia findings to solid tumors. There is no question (from mouse models to anecdotal responses in clinical trials) that hypomethylating drugs can induce responses in patients with solid tumors (Table 1). However, an important issue is delivering sustained levels of drug and overcoming the S-phase dependency of the therapy. Thus, in tumors with a low replicating pool of cells, drug incorporation will likely be less than that in normal hematopoietic cells, exposing patients to toxicity with little chances of a response. Indeed, in a phase I study, we observed little correlation between hypomethylation induction in blood and in (solid) tumor biopsies. Current efforts to improve on this situation include alternate delivery methods for the nucleoside analogs, oral preparations (47), a DAC-containing dinucleotide with improved stability (48), and, potentially, small molecules that can inhibit DNA methylases without requiring DNA incorporation (20).

Even if drugs were identified with optimal penetration into solid tumors (or perhaps cancers identified with a high enough S phase for current drugs to work), the issue of optimal clinical testing needs careful thought. Based on the MDS experience, patience and front-line testing are required to optimize the chances of observing clinical efficacy. Unfortunately, many solid tumor phase I/II study designs rely on heavily pretreated patients and single course of administration for evaluation. This strategy has failed before for hypomethylating agents and may fail again if the same designs are utilised. There is no reason why multidrug resistant cancers would not also be resistant to hypomethylating drugs. It is therefore important to identify populations of patients where such therapy can be tested relatively early, and over at least two to four courses. Possibilities include an adjuvant setting, or testing this approach prior to standard therapy in incurable cancers where conventional therapy offers relatively little prolongation of life.

An issue that has not yet been given enough attention is the mechanism of primary and secondary resistance to existing DNA methylation inhibitors. It is clear that not all patients respond equally to AZA or DAC. We do not understand why some patients are resistant to these drugs. In vitro, resistance seems to be primarily pharmacologic (49) and this needs to be tested in vivo as well. Interestingly, the correlation between gene expression induction and response also identifies another potential source of resistance (40). It is at least theoretically possible that hypomethylation does not lead to gene reactivation because of some other downstream defect intrinsic to the cancers. A detailed understanding of in vitro and in vivo links between

Male pregnancy.


Clinical Cancer Research

Targeting DNA Methylation
Jean-Pierre J. Issa and Hagop M. Kantarjian


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/15/12/3938

Cited articles
This article cites 56 articles, 30 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/15/12/3938.full.html#ref-list-1

Citing articles
This article has been cited by 24 HighWire-hosted articles. Access the articles at:
/content/15/12/3938.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.