Nucleoside analogs count among the most active components of anticancer therapies. Through DNA incorporation and inhibition of ribonucleotide reductase (RnR), an enzyme involved in the recycling of the cellular nucleotide pool, their effects result in termination of DNA chain elongation, inhibition of DNA synthesis, interference with DNA repair mechanisms, and finally apoptosis. Several nucleoside analogs have become an integral part of treatment for a diverse range of malignancies. Cytarabine (1-β-D-arabinosylcytosine, ara-C) is used predominantly for therapy of patients with acute myeloid leukemias (AML) whereas the main spectrum of activity of gemcitabine (2',2'-difluorodeoxycytidine), is in solid tumor malignancies. Fludarabine (9-β-D-arabinofuranosyl-2-fluoro-adenine 5'-monophosphate) and cladribine (2-chlorodeoxyadenosine, 2-CdA) have been among the first clinically useful purine nucleoside analogs and both are highly active in indolent lymphoproliferative disorders. An intriguing aspect of nucleoside analogs thus remains how apparently minor alterations in structure can result in major differences with respect to pharmacology, metabolism, and spectrum of activity.

Clofarabine (2-chloro-2'-fluoro-2'-deoxy-9-β-D-arabinofuranosyladenine) is a prime example to illustrate this point. It is a next generation deoxyadenosine analog, which was synthesized as a rational extension of the experience with fludarabine and cladribine, with the objective to combine favorable pharmacokinetic properties of the congener compounds and at the same time overcoming some of their pharmacologic limitations. Partly in an effort to create compounds with higher resistance to the deactivation mechanisms of purine nucleoside analogs, a series of analogs were synthesized that incorporated a halogen group at the 2'-position of the adenine. These modifications in the structure led to the synthesis of a series of 2-halo-2' halo-2'-deoxyarabinofuranosyladenine analogs, where further substitution of fluorine at the arabinosyl configuration at the critical 2'-position of the carbohydrate (and retaining the 2-chloro adenine aglycone of cladribine) was found to be more cytotoxic in cell cultures than substitution with other halogen groups. Of several candidate molecules, clofarabine proved to be most cytotoxic in preclinical models, and was chosen to be developed further.

Similar to other nucleoside analogs, clofarabine requires intracellular phosphorylation by deoxycytidine (dCyd) kinase to its active triphosphate compound. It also shares similar mechanisms of action: 1) inhibition of DNA polymerases; 2) inhibition of ribonucleotide reductase (RnR); and 3) induction of apoptosis via induction of DNA strand breaks and disruption of mitochondrial membranes. Unlike the other nucleoside analogs, clofarabine is a more efficient substrate for dCyd kinase, exceeding the affinity of dCyd kinase for other nucleoside analogs such as cladribine. Retention of the triphosphate form of clofarabine in cells is longer than that of fludarabine or cladribine. Further distinguishing features from fludarabine or cladribine include: 1) the triphosphate of fludarabine primarily inhibits DNA polymerases after incorporation into DNA; 2) cladribine acts mainly by inhibition of RnR; 3) although antipapoptotic effects of all three purine nucleoside analogs occur by induction of DNA strand breaks, only clofarabine and cladribine have direct impact on mitochondrial integrity and activity.

Clofarabine has shown anticancer activity in a variety of human tumor cell lines derived from hematologic and solid tumor malignancies. In 1992, the group at MD Anderson Cancer Center recognized the potential of clofarabine, and conducted the animal toxicology studies necessary to define the starting dose in human trials (H. Kantarjian - unpublished data) and to further proceed with clinical studies.

The objective of the phase I studies was
to determine dose-limiting toxicities (DLT) and the maximum tolerated doses (MTD) of clofarabine for a broad range of malignancies including acute leukemias, chronic lymphoproliferative disorders (LPD), and solid tumors in both adults and children.\textsuperscript{18,19,20} Clofarabine was given as a 1-hour infusion daily for 5 days at a starting dose of 15 mg/m\textsuperscript{2}/day in adults and 11.25 mg/m\textsuperscript{2}/day in children. Myelosuppression turned out to be the DLT for solid tumor and LPD patients and a dose of 2 mg/m\textsuperscript{2} daily x 5 was suggested for phase II studies in those patients. Dose escalation in leukemias proceeded up to a maximum of 55 mg/m\textsuperscript{2} daily x 5, at which point hepatic toxicity precluded further escalations and the next lower dose schedule of 40 mg/m\textsuperscript{2} daily for 5 days has been recommended as the dose and schedule for phase II studies in adult acute leukemias. The pediatric phase I study was restricted to children with advanced acute leukemias.\textsuperscript{20} The treatment schedule was identical to the adult study. Reversible DLTs (hyperbilirubinemia and transaminitis) at 70 mg/m\textsuperscript{2} required expansion of the experience at the next lower dose level of 52 mg/m\textsuperscript{2}, which appeared safe and was thus recommended as the phase 2 dose for children. Based on the initial experience during the phase 1 studies, the emphasis of further clinical development of clofarabine has shifted to the acute leukemias. However, its role in other malignancies including LPD and chronic lymphocytic leukemia (CLL) remains heavily investigated.

A phase II study of clofarabine was subsequently conducted in 62 patients with relapsed and refractory acute leukemias (AML 31, myelodysplastic syndrome [MDS] 8, acute lymphoblastic leukemia [ALL] 12, myeloid blast phase CML 1).\textsuperscript{21} All patients received clofarabine at a dose of 40 mg/m\textsuperscript{2} intravenously daily for 5 days, every 3 to 6 weeks. The overall response rate was 48\% including 32\% CR (and 16\% with PR or hematologic improvement (CRp)). Of the 31 AML patients, 13 (42\%) achieved CR and 4 (13\%) CRp for an overall response of 55\%. Of the 8 patients with MDS, 2 achieved CR and 2 CRp, for an overall response rate of 50\%. Common adverse events were transient liver dysfunction, skin rashes, palmoplantar erythrodysesthesia, and mucositis. A multicenter phase II study of patients with refractory and relapsed AML reported only 1 CR among 15 patients.\textsuperscript{22} Differences in response and outcome between the phase II studies require further confirmation, but may be attributed to different eligibility criteria and patient selection.

In the pediatric phase II studies, clofarabine was given at 52 mg/m\textsuperscript{2}/dose daily for 5 days every 2 to 6 weeks.\textsuperscript{23} Thirty-five children with AML have been treated. Their median age was 11 years (range 2 to 22); all patients were heavily pretreated (median number of 3 prior regimens; range 1 to 6). Almost half of all children had undergone and failed prior stem cell transplantation. The overall response rate was 26\% (1 CRp and 8 PR). Among 49 children with ALL, the median age was 12 years (range 1 to 19). Similar to the AML study group, patients with ALL were heavily pretreated (median of 3 prior regimens; range 1 to 6) and 20\% had undergone prior stem cell transplantation. Six patients achieved CR, 4 had CRp, and 5 had PR for an overall response rate of 31\%. Median survival was 42 weeks (range 7 to 63.1+) for responding ALL patients and 39 weeks (range 7.7 to 93.6+) for responding AML patients. Overall, 13 of 24 responding patients (54\%) were able to receive a subsequent stem cell transplant. Toxicities were comparable in both disease groups and consisted mainly of nausea, vomiting, fever, myelosuppression, skin rashes, hand-foot syndrome, and transient elevations of liver transaminases. Based on these positive results, clofarabine received FDA approval in December of 2004 for the treatment of children with ALL in relapse who have at least received 2 prior regimens.

An important extension of the single agent trials has been combinations of clofarabine with other active anti-leukemia agents such as cytarabine. Cytarabine remains the most active antileukemic agents and is the backbone of many combination regimens in patients with AML. Biochemical modulation strategies that aim at increasing intracellular concentrations of cytarabine triphosphate (ara-CTP) have been demonstrated in vitro and have been tested and validated clinically in combinations of cytarabine with fludarabine in adult AML, and cladribine in children with AML.\textsuperscript{24,25} Clofarabine thus not only has antileukemic activity by itself, the activity of clofarabine and cytarabine will also be enhanced in leukemic cells by a biochemical synergy between these two agents.

The first study combining clofarabine plus cytarabine was a phase I/2 study in adults with acute leukemias and high-risk MDS in first relapse or who were primary refractory.\textsuperscript{26} Cytarabine was administered at 1 g/m\textsuperscript{2}/day intravenously daily on 5 days and clofarabine eventually at 40 mg/m\textsuperscript{2} daily on 5 days as well. Of the 29 patients with AML and MDS, 7 (24\%) achieved CR and 5 (17\%) had CRp for an overall response rate of 41\%. With a median follow up of 14 months (range 5.5–15), the median remission duration (CR+CRp) was 3.2 months (0.5–14), median survival was 5.5 months (0.2 to 15+) overall and 8 months (2–8+) in responders. The combination regimen was safe without additional or unexpected adverse events, and a low early death rate (i.e. within 4 weeks of therapy) of 3\%. Liver function abnormalities of any grade were observed in up to 90\% of the patients, but were usually transient and < grade 3 by NCI Common Toxicity Criteria. Plasma and cellular pharmacokinetic studies...
confirmed that: 1) plasma clofarabine levels increased with the clofarabine dose without evidence of nonlinearity; 2) the infusion of cytarabine did not significantly affect plasma clofarabine levels; and 3) 5 of 8 patients studied demonstrated an increase in ara-CTP levels following the clofarabine infusion.

Establishing the combination as effective with a manageable toxicity profile, clofarabine/cytarabine combinations have since moved to frontline AML therapy. The first study to do so was completed at MDACC and used the combination as induction regimen for older AML patients. Of 27 evaluable subcutaneously x 5 with prior malignancy; 28 patients (47%) had poor-prognosis karyotypes. Two-thirds of patients had an antecedent hematologic disorder (MDS) or another prior malignancy; 28 patients (47%) had poor-prognosis karyotypes. Two-thirds of patients were ≥60 years. Overall, 36 patients (60%) responded (52% CR, 8% CRp). The response rate was higher in patients with diploid cytogenetics (60% CR versus 43%). Most responses occurred following one induction course. Of 11 patients who received a second induction course, 2 patients achieved CR and 2 CRp. Four patients died during induction (induction mortality 7%). The most common adverse events included transient liver function abnormalities, rashes, and palmo-plantar dysesthesias. The combined experiences from the salvage and frontline combinations studies thus confirmed these combinations to be safe and active. To validate the position of clofarabine and cytarabine combinations in AML therapy, further follow up and comparison with standard AML induction regimens is eventually needed.

This led to further innovative approaches in adult AML. Burnett et al. developed single agent clofarabine in elderly AML, using clofarabine at the lower dose of 30 mg/m² intravenously daily for 5 days every 28 days in patients not suitable for intensive chemotherapy and any patient over age 70 years. Of 27 evaluable patients, 16 patients (59%) achieved CR making clofarabine probably one of the most active anti-AML agents in this group of patients. To further validate this approach, the group at MDACC initiated a randomized study of clofarabine 30 mg/m² daily x 5 with or without low-doses of cytarabine (20 mg/m² subcutaneously daily x 7 to 14). The rationale for using low-dose cytarabine was based on data from the leukemia group in the United Kingdom showing in a randomized trial a superior CR rate (15% versus 0%) and survival rates (30% at 1-year versus <5%) with low-dose cytarabine versus hydroxyurea (the proposed standard of care). The study is ongoing. Of 50 patients enrolled so far, response rates remain at >50% with the combination. An update of the randomized frontline study will be presented.

Clofarabine is the first of a new generation of nucleoside analogs with established single agent antileukemic activity at tolerable doses. Development of clofarabine has since been carried forward through adult and pediatric phase 2 studies, and in combination strategies based on biochemical modulation with cytarabine. In addition to further defining the optimal dose and combination of clofarabine in adult AML, further combination studies (e.g. with anthracyclines +/− cytarabine) are underway to even better exploit the activity of clofarabine in hematologic malignancies and particularly the acute leukemias.

References