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Clofarabine: From Design to Approval

John A. Secrist III, Jaideep V. Thottassery, and William B. Parker

25.1
Introduction

Many different classes of compounds have been found to have utility in treating a variety of different types of cancers. These compounds act through a variety of different mechanisms, in many cases targeting metabolic differences between normal and cancerous cells, and more recently targeting cancer-specific targets and pathways. An examination of the drugs that have been approved by governments worldwide demonstrates that antimetabolites – compounds that affect the pathways leading to nucleic acids – represent a rich source of anticancer drugs. Almost all of those approved drugs are either nucleosides or compounds that are converted to nucleosides or nucleotides after administration to patients. In the United States, the list of FDA-approved antimetabolites of this type includes 5-fluorouracil (colorectal, breast, stomach, and pancreatic carcinomas), 6-thioguanine (acute non-lymphocytic leukemias), 1-β-D-arabinofuranosylcytosine [acute lymphocytic leukemia (ALL), and acute myelocytic leukemia (AML)], 5-fluoro-2′-deoxyuridine (metastatic colon cancer), fludarabine phosphate [chronic lymphocytic leukemia (CLL)], 2-deoxycoformycin (hairy cell leukemia), cladribine (hairy cell leukemia), gemcitabine [pancreatic cancer, non-small cell lung cancer (NSCLC)], capecitabine (metastatic colorectal and breast cancer), nelarabine (T-cell acute lymphoblastic leukemia and lymphoma), decitabine (myelodysplastic syndrome), and clofarabine (pediatric ALL). Both fludarabine phosphate and clofarabine were discovered and pushed forward preclinically at the Southern Research Institute, and both are products of our preclinical optimization process for nucleoside analogues.

This chapter will focus on the development of clofarabine, and will present that development from our viewpoint as preclinical scientists. A recent review covering many aspects of the development of clofarabine is recommended to the reader for additional details [1].

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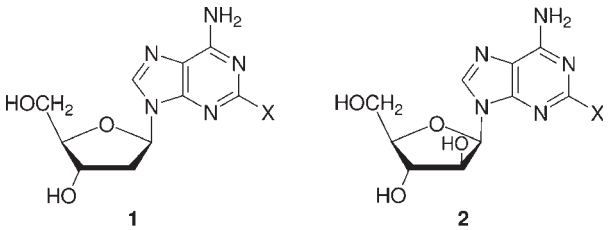
25.2
Clofarabine: The Background

Before examining the chronology of the discovery and development of clofarabine, it is important first to identify its current and potential future uses, as well as the companies involved. Clofarabine was approved for the treatment of pediatric ALL in the US in December 2004, and in Europe in May 2006. It has been granted orphan drug status in both the US and Europe. Early efforts toward pushing the drug into clinical trials, beginning in 1992, were initiated at the M. D. Anderson Cancer Center (MDACC) in Houston, Texas, and included clinicians Drs. J. Freireich, M. Keating and H. Kantarjian, as well as pharmacologists Drs. W. Plunkett and V. Gandhi. The initial licensing of the drug by Southern Research Institute was to the Eurobiotech Group in 1998, which utilized MDACC for the clinical trials. The lag time evident in the above dates is truly unfortunate from the standpoint of cancer patients who might have benefited from the drug. The difficulties in licensing clofarabine stemmed from two views prevalent in the pharmaceutical industry at the time: (i) clofarabine was just another fludarabine, and had little chance of making a mark on its own; and (ii) future cancer drugs needed to focus on solid tumors such as colorectal, breast, prostate and lung cancer, and nucleosides were of little interest in that regard. Moreover, even if they had some activity, the market size was too small to be of interest to the larger companies. The CEO of Eurobiotech (most recently called Bioenvision), Dr. Christopher Wood, understood the properties of fludarabine phosphate, and believed that clofarabine had properties that might make it significantly better, and on that basis he was eager to proceed. Similarly, the team at MDACC also believed that clofarabine had the potential to take its own place among anticancer treatments. The eventual approval of clofarabine was aided immeasurably by the commitment of these people.

25.3
The Beginnings

During the early 1980s, two types of nucleoside were found to have very promising selectivity in animal models. These compounds were the 2-halo-2′-deoxyadenosines (**1**), with the halogen being fluorine, chlorine or bromine, and the corresponding β-D-arabinofuranosyl analogues (**2**). The 2′-deoxy compounds had been prepared in several different laboratories [2–4] and examined in various cell lines as potential anticancer drugs. In our laboratories, we examined all three of the 2′-deoxy compounds in a series of experiments in the then-standard L1210 mouse leukemia model system. Interestingly, all three compounds had excellent selectivity, and a summary of that previously unpublished data is shown in Table 25.1 [5]. Although it is easy to see that all three had some selectivity, the chloro and bromo analogues appeared to be the most promising, with some cures seen, and these results warranted further investigation.



1
X = F, Cl (Cladribine), Br

2
X = F (Fludarabine), Cl, Br

Over the next few years, the 2-chloro compound was examined in further detail through a collaboration between John Montgomery at Southern Research Institute and Dennis Carson at the University of California at San Diego, while the 2-bromo compound was further examined by Raymond Blakley and his colleagues at St. Jude Children's Hospital. As events unfolded, the 2-chloro compound (cladribine) was eventually approved in 1992 by the FDA for the treatment of hairy cell leukemia.

In the 2-halo-ara-A series, the fluoro, chloro, and bromo compounds were prepared at Southern Research Institute and elsewhere [2, 6–10], and all three had some activity [11, 12]. The data demonstrated that the fluoro compound was significantly better than the other two, and consequently it was carried forward, eventually being approved in 1991 as the 5′-phosphate (fludarabine phosphate), a prodrug form developed to aid solubility, for the treatment of CLL.

During the first half of the 1980s, some preliminary data were acquired on these compounds which, when combined with previous information regarding the physical properties of the two series, suggested that some improvements in the structures could be made that might have a significant effect on their potential clinical utility. At the time, it was of course not known whether any of these compounds would become approved, and the quest was to prepare compounds that would have enhanced properties that might either achieve approval if the earlier compounds did not, or might be next-generation compounds with more attractive properties than any earlier compounds that did achieve approval.

Table 25.1 Response of intraperitoneally (i.p.) implanted L1210 leukemia to 2-halo-2′-deoxyadenosines.

Compound	Optimal i.p. dose ($\leq LD_{10}$, mg kg^{-1} $dose^{-1}$)[a]	Total dose (mg kg^{-1})	Median % ILS[b] (dying mice only)	Net log_{10} cell kill[c]	Tumor-free survivors/ total
F-dAdo	25	600	+118	+0.5	0/10
Cl-dAdo	20	480	+150	+2.9	5/10
Br-dAdo	40	960	+125	+1.1	3/6

[a] Treatment schedule was q3 h × 8, Days 1, 5, and 9.
[b] Median day of death of tumored control mice (10^5 cells) was 8 days. ILS, increase in lifespan.
[c] Net log_{10} reduction in the tumor cell population between the beginning and the end of therapy, based on the median day of death of the mice that died.

Thus, attention was focused on three properties while seeking to improve on the two series presented above. It is easiest to consider the characteristics that might be improved by focusing on the two compounds that were eventually approved, fludarabine and cladribine. In the case of fludarabine phosphate, when administered to an animal it is rapidly cleaved to fludarabine, which enters the cells and is further metabolized [13].

In the case of cladribine, it is well known that 2′-deoxy compounds in the purine series are susceptible to chemical cleavage at low pH, and thus a loss of potency through hydrolytic cleavage is clearly an issue with cladribine. In addition, cleavage of the glycosidic bond by phosphorylases is another means of loss of potency. With both types of cleavage, 2-chloroadenine would be generated, which is a compound with only modest toxicity.

For fludarabine, the chemical hydrolysis of the glycosidic bond is not a significant problem as the presence of the 2′-hydroxyl group provides significant stability, although there is a loss of potency through some phosphorylase cleavage [13–16]. In the case of fludarabine, this process generates 2-fluoroadenine, which is a highly undesirable metabolite. This purine is readily metabolized up to 2-fluoro-ATP, which is an extremely toxic but unselective compound, and so its systemic generation could present a concern. In recent years, attention has been focused on utilizing gene therapy approaches to generate this toxin in tumor cells in a selective manner [17].

The other mechanism of loss of potency that can occur with adenine derivatives is enzymic deamination, which is carried out by adenosine deaminase at the nucleoside level, and by AMP deaminase at the monophosphate level. The incorporation of a 2-halogen into the adenine ring of a nucleoside confers significant resistance to deamination as compared to the parent adenine compounds [11, 12]. Many evaluations have been carried out examining the ability of the 2-haloadenine nucleosides to serve as substrates for adenosine deaminase, and although they are highly resistant to deamination, all have at least some substrate activity. The order of deamination is F > Cl > Br, and within that order the 2-fluoro compounds are significantly more susceptible to deamination than the other two. Thus, in the case of fludarabine there is a minor loss of potency through some deamination [13–16], a pathway that is not a significant problem with cladribine.

The other key issue with regard to nucleoside analogues is their activation to an active metabolite which, in the vast majority of cases, is the nucleoside triphosphate (NTP). In general, nucleosides exert their effects on the biosynthetic pathway leading to DNA, and thus, analogues of 2′-deoxynucleosides are typically of more interest than the building blocks of RNA, although ribonucleoside analogues were also prepared. It was determined that the addition of a 2-halogen did not prevent the phosphorylation of some 2′-deoxynucleoside analogues, and many laboratories have determined that the major enzyme carrying out the initial phosphorylation is generally deoxycytidine kinase [18–20]. Another key observation was that nucleosides with an *arabino* configuration often were also substrates of deoxycytidine kinase. The other enzymes carrying out conversion of monophosphates to their di- and triphosphate metabolites were in general less discriminating, and the majority of nucleosides that could be metabolized to the 5′-monophosphate were converted at a meaningful rate to the triphosphate.

The above-described information relates to the situation during the early to mid-1980s as ways were sought to improve on the activity of this class of potential anticancer nucleosides. The set of simple conclusions drawn from the above information can be summarized as follows:

- A 2-halogen in an adenine ring analogue dramatically reduces deamination, but in general will allow phosphorylation, depending upon the carbohydrate attached.
- A 2-chloro or 2-bromoadenine ring is more desirable than a 2-fluoro, based upon the high toxicity of any 2-fluoroadenine that may be generated, and also based upon its increased ability of 2-fluoroadenine-containing nucleosides to serve as substrates for adenosine deaminase.
- A stabilizing group at C-2′ in the *arabino* configuration – one that will significantly reduce both phosphorylase cleavage and hydrolytic cleavage of the glycosidic bond – is highly desirable.

25.4 The Next Generation of Compounds

Over the years, a highly efficient system was developed for the rapid examination of new compounds in our anticancer drug discovery program, which was strongly supported by the US National Institutes of Health. Whenever a new compound had been prepared and properly characterized, it was submitted for an evaluation of its cytotoxicity in a small series of cancer cell lines, with generally six or seven such lines being derived from various types of human tumors. Typically, the results were available in a few weeks. In all of these cases, the corresponding human tumor xenograft mouse model was available if a compound exhibited significant cytotoxicity. The main challenge was to prepare sufficient material for evaluation in a mouse model, once it had been learned that such an examination was warranted based upon the cytotoxicity profile. When sufficient material became available for such an initial evaluation, the compound was submitted and generally placed into a test system within a month. In parallel, the mechanistic evaluation of compounds of potential interest was started in our biochemistry laboratories. Together, this research effort provided us with the basic information on the activation of new nucleosides to the various phosphorylated metabolites, their effects on DNA, RNA and protein synthesis, and also specific information on key enzymes. Feedback from both the *in-vitro* and *in-vivo* evaluations was thus rapidly available, and we were able quickly to adjust our target structure list based upon this iterative feedback. This simple system prevented us from spending too much time on the synthesis of series of compounds that did not show promise as anticancer drugs.

By utilizing this efficient system, a variety of compounds was evaluated relatively rapidly. The major efforts revolved around carbohydrate modifications with the 2-haloadenines as the bases, and on similar compounds with the nitrogen base somewhat altered.

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