

Physico-chemical stability of nelarabine infusion solutions in EVA infusion bags

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Jeanette Kaiser, PharmD; Professor Irene Krämer, PhD

ABSTRACT

Study objectives: To determine the physico-chemical stability of ready-to-use nelarabine infusion solutions in ethylene vinyl acetate (EVA) infusion bags stored under refrigeration (2°C–8°C) or at room temperature, and either protected from light or exposed to light.

Methods: Stability of nelarabine infusion solutions was determined by a stability-indicating reversed-phase high-performance liquid chromatography assay with ultraviolet detection. Undiluted nelarabine solution for infusion (Atriance, 5 mg/mL) was transferred to EVA infusion bags and the physico-chemical stability of the infusion solutions assessed over a four-week storage period. Test solutions were stored under refrigeration (2°C–8°C) or at room temperature, and either protected from or exposed to light. Nelarabine concentrations were determined after preparation on days 0 (initial), 1, 3, 7, 14, 21, and 28. In addition, pH values were measured once a week during the storage period and test solutions were visually checked for colour change and precipitation.

Results: The stability tests revealed that nelarabine infusion solutions are physico-chemically stable for a minimum of four weeks. Nelarabine concentrations remained at a level of > 95% of the initial concentration independent of the storage conditions. Exposure to daylight did not influence the stability of the nelarabine infusion solutions. No colour change or precipitation occurred. The pH of the infusion solutions varied between 5.7 and 6.5.

Conclusion: Ready-to-use nelarabine infusion solutions in EVA infusion bags are physico-chemically stable over a four-week period even when stored at room temperature without protection from light.

KEYWORDS

Degradation, high-performance liquid chromatography (HPLC), infusion solution, nelarabine, physico-chemical stability

INTRODUCTION

Nelarabine (code 506U78 and GI26250), a new purine nucleoside analogue, was approved in 2005 by the FDA (brand name Arranon) and in 2007 by the EMA (brand name Atriance) for the treatment of T-cell acute lymphoblastic leukaemia and T-cell lymphoblastic lymphoma. Nelarabine is indicated when patients have failed to respond or have stopped responding to at least two types of chemotherapy. The T-cell phenotype occurs in only about 15–20% of children and 25% of adults with acute lymphoblastic leukaemia and in approximately 10–20% of patients with non-Hodgkin's lymphoma. Because of the rarity of the condition, nelarabine was

authorised under exceptional circumstances and has been granted orphan drug status [1].

Nelarabine is a water-soluble prodrug of a cytotoxic deoxyguanosine analogue named 9-β-D-arabinofuranosylguanine (ara-G). *In vivo*, nelarabine is rapidly converted to ara-G and subsequently into the active 5'-triphosphate (ara-GTP) [1-5].

The recommended dose of nelarabine in adults and adolescents (16 years and older) is 1,500 mg/m² to be administered intravenously over two hours on days 1, 3 and 5, repeated every 21 days. In children and adolescents (21 years and younger) the recommended dose is 650 mg/m² on five consecutive days, repeated every 21 days. The prescribing physician should consider which regimen is appropriate when treating patients in the age range of 16–21 years.

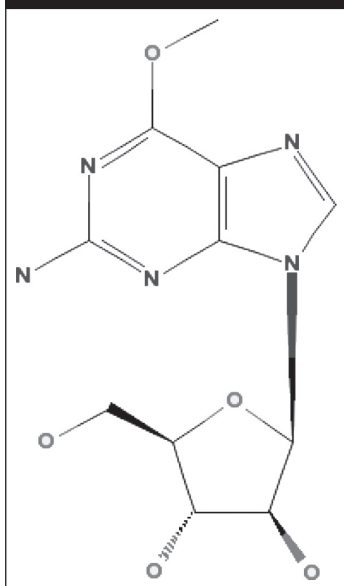
Nelarabine is chemically designated as 9-β-D-arabino-furanosyl-6-methoxy-9H-purine-2-amine, see Figure 1. It is an ampholyte with an aqueous solubility of 8–9 mg/mL at 25°C over the pH range of 4 to 10 and has a molecular weight of 297.27 [1, 6]. Nelarabine is manufactured by GlaxoSmithKline and marketed under the names of Atriance

Contact for correspondence:

Professor Irene Krämer, PhD
Department of Pharmacy
Johannes Gutenberg-University Hospital
1 Langenbeckstraße
DE-55131 Mainz, Germany
Tel: +49 6131 177209
Fax: +49 6131 175525
irene.kraemer@unimedizin-mainz.de

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Figure 1: Chemical structure of nelarabine



or Arranon, depending on the country. It is commercially available as a solution for infusion and supplied as a clear, colourless, sterile solution in glass vials. Each ready-to-use liquid injectable dosage form contains 250 mg of the active substance, nelarabine, dissolved in 50 mL water for injection. Sodium chloride is used as an excipient to adjust the tonicity of the formulation, and sodium hydroxide or hydrochloric acid to adjust the pH. In aqueous solution, nelarabine is most stable between pH 5 and 7 [1, 7].

Nelarabine is intended for IV use only. The commercially available solution for infusion is not diluted before administration. The appropriate volume of nelarabine solution for infusion is transferred into ethinyl vinyl acetate (EVA) or polyvinylchloride (PVC) infusion bags or into glass containers and administered as a two-hour infusion in adult patients and as a one-hour infusion in paediatric patients [7, 8].

When nelarabine was stored at 30°C/65% relative humidity (RH) for 24 months or at 40°C/75% RH for six months, the results of all studies complied with the manufacturer's product specification. No changes were observed under photostability, stress or freeze/thaw conditions [1, 7]. According to the summary of product characteristics, Atriance solution is stable for up to eight hours at up to 30°C once the vial is opened [9]. No further information, either on the stability of nelarabine infusion solution in the glass vial or on the ready-to-use nelarabine infusion solutions in infusion bags, was found in the literature. Therefore a systematic study was initiated to determine the stability of nelarabine infusion solution in EVA infusion bags under different storage conditions. The results can be used to set storage-period limits for drugs prepared in pharmacy-based cytotoxic preparation units.

MATERIALS AND METHODS

Preparation of test solutions

Stability studies were performed on the commercially available Atriance liquid injectable dosage form (nelarabine 5 mg/mL, 250 mg per vial; excipients: sodium chloride, hydrochloric acid, sodium hydroxide and water for

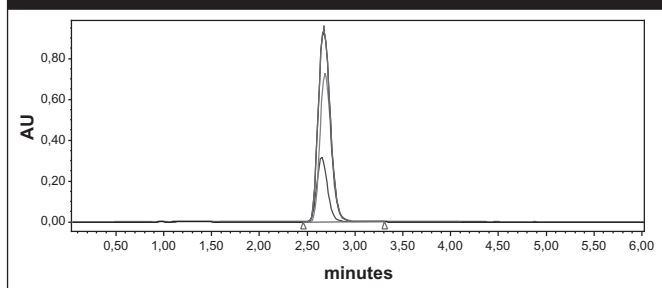
injection [1] lot C370069, GlaxoSmithKline, Munich, Germany). Nelarabine test solutions were prepared aseptically by transferring 50 mL of nelarabine infusion solution to empty EVA infusion bags (nominal volume 100–300 mL, product number: SN2020P, lot: 01O001, Neocare, Lüdenscheid, Germany). Test solutions were stored under refrigeration (2°C–8°C) or at room temperature (20°C–25°C), either protected from or exposed to light for a period of 28 days. For each storage condition, three test solutions were prepared (a total of nine test solutions).

HPLC Assay

Nelarabine concentrations were analysed by a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) assay with photodiode array detection adopted from Reichelova [10]. The HPLC system consisted of a Waters 717 plus autosampler, a Waters photodiode array (PDA) detector 996 and a Waters 510 HPLC-pump. Data were acquired and integrated by using Waters Empower Pro, Empower 2 software, version 6.10.01.00 (Waters, Eschborn, Germany). The column used was the Spherisorb ODS-2 C18, end-capped, 80 Å x 4.6 mm, particle size 3 µm (MZ-Analysentechnik, Mainz, Germany). The mobile phase consisted of 80% 0.01 M potassium dihydrogen phosphate solution [1.36 g anhydrous potassium dihydrogen phosphate (Merck, Darmstadt, Germany) dissolved in 1 L of water HPLC gradient grade (Mallinckrodt Baker, Phillipsburg, New Jersey, US)] pH 6.8 and 20% methanol [methanol HPLC gradient grade (Fisher Scientific, Schwerte, Germany)]. The pH of the potassium dihydrogen phosphate solution was adjusted to pH 6.8 with 1.0 M potassium hydroxide solution [55.61 g potassium hydroxide pellets (Merck, Darmstadt, Germany) dissolved in 1 L of water HPLC gradient grade (Mallinckrodt Baker, Phillipsburg, New Jersey, US)] using a pH 210 Microprocessor pH Meter (Hanna Instruments, Kehl am Rhein, Germany). The flow rate was set at 1.0 mL/minute, with an injection volume of 10 µL. The detection wavelength was set at 265 nm. Under these conditions, the retention time of nelarabine was about 2.5 minutes, see Figure 2. Therefore the run time was set at six minutes. The calibration curve was constructed from plots of peak area versus concentration. The linearity of the method was evaluated at seven nelarabine concentrations varying from 0.05 mg/mL to 1.0 mg/mL and five-fold injections. Nelarabine stock solution (Atriance 5 mg/mL) was diluted with 0.9% sodium chloride solution to the required concentration. The calibration curve was linear, the correlation coefficient was 0.999.

The accuracy of the assay was proved by analysing three different concentrations (0.2 mg/mL, 0.5 mg/mL and

Figure 2: Sample RP-HPLC chromatogram of nelarabine solutions (200, 500 and 700 µg/mL)



0.7 mg/mL, 10 injections per concentration). The percentage rate of closeness to the nominal value was calculated for each sample injected. Mean accuracy was $100\% \pm 1.7$ ($n = 30$). The intraday precision, expressed as relative standard deviation (RSD), was 1.8% for 0.2 mg/mL solutions, 1.9% for 0.5 mg/mL solutions, and 1.3% for 0.7 mg/mL solutions. Interday precision was determined with three different nelarabine concentrations assayed on five different days. Triplicate injections of each concentration yielded interday RSDs of 1.4% for the 0.2 mg/mL, 0.3% for the 0.5 mg/mL and 0.4% for the 0.7 mg/mL concentrations.

The assay was validated as stability-indicating by analysing forced degraded nelarabine solutions. Samples were diluted with 0.9% sodium chloride solution to a nominal concentration of 1.0 mg/mL nelarabine and heated for three hours at 85°C or adjusted to acidic (pH 1 with HCl) or alkaline (pH 13 with NaOH) conditions without heating for one day or with heating for three hours at 85°C.

In addition, forced degradation samples were analysed by using a mobile phase that consisted of 90% 0.05 M potassium dihydrogen phosphate solution and 20% methanol. Run time was set at 15 minutes because of the nelarabine retention time of 6.8 minutes. Degradation peaks were clearly separated from the original nelarabine peak, see Figures 3b and 4b.

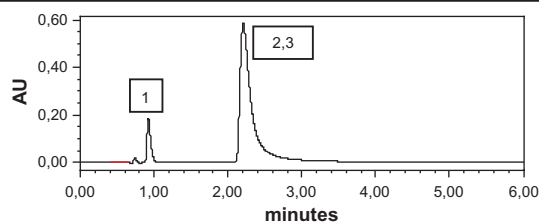
Sample Preparation

On days 0, 1, 3, 7, 14, 21 and 28, 1 mL samples were withdrawn from each test solution and diluted 1:10 with 0.9% sodium chloride solution [100 mL Freeflex infusion bag (lot 13BAS091, Fresenius Kabi, Bad Homburg, Germany)] in order to fit the calibration curve. Immediately after dilution, the samples were analysed in triplicate by RP-HPLC.

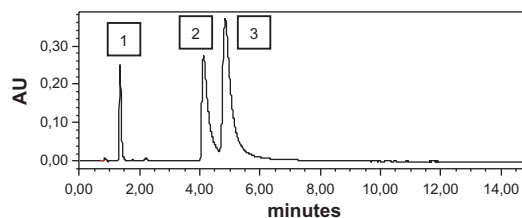
Physical Stability

Physical stability was determined by visual inspection and pH measurement. Test solutions were visually

Figure 3: HPLC chromatograms of nelarabine solution adjusted to pH 1 and heated for three hours at 85°C



a: with a mobile phase of 20% MeOH and 80% K_2HPO_4 0.01 M



b: with a mobile phase of 10% MeOH and 90% K_2HPO_4 0.05 M
 (1 + 2: unknown degradation products, 3: 2-amino-6-mercapto-purine)

examined in normal laboratory light whenever samples were withdrawn. Test solutions with no colour change or any precipitation were defined as physically stable.

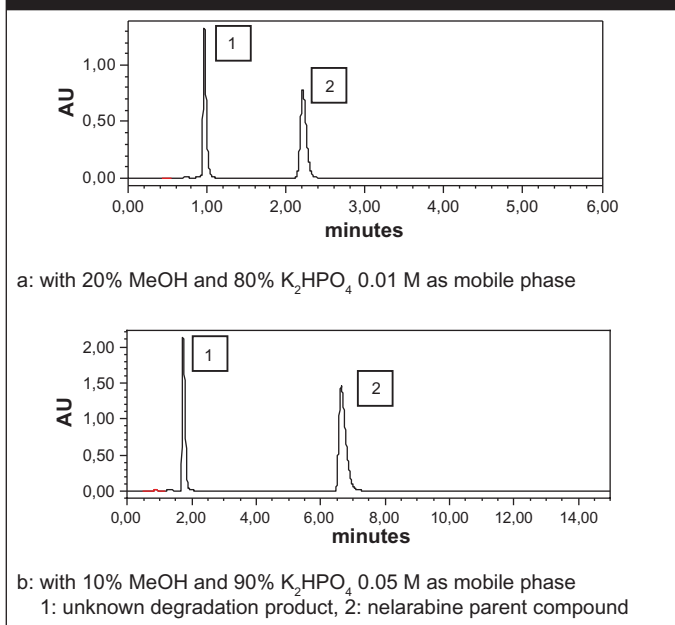
Once a week, samples of nelarabine test solutions were withdrawn. Values of pH were measured, using a pH 210 Microprocessor pH Meter (Hanna Instruments, Kehl am Rhein, Germany) equipped with an InLab Micro pH glass electrode (Mettler Toledo, Giessen, Germany). The pH meter was calibrated with standard buffer solutions (pH 4.01 and 7.01) from Hanna Instruments. PH values were stated as stable between pH 5 and 7 [11].

RESULTS

Reversed Phase HPLC

The HPLC method adopted from Reichelova et al. [10] was shown to be suitable for stability studies of the guanosine analogue, nelarabine, although the method was originally developed to study deoxyadenosine analogues. By using this method, degradation products could only be detected when the pH-modified samples were heated for three hours at 85°C, see Figures 3a and 4a. Analysing the forced degradation samples with an adapted mobile phase, three main degradation products (after retention times (t_R) of 1.4, 4.0 and 4.8 minutes) were recognised in the chromatograms of the acidulated and heat-degraded samples. Intact nelarabine (after t_R 6.8 minutes) was no longer detectable. Chromatograms of the alkalisied and heated samples revealed one degradation product peak (t_R : 1.8 minutes)

Figure 4: HPLC chromatograms of nelarabine solution adjusted to pH 13 and heated for three hours at 85°C



and a decreased parent peak (t_R : 6.8 minutes), see Figures 3b and 4b. The additional peak with the retention time of 4.8 minutes results from acid-catalysed cleavage of the N-glycosidic bond. The degradation product corresponds to the respective free purine base as was demonstrated by chromatography of pure 2-amino-6-methoxy-(9H)-purine (CAS-Nr. 20535-83-5, Chemos, Regenstauf, Germany).

Ready-to-use nelarabine infusion solutions in EVA bags were found to be chemically stable for a minimum of four weeks, independent of temperature or light conditions. Nelarabine concentrations declined less than 5% over the entire test period, see Table 1, and no peaks of degradation products were detected in the PDA chromatograms. In none of the chromatograms was tailing of the nelarabine parent peak or peaks of degradation products detected after a storage period of 28 days, see Figure 5. Marginal variations in nelarabine concentration can be explained by the dilution step before HPLC assay and by standard deviations in the HPLC method.

Physical stability

Nelarabine infusion solutions in EVA infusion bags are physically stable for at least four weeks. No colour changes and no haze or precipitation were observed in the test solutions during the entire storage period.

The pH values measured over the four-week period remained between 5.7 and 6.5, see Table 2.

DISCUSSION

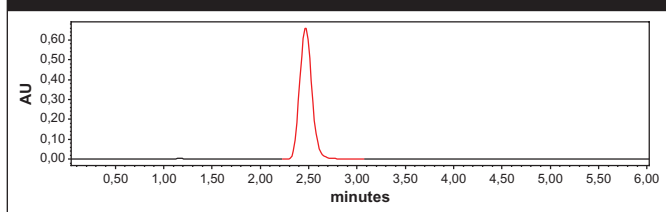
Nelarabine is commercially available as a solution for injection. The medicinal product Atriance undergoes terminal sterilisation after filling the filtered solution in sterile vials. Its stability is not affected under long-term storage conditions and accelerated conditions including exposure to light. These facts indicate the thermal stability and photostability of nelarabine in aqueous

Table 1: Stability of nelarabine infusion solutions in EVA infusion bags over a 28-day period (determined with RP-HPLC) under different storage conditions

Storage conditions	Infusion bag number	Initial drug concentration (mg/mL)		Initial concentration remaining (%)					
		Nominal	Actual	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28
Room temperature Exposed to light	1	5.0	4.88 ± 0.03	102.28 ± 0.90	101.88 ± 1.08	102.33 ± 0.72	98.19 ± 0.81	103.26 ± 2.37	102.88 ± 0.31
	2	5.0	5.05 ± 0.13	96.80 ± 2.49	98.07 ± 1.48	97.98 ± 2.19	94.86 ± 2.60	98.38 ± 2.33	97.47 ± 2.74
	3	5.0	5.02 ± 0.08	98.85 ± 1.37	96.84 ± 0.77	97.74 ± 1.51	96.78 ± 1.83	98.24 ± 1.22	97.99 ± 1.22
2°C–8°C Protected from light	1	5.0	5.08 ± 0.21	96.81 ± 3.25	99.09 ± 3.93	95.52 ± 3.52	96.15 ± 4.10	96.92 ± 5.90	95.93 ± 4.02
	2	5.0	4.98 ± 0.09	99.48 ± 0.58	101.46 ± 1.76	99.55 ± 1.98	97.83 ± 1.57	97.26 ± 1.94	97.36 ± 1.83
	3	5.0	5.07 ± 0.08	98.72 ± 0.65	98.49 ± 1.99	97.26 ± 1.09	96.80 ± 0.64	95.68 ± 0.59	96.82 ± 2.38
Room temperature Protected from light	1	5.0	5.00 ± 0.09	98.13 ± 2.24	102.51 ± 1.27	99.32 ± 1.94	100.01 ± 2.11	95.70 ± 2.00	98.78 ± 1.94
	2	5.0	5.01 ± 0.09	98.14 ± 1.05	101.86 ± 2.39	98.61 ± 2.07	98.62 ± 1.63	95.87 ± 1.23	99.13 ± 1.68
	3	5.0	4.96 ± 0.07	99.98 ± 1.51	102.46 ± 1.32	98.92 ± 1.18	100.03 ± 1.78	102.65 ± 1.32	99.15 ± 1.12

Concentration expressed as mean ± standard deviation of triplicate assays of one test solution. Drug concentrations in samples taken at time zero were designated as 100%.

Figure 5: Sample RP-HPLC chromatogram of nelarabine infusion solution after a storage period of 28 days



solution. In addition, during the manufacturing process no loss in content was seen at any point [1]. In the study, nelarabine concentrations remained consistently above 95% of the initial concentration over the four-week test period, independent of storage, temperature and light conditions. Loss of nelarabine because of adsorption on to EVA, the primary packaging material, can be excluded. No degradation products could be detected in PDA chromatograms of the test solutions at any time.

Test solutions, in primary packing material at the standard available concentration, and storage conditions were selected to observe normal clinical practice. Because the commercially available infusion solution is not diluted before administration, the influence of different vehicles with different ionic strengths and pH values was not assessed. The pH of Atriance infusion solution is adjusted to pH 5–7 because it is the optimum pH range for the stability of aqueous nelarabine solutions. During

the entire test period, the pH of test solutions remained in the range given by the manufacturer (GlaxoSmithKline), thereby supporting the chemical stability of nelarabine. Moreover, nelarabine was revealed to be fairly stable in acid and base conditions. In the forced degradation studies, sole acidification or alkalinisation of nelarabine solutions did not induce degradation. Only maximum forced conditions (pH modification plus prolonged heating at 85°C) caused significant degradation. Purine nucleosides are known to undergo acid-catalysed hydrolysis of the N-glycosylic bond resulting in the respective sugar and purine moiety [10, 12]. The acid-catalysed hydrolysis rates depend on the structure of the purine base and the structure of the sugar moiety. Guanine nucleosides are slightly less stable than adenine nucleosides. The 2'-hydroxyl component on the sugar moiety was shown to have a very strong stabilising effect on the rate of acid hydrolysis thereby explaining the stability of nelarabine. The configuration of the hydroxyl group on the 2'-carbon atom has only minor influence [12]. Consequently, the arabinoside configuration decreases the hydrolysis rate and favours the stability of nelarabine. Liquid chromatographic studies of acid stability of adenosine analogues also revealed unknown products which were different from the expected adenine analogues [10]. This was also the case in this study, which revealed two unknown degradation products in addition to the expected purine base.

In general, nucleosides are even more resistant to alkaline nucleoside hydrolysis. Elevated temperatures (60°C–80°C)

are necessary to obtain reasonable rates of hydrolysis independent from the structure of the sugar moiety [13]. In addition to the cleavage of the glycosyl bond, certain purine nucleosides undergo alkaline-induced cleavage of the imidazole ring. Alkaline-based forced degradation of nelarabine resulted in only one additional peak in the HPLC chromatogram, see Figures 4a and 4b, which corresponds most probably to ring opening of the imidazole ring. Solvolysis of nelarabine to the respective purine was not observed. Alkaline solvolysis by two parallel routes has been thoroughly investigated for adenine nucleosides [11] and shown that ring opening is favoured at higher alkalinities. These results support the assumption in this study of a ring-opened degradation

Table 2: pH values of nelarabine infusion solutions in EVA infusion bags under different storage conditions. Mean of three infusion bags is calculated from (H⁺) concentrations

Storage conditions	Infusion bag number	Day 0	Day 7	Day 14	Day 21	Day 28
Room temperature Exposed to light	1	5.64	6.53	5.79	5.15	6.09
	2	5.67	6.50	5.65	5.42	6.23
	3	5.80	6.16	5.71	5.54	6.38
	Mean	5.70	6.36	5.71	5.34	6.22
2°C–8°C Protected from light	1	5.62	6.00	5.78	5.67	6.46
	2	5.74	5.89	5.77	5.66	6.54
	3	5.78	5.81	5.82	5.76	6.45
	Mean	5.71	5.89	5.79	5.69	6.48
Room temperature Protected from light	1	5.82	5.97	5.82	5.94	6.50
	2	5.82	5.96	5.89	5.93	6.43
	3	6.03	6.04	5.75	5.83	6.36
	Mean	5.88	5.99	5.82	5.90	6.43

product of nelarabine (as a guanosine analogue) under strong alkaline conditions. The fact that during the stability tests no tailing of the nelarabine parent peak or additional peaks of degradation products (t_R : 1.2 minutes) were to be found at any time gives proof of the stability of nelarabine.

With regard to physico-chemical stability, pharmacy-based centralised preparation of ready-to-use nelarabine infusion solutions has proved unproblematic and cost-saving. However, despite its pronounced physico-chemical stability, the risk of microbiological contamination should be considered when preparing the solutions and assigning extended expiry dates. The tests carried out in this study revealed physico-chemical stability of nelarabine infusion solution in EVA

bags. Considering that there is no preservative in the commercially available product, handling under strict aseptic conditions and storage of all products under refrigeration is crucial.

CONCLUSION

Ready-to-use nelarabine infusion solutions in EVA infusion bags are physico-chemically stable for at least four weeks, either refrigerated or at ambient temperature, and with or without protection from light.

ACKNOWLEDGEMENT

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REFERENCES

1. European Medicines Agency, London: Scientific discussion on Atriance. 2007 [cited 2010 November 16]. Available from: www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000752/WC500027915.pdf
2. Lambe CU, Averett DR, Paff MT, Reardon JE, Wilson JG, Krenitsky TA. 2-Amino-6-methoxypurine arabinoside: an agent for T-cell malignancies. *Cancer Res.* 1995;55(15):3352-6.
3. Gandhi V, Plunkett W, Weller S. Evaluation of the combination of nelarabine and fludarabine in leukemias: clinical response, pharmacokinetics, and pharmacodynamics in leukemia cells. *J Clin Oncol.* 2001;19(8):2142-52.
4. Cohen MH, Johnson JR, Massie T, et al. Approval summary: nelarabine for the treatment of T-cell lymphoblastic leukemia/lymphoma. *Clin Cancer Res.* 2006;12(18):5329-35.
5. Parker WB, Secrist JA 3rd, Waud WR. Purine nucleoside antimetabolites in development for the treatment of cancer. *Curr Opin Investig Drugs.* 2004;5(6):592-6.
6. GlaxoSmithKline. Arranon prescribing information. Research Triangle Park, NC. Revised December 2009 [cited 2010 November 16]. Available from: http://us.gsk.com/products/assets/us_arranon.pdf
7. Center for Drug Evaluation and Research. Application number: 21-877, Pharmacology Review of Arranon. October 28 2005 [cited 2010 November 22]. Available from: www.accessdata.fda.gov/drugsatfda_docs/nda/2005/021877_s000_Arranon_Pharmr.pdf
8. European Medicines Agency. London: Product Information for Atriance. Updated 2009 February 24 [cited 2010 November 16]. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000752/human_med_000656.jsp&menu=menus/medicines/medicines.jsp&jsenabled=false
9. GlaxoSmithKline, München. Summary of product characteristics for Atriance, January 2010 [cited 2010 November 22]. Available from: www.fachinfo.de/FachInfo/data/fi/pdf/01/05/010503.pdf
10. Reichelova V, Liliemark J, Albertioni F. Liquid chromatographic study of acid stability of 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine, 2-chloro-2'-deoxyadenosine and related analogues. *J Pharm Biomed Anal.* 1995;13(4-5):711-4.
11. US National Library of Medicine, Bethesda, MD. Current medication information for Arranon. December 2009 [cited 2010 November 22]. Available from: <http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=14253&CFID=56057883&CFTOKEN=1970cd3ab443c5b6-7433826E-B334-2078-68D55E99BE1C&jsessionid=ca304b87464e5a7d4446>
12. Garrett ER, Mehta PJ. Solvolysis of adenine nucleosides. I. Effects of sugars and adenine substituents on acid solvolyses. *J Am Chem Soc.* 1972;94(24):8532-41.
13. Garrett ER, Mehta PJ. Solvolysis of adenine nucleosides. II. Effects of sugars and adenine substituents on alkaline solvolyses. *J Am Chem Soc.* 1972;94(24):8542-7.