Simple and sensitive HPLC-UV method for determination of bexarotene in rat plasma

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ABSTRACT

Bexarotene is currently marketed for treatment of cutaneous T-cell lymphoma and there has been growing interest in its therapeutic effectiveness for other cancers. Neuroprotective effects of bexarotene have also been reported. In this study, a simple, sensitive and cost-efficient bioanalytical method for determination of bexarotene in rat plasma was developed and fully validated. The method utilises protein precipitation with acetonitrile and liquid-liquid extraction with n-hexane-ethyl acetate (10:1, v/v). An HPLC-UV system with a Waters Atlantis C18 column and a mobile phase of acetonitrile-ammonium acetate buffer (10 mM, pH 4.1) at a ratio of 75:25 (v/v), flow rate 0.2 mL/min was used. Chromatograms were observed by a UV detector with wavelength set to 259 nm. Intra- and inter-day validations were performed and sample stability tests were conducted at various conditions. The applicability of the method was demonstrated by a pharmacokinetic study in rats. Intravenous bolus dose of 2.5 mg/kg was administered to rats and samples were obtained at predetermined time points. As a result, pharmacokinetic parameters of AUC_{inf} (4668 ± 452 h·ng/mL), C_0 (6219 ± 1068 ng/mL) and $t_{1/2}$ (1.15 ± 0.02 h) were obtained. In addition, the developed method was further applied to human and mouse plasma to assess the suitability of the method for samples from other species.

KEYWORDS

Bexarotene, HPLC-UV, preclinical pharmacokinetics, rat plasma, human plasma, mouse plasma

1. Introduction

Bexarotene is an orally bioavailable synthetic rexinoid that selectively binds to retinoid X receptors [1]. It was the first selective retinoid that entered clinical trials [2] and it is currently approved for treatment of cutaneous T-cell lymphoma (CTCL) in patients whose disease is refractory to at least one systemic chemotherapy [3]. Following the approval, the efficacy and safety of bexarotene was shown in refractory early-stage CTCL [4] and other related conditions such as panniculitis-like T-cell lymphoma [5]. Bexarotene also showed certain promise in non-small cell lung cancer [6-9]. In addition, clinical trials are currently ongoing for efficacy in acute myeloid leukaemia [10, 11] and other tumours such as aerodigestive tract cancer [12], breast cancer [13], metastases of differentiated thyroid carcinoma [14] and keratoacanthomas [15].

In the past few years, additional attention was drawn to bexarotene as it was reported to have neuroprotective effects [16, 17]. Preclinical studies have shown that bexarotene reverses cognitive and neuronal impairments and improves neural circuit function with a mechanism related to apolipoprotein E, therefore providing a new strategy for treatment of Alzheimer's disease [18, 19]. These results were followed by additional studies which showed neuroprotective effects of bexarotene in Parkinson's disease [20]. Although some debate on its effectiveness in neurodegenerative diseases still remains [21, 22], a clinical trial of bexarotene in Alzheimer's disease is ongoing and a clinical trial in healthy volunteers to elucidate its neuroprotective mechanism is also being conducted [17]. Other clinical trials investigating potential indications of bexarotene include trials in chronic severe hand dermatitis [23], lymphomatoid papulosis [24], psoriasis [25], alopecia areata [26], schizophrenia [27] and Cushing's disease [28].

In response to the extensive clinical research of bexarotene, interest in mechanistic preclinical studies to explore its benefits in cancer and other diseases persists. Apart from the already known anticancer effects such as inhibition of cell cycle progressions and induction of apoptosis [29], studies have demonstrated that bexarotene induces differentiation of cells, which can lead to treatment of cancer [30, 31]. It also prevents multidrug resistance, which is a major problem in chemotherapies [32] and other studies have shown that it has anticancer effects by inhibition of angiogenesis and metastasis [33]. Preventive effects of bexarotene have also been elucidated in lung cancer [34, 35], breast cancer [36] and intestinal cancer [37]. Other preclinical studies include research in neurodegenerative diseases as previously mentioned [38, 39], and also research in cholesterol homeostasis [40, 41] and pharmacokinetic studies to interpret relationships between exposure and effects [6]. Additionally, recent pharmaceutical research of nano-crystalisation of bexarotene has been reported with bioanalysis of plasma samples for pharmacokinetics [42, 43]. Unfortunately, these studies have not included a validation of the analytical methods used.

Previously published analytical methods for quantification of bexarotene in biological matrices include an assay that involves high-performance liquid chromatography (HPLC) with fluorescence detection [44]. This method achieved good sensitivity with lower limit of quantification (LLOQ) of 0.5 ng/mL and has been applied to clinical pharmacokinetic studies. However, it is limited by the fact that it requires a high volume of plasma (1 mL) to achieve this sensitivity. This could be a major limitation for preclinical studies in rodents as sample volume is usually substantially lower in these studies. Furthermore, the calibration curve of this method was separated into a low-range and a high-range, and the two ranges utilised different instruments for analysis. Additionally, there was no full validation of that analytical method to meet the guidelines proposed by the US Food and Drug Administration (FDA) [45].

Another published method employed gas chromatography-mass spectrometry to achieve LLOQ of 1 ng/mL, but requires a chemical derivatisation step which makes the method time-consuming, complicated and expensive [46]. A more simple analytical method using HPLC-UV has been reported but this study was focused on the metabolic pathway of bexarotene [47]. Therefore it did not report specific details on the analytical procedure such as LLOQ, quantification range, use of an internal standard and validation.

Therefore, the aim of this study was to develop a simple, sensitive and cost-efficient bioanalytical method for determination of bexarotene in rat plasma, utilising low-volume samples and a high-range calibration curve. The assay was developed using HPLC coupled with ultra-violet (UV) detection and full validation of the method was performed to meet the regulations of the FDA. The developed and validated method was applied to a pharmacokinetic study of intravenous bolus administration in rats. The suitability of the method for mouse and human plasma was also assessed in this study.

2. Materials and methods

2.1. Chemicals and reagents

Bexarotene (CAS: 153559-49-0) was purchased from LC Laboratories (Woburn, MA, USA). For internal standard (IS), Δ^9-tetrahydrocannabinol (CAS: 1972-08-3) was used (from THC Pharm GmbH (Frankfurt, Germany)). Rat and mouse plasma were purchased from Sera Laboratories (West Sussex, UK) and human plasma from TCS Biosciences (Buckingham, UK). All solvents were of HPLC grade and were purchased from Fisher Scientific (Leicestershire, UK). All other reagents were purchased from commercial sources and were of HPLC grade or higher.

2.2. Instrumentation

2.2.1. Analytical equipment

The HPLC-UV system consisted of a Waters Alliance 2695 separations module coupled with a Waters 996 photodiode array detector. The samples in the autosampler were maintained at 4°C and a column oven was used to control the column temperature at 40°C. Empower™ 2 software was used for data processing.

2.2.2. Analytical conditions

Chromatographic separation was achieved by Waters Atlantis C18 2.1 × 150 mm, 5 µm particle size column (Milford, MA, USA) equipped with a SecurityGuard 2 × 4 mm, 3 µm particle size (Phenomenex, Macclesfield, UK). A mobile phase mixture of acetonitrile/ammonium acetate buffer (10 mM, pH modified to 4.1 with glacial acetic acid) was used in a ratio of 75:25 (v/v). The mobile phase was eluted with isocratic conditions at 0.2 mL/min. The analytes were detected at 259 nm.

2.3. Sample preparation

2.3.1. Preparation of calibration curve standards and quality control samples

Stock standard solutions of bexarotene and IS were prepared at concentration of 1 mg/mL in acetonitrile and were stored at -20°C. Working standard solutions of bexarotene were prepared by diluting the stock standard solution with acetonitrile to yield concentrations of 0.1, 0.2, 0.5, 1, 5, 10, 50 and 100 µg/mL. Working standard solution of the IS was prepared in the same manner to yield a concentration of 100 µg/mL. Plasma calibration curve samples were prepared by spiking 100 µL of plasma with 10 µL of corresponding working standard solutions of bexarotene to yield concentrations of 10, 20, 50, 100, 500, 1000, 5000 and 10000 ng/mL.

147 Working standard solutions of bexarotene for quality control samples were prepared in a
148 similar procedure at concentrations of 0.1, 0.25, 4 and 80 µg/mL. Ten µL of working standard
149 solutions were spiked to 100 µL of plasma to give lower limit of quantification (LLOQ), low
150 quality control (LQC), medium quality control (MQC) and high quality control (HQC) samples
151 at concentrations of 10, 25, 400 and 8000 ng/mL, respectively. These calibration curve and
152 quality control samples then underwent sample preparation procedure as described below.
153
154 *2.3.2. Sample preparation procedure*
155 For the sample preparation, 10 µL of IS solution (100 µg/mL) was added to 100 µL of plasma
156 sample. Protein precipitation of the samples was performed by adding 300 µL of acetonitrile.
157 For pH modification, 300 µL of 0.1 M HCl was then added. The samples were briefly vortex-
158 mixed for 1 min and extraction solvent (3.3 mL) consisting of n-hexane-ethyl acetate (10:1,
159 v/v) was added. The samples were vortex-mixed for 10 min and were centrifuged at 1160 *g* for
160 10 min at 10°C (Harrier 18/80R, UK). The upper organic layer was transferred and evaporated
161 to dryness under gentle stream of N_2 gas at 40°C (Techne DRI-Block type DB-3D, Cambridge,
162 UK). Reconstitution solvent (100 µL) of acetonitrile-water (1:1, v/v) was added to the residue
163 and the samples were vortex-mixed for 10 min. A volume of 40 µL was injected into the HPLC-
164 UV system for analysis.
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166 ***2.4. Bioanalytical method validation***
167 Full validation of the bioanalytical method was conducted in accordance with the guideline
168 established by the FDA [45].
169
170 *2.4.1. Selectivity*

171 The selectivity of the developed bioanalytical method was assessed by comparing the
172 chromatograms of plasma samples spiked with bexarotene (n = 6) at the LLOQ with the
173 chromatograms of blank plasma samples. It was also assessed in plasma samples obtained from
174 the pharmacokinetic experiment in rats [45].
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176
177 *2.4.2. Accuracy and precision*
178 Accuracy was the bias from nominal concentration and was expressed as relative error (RE).
179 Precision was the coefficient of variation and was expressed as relative standard deviation
180 (RSD). The intra-day accuracy and precision were validated by preparing and analysing six
181 replicates of QC samples (LLOQ, LQC, MQC and HQC) on the same day. The inter-day
182 accuracy and precision were validated by preparing and analysing six replicates of QC samples
183 on six different days. The acceptable values for accuracy and precision were RE within ±15%
184 and RSD ≤15%, respectively, with the exception of RE within ±20% and RSD ≤20%,
185 respectively, for the LLOQ.
186
187 *2.4.3. Sensitivity*
188 The LLOQ was determined as the lowest tested concentration of bexarotene in spiked plasma
189 that had acceptable accuracy and precision of RE within ±20% and RSD ≤20%, respectively,
190 from intra-day and inter-day analyses [45].
191
192 *2.4.4. Linearity*
193 Calibration curves were constructed at the range of 10 – 10000 ng/mL as described above with
194 10 ng/mL as LLOQ. A double blank sample (unspiked plasma) and a blank sample (plasma
195 spiked with IS solution only) were included in each calibration curve. The peak ratio between

196 bexarotene and the IS was plotted against nominal concentration to obtain calibration curves.
197 Calibration curves with correlation coefficient (r^2) values of >0.99 and accuracy of ±15%
198 (except for LLOQ where ±20% was applied) were considered to be acceptable.
199
200 *2.4.5. Recovery*
201 The recovery of bexarotene was determined by comparing the peak areas of processed samples
202 with peak areas of solutions of equivalent concentration in the reconstitution solvent mixture
203 [45]. It was expressed as the mean ± SD from three concentrations tested (LQC, MQC and
204 HQC). The recovery of IS was determined in the same manner at a concentration of 10 µg/mL.
205
206 *2.4.6. Freeze and thaw stability*
207 Three freeze-thaw cycles (freezing at -80°C for 24 h and thawing at room temperature) were
208 applied to six replicates of QC samples (LQC, MQC and HQC). Following the third freeze-
209 thaw cycle the samples were processed and a freshly prepared set of calibration curve was used
210 for quantification.
211
212 *2.4.7. Bench-top, short-term and long-term stability*
213 Sample stability studies were performed with six replicates of QC samples (LQC, MQC and
214 HQC). Bench-top stability was assessed by preparing the QC samples and storing them at room
215 temperature for 6 h. The QC samples for short-term stability were prepared and stored at -20°C
216 for 24 h. Long-term stability was tested by storing the QC samples for 2, 4 and 8 weeks at -
217 80°C. After desired storage time, the samples underwent sample preparation procedure as
218 described in section 2.3.2. Quantification was performed using a calibration curve freshly
219 prepared each time.
220

221 *2.4.8. Autosampler stability*

222 Six replicates of QC samples (LQC, MQC and HQC) were prepared and processed according
223 to the sample preparation procedure described above. These processed samples were stored at
224 4°C for 24 h to mimic the condition inside the autosampler of HPLC-UV system. After 24 h,
225 the analysis was performed and the samples were quantified using a freshly prepared
226 calibration curve.

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228 *2.4.9. Stock solution stability*

229 Working standard solutions for QC samples were prepared at concentrations of 0.25, 4 and 80
230 µg/mL and were stored in room temperature for 6 h. These solutions were then used to prepare
231 six replicates of QC samples (LQC, MQC and HQC) and they were submitted for sample
232 preparation procedure. Calibration curve samples used for quantification of these QC samples
233 were prepared at the time of sample preparation procedure.

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235 ***2.5. Pharmacokinetic study***

236 *2.5.1. Animals*

237 All procedures of the *in vivo* pharmacokinetic study were reviewed and approved by the
238 University of Nottingham Ethical Review Committee in accordance with the Animals
239 [Scientific Procedures] Act 1986. Male Sprague Dawley rats (Charles River Laboratories, UK)
240 weighing 350-380 g were used for the experiment. They were housed in the Bio Support Unit,
241 University of Nottingham, with controlled temperature, 12 h light/dark cycle and free access
242 to food and water. The acclimatisation of the animals was at least for six days. On the day of
243 surgery, general anaesthesia was induced by intraperitoneal administration of a mixture of
244 ketamine (90 mg/kg) and xylazine (8 mg/kg) and right jugular vein cannulation was performed.
245 The rats were then allowed to recover for two nights before the experimental procedures.

246 *2.5.2. Experimental procedure*

247 A dosing formulation of bexarotene was prepared at 2.5 mg/mL in polyethylene glycol 400.
248 Intravenous bolus dose of 2.5 mg/kg was administered to the rats and blood samples (0.25 mL)
249 were collected before administration, and at 5, 15, 30, 45, 60, 120, 240 and 480 min after the
250 administration via the jugular vein cannula. Plasma samples were obtained by centrifugation
251 (3000 *g*, 10 min) and were stored at -80°C until analysis. Non-compartmental pharmacokinetic
252 analysis of the plasma concentration profiles were performed using Phoenix WinNonlin 6.3
253 software (Pharsight, Mountain View, CA, USA).

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3. Results and discussion

3.1. Method development

As bexarotene is a weak acid (pK_a = 4.08, predicted by ACD/Labs, Toronto, Canada), to improve the extraction efficiency, the pH of plasma samples was modified using 0.1 M HCl following protein precipitation with acetonitrile. When 0.1 M HCl was not added to plasma, major fraction of bexarotene stayed in the ionised form, resulting in poor recovery. After acidification of the samples, bexarotene was easily extracted. Since bexarotene is a highly lipophilic compound (Log P = 8.55, predicted by ACD/Labs, Toronto, Canada), non-polar organic solvents were considered to be suitable for the extraction step [48-50]. Extraction using n-hexane was attempted initially, but interestingly addition of ethyl acetate, a relatively polar extraction solvent, improved recovery of bexarotene. However, increasing the ratio of ethyl acetate further decreased the recovery and finally n-hexane-ethyl acetate with the ratio of 10:1 (v/v) was selected as the extraction solvent.

For the mobile phase mixture, water was initially tried as the aqueous component. However, bexarotene was not efficiently retained in the C18 column as it had an ionised form in water. Therefore 10 mM ammonium acetate buffer was used instead of water. The pH of the buffer was tested at a range of 4.0-5.5 and from the shift of retention time, it was found that bexarotene was better retained at lower pH. The final pH of the buffer (pH 4.1) and the composition ratio with acetonitrile were selected to provide optimal selectivity and sensitivity.

3.2. Method validation

3.2.1. Selectivity

The selectivity of the method was demonstrated as interference peaks from blank rat plasma samples were effectively separated from the peak of bexarotene (Figure 1). It is also shown in

Figure 1D that endogenous peaks that originate from plasma of rats used in pharmacokinetic experiments did not interfere with the peaks of interest. Therefore the current method provides selective determination of bexarotene in rat plasma. The inability of UV-based analytical methods to detect metabolites can be a limitation of this method.

3.2.2. Accuracy and precision

Accuracy and precision, expressed as RE and RSD, respectively, for intra- and inter-day validations are shown in Table 1. The values for all QC samples (LQC, MQC and HQC) were within the acceptable range (RE within ±15% and RSD <15%). The values for LLOQ samples were also within the criteria (RE within ±20% and RSD <20%), indicating that the developed method was able to quantify bexarotene with appropriate accuracy and precision [45].

3.2.3. Sensitivity and linearity

As mentioned in the previous section, the LLOQ samples had acceptable accuracy and precision in intra- and inter-day validations and therefore 10 ng/mL was determined to be the LLOQ for the developed method (Table 1). The linearity of the calibration curves was established over a range of 10-10000 ng/mL as all the points within the calibration curve, except LLOQ, were within the criteria of RE within ±15% and RSD <15% and the correlation coefficient (r^2) values were >0.99. The LLOQ of 10 ng/ml is sufficiently sensitive to support preclinical pharmacokinetic studies in rats.

321 *3.2.4. Recovery*

322 The absolute recoveries (mean ± SD) of bexarotene from rat plasma were 96.2 ± 0.6%, 97.2 ±
323 1.1%, and 96.0 ± 2.8% for the LQC, MQC and HQC samples, respectively. Such high recovery
324 was reached by optimising the extraction solvent. The high recovery of this method contributed
325 to achieving good sensitivity. The recovery of the IS was 89.4 ± 2.7%.

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327 *3.2.5. Stability*

328 The stability of spiked rat plasma samples under different storage conditions is shown in Table
329 2. The RE and RSD values were within acceptable limits for all conditions tested. The bench-
330 top stability and stock solution stability ensures that the samples and stock solutions are stable
331 during sample preparation procedures, respectively. Short- and long-term stability results
332 indicate that these samples can be stored under these conditions without compromising the
333 accuracy and precision. Samples that had undergone freeze-thaw cycles have demonstrated that
334 stability is not affected up to three cycles. Also, autosampler stability indicates that processed
335 samples can be stored in the autosampler for up to 24 h. Although some stability data in human
336 plasma samples with bexarotene has been previously reported, it only included bench-top,
337 freeze-thaw cycles and autosampler stabilities [44]. To the best of our knowledge, the present
338 study is the first report of full validation in rat plasma, including all the stability tests required
339 by the FDA [45].

340

341 ***3.3. Pharmacokinetic study in rats***

342 The applicability of the analytical method was demonstrated in a pharmacokinetic study in rats
343 following IV bolus administration. The plasma concentration-time profiles of bexarotene are
344 shown in Figure 2 and pharmacokinetic parameters obtained by non-compartmental analysis
345 are shown in Table 3. The plasma concentration profiles showed consistency across the animals

(Figure 2) which is also reflected in the variability of the obtained pharmacokinetic parameters (Table 3). The selectivity, sensitivity and range of the calibration curve of the developed method are sufficient to be utilised in pharmacokinetic studies in rats.

3.4. Assessment of the suitability of the analytical method to human and mouse plasma.

Following the development and validation of the bioanalytical method for rat plasma, it was further applied to human and mouse plasma to assess the potential suitability of the methodology for studies in additional species. The same sample preparation method was applied to both human and mouse plasma for the levels of LQC, MQC and HQC. The validity was assessed by inter-day validation and the accuracy and precision are shown in Table 4. Good selectivity was also exhibited by comparing chromatograms of spiked samples and blank plasma samples for both human and mouse plasma (Figures 3 and 4, respectively). The only remarkable point was that for human plasma, an interference peak appeared at the retention time of IS (Figure 3). The problem was solved by observing the IS peak at 220 nm for human plasma only (Figure 3). These results show that the current method has good potential to be utilised in studies involving both human subjects and mice. A bioanalytical method for determination of bexarotene in mouse plasma has not been previously reported.

4. Conclusion

The present study describes development and full validation of a simple, sensitive and cost-efficient bioanalytical method using HPLC-UV for the determination of bexarotene in rat plasma. This method utilises 100 µL volume of samples to achieve LLOQ adequate to support preclinical pharmacokinetic studies. A full validation of a bioanalytical method for bexarotene in rat plasma is reported here for the first time. It was also shown that the method can be applied to studies involving human subjects and mice.

371 **REFERENCES**

372

373 [1] L.T. Farol, K.B. Hymes, Bexarotene: a clinical review, Expert Rev. Anticancer Ther. 4
374 (2004) 180-188.
375 [2] M.F. Boehm, L. Zhang, B.A. Badea, S.K. White, D.E. Mais, E. Berger, C.M. Suto, M.E.
376 Goldman, R.A. Heyman, Synthesis and structure-activity relationships of novel retinoid X
377 receptor-selective retinoids, J. Med. Chem. 37 (1994) 2930-2941.
378 [3] R.A. Abbott, S.J. Whittaker, S.L. Morris, R. Russell-Jones, T. Hung, S.J. Bashir, J.J.
379 Scarisbrick, Bexarotene therapy for mycosis fungoides and Sezary syndrome, Br. J. Dermatol.
380 160 (2009) 1299-1307.
381 [4] M. Duvic, A.G. Martin, Y. Kim, E. Olsen, G.S. Wood, C.A. Crowley, R.C. Yocum, G.
382 Worldwide Bexarotene Study, Phase 2 and 3 clinical trial of oral bexarotene (Targretin
383 capsules) for the treatment of refractory or persistent early-stage cutaneous T-cell lymphoma,
384 Arch. Dermatol. 137 (2001) 581-593.
385 [5] N. Mehta, A.S. Wayne, Y.H. Kim, G.A. Hale, C.S. Alvarado, P. Myskowski, E.S. Jaffe,
386 K.J. Busam, M. Pulitzer, J. Zwerner, S. Horwitz, Bexarotene is active against subcutaneous
387 panniculitis-like T-cell lymphoma in adult and pediatric populations, Clin. Lymphoma
388 Myeloma Leuk. 12 (2012) 20-25.
389 [6] K.H. Dragnev, W.J. Petty, S.J. Shah, L.D. Lewis, C.C. Black, V. Memoli, W.C. Nugent, T.
390 Hermann, A. Negro-Vilar, J.R. Rigas, E. Dmitrovsky, A proof-of-principle clinical trial of
391 bexarotene in patients with non-small cell lung cancer, Clin. Cancer Res. 13 (2007) 1794-1800.
392 [7] J.R. Rigas, K.H. Dragnev, Emerging role of rexinoids in non-small cell lung cancer: focus
393 on bexarotene, Oncologist 10 (2005) 22-33.
394 [8] H.A. Wakelee, G. Middleton, D. Dunlop, R. Ramlau, N. Leighl, D. Hao, A. Lopez-Anaya,
395 P. Zatloukal, C.D. Jacobs, A phase I pharmacokinetic study of bexarotene with vinorelbine and

cisplatin in patients with advanced non-small-cell lung cancer (NSCLC), Cancer Chemother. Pharmacol. 69 (2012) 815-824.

[9] G.R. Blumenschein, Jr., F.R. Khuri, J. von Pawel, U. Gatzemeier, W.H. Miller, Jr., R.M. Jotte, J. Le Treut, S.L. Sun, J.K. Zhang, Z.E. Dziewanowska, A. Negro-Vilar, Phase III trial comparing carboplatin, paclitaxel, and bexarotene with carboplatin and paclitaxel in chemotherapy-naive patients with advanced or metastatic non-small-cell lung cancer: SPIRIT II, J. Clin. Oncol. 26 (2008) 1879-1885.

[10] J.S. Welch, H. Niu, G.L. Uy, P. Westervelt, C.N. Abboud, R. Vij, K.E. Stockerl-Goldstein, M. Jacoby, I. Pusic, M.A. Schroeder, J.F. Dipersio, A.F. Cashen, A phase I dose escalation study of oral bexarotene in combination with intravenous decitabine in patients with AML, Am. J. Hematol. 89 (2014) E103-108.

[11] D.E. Tsai, S.M. Luger, C. Andreadis, D.T. Vogl, A. Kemner, M. Potuzak, A. Goradia, A.W. Loren, A.E. Perl, S.J. Schuster, D.L. Porter, E.A. Stadtmauer, S.C. Goldstein, J.E. Thompson, C. Swider, A. Bagg, A.R. Mato, M. Carroll, A phase I study of bexarotene, a retinoic X receptor agonist, in non-M3 acute myeloid leukemia, Clin. Cancer Res. 14 (2008) 5619-5625.

[12] K.H. Dragnev, W.J. Petty, S. Shah, A. Biddle, N.B. Desai, V. Memoli, J.R. Rigas, E. Dmitrovsky, Bexarotene and erlotinib for aerodigestive tract cancer, J. Clin. Oncol. 23 (2005) 8757-8764.

[13] F.J. Esteva, Multicenter Phase II Study of Oral Bexarotene for Patients With Metastatic Breast Cancer, J. Clin. Oncol. 21 (2003) 999-1006.

[14] Y.Y. Liu, M.P. Stokkel, A.M. Pereira, E.P. Corssmit, H.A. Morreau, J.A. Romijn, J.W. Smit, Bexarotene increases uptake of radioiodide in metastases of differentiated thyroid carcinoma, Eur. J. Endocrinol. 154 (2006) 525-531.

[15] C.B. Marquez, E.E. Smithberger, S.M. Bair, R.M. Wenham, N.A. Fenske, L.F. Glass, B.S. Cherpelis, Multiple keratoacanthomas arising in the setting of sorafenib therapy: novel chemoprophylaxis with bexarotene, Cancer Control 16 (2009) 66-69.

[16] G. Aicardi, New hope from an old drug: fighting Alzheimer's disease with the cancer drug bexarotene (targretin)?, Rejuvenation Res. 16 (2013) 524-528.

[17] B. Tousi, The emerging role of bexarotene in the treatment of Alzheimer's disease: current evidence, Neuropsychiatr. Dis. Treat. 11 (2015) 311-315.

[18] P.E. Cramer, J.R. Cirrito, D.W. Wesson, C.Y. Lee, J.C. Karlo, A.E. Zinn, B.T. Casali, J.L. Restivo, W.D. Goebel, M.J. James, K.R. Brunden, D.A. Wilson, G.E. Landreth, ApoE-directed therapeutics rapidly clear beta-amyloid and reverse deficits in AD mouse models, Science 335 (2012) 1503-1506.

[19] V. Bomben, J. Holth, J. Reed, P. Cramer, G. Landreth, J. Noebels, Bexarotene reduces network excitability in models of Alzheimer's disease and epilepsy, Neurobiol. Aging 35 (2014) 2091-2095.

[20] A. Schafer, E.S. Burstein, R. Olsson, Bexarotene prodrugs: targeting through cleavage by NQO1 (DT-diaphorase), Bioorg. Med. Chem. Lett. 24 (2014) 1944-1947.

[21] I. Tesseur, B. De Strooper, When the dust settles: what did we learn from the bexarotene discussion?, Alzheimers. Res. Ther. 5 (2013) 54.

[22] K.D. LaClair, K.F. Manaye, D.L. Lee, J.S. Allard, A.V. Savonenko, J.C. Troncoso, P.C. Wong, Treatment with bexarotene, a compound that increases apolipoprotein-E, provides no cognitive benefit in mutant APP/PS1 mice, Mol. Neurodegener. 8 (2013) 18.

[23] J.M. Hanifin, V. Stevens, P. Sheth, D. Breneman, Novel treatment of chronic severe hand dermatitis with bexarotene gel, Br. J. Dermatol. 150 (2004) 545-553.

[24] R.A. Krathen, S. Ward, M. Duvic, Bexarotene Is a New Treatment Option for Lymphomatoid Papulosis, Dermatology 206 (2003) 142-147.

445 [25] J.V. Smit, M.E. Franssen, E.M. de Jong, J. Lambert, D.I. Roseeuw, J. De Weert, R.C.
446 Yocum, V.J. Stevens, P.C. van De Kerkhof, A phase II multicenter clinical trial of systemic
447 bexarotene in psoriasis, J. Am. Acad. Dermatol. 51 (2004) 249-256.
448 [26] R. Talpur, J. Vu, R. Bassett, V. Stevens, M. Duvic, Phase I/II randomized bilateral half-
449 head comparison of topical bexarotene 1% gel for alopecia areata, J. Am. Acad. Dermatol. 61
450 (2009) 592 e591-599.
451 [27] M. George, R. Amrutheshwar, R.P. Rajkumar, S. Kattimani, S.A. Dkhar, Newer
452 antipsychotics and upcoming molecules for schizophrenia, Eur. J. Clin. Pharmacol. 69 (2013)
453 1497-1509.
454 [28] N.A. Tritos, B.M. Biller, Advances in medical therapies for Cushing's syndrome, Discov.
455 Med. 13 (2012) 171-179.
456 [29] L. Qu, X. Tang, Bexarotene: a promising anticancer agent, Cancer Chemother. Pharmacol.
457 65 (2010) 201-205.
458 [30] M. Kizaki, M.I. Dawson, R. Heyman, E. Elster, R. Morosetti, S. Pakkala, D.L. Chen, H.
459 Ueno, W. Chao, M. Morikawa, Y. Ikeda, D. Heber, M. Pfahl, H.P. Koeffler, Effects of novel
460 retinoid X receptor-selective ligands on myeloid leukemia differentiation and proliferation in
461 vitro, Blood 87 (1996) 1977-1984.
462 [31] R.M. Cesario, J. Stone, W.C. Yen, R.P. Bissonnette, W.W. Lamph, Differentiation and
463 growth inhibition mediated via the RXR:PPARgamma heterodimer in colon cancer, Cancer
464 Lett. 240 (2006) 225-233.
465 [32] W.C. Yen, W.W. Lamph, A selective retinoid X receptor agonist bexarotene (LGD1069,
466 Targretin) prevents and overcomes multidrug resistance in advanced prostate cancer, Prostate
467 66 (2006) 305-316.

468 [33] W.C. Yen, R.Y. Prudente, M.R. Corpuz, A. Negro-Vilar, W.W. Lamph, A selective
469 retinoid X receptor agonist bexarotene (LGD1069, targretin) inhibits angiogenesis and
470 metastasis in solid tumours, Br. J. Cancer 94 (2006) 654-660.
471 [34] Y. Wang, Z. Zhang, R. Yao, D. Jia, D. Wang, R.A. Lubet, M. You, Prevention of lung
472 cancer progression by bexarotene in mouse models, Oncogene 25 (2006) 1320-1329.
473 [35] Y. Wang, W. Wen, Y. Yi, Z. Zhang, R.A. Lubet, M. You, Preventive effects of bexarotene
474 and budesonide in a genetically engineered mouse model of small cell lung cancer, Cancer
475 Prev. Res. (Phila) 2 (2009) 1059-1064.
476 [36] H.T. Kim, G. Kong, D. Denardo, Y. Li, I. Uray, S. Pal, S. Mohsin, S.G. Hilsenbeck, R.
477 Bissonnette, W.W. Lamph, K. Johnson, P.H. Brown, Identification of biomarkers modulated
478 by the rexinoid LGD1069 (bexarotene) in human breast cells using oligonucleotide arrays,
479 Cancer Res. 66 (2006) 12009-12018.
480 [37] N.B. Janakiram, A. Mohammed, L. Qian, C.-I. Choi, V.E. Steele, C.V. Rao,
481 Chemopreventive Effects of RXR-Selective Rexinoid Bexarotene on Intestinal Neoplasia of
482 ApcMin/+ Mice, Neoplasia 14 (2012) 159-168.
483 [38] L. Xu, F. Cao, F. Xu, B. He, Z. Dong, Bexarotene reduces blood-brain barrier permeability
484 in cerebral ischemia-reperfusion injured rats, PLoS One 10 (2015) e0122744.
485 [39] K. McFarland, T.A. Spalding, D. Hubbard, J.N. Ma, R. Olsson, E.S. Burstein, Low dose
486 bexarotene treatment rescues dopamine neurons and restores behavioral function in models of
487 Parkinson's disease, ACS Chem. Neurosci. 4 (2013) 1430-1438.
488 [40] F. Lalloyer, C. Fievet, S. Lestavel, G. Torpier, J. van der Veen, V. Touche, S. Bultel, S.
489 Yous, F. Kuipers, R. Paumelle, J.C. Fruchart, B. Staels, A. Tailleux, The RXR agonist
490 bexarotene improves cholesterol homeostasis and inhibits atherosclerosis progression in a
491 mouse model of mixed dyslipidemia, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 2731-2737.

492 [41] F. Lalloyer, T.A. Pedersen, B. Gross, S. Lestavel, S. Yous, E. Vallez, J.A. Gustafsson, S.
493 Mandrup, C. Fievet, B. Staels, A. Tailleux, Rexinoid bexarotene modulates triglyceride but not
494 cholesterol metabolism via gene-specific permissivity of the RXR/LXR heterodimer in the
495 liver, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 1488-1495.
496 [42] L. Chen, Y. Wang, J. Zhang, L. Hao, H. Guo, H. Lou, D. Zhang, Bexarotene nanocrystal-
497 Oral and parenteral formulation development, characterization and pharmacokinetic evaluation,
498 Eur. J. Pharm. Biopharm. 87 (2014) 160-169.
499 [43] L. Li, Y. Liu, J. Wang, L. Chen, W. Zhang, X. Yan, Preparation, in vitro and in vivo
500 evaluation of bexarotene nanocrystals with surface modification by folate-chitosan conjugates,
501 Drug Deliv. 23 (2016) 79-87.
502 [44] N.C. van de Merbel, J.H. van Veen, G. Wilkens, G. Loewen, Validated liquid
503 chromatographic method for the determination of bexarotene in human plasma, J. Chromatogr.
504 B: Anal. Technol. Biomed. Life Sci. 775 (2002) 189-195.
505 [45] U.S. Food and Drug Administration, Guidance for Industry, Bioanalytical Method
506 Validation, (2001).
507 [46] V.A. Miller, F.M. Benedetti, J.R. Rigas, A.L. Verret, D.G. Pfister, D. Straus, M.G. Kris,
508 M. Crisp, R. Heyman, G.R. Loewen, J.A. Truglia, R.P. Warrell, Jr., Initial clinical trial of a
509 selective retinoid X receptor ligand, LGD1069, J. Clin. Oncol. 15 (1997) 790-795.
510 [47] S.R. Howell, M.A. Shirley, T.A. Grese, D.A. Neel, K.E. Wells, E.H. Ulm, Bexarotene
511 metabolism in rat, dog, and human, synthesis of oxidative metabolites, and in vitro activity at
512 retinoid receptors, Drug Metab. Dispos. 29 (2001) 990-998.
513 [48] P. Gershkovich, B. Qadri, A. Yacovan, S. Amselem, A. Hoffman, Different impacts of
514 intestinal lymphatic transport on the oral bioavailability of structurally similar synthetic
515 lipophilic cannabinoids: dexanabinol and PRS-211,220, Eur. J. Pharm. Sci. 31 (2007) 298-305.

[49] P. Gershkovich, F. Ibrahim, O. Sivak, J.W. Darlington, K.M. Wasan, A simple and sensitive method for determination of vitamins D3 and K1 in rat plasma: application for an in vivo pharmacokinetic study, Drug. Dev. Ind. Pharm. 40 (2014) 338-344.

[50] A. Zgair, J.C. Wong, A. Sabri, P.M. Fischer, D.A. Barrett, C.S. Constantinescu, P. Gershkovich, Development of a simple and sensitive HPLC-UV method for the simultaneous determination of cannabidiol and Delta(9)-tetrahydrocannabinol in rat plasma, J. Pharm. Biomed. Anal. 114 (2015) 145-151.

FIGURE CAPTIONS

Figure 1. Representative chromatograms from rat plasma. **A**, Rat plasma spiked with 10 μg/mL of IS; **B**, rat plasma spiked with 10 ng/mL bexarotene and 10 μg/mL of IS (LLOQ sample); **C**, rat plasma spiked with 100 ng/mL bexarotene and 10 μg/mL of IS (calibration curve sample); **D**, rat plasma sample obtained in pharmacokinetic study 1 h following intravenous bolus administration of 2.5 mg/kg bexarotene. All observed at λ = 259 nm.

Figure 2. Plasma concentration-time profiles of bexarotene following intravenous bolus administration at 2.5 mg/kg in rats (n = 3).

Figure 3. Representative chromatograms from human plasma. **A**, blank human plasma observed at λ = 259 nm; **B**, human plasma spiked with 400 ng/mL bexarotene observed at λ = 259 nm (MQC sample); **C**, blank human plasma observed at λ = 220 nm; **D**, human plasma spiked with 10 μg/mL of IS observed at λ = 220 nm.

Figure 4. Representative chromatograms from mouse plasma. **A**, blank mouse plasma; **B**, mouse plasma spiked with 400 ng/mL bexarotene and 10 μg/mL of IS (MQC sample). All observed at λ = 259 nm.

Table 1. Intra- and inter-day validation results of the analytical method of bexarotene in rat plasma using HPLC-UV (n = 6)

	Intra-day		Inter-day	
Concentration levels	**Accuracy (RE, %)**	**Precision (RSD, %)**	**Accuracy (RE, %)**	**Precision (RSD, %)**
LLOQ (10 ng/mL)	4.67	6.10	-2.73	14.30
LQC (25 ng/mL)	-5.06	10.00	-0.27	7.57
MQC (400 ng/mL)	7.57	3.00	-0.82	12.27
HQC (8000 ng/mL)	7.93	3.50	0.54	9.63

LLOQ, lower limit of quantification; LQC, low quality control; MQC, medium quality control; HQC, high quality control.

Table 2. Stability results of rat plasma samples of bexarotene under various storage conditions (n = 6)

	Benchtop stability (25°C, 6 h)		**Short term stability (-20°C, 24 h)**		**Autosampler stability (4°C, 24 h)**		**Freeze-thaw stability (-80°C, 3 cycles)**		**Stock solution stability (25°C, 6 h)**		**Long term stability (-80°C, 2 wks)**		**Long term stability (-80°C, 4 wks)**		**Long term stability (-80°C, 8 wks)**	
Concentration levels	**RE (%)**	**RSD (%)**	**RE (%)**	**RSD (%)**	**RE (%)**	**RSD (%)**	**RE (%)**	**RSD (%)**	**RE (%)**	**RSD (%)**	**RE (%)**	**RSD (%)**	**RE (%)**	**RSD (%)**	**RE (%)**	**RSD (%)**
LQC (25 ng/mL)	0.14	7.05	-3.99	3.78	7.88	4.27	-2.86	13.14	-4.14	4.08	-10.18	6.78	-8.21	4.07	-3.73	6.49
MQC (400 ng/mL)	7.81	4.84	-2.41	10.46	12.20	2.69	-1.83	12.17	-2.48	1.68	-13.94	5.72	-7.91	5.52	-8.32	2.64
HQC (8000 ng/mL)	7.58	6.07	-1.67	6.63	-5.83	5.75	-3.30	8.78	-8.53	11.56	-11.39	9.79	-1.65	6.54	-7.61	2.50

RE, relative error; RSD, relative standard deviation; LQC, low quality control; MQC, medium quality control; HQC, high quality control.

Table 3. Pharmacokinetic parameters of bexarotene obtained following intravenous bolus administration of 2.5 mg/kg bexarotene in rats (n = 3)

Parameters	Mean	SD
$AUC_{0\rightarrow t}$ (h·ng/mL)	4631	453
AUC_{inf} (h·ng/mL)	4668	452
C_0 (ng/mL)	6219	1068
$t_{1/2}$ (h)	1.15	0.02
V_{ss} (mL/kg)	734	104
CL (mL/h/kg)	539	55

AUC_{inf}, area under the curve from time zero to infinity; $AUC_{0\rightarrow t}$, area under the curve from time zero to the last sampling time point; C_0, concentration extrapolated to time zero; $t_{1/2}$, half-life; V_{ss}, volume of distribution at steady state; CL, clearance.

Table 4. Intra-day validation results of the analytical method of bexarotene in human and mouse plasma using HPLC-UV (n = 6)

Concentration levels	Human plasma		Mouse plasma	
	Accuracy (RE, %)	**Precision (RSD, %)**	**Accuracy (RE, %)**	**Precision (RSD, %)**
LQC (25 ng/mL)	-2.67	8.52	5.59	7.08
MQC (400 ng/mL)	-1.69	13.04	10.75	5.87
HQC (8000 ng/mL)	-5.49	14.65	6.96	3.54

RE, relative error; RSD, relative standard deviation; LQC, low quality control; MQC, medium quality control; HQC, high quality control.