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NOVEL FORMS OF BENDAMUSTINE FREE BASE

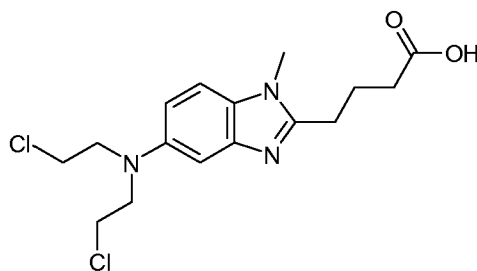
FIELD OF THE INVENTION

10 This invention pertains to bendamustine free base-containing compositions, pharmaceutical compositions comprising bendamustine free base, processes to reproducibly make them, and methods of treating patients using them.

BACKGROUND OF THE INVENTION

15 Active pharmaceutical ingredients (APIs) can be prepared in a variety of different forms, for example, chemical derivatives, solvates, hydrates, co-crystals, or salts. APIs may also be amorphous, may have different crystalline polymorphs, or may exist in different solvation or hydration states. By varying the form of an API, it is possible to vary the physical properties thereof. For instance, crystalline polymorphs typically have different solubilities such that a more thermodynamically stable polymorph is less soluble
20 than a less thermodynamically stable polymorph. Polymorphs can also differ in properties such as shelf-life, bioavailability, morphology, vapor pressure, density, color, and compressibility. Accordingly, variation of the crystalline state of an API is one of many ways in which to modulate the physical and pharmacological properties thereof.

25 Bendamustine, 4-{5-[Bis(2-chloroethyl)amino]-1-methyl-2-benzimidazolyl} butyric acid :



Bendamustine

was initially synthesized in 1963 in the German Democratic Republic (GDR) and was
5 available from 1971 to 1992 there, as the hydrochloride salt, under the tradename
Cytostasan®. Since that time, it has been marketed in Germany under the tradename
Ribomustin®. Ribomustin® is an amorphous, non-crystalline powder. Bendamustine
Hydrochloride for injection is available in the United States under the tradename
Treanda®.

10 Bendamustine is an alkylating agent that has been shown to have therapeutic utility
in treating diseases such as chronic lymphocytic leukemia, Hodgkin's disease, non-
Hodgkin's lymphoma, multiple myeloma, and breast cancer. It is supplied as a
lyophilized cake of bendamustine hydrochloride salt. Immediately before treatment, the
lyophilized cake is dissolved in a pharmaceutically acceptable diluent, preferably Sodium
15 Chloride for Injection.

It is generally desirable that injectable compositions be supplied as the free base
form, rather than a salt form, to minimize any side effects that the counterion can produce.
Previously described forms of bendamustine free base, however, were unstable and not
suitable for commercial preparation, distribution, and administration. As a result, stable
20 forms of bendamustine free base are needed.

SUMMARY OF THE INVENTION

Fifteen novel, crystalline polymorphic forms of bendamustine free base are
described herein. Pharmaceutical compositions comprising one or more of the described
polymorphic forms of bendamustine free base are also described, as well as
25 pharmaceutical compositions further comprising amorphous bendamustine free base.
Methods of using the described forms and compositions for the treatment of leukemia,
lymphoma, myeloma, and breast cancer, for example, are also described.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an X-Ray Powder Diffractogram (XRPD) spectrum of bendamustine free base Form 1.

FIG. 1A Differential Scanning Calorimetry/Thermogravimetric Analysis

5 (DSC/TGA) Overlay Data for Form 1.

FIG. 2 is an XRPD spectrum of bendamustine free base Form 2.

FIG. 3 is an overlay of the DSC/TGA data for bendamustine free base Form 2.

FIG. 4 is an XRPD spectrum of bendamustine free base Form 3.

FIG. 5 is an overlay of the DSC/TGA data for bendamustine free base Form 3.

10 FIG. 6 is an XRPD spectrum of bendamustine free base Form 4.

FIG. 7 is an XRPD spectrum of bendamustine free base Form 5.

FIG. 8 is an overlay of the DSC/TGA data for bendamustine free base Form 5.

FIG. 9 is an XRPD spectrum of bendamustine free base Form 6.

FIG. 10 is an overlay of the DSC/TGA data for bendamustine free base Form 6

15 FIG. 11 is an XRPD spectrum of bendamustine free base Form 7.

FIG. 12 is an overlay of the DSC/TGA data for bendamustine free base Form 7.

FIG. 13 is an XRPD spectrum of bendamustine free base Form 8.

FIG. 14 is an overlay of the DSC/TGA data for bendamustine free base Form 8.

FIG. 15 is an XRPD spectrum of bendamustine free base Form 9.

20 FIG. 16 is an overlay of the DSC/TGA data for bendamustine free base Form 9.

FIG. 17 is an XRPD spectrum of bendamustine free base Form 10.

FIG. 18 is an overlay of the DSC/TGA data for bendamustine free base Form 10.

FIG. 19 is an XRPD spectrum of bendamustine free base Form 11.

FIG. 20 is an overlay of the DSC/TGA data for bendamustine free base Form 11.

25 FIG. 21 is an XRPD spectrum of bendamustine free base Form 12.

FIG. 22 is an overlay of the DSC/TGA data for bendamustine free base Form 12.

FIG. 23 is an XRPD spectrum of bendamustine free base Form 13.

FIG. 24 is an overlay of the DSC/TGA data for bendamustine free base Form 13.

FIG. 25 is an XRPD spectrum of bendamustine free base Form 14.

30 FIG. 26 is an overlay of the DSC/TGA data for bendamustine free base Form 14.

FIG. 27 is an XRPD spectrum of bendamustine free base Form 15.

FIG. 28 is TGA data for bendamustine free base Form 15, with a sample size of 1.4590 mg, running from ambient to 200 °C at 10 °C per minute.

FIG. 29 is a ¹H NMR of bendamustine free base Form 15.

FIG. 30 is a Variable Temperature XRPD for Form 3.

FIG. 31 is an XPPD spectrum of amorphous bendamustine free base.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Stable forms of bendamustine free base have now been discovered. Six polymorphic forms of anhydrous bendamustine free base are described herein (Form 1, Form 2, Form 4, Form 6, Form 7, Form 10), as well as four hydrate forms (Form 3, Form 5, Form 13, Form 14) and four solvate forms of bendamustine free base (Form 8, Form 9, Form 11, Form 12, Form 15). Amorphous bendamustine free base is also within the scope of the present invention.

In preferred embodiments are crystalline forms of bendamustine free base that are Form 1, Form 2, Form 3, Form 4, Form 5, Form 6, Form 7, Form 8, Form 9, Form 10, Form 11, Form 12, Form 13, Form 14, Form 15, or mixtures thereof. These polymorphs may be identified by X-ray powder diffraction and characterized by one, two, three, four, five or more reflection peaks that are characteristic of each polymorphic form. The crystalline polymorphs (Form 1, Form 2, Form 3, Form 4, Form 5, Form 6, Form 7, Form 8, Form 9, Form 10, Form 11, Form 12, Form 13, Form 14, Form 15) can also be identified by reference to their X-ray powder diffractograms (XRPD), Differential Scanning Calorimetry (DSC) thermograms, Thermogravimetric Analysis (TGA) thermograms, and/or Gravimetric Vapor Sorption (GVS) traces, which are set forth in FIGS. 1-29. Methods of making each polymorph, or a mixture of polymorphs, can be preformed using the techniques described herein.

In another embodiment of the invention are pharmaceutical compositions comprising Form 1, Form 2, Form 3, Form 4, Form 5, Form 6, Form 7, Form 8, Form 9, Form 10, Form 11, Form 12, Form 13, Form 14, Form 15, or mixtures thereof, of bendamustine free base. In other embodiments of the invention are pharmaceutical compositions comprising one or more of Form 1, Form 2, Form 3, Form 4, Form 5, Form 6, Form 7, Form 8, Form 9, Form 10, Form 11, Form 12, Form 13, Form 14, or Form 15 with amorphous (*i.e.*, non-crystalline) bendamustine.

In yet another embodiment of the invention are pharmaceutical compositions comprising Form 1, Form 2, Form 3, Form 4, Form 5, Form 6, Form 7, Form 8, Form 9, Form 10, Form 11, Form 12, Form 13, Form 14, Form 15, or mixtures thereof, of bendamustine free base, and at least one pharmaceutically acceptable excipient or carrier. In other embodiments of the invention are pharmaceutical compositions comprising one or

more of Form 1, Form 2, Form 3, Form 4, Form 5, Form 6, Form 7, Form 8, Form 9, Form 10, Form 11, Form 12, Form 13, Form 14, or Form 15 with amorphous (*i.e.*, non-crystalline) bendamustine, and at least one pharmaceutically acceptable excipient or carrier.

Techniques such as thermal analysis (DSC, TGA/MS) and X-ray diffraction are well established for both characterization and quantification of different crystalline forms. It is generally accepted that complementary analytical techniques should be used to properly characterize all different crystalline forms. A series of crystallization experiments were performed in order to explore the effects of solvent, crystallization method and temperature in order to identify and characterize the polymorphs and solvates of bendamustine free base.

Form 1 bendamustine free base can be prepared by slurrying Form 2 bendamustine free base in 2-butanone, acetonitrile, cyclohexane, ethyl acetate, methyl tert-butyl ether, n-butyl acetate, propionitrile, or tetrahydropyran, heating the sample, slow cooling the sample, and isolating the solids. Alternatively, Form 1 bendamustine free base can be prepared by slurrying Form 2 bendamustine free base in 1-butanol, 1,4-dioxane, 1-propanol, acetone, chloroform, cyclohexane, ethanol, methyl isobutyl ketone, methyl tert-butyl ether, n-butyl acetate, propionitrile, tetrahydropyran, or toluene. The sample was slurried for 48 hours with alternating 4 hour periods at 50 °C and 5 °C and the solid isolated. The X-ray diffraction pattern characteristic of the crystalline Form 1 is shown in Table 1 and FIG. 1.

Table 1: Most Important Two Theta Positions (2θ), D -spacings (d) and Relative Intensities (I) of XRPD of Form 1

No.	Pos. [2θ]	d -spacing [$\text{\AA}$]	Rel. Int. [%]	No.	Pos. [2θ]	d -spacing [$\text{\AA}$]	Rel. Int. [%]
1	9.54	9.27	12	16	22.67	3.92	51
2	10.34	8.55	62	17	24.03	3.70	100
3	10.40	8.50	45	18	24.88	3.58	24
4	13.12	6.74	12	19	25.77	3.45	20
5	15.93	5.56	43	20	26.03	3.42	51
6	16.69	5.31	45	21	27.64	3.22	21
7	16.84	5.26	36	22	27.80	3.21	17
8	17.52	5.06	33	23	28.43	3.14	73
9	19.59	4.53	30	24	28.54	3.12	20
10	20.53	4.32	49	25	29.50	3.03	72
11	20.77	4.27	29	26	30.62	2.92	36
12	20.80	4.27	25	27	30.70	2.91	17
13	21.48	4.13	15	28	33.70	2.66	15
14	21.97	4.04	25	29	36.73	2.44	15
15	22.30	3.98	71	30	36.77	2.44	14

Form 2 bendamustine free base can be prepared from bendamustine hydrochloride. Treatment of an aqueous solution of bendamustine hydrochloride with NaOH (1M in water), causes Form 2 bendamustine free base to precipitate and the precipitate can be filtered to isolate Form 2. The X-ray diffraction pattern characteristic of the crystalline Form 2 is shown in Table 2 and FIG. 2.

Table 2: Most Important Two Theta Positions (2θ), D -spacings (d) and Relative Intensities (I) of XRPD of Form 2

No.	Pos. [2θ .]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
1	10.28	8.60	100
2	12.54	7.05	8
3	13.51	6.55	21
4	15.40	5.75	20
5	19.81	4.48	5
6	20.59	4.31	60
7	21.55	4.12	26
8	21.69	4.09	29
9	22.39	3.97	11
10	24.78	3.59	11
11	26.65	3.34	9
12	27.62	3.23	5
13	31.36	2.85	5
14	36.38	2.47	5
15	37.51	2.40	6

Form 3 bendamustine free base can be obtained by the base-mediated hydrolysis of bendamustine ethyl ester. The X-ray diffraction pattern characteristic of the crystalline Form 3 is shown in Table 3 and FIG. 4.

Table 3: Most Important Two Theta Positions (2θ), D -spacings (d) and Relative Intensities (I) of XRPD of Form 3

No.	Pos. [2θ .]	d-spacing [$\text{\AA}$]	Rel. Int. [%]	No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
1	4.78	18.46	5	16	27.63	3.23	6
2	9.41	9.40	100	17	27.86	3.20	6
3	9.46	9.34	88	18	31.71	2.82	8
4	14.15	6.26	30				
5	15.47	5.72	14				
6	18.78	4.72	11				
7	19.50	4.55	14				
8	20.31	4.37	5				
9	21.24	4.18	21				
10	22.12	4.02	15				
11	23.42	3.79	28				
12	23.65	3.76	32				
13	24.04	3.70	10				
14	24.83	3.58	34				
15	25.79	3.45	6				

Form 4 bendamustine free base can be obtained by exposing Form 3 bendamustine free base to 0% relative humidity. The X-ray diffraction pattern characteristic of the crystalline Form 4 is shown in Table 4 and FIG. 6.

Table 4: Most Important Two Theta Positions (2θ), D -spacings (d) and Relative Intensities (I) of XRPD for Form 4

No.	Pos. [2θ .]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
1	9.88	8.96	100
2	14.88	5.95	15
3	15.13	5.86	35
4	18.05	4.91	6
5	19.44	4.57	12
6	19.92	4.46	23
7	20.70	4.29	7
8	20.91	4.25	8
9	22.99	3.87	17
10	24.72	3.60	35
11	24.98	3.56	31

Form 5 bendamustine free base can be obtained by exposing Form 3 bendamustine free base to about 85% relative humidity for about 1 day. The X-ray diffraction pattern characteristic of the crystalline Form 5 is shown in Table 5 and FIG. 7.

Table 5: Most Important Two Theta Positions (2θ), D -spacings (d) and Relative Intensities (I) of XRPD of Form 5

No.	Pos. [2θ .]	d-spacing [$\text{\AA}$]	Rel. Int. [%]	No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
1	8.94	9.88	100	16	23.74	3.75	14
2	12.07	7.33	5	17	23.96	3.71	13
3	13.39	6.61	71	18	24.18	3.68	11
4	14.84	5.96	8	19	25.37	3.51	19
5	16.04	5.52	58	20	25.82	3.45	6
6	16.28	5.44	8	21	26.95	3.31	6
7	17.20	5.15	5	22	27.38	3.26	11
8	17.90	4.95	44	23	28.37	3.14	12
9	18.16	4.88	13	24	29.75	3.00	10
10	19.29	4.60	29	25	31.57	2.83	10
11	20.76	4.28	12	26	31.96	2.80	5
12	21.31	4.17	49	27	32.81	2.73	13
13	21.50	4.13	16				
14	22.38	3.97	99				
15	23.41	3.80	9				

Form 6 bendamustine free base can be prepared by slurrying Form 3 bendamustine free base in 1-butanol or 1-propanol, heating the sample, slow cooling the sample, and then isolating the solids. Alternatively, Form 6 bendamustine free base can be prepared by slurrying Form 3 bendamustine free base in 1-butanol, exposing the sample to alternative

4 hour periods at 50 °C and 5 °C, and isolating the solids. The X-ray diffraction pattern characteristic of the crystalline Form 6 is shown in Table 6 and FIG. 9.

Table 6: Most Important Two Theta Positions (2 θ), *D*-spacings (*d*) and Relative Intensities (*I*) of XRPD of Form 6

No.	Pos. [2 θ .]	d-spacing [Å]	Rel. Int. [%]	No.	Pos. [2 θ]	d-spacing [Å]	Rel. Int. [%]
1	8.67	10.19	98	12	26.21	3.40	42
2	10.29	8.59	18	13	27.74	3.21	44
3	11.48	7.70	61	14	28.68	3.11	41
4	14.23	6.22	41	15	29.88	2.99	16
5	17.23	5.14	48	16	31.57	2.83	18
6	18.15	4.88	78	17	34.62	2.59	40
7	19.40	4.57	48				
8	20.94	4.24	60				
9	22.55	3.94	100				
10	22.95	3.87	52				
11	25.46	3.50	98				

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Form 7 bendamustine free base can be prepared by slurrying Form 3 bendamustine free base in N,N dimethylformamide or isopropyl acetate. The samples were heated, slow cooled, and the solid isolated. Alternatively, Form 7 bendamustine free base can be prepared by slurrying Form 3 bendamustine free base in N,N dimethylformamide, exposing the sample to alternating 4 hour periods at 50 °C and 5 °C, and isolating the solids. The X-ray diffraction pattern characteristic of the crystalline Form 7 is shown in Table 7 and FIG. 11.

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Table 7: Most Important Two Theta Positions (2 θ), *D*-spacings (*d*) and Relative Intensities (*I*) of XRPD of Form 7

No.	Pos. [2 θ .]	d-spacing [Å]	Rel. Int. [%]	No.	Pos. [2 θ]	d-spacing [Å]	Rel. Int. [%]
1	7.06	12.51	6	12	22.87	3.89	13
2	8.51	10.39	95	13	23.29	3.82	36
3	8.56	10.32	100	14	24.04	3.70	24
4	11.42	7.74	20	15	25.13	3.54	7
5	14.23	6.22	25	16	27.39	3.25	12
6	17.10	5.18	13	17	28.09	3.17	17
7	17.62	5.03	17	18	28.94	3.08	18
8	17.97	4.93	25	19	32.33	2.77	14
9	20.53	4.32	10	20	34.50	2.60	16
10	21.25	4.18	49	21	36.31	2.47	20
11	22.08	4.02	13				

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Form 8 bendamustine free base can be prepared by slurrying Form 3 bendamustine free base in ethanol, exposing the sample to alternating 4 hour periods at 50 °C and 5 °C, and isolating the solids. The X-ray diffraction pattern characteristic of the crystalline Form 8 is shown in Table 8 and FIG. 13.

Table 8: Most Important Two Theta Positions (2θ), D -spacings (d) and Relative Intensities (I) of XRPD of Form 8

No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]	No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
1	5.25	16.82	9	16	23.33	3.81	5
2	8.60	10.27	5	17	23.88	3.72	6
3	9.34	9.46	12	18	24.36	3.65	8
4	10.45	8.46	45	19	24.98	3.56	95
5	11.17	7.91	58	20	25.56	3.48	7
6	13.97	6.34	9	21	26.40	3.37	67
7	15.32	5.78	60	22	27.58	3.23	16
8	15.72	5.63	35	23	30.87	2.89	20
9	16.25	5.45	5	24	35.31	2.54	12
10	17.16	5.16	9				
11	20.22	4.39	9				
12	21.01	4.23	34				
13	21.30	4.17	31				
14	21.88	4.06	10				
15	22.48	3.95	100				

Form 9 bendamustine free base can be prepared by heating Form 2 bendamustine free base in 3-pentanone, slow cooling the sample, and isolating the solids. The X-ray diffraction pattern characteristic of the crystalline Form 9 is shown in Table 9 and FIG. 15.

Table 9: Most Important Two Theta Positions (2θ), D -spacings (d) and Relative Intensities (I) of XRPD of Form 9

No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]	No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
1	4.44	19.88	28	16	21.89	4.06	14
2	8.88	9.95	22	17	22.32	3.98	100
3	9.13	9.68	28	18	22.66	3.92	25
4	12.67	6.98	15	19	22.98	3.87	29
5	13.34	6.63	44	20	23.45	3.79	27
6	16.56	5.35	32	21	23.69	3.75	18
7	16.73	5.30	64	22	24.80	3.59	24
8	17.50	5.06	5	23	25.92	3.43	9
9	17.82	4.97	23	24	26.28	3.39	17
10	18.31	4.84	13	25	26.80	3.32	11
11	19.15	4.63	14	26	29.26	3.05	5
12	19.33	4.59	18	27	30.71	2.91	8
13	19.54	4.54	43	28	31.38	2.85	13
14	20.36	4.36	10	29	31.64	2.83	9
15	20.90	4.25	8	30	36.09	2.49	10

Form 10 bendamustine free base can be prepared by heating Form 2 bendamustine free base in toluene, slow cooling the sample, and isolating the solids. The X-ray diffraction pattern characteristic of the crystalline Form 10 is shown in Table 10 and FIG. 17.

Table 10: Most Important Two Theta Positions (2θ), D -spacings (d) and Relative Intensities (I) of XRPD of Form 10

No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]	No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
1	9.76	9.05	11	16	22.87	3.89	50
2	10.59	8.34	14	17	24.03	3.70	51
3	16.13	5.49	35	18	24.24	3.67	100
4	16.82	5.27	65	19	25.08	3.55	9
5	16.93	5.23	85	20	25.75	3.46	23
6	17.06	5.19	44	21	26.27	3.39	17
7	17.72	5.00	13	22	26.58	3.35	12
8	18.90	4.69	15	23	27.85	3.20	29
9	19.31	4.59	13	24	28.03	3.18	18
10	19.68	4.51	51	25	28.63	3.12	32
11	19.83	4.47	41	26	29.72	3.00	37
12	20.72	4.28	26	27	30.70	2.91	26
13	21.67	4.10	17	28	30.82	2.90	26
14	22.14	4.01	35	29	33.90	2.64	18
15	22.51	3.95	22	30	37.71	2.38	17

Form 11 bendamustine free base can be prepared by slurring Form 3

- 5 bendamustine free base in 1-butanol, 1,4-dioxane, or isopropyl acetate at ambient temperature for about 48 hours and isolating the solids. The X-ray diffraction pattern characteristic of the crystalline Form 11 is shown in Table 11 and FIG. 19.

Table 11: Most Important Two Theta Positions (2θ), D -spacings (d) and Relative Intensities (I) of XRPD of Form 11

No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]	No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
1	4.64	19.02	10	16	22.36	3.97	100
2	8.90	9.93	86	17	23.33	3.81	73
3	9.28	9.52	94	18	24.02	3.70	7
4	13.36	6.62	59	19	24.72	3.60	20
5	13.94	6.35	87	20	25.38	3.51	24
6	15.11	5.86	7	21	25.79	3.45	10
7	16.01	5.53	39	22	26.05	3.42	10
8	17.15	5.17	5	23	26.92	3.31	7
9	17.86	4.96	33	24	27.36	3.26	8
10	18.22	4.86	11	25	28.38	3.14	14
11	18.59	4.77	8	26	29.71	3.00	9
12	19.30	4.59	26	27	31.53	2.84	11
13	20.03	4.43	5	28	32.77	2.73	18
14	20.76	4.28	13				
15	21.29	4.17	51				

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Form 12 bendamustine free base can be prepared by slurring Form 3

bendamustine free base in N,N dimethylformamide at ambient temperature for about 48 hours and isolating the solids. The X-ray diffraction pattern characteristic of the crystalline Form 12 is shown in Table 12 and FIG. 21.

Table 12: Most Important Two Theta Positions (2θ), D -spacings (d) and Relative Intensities (I) of XRPD of Form 12

No.	Pos. [2θ .]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
1	4.71	18.76	10
2	8.57	10.31	5
3	9.31	9.49	99
4	9.35	9.45	87
5	13.97	6.33	100
6	14.03	6.31	82
7	18.68	4.75	9
8	20.79	4.27	5
9	21.14	4.20	9
10	22.20	4.00	5
11	22.80	3.90	5
12	23.38	3.80	80
13	24.75	3.59	11
14	25.39	3.51	6
15	26.06	3.42	7

Form 13 can be prepared by heating Form 3 bendamustine free base in methyl tert-butyl ether or 3-pentanone, slow cooling the sample, and isolating the solids. The X-ray diffraction pattern characteristic of the crystalline Form 13 is shown in Table 13 and FIG. 23.

Table 13: Most Important Two Theta Positions (2θ), D -spacings (d) and Relative Intensities (I) of XRPD of Form 13

No.	Pos. [2θ .]	d-spacing [$\text{\AA}$]	Rel. Int. [%]	No.	Pos. [2θ .]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
1	4.99	17.68	6	16	25.17	3.54	12
2	9.84	8.98	85	17	26.02	3.42	11
3	12.51	7.07	18	18	26.91	3.31	20
4	12.85	6.89	34	19	27.36	3.26	5
5	14.76	6.00	100	20	28.30	3.15	13
6	15.38	5.76	5	21	28.71	3.11	6
7	16.89	5.25	8	22	29.81	2.99	11
8	17.29	5.12	6	23	30.79	2.90	18
9	19.67	4.51	76	24	37.06	2.42	5
10	20.32	4.37	9				
11	21.35	4.16	22				
12	21.67	4.10	14				
13	22.21	4.00	20				
14	23.20	3.83	19				
15	24.62	3.61	40				

Form 14 bendamustine free base can be prepared by slurrying Form 3 bendamustine free base in acetonitrile at ambient temperature for about 48 hours and isolating the solids. The X-ray diffraction pattern characteristic of the crystalline Form 14 is shown in Table 14 and FIG. 25.

Table 14: Most Important Two Theta Positions (2θ), D -spacings (d) and Relative Intensities (I) of XRPD of Form 14

No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]	No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
1	8.74	10.11	74	16	27.16	3.28	18
2	9.14	9.67	70	17	28.15	3.17	7
3	11.89	7.44	7	18	29.57	3.02	11
4	13.20	6.70	44	19	31.44	2.84	18
5	13.68	6.47	45	20	32.59	2.75	19
6	14.69	6.02	20				
7	15.85	5.59	50				
8	17.72	5.00	45				
9	19.19	4.62	70				
10	21.13	4.20	100				
11	22.10	4.02	88				
12	23.12	3.84	79				
13	23.61	3.76	36				
14	24.59	3.62	25				
15	25.28	3.52	31				

Form 15 bendamustine free base can be prepared by recrystallization of Form 1
 5 bendamustine free base in tetrahydrofuran and isolating the solids. The X-ray diffraction pattern characteristic of the crystalline Form 15 is shown in Table 15 and FIG. 27.

Table 15: Most Important Two Theta Positions (2θ), D -spacings (d) and Relative Intensities (I) of XRPD of Form 15

No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]	No.	Pos. [2θ]	d-spacing [$\text{\AA}$]	Rel. Int. [%]
1	4.09	21.60	59	16	29.43	3.03	17
2	8.10	10.91	63				
3	10.26	8.62	38				
4	12.15	7.28	5				
5	16.46	5.38	6				
6	17.36	5.11	9				
7	17.96	4.94	10				
8	19.92	4.46	28				
9	20.58	4.32	14				
10	22.19	4.01	37				
11	23.83	3.73	27				
12	24.56	3.62	100				
13	26.01	3.43	25				
14	26.83	3.32	16				
15	28.39	3.14	30				

10 Also within the scope of the invention are pharmaceutical compositions comprising amorphous, non-crystalline bendamustine free base. The bendamustine free base may be provided as compositions consisting primarily of an amorphous form of bendamustine free base or as compositions comprising amorphous bendamustine free base as well as at least one crystalline form, such as crystalline bendamustine free base Form 1,

Form 2, Form 3, Form 4, Form 5, Form 6, Form 7, Form 8, Form 9, Form 10, Form 11, Form 12, Form 13, Form 14, Form 15, or mixtures thereof.

In preferred embodiments, pharmaceutical compositions comprising at least one of Form 1, Form 2, Form 3, Form 4, Form 5, Form 6, Form 7, Form 8, Form 9, Form 10, 5 Form 11, Form 12, Form 13, Form 14, or Form 15, and optionally, amorphous bendamustine free base, as well as at least one pharmaceutically acceptable excipient, are provided. Pharmaceutically acceptable excipients are known in the art and include those described in, for example, U.S. Application No. 11/267,010. These pharmaceutical compositions may be prepared as injectables, either as liquid solutions or suspensions, as 10 well as solid forms, for example, capsules, tablets, lozenges, pastilles, powders, suspensions, and the like.

In preferred embodiments, the pharmaceutical compositions are sublimed, preferably freeze-dried or lyophilized, compositions. In other embodiments, methods of preparing such sublimed, preferably freeze-dried or lyophilized, preparations of 15 bendamustine free base that contain Form 1, Form 2, Form 3, Form 4, Form 5, Form 6, Form 7, Form 8, Form 9, Form 10, Form 11, Form 12, Form 13, Form 14, Form 15, or a mixture thereof, are provided. Lyophilization involves the addition of water to a compound, followed by freezing of the resultant suspension or solution, and sublimation of the water from the compound. In preferred embodiments, at least one organic solvent is 20 added to the suspension/solution. In other preferred embodiments, the suspension/solution further comprises a lyophilization excipient. The lyophilized preparations of bendamustine free base of the present invention may further comprise amorphous bendamustine free base.

In a typical lyophilization procedure, water, a pharmaceutically acceptable 25 lyophilizing excipient, an organic solvent, and bendamustine free base are combined, preferably under sterile conditions, to form a solution. Preferably, the solution is prepared and sterilized by filtration. This solution is then lyophilized using standard, sterile lyophilization equipment.

While preferred embodiments of the present invention include lyophilization of 30 bendamustine free base, it is envisioned that other sublimation techniques may also be used. For example, one or more of the described forms of bendamustine free base may be dissolved, dispersed or suspended in a solvent, the resulting mixture (be it a solution, dispersion or suspension) frozen, and the solvent removed by sublimation.

A lyophilization excipient can be any pharmaceutically acceptable excipient that, when used during the lyophilization process, results in a lyophilized product that has improved properties, for example, improved handling properties, solubility properties, and the like. A lyophilization excipient can be, for example, a bulking agent; suitable bulking agents are known in the art. Examples of suitable lyophilization excipients include, for example, sodium phosphate, potassium phosphate, citric acid, tartaric acid, gelatin, glycine, mannitol, lactose, sucrose, maltose, glycerin, dextrose, dextran, trehalose, hetastarch, or mixtures thereof. A lyophilization excipient may also comprise a pharmaceutically acceptable antioxidant, such as, for example, ascorbic acid, acetylcysteine, cysteine, sodium hydrogen sulfite, butyl-hydroxyanisole, butyl-hydroxytoluene, or alpha-tocopherol acetate. A preferred lyophilization excipient is mannitol.

Solvents for use in the present invention include water and organic solvents that form stable solutions with bendamustine free base without appreciably degrading the bendamustine, and which are capable of being removed through lyophilization. Examples of suitable organic solvents include, for example, methanol, ethanol, n-propanol, isopropanol, n-butanol, tert-butanol, or mixtures thereof. A preferred organic solvent is tert-butanol.

Also within the scope of the invention are methods of treating diseases, such as, for example, chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, or breast cancer, with a pharmaceutical composition of the present invention. In certain embodiments, the method comprises administering a therapeutically effective amount of a pharmaceutical composition of the present invention directly to the patient. In other embodiments, the method comprises modifying a pharmaceutical composition of the present invention before administration, such as by dissolving the composition in water or another solvent prior to administration. In these embodiments, the method comprises administering to the patient a therapeutically effective amount of a preparation prepared from a pharmaceutical composition of the present invention. Preferably, the preparation is an injectable preparation. The injectable preparation may be administered subcutaneously, intracutaneously, intravenously, intramuscularly, intra-articularly, intrasynovially, intrasternally, intrathecally, intralesionally, intracranially or via infusion. Other conditions amenable to treatment utilizing the compositions and injectable preparations of the present invention include small cell lung cancer,

hyperproliferative disorders, and autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, and lupus.

The injectable preparations described herein are in the form of a sterile injectable preparation, for example, as a sterile, injectable aqueous or oleaginous suspension formulated according to techniques known in the art. Typically, the pharmaceutical compositions of the present invention, containing at least one of Form 1, Form 2, Form 3, Form 4, Form 5, Form 6, Form 7, Form 8, Form 9, Form 10, Form 11, Form 12, Form 13, Form 14, Form 15, or amorphous bendamustine free base, are formulated as lyophilized powders which may be provided, for example, in vials containing 100 mg of drug per 50 mL vial. The injectable preparation may be prepared by reconstitution of a freeze-dried or lyophilized composition with Sterile Water for Injection and then further dilution with a pharmaceutically acceptable intravenous solution, such as, for example, 0.9% sodium Chloride, 5% dextrose in water (D5W), Lactated Ringers solution, or 0.45% Sodium Chloride/2.5% dextrose.

Preferably, the pharmaceutical compositions of bendamustine free base described herein are reconstituted into an injectable preparation, for example, with sterile water, in less than about 20 minutes. More preferably, reconstitution occurs in less than about 10 minutes, most preferably about 5 minutes.

A typical reconstitution process would include reconstituting, preferably aseptically, 100 mg bendamustine free base with 20 mL Sterile Water for Injection. This yields a clear, colorless to pale yellow solution having a bendamustine concentration of 5 mg/mL. If lyophilized bendamustine free base is being reconstituted, the bendamustine free base should completely dissolve in about 5 minutes. The volume needed for the required dose (based on 5 mg/mL concentration) can be aseptically withdrawn and transferred to a 500 mL infusion bag of 0.9% Sodium Chloride (or other pharmaceutically acceptable intravenous solution) for injection. Preferably, the reconstituted solution is transferred to the infusion bag within 30 minutes of reconstitution. After transfer, the contents of the infusion bag are thoroughly mixed. Administration by intravenous infusion is typically provided over a time period of from about 30 to about 60 minutes.

It is envisioned that the pharmaceutical compositions of the present invention can be administered in combination with one or more anti-neoplastic agents where the anti-neoplastic agent is given prior to, concurrently with, or subsequent to the administration of the composition of the present invention. Pharmaceutically acceptable anti-neoplastic agents are known in the art. Preferred anti-neoplastic agents are those disclosed in co-

pending U.S. Application No. 11/330,868, filed January 12, 2006, the entirety of which is incorporated herein by reference.

Therapeutically effective amounts of bendamustine can be readily determined by an attending diagnostician by use of conventional techniques. The effective dose can vary depending upon a number of factors, including type and extent of progression of the disease or disorder, overall health of a particular patient, biological efficacy of the bendamustine, formulation of the bendamustine, and route of administration of the forms of bendamustine. Bendamustine can also be administered at lower dosage levels with gradual increases until the desired effect is achieved.

TERMINOLOGY

“Polymorphism,” as used herein, is defined as the occurrence of different crystalline arrangements for the same molecules. A “solvate,” as used herein, is a crystalline material that contains solvent molecules, for example, water, ethanol, 3-pentanone, ethyl acetate, dichloromethane, diethyl ether, and the like, within the crystal structure. The term “solvent,” as used herein, means a substance, typically a liquid, that is capable of completely or partially dissolving another substance, typically a solid. The following solvents were used in the experiments described herein:

1- butanol	Heptane
1,4-dioxane	Isopropyl acetate
1-propanol	Methanol
2-butanone	Methyl isobutyl ketone
3-pentanone	Methyl tert-butyl ether
Acetone	N,N-dimethylformamide
Acetonitrile	N-butyl acetate
Chloroform	Propanonitrile
Cyclohexane	Tetrahydrofuran
Dichloromethane	Tetrahydropyran
Ethanol	Toluene
Ethyl acetate	Water

The term “crystalline,” as used herein, means having a regularly repeating arrangement of molecules or external face planes. “Non-crystalline,” as used herein, refers to amorphous material having no detectible regularly repeating arrangement of molecules or external face planes.

The term “crystalline composition,” as used in herein, refers to a solid chemical compound or mixture of compounds that provides a characteristic pattern of peaks when

analyzed by x-ray powder diffraction; this includes, but is not limited to, polymorphs, solvates, hydrates, co-crystals, and desolvated solvates.

The term “isolating” as used herein, means separating a compound from a solvent to provide a solid, semisolid or syrup. This is typically accomplished by means such as centrifugation, filtration with or without vacuum, filtration under positive pressure, distillation, evaporation or a combination thereof. Isolating may or may not be accompanied by purifying during which the chemical, chiral or chemical and chiral purity of the isolate is increased. Purifying is typically conducted by means such as crystallization, distillation, extraction, filtration through acidic, basic or neutral alumina, filtration through acidic, basic or neutral charcoal, column chromatography on a column packed with a chiral stationary phase, filtration through a porous paper, plastic or glass barrier, column chromatography on silica gel, ion exchange chromatography, recrystallization, normal-phase high performance liquid chromatography, reverse-phase high performance liquid chromatography, trituration and the like.

The term “pharmaceutically acceptable excipient,” as used herein, includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like, acceptable for pharmaceutical use, for example, those that have been accorded Generally Regarded as Safe (GRAS) status by the U.S. Food and Drug Administration. The use of such media and agents for pharmaceutical active substances is well known in the art, such as in *Remington: The Science and Practice of Pharmacy*, 20th ed.; Gennaro, A. R., Ed.; Lippincott Williams & Wilkins: Philadelphia, PA, 2000. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The term “solution,” as used herein, refers to a mixture containing at least one solvent and at least one compound that is at least partially dissolved in the solvent.

The term “sublimation,” as used herein, refers to the transition from the solid phase to the gas phase with no intermediate liquid stage.

The term “substantially free,” as used herein with regard to compositions that contain a particular form of bendamustine while being “substantially free” of other forms of the compound, means that the recited form is associated with less than 10%, preferably less than 5%, in particular less than 2% and most preferably less than 1% of the other recited forms of bendamustine.

The term “therapeutically effective amount,” as used herein, refers to the amount determined to be required to produce the physiological effect intended and associated with a given drug, as measured according to established pharmacokinetic methods and techniques, for the given administration route. Appropriate and specific therapeutically effective amounts can be readily determined by the attending diagnostician, as one skilled in the art, by the use of conventional techniques. The effective dose will vary depending upon a number of factors, including the type and extent of progression of the disease or disorder, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the active agent with appropriate excipients, and the route of administration.

Experimental Section

INSTRUMENTATION

X-Ray Powder Diffraction

PANalytical X Pert Pro

Powder X-Ray Diffraction patterns were recorded on a PANalytical X Pert Pro diffractometer equipped with an X celerator detector using Cu K α radiation at 40 kV and 40 mA. K α 1 radiation is obtained with a highly oriented crystal (Ge111) incident beam monochromator. A 10mm beam mask, and fixed (1/4°) divergence and anti-scatter (1/8°) slits were inserted on the incident beam side. A fixed 0.10mm receiving slit was inserted on the diffracted beam side. The X-ray powder pattern scan was collected from ca. 2 to 40° 2 θ with a 0.0080° step size and 96.06 sec counting time which resulted in a scan rate of approximately 0.5°/min. The samples were spread on silicon zero background (ZBG) plate for the measurement. The sample was rotated at 4°/min on a PANalytical PW3064 Spinner. Measurement of the Si reference standard before the data collection resulted in values for 2 θ and intensity that were well within the tolerances of 28.44 < 2 θ < 28.50 and significantly greater than the minimum peak height of 150cps.

Bruker AXS/Siemens D5000

X-Ray Powder Diffraction patterns were collected on a Siemens D5000 diffractometer using Cu Ka radiation (40kV, 40mA), 600 nm goniometer, automatic divergence and receiving slits, a graphite secondary monochromator and a scintillation counter. The instrument is performance checked using a certified corundum standard (NIST 1976).

Ambient conditions

Samples run under ambient conditions were prepared as flat plate specimens using powder as received. Approximately 35mg of the sample was gently packed into a cavity cut into polished, zero-background (510) silicon wafer and a MYLAR® cover was placed over the sample. The sample was rotated in its own plane during analysis.

Bruker AXS C2 GADDS

X-Ray Powder Diffraction patterns were collected on a Bruker AXS C2 GADDS diffractometer using Cu Ka radiation (40kV, 40mA), automated XYZ stage, laser video microscope for autosample positioning and a HiStar 2-dimensional area detector. X-ray optics consists of a single Göbel multilayer mirror coupled with a pinhole collimator of 0.3mm. The beam divergence, i.e. the effective size of the X-ray beam on the sample, was approximately 5mm. A θ - θ continuous scan mode was employed with a sample - detector distance of 20cm which gives an effective 2θ range of $3.2^\circ - 29.7^\circ$. Typically, the sample would be exposed to the X-ray beam for 120 seconds.

Ambient conditions

Samples run under ambient conditions were prepared as flat plate specimens using powder as received without grinding. Approximately 1-2mg of the sample was lightly pressed on a glass slide to obtain a flat surface.

Variable Temperature X-Ray Powder Diffraction (VT-XRPD)

Variable temperature studies were performed with an Anton Paar TTK450 temperature chamber under computer control through an Anton Paar TCU100 temperature control unit. Typically, the measurements were done with a nitrogen flow through the camera. Two measurement schemes were used, restricted and continuous. In the restricted mode, measurements were made only after the TK450 chamber reached the requested temperature. In the continuous mode, the sample was heated at $10^\circ\text{C}/\text{minute}$ and fast scans were measured as the temperature changed. After the pre-selected temperature was reached, the sample was cooled at $35^\circ\text{C}/\text{minute}$. To monitor for changes during the cooling, fast scans were again measured. At 25°C , a slow scan was measured. The temperatures chosen were based on DSC results. For the diffractometer set-up a 10mm beam mask, 0.04 radian Soller slits and fixed ($1/4^\circ$) divergence and anti-scatter ($1/8^\circ$) slits were inserted on the incident beam side. A fixed 0.10 mm receiving slit, 0.04 radian Soller

slits and a 0.02 mm Nickel filter were inserted on the diffracted beam side. The slow scans were collected from ca. 3 to 30° 2 θ with a 0.0080° step size and 100.97 sec counting time which resulted in a scan rate of approximately 0.5°/min. The fast scans were collected from ca. 3 to 30° 2 θ with a 0.0167° step size and 1.905 sec counting time which resulted in a scan rate of approximately 44°/min. With the nitrogen gas supply turned off, the camera returned to ambient conditions. The humidity in the ambient state varies with the humidity in the laboratory, typically 30 to 40%, and with the temperature in the camera. As the temperature is raised, the relative humidity will drop.

Differential Scanning Calorimetry (DSC)

Thermal curves were acquired using a Perkin-Elmer Sapphire DSC unit equipped with an autosampler running Pyris software version 6.0 calibrated with Indium prior to analysis. Solid samples of 1-11 mg were weighed into 20 μ L aluminum open samples pans. The DSC cell was then purged with nitrogen and the temperature heated from 0° to 275°C at 10°C/min.

Thermogravimetric Mass Spectrometry - (TGA/MS)

Thermal curves were acquired using a Perkin-Elmer Pyris 1 TGA unit running Pyris software version 6.0 calibrated with calcium oxalate monohydrate. TGA samples between 1-5 mg were monitored for percent weight loss as heated from 25° to 300°C at 10°C/min in a furnace purged with Helium at ca. 50 mL/min. To simultaneously follow the evolution of the gaseous decomposition products over the temperature range investigated, the thermobalance was connected to a ThermoStar Mass Spectrometer (Asslar, Germany). The transfer line to introduce gaseous decomposition products into the mass spectrometer was a deactivated fused silica capillary temperature controlled to 200°C to avoid possible condensation of the evolved gases. In this way the thermogravimetric (TG) and mass spectrometric ion intensity curves of the selected ionic species could be recorded simultaneously.

Thermogravimetric analysis- (TGA)

TGA data were collected on a TA Instruments Q500 TGA, equipped with a 16 position autosampler. The instrument was temperature calibrated using certified Alumel. Typically 1-2mg of each sample was loaded into a pin-holed hermetically sealed aluminum DSC pan on a pre-tared platinum crucible, and was heated at 10°C.min⁻¹ from ambient temperature to 200°C. A nitrogen purge at 60ml.min⁻¹ was maintained over the

sample. The instrument control software was Thermal Advantage v4.6.6 and the data were analyzed using Universal Analysis v4.3A.

¹H NMR

5 ¹H NMR spectra were collected on a Bruker 400MHz instrument equipped with an auto-sampler and controlled by a DRX400 console. Automated experiments were acquired using ICON-NMR v4.0.4 (build 1) running with Topspin v 1.3 (patch level 6) using the standard Bruker loaded experiments. For non-routine spectroscopy, data were acquired through the use of Topspin alone. Samples were prepared in d₆-DMSO, unless otherwise
10 stated. Off-line analysis was carried out using ACD SpecManager v 9.09 (build 7703).

Polymorph Screening

Crystallizations studies were performed on bendamustine free base to investigate polymorphism in 24 different solvents. Solvents were selected on the basis of acceptability
15 (ICH Class 3 and 2) and to give a range of dielectric constants, dipole moments and functional groups. Maturation, slow cool and slurry crystallization were also employed to obtain different forms of the bendamustine free base. When possible, full characterization was performed on the new forms that were generated during the polymorphism screening on bendamustine free base. This characterization consisted of: X-ray powder diffraction;
20 thermal analysis; GVS; and purity by HPLC.

Crystallization by Maturation Experiments

Approximately 40 mg of bendamustine free base (Form 2 bendamustine free base or Form 3 bendamustine free base) in 800 µL of solvent (25 volumes) was slurried in the
25 24 diverse solvents. These mixtures were slurried for 48 hours with alternating 4 hour periods at 50°C and 5°C (-0.5°C/min). The solid material was isolated by filtration and analyzed by XRPD and thermal analysis. The material was dried at 40°C during 3 hour. Results are shown in Table 16 and Table 17 below.

Table 16. Maturation with Form 3

Solvent	XRPD Status	Solvent	XRPD Status
1- butanol	Form 6	Heptane	Form 3
1-propanol	Form 13	Methyl tert-butyl ether	Form 13
3-pentanone	Form 13	N,N-dimethylformamide	Form 7
Chloroform	Form 13	Tetrahydropyran	Form 3
Cyclohexane	Form 3	Toluene	Form 3
Dichloromethane	Form 3	Water	No crystallization

Ethanol

Form 8

Table 17. Maturation with Form 2

Solvent	XRPD Status	Solvent	XRPD Status
1- butanol	Form 1	Methyl isobutyl ketone	Form 1
1,4-dioxane	Form 1	Methyl tert-butyl ether	Form 1
1-propanol	Form 1	N-butyl acetate	Form 1
Acetone	Form 1	Propanonitrile	Form 1
Chloroform	Form 1	Tetrahydropyran	Form 1
Cyclohexane	Form 1	Toluene	Form 1
Ethanol	Form 1		
Heptane	Form 2		

Crystallization by Slow Cool

- 5 Approximately 40 mg of bendamustine free base (Form 2 or Form 3) in 800 μ L of solvent (25 volumes) were slurried in the 24 diverse solvents. The samples were heated from 20°C to 80°C at a rate of 4.8°C/min and after 30 minutes cooled at a slow rate (0.25°C/min) to a final temperature of 5°C and kept at that temperature for 18 h. The solid material from each vial was isolated by filtration and evaluated by XRPD and thermal
- 10 analysis. The material was dried at 40°C during 3 hour. Results are shown in Table 18 and Table 19 below.

Table 18: Slow Cool with Form 3

Solvent	XRPD Status	Solvent	XRPD Status
1- butanol	Form 6	Isopropyl acetate	Form 7
1-propanol	Form 6	N,N-dimethylformamide	Form 7
		Toluene	Form 3

Table 19: Slow Cool with Form 2

Solvent	XRPD Status	Solvent	XRPD Status
2-butanone	Form 1	Methyl tert-butyl ether	Form 1
3-pentanone	Form 9	N-butyl acetate	Form 1
Acetonitrile	Form 1	Propanonitrile	Form 1
Cyclohexane	Form 1	Tetrahydropyran	Form 1
Ethyl acetate	Form 1	Toluene	Form 10

Crystallization by Slurry Experiments

The slurries (40 mg of bendamustine free base in 400 μ L) were shaken at 25°C during 48 hours. The solid was isolated by filtration and dried at 40°C during 3 hours. The XRPD results from the isolated solid are recorded in Table 20.

Table 20: Slurry Experiments with Form 3

Solvent	XRPD Status	Solvent	XRPD Status
1- butanol	Form 11	Isopropyl acetate	Form 11
1,4-dioxane	Form 11	Methyl isobutyl ketone	Form 3
2-butanone	Form 3	Methyl tert-butyl ether	Form 3
3-pentanone	Form 3	N,N-dimethylformamide	Form 12
Acetone	Form 3	N-butyl acetate	Form 3
Acetonitrile	Form 7	Propanonitrile	Form 3
Chloroform	Form 3	Tetrahydropyran	Form 3
Cyclohexane	Form 3	Toluene	Form 3
Dichloromethane	Form 3		
Ethyl acetate	Form 3		

Form 1*Preparation*5 Slow Cool Experiments

Approximately 40 mg of Form 2 bendamustine free base in 800 μ L of solvent (20 volumes) were slurried in 2-butanone, acetonitrile, cyclohexane, ethyl acetate, methyl tert-butyl ether, n-butyl acetate, propionitrile and tetrahydropyran. The samples were heated from 20°C to 80°C at a rate of 4.8°C/min and after 30 minutes cooled at a slow rate (0.25°C/min) to a final temperature of 5°C and kept at that temperature for 18 h. The solid material was isolated by filtration and dried at 40 °C over 3 hours. The material was analyzed by XRPD and thermal analysis..

Crystallization by Maturation Experiments

Approximately 40 mg of Form 2 bendamustine free base in 800 μ L of solvent (20 volumes) were slurried in 1-butanol, 1-4 dioxane, 1-propanol, acetone, chloroform, cyclohexane, ethanol, methyl isobutyl ketone, methyl tert-butyl ether, n-butyl acetate, propionitrile, tetrahydropyran and toluene. These mixtures were slurried for 48 hours with alternating 4 hour periods at 50°C and 5°C (-0.5°C/min) The solid material was isolated by filtration and dried at 40 °C over 3 hours. The material was analyzed by XRPD and thermal analysis.

The X-ray diffraction pattern characteristic of the crystalline Form 1 is shown in Table 1 (above) and FIG. 1.

Characterization of Form 1 by Thermal Analysis

Form 1 shows a single peak at ca. 142.3 °C with an enthalpy of fusion (ΔH_{Fus}) of 114.3 J/g. No loss of mass is detected by TGA. The existence of a desolvation process was discounted because no loss of weight was detected by TGA (FIG. 1A).

Form 2*Preparation*Slow Cool Experiments

5 1.08g of Bendamustine Hydrochloride was dissolved in 100 mL of deionised water. One mole equivalent of NaOH (1 molar in water) was added drop-wise causing precipitation to occur. Solid was then rapidly isolated by filtration and washed on the filter with 2 portions of deionised water and 2 portions of heptane to remove excess water. The solid was dried under vacuum at ambient temperature for 18 hours. 752mg of solid was
10 obtained and analyzed by XRPD.

 The X-ray diffraction pattern characteristic of the crystalline Form 2 is shown in Table 2 (above) and FIG. 2.

Characterization of Form 2 by Thermal Analysis

15 Form 2 bendamustine free base shows a single peak at ca. 132.8°C with an enthalpy of fusion (ΔH_{Fus}) of 89.1 J/g. No loss of mass is detected by TGA. The existence of a desolvation process was discounted because no loss of weight was detected by TGA (FIG. 3)

Form 3*Preparation*Slow Cool Experiments

 Bendamustine free base was prepared by the base-mediated hydrolysis of the ethyl ester of bendamustine. After the hydrolysis was completed, the reaction mixture was
25 neutralized by aqueous hydrochloric acid. Around pH 6-7, the product, precipitating as a solid, was collected by filtration and washed by water. The crude product was triturated by a mixture of acetone (20ml) and Methyl tert-butyl ether (5ml). Following filtration, the solid was dried at 60°C.

 The X-ray diffraction pattern characteristic of the crystalline Form 3 is shown in
30 Table 3 (above) and FIG. 4.

Characterization of Form 3 by Thermal Analysis

Form 3 shows a multiple onset at ca. 29.1 and 101.8 with an enthalpy of fusion (ΔH_{Fus}) of 142.5 and 76.2 J/g (FIG. 23). TGA experiment loses an average weight of 8.8 % between 20 and 150 °C. The theoretical value for incorporation of two moles of water with one mole of bendamustine free base is 9.1% (FIG. 5).

Form 4

Preparation by solid-solid transition

Approximately 100 mg of Form 3 bendamustine free base was exposed to 0% RH at 25°C during 30 minutes. The X-ray diffraction pattern characteristic of the crystalline Form 4 is shown in Table 4 (above) and FIG. 6.

Form 5

Preparation by solid-solid transition

Approximately 100 mg of Form 3 bendamustine free base was exposed to 85% RH at 25 °C during 1 day. The X-ray diffraction pattern characteristic of the crystalline Form 5 is shown in Table 5 (above) and FIG. 7.

Characterization of Form 5 by Thermal Analysis

Form 5 shows a multiple onset at ca. 25.9, 82.6 and 123.0 with an enthalpy of fusion (ΔH_{Fus}) of 255.1, 20.6 and 23.4 J/g (FIG. 23). TGA experiment loses an average weight of 15.6 % between 20 and 150 °C. The theoretical value for incorporation of four moles of water with one mole of bendamustine free base is 16.7% (FIG. 8).

Form 6

Preparation

Slow Cool Experiments

Approximately 40 mg of Form 3 bendamustine free base in 800 μL of solvent (20 volumes) was slurried in the 1-butanol or 1-propanol. The samples were heated from 20 °C to 80 °C at a rate of 4.8 °C/min and after 30 minutes cooled at a slow rate (0.25 °C/min) to a final temperature of 5 °C and kept at that temperature for 18 h. The solid material was isolated by filtration and dried at 40 °C over 3 hours. The material was analyzed by XRPD and thermal analysis.

Crystallization by Maturation Experiments

Approximately 40 mg of Form 3 bendamustine free base in 800 μ L of solvent (20 volumes) were slurried in 1-butanol. The mixtures was slurried for 48 hours with alternating 4 hour periods at 50°C and 5°C (-0.5°C/min) The solid material was isolated by filtration and dried at 40 °C over 3 hours. The material was analyzed by XRPD and thermal analysis.

The X-ray diffraction pattern characteristic of the crystalline Form 6 is shown in Table 6 (above) and FIG. 9.

Characterization of Form 6 by Thermal Analysis

Form 6 shows a single onset at ca. 207.8°C with an enthalpy of fusion (ΔH_{Fus}) of 28.0 J/g. No loss of mass is detected by TGA. The existence of a desolvation process was discounted because no loss of weight was detected by TGA (FIG. 10).

Form 7

Preparation

Slow Cool Experiments

Approximately 40 mg of Form 3 bendamustine free base in 800 μ L of solvent (20 volumes) were slurried in N,N dimethylformamide or isopropyl acetate. The samples were heated from 20°C to 80°C at a rate of 4.8 °C/min and after 30 minutes cooled at a slow rate (0.25 °C/min) to a final temperature of 5 °C and kept at that temperature for 18 h. The solid material was isolated by filtration and dried at 40 °C over 3 hours. The material was analyzed by XRPD and thermal analysis.

Crystallization by Maturation Experiments

Approximately 40 mg of Form 3 bendamustine free base in 800 μ L of solvent (20 volumes) were slurried in N,N dimethylformamide. These mixtures were slurried for 48 hours with alternating 4 hour periods at 50 °C and 5 °C (-0.5 °C/min) The solid material was isolated by filtration and dried at 40 °C over 3 hours. The material was analyzed by XRPD and thermal analysis.

The X-ray diffraction pattern characteristic of the crystalline Form 7 is shown in Table 7 (above) and FIG. 11.

Characterization of Form 7 by Thermal Analysis

Form 7 shows a single onset at ca. 200.6°C with an enthalpy of fusion (ΔH_{Fus}) of 12.7 J/g. No loss of mass is detected by TGA. The existence of a desolvation process was discounted because no loss of weight was detected by TGA (FIG. 12).

5

Form 8

Preparation

Crystallization by Maturation Experiments

Approximately 40 mg of Form 3 bendamustine free base was added in 800 μL of ethanol (20 volumes). The mixture was slurried for 48 hours with alternating 4 hour periods at 50°C and 5°C (-0.5°C/min) The solid material was isolated by filtration and dried at 40 C over 3 hours. The material was analyzed by XRPD and thermal analysis.

The X-ray diffraction pattern characteristic of the crystalline Form 8 is shown in Table 8 (above) and FIG. 13.

15

Characterization of Form 8 by Thermal Analysis

Form 8 shows a single onset at ca. 77.6 °C with an enthalpy of fusion (ΔH_{Fus}) of 94.6 J/g. Form 8 in TGA experiment loses an average weight of 5.5 % between 20 and 150°C. The theoretical value for incorporation of one moles of ethanol with two moles of bendamustine free base is 5.4%. (FIG. 14).

20

Form 9

Preparation

Slow Cool Experiments

Approximately 40 mg of Form 2 bendamustine free base was added in 800 μL of 3-pentanone (20 volumes). The sample were heated from 20°C to 80°C at a rate of 4.8°C/min and after 30 minutes cooled at a slow rate (0.25°C/min) to a final temperature of 5°C and kept at that temperature for 18 The solid material was isolated by filtration and dried at 40 C over 3 hours. The material was analyzed by XRPD and thermal analysis.

25

The X-ray diffraction pattern characteristic of the crystalline Form 9 is shown in Table 9 (above) and FIG. 15.

30

Characterization of Form 9 by Thermal Analysis

Form 9 shows a single onset at ca. 138.3°C with an enthalpy of fusion (ΔH_{Fus}) of 37.0 J/g. Form 9 in TGA experiment loses an average weight of 5.5 % between 20 and 140°C. The theoretical value for incorporation of one moles of 3-pentanone with four moles of bendamustine free base is 5.7%. (FIG. 16)

5

Form 10

Preparation

Slow Cool Experiments

Approximately 40 mg of Form 2 bendamustine free base was added in 800 μL of toluene (20 volumes). The sample were heated from 20°C to 80°C at a rate of 4.8°C/min and after 30 minutes cooled at a slow rate (0.25°C/min) to a final temperature of 5°C and kept at that temperature for 18 h. The solid material was isolated by filtration and dried at 40 C over 3 hours. The material was analyzed by XRPD and thermal analysis.

The X-ray diffraction pattern characteristic of the crystalline Form 10 is shown in Table 10 (above) and FIG. 17.

Characterization of Form 10 by Thermal Analysis

Form I₀ shows two onsets at ca. 109.3 and 144.9°C with an enthalpy of fusion (ΔH_{Fus}) of 5.6 and 98.3 J/g. (FIG.18). No loss of mass is detected by TGA. The existence of a desolvation process was discounted because no loss of weight was detected by TGA.

Form 11

Preparation

Crystallization by Slurry Experiments

Approximately 40 mg of Form 3 bendamustine free base in 400 μL of 1 butanol or 1-4 dioxane or isopropyl acetate was stirred at 25°C during 48 h. The solid was isolated by filtration and dried at 40°C over 3 hours. The material was analyzed by XRPD and thermal analysis.

The X-ray diffraction pattern characteristic of the crystalline Form 11 is shown in Table 11 (above) and FIG. 19.

Characterization of Form 11 by Thermal Analysis

Form 11 shows a multiple onset at ca. 30.5, 70.9 and 112.3°C with an enthalpy of fusion (ΔH_{Fus}) of 49.3, 51.0 and 74.9 J/g. (FIG. 20)

5 **Form 12**

Preparation

Crystallization by Slurry Experiments

Approximately 40 mg of Form 3 bendamustine free base was added in 400 μL of N,N-dimethylformamide. The slurry was stirred at 25°C during 48 hours. The solid was
10 isolated by filtration and dried at 40°C during 3 hours. The material was analyzed by XRPD and thermal analysis.

The X-ray diffraction pattern characteristic of the crystalline Form 12 is shown in Table 12 (above) and FIG. 21.

15 Characterization of Form 12 by Thermal Analysis

Form 12 shows a multiple onset at ca. 56.8, 72.0, 111.5 and 206.7 with an enthalpy of fusion (ΔH_{Fus}) of 3.3, 1.6, 1.7 and 13.2 J/g (FIG. 22).

Form 13

20 *Preparation*

Slow Cool Experiments

Approximately 40 mg of Form 3 bendamustine free base in 800 μL of solvent (20 volumes) were slurried in the methyl tert-butyl ether, 3-pentanone and 1-propanol. The samples were heated from 20°C to 80°C at a rate of 4.8°C/min and after 30 minutes cooled
25 at a slow rate (0.25°C/min) to a final temperature of 5°C and kept at that temperature for 18 h. The solid material was isolated by filtration and dried at 40°C over 3 hours. The material was analyzed by XRPD and thermal analysis.

The X-ray diffraction pattern characteristic of the crystalline Form 13 is shown in Table 13 (above) and FIG. 23.

30

Characterization of Form 13 by Thermal Analysis

Form 13 shows a single onset at ca. 93.4°C with an enthalpy of fusion (ΔH_{Fus}) of 118.2 J/g.. TGA experiment loses an average weight of 9.4 % between 20 and 150 °C. The

theoretical value for incorporation of two moles of water with one mole of bendamustine free base is 9.1% (FIG. 24).

Form 14

5 *Preparation*

Crystallization by Slurry Experiment

Approximately 40 mg of Form 3 bendamustine free base in 400 μ L of acetonitrile was shaken at 25°C during 48 hours. The solid was isolated by filtration and dried at 40°C over 3 hours. The material was analyzed by XRPD and thermal analysis.

10 The X-ray diffraction pattern characteristic of the crystalline Form 14 is shown in Table 14 (above) and FIG. 25.

Characterization of Form 14 by Thermal Analysis

Form 14 shows a multiple onset at ca. 25.8, 83.2 and 123.2 with an enthalpy of fusion (ΔH_{Fus}) of 215.4, 51.9 and 30.0 J/g. TGA experiment loses an average weight of 11.1 % between 20 and 150 °C. The theoretical value for incorporation of five moles of water with two moles of bendamustine free base is 11.2% (FIG. 26).

Form 15

20 *Preparation*

Crystallization by Slurry Experiments

Approximately 25 mg of Form 1 bendamustine free base in 1.25 mL of tetrahydrofuran was dissolved at 50°C. On cooling to ambient temperature, precipitation occurred. The solid was isolated and rapidly analyzed by XRPD.

25 The X-ray diffraction pattern characteristic of the crystalline Form 15 is shown in Table 15 (above) and FIG. 27.

Characterization of Form 15 by thermogravimetry analysis

Thermogravimetric analysis is consistent with the THF solvate of Bendamustine Free Base (FIG. 28).

Characterization of Form 15 by 1 NMR

The 1 H NMR spectrum confirmed that THF (0.05 equivalents) is present (FIG 29).

Amorphous Bendamustine Free Base*Preparation*

During the characterization of Form 3 by VT-XRPD, amorphous bendamustine free base was formed. No significant, solid-solid transformations take place in the temperature range of 25 °C to 50 °C for Form 3. Dehydration of the hydrate to Form 4 takes place in the temperature range 50 °C to 66 °C. The crystallinity disappears in the temperature range 124 °C to 133 °C. FIG. 30. After 133 °C, the sample converts to amorphous material. FIG. 31.

A first embodiment of the present invention provides a pharmaceutical composition comprising bendamustine free base selected from the group consisting of amorphous bendamustine free base, bendamustine free base Form 1, bendamustine free base Form 2, bendamustine free base Form 3, bendamustine free base Form 4, bendamustine free base Form 5, bendamustine free base Form 6, bendamustine free base Form 7, bendamustine free base Form 8, bendamustine free base Form 9, bendamustine free base Form 10, bendamustine free base Form 11, bendamustine free base Form 12, bendamustine free base Form 13, bendamustine free base Form 14, bendamustine free base Form 15, or a mixture thereof.

A second embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is amorphous bendamustine free base.

A third embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 1.

A fourth embodiment provides a pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 2.

A fifth embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 3.

A sixth embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 4.

A seventh embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 5.

5 An eighth embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 6.

A ninth embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 7.

10 A tenth embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 8.

An eleventh embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 9.

A twelfth embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 10.

15 A thirteenth embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 11.

A fourteenth embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 12.

20 A fifteenth embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 13.

A sixteenth embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 14.

A seventeenth embodiment provides the pharmaceutical composition of the first embodiment wherein the bendamustine free base is bendamustine free base Form 15.

An eighteenth embodiment of the present invention provides a crystalline form of bendamustine free base said bendamustine free base selected from the group consisting of bendamustine free base Form 1, bendamustine free base Form 2, bendamustine free base Form 3, bendamustine free base Form 4, bendamustine free base Form 5, bendamustine free base Form 6, bendamustine free base Form 7, bendamustine free base Form 8, bendamustine free base Form 9, bendamustine free base Form 10, bendamustine free base Form 11, bendamustine free base Form 12, bendamustine free base Form 13, bendamustine free base Form 14, bendamustine free base Form 15, or a mixture thereof.

A nineteenth embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 1.

A twentieth embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 2.

A twenty-first embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 3.

A twenty-second embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 4.

A twenty-third embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 5.

A twenty-fourth embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 6.

A twenty-fifth embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 7.

A twenty-sixth embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 8.

A twenty-seventh embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 9.

A twenty-eighth embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 10.

A twenty-ninth embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 11.

5 A thirtieth embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 12.

A thirty-first embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 13.

10 A thirty-second embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 14.

A thirty-third embodiment provides the crystalline form of the eighteenth embodiment, wherein the bendamustine free base is bendamustine free base Form 15.

A thirty-fourth embodiment provides the crystalline form of any of the eighteenth to thirty-third embodiments, further comprising amorphous bendamustine free base.

15 A thirty-fifth embodiment of the present invention provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 10.34, 22.30, 24.03, 28.43, and 29.50 ± 0.2 degrees 2θ .

20 A thirty-sixth embodiment provides the crystalline form of bendamustine free base according to the thirty-fifth embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 16.69, 20.53, and 22.67 ± 0.2 degrees 2θ .

25 A thirty-seventh embodiment of the present invention provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 1.

A thirty-eighth embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of the thirty-fifth to thirty-seventh embodiments.

5 A thirty-ninth embodiment of the present invention provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 10.28, 20.59, 21.55, 21.69, and 24.78 ± 0.2 degrees 2θ .

10 A fortieth embodiment provides the crystalline form of bendamustine free base according to the thirty-ninth embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 12.54, 13.51, 15.40, and 22.39 ± 0.2 degrees 2θ .

A forty-first embodiment of the present invention provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 2.

15 A forty-second embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of the thirty-eight to forty-first embodiments.

20 A forty-third embodiment of the present invention provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 9.41, 9.46, 14.15, 23.42, and 23.65 ± 0.2 degrees 2θ .

A forty-fourth embodiment provides the crystalline form of bendamustine free base according to the forty-third embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 14.15, 18.78, and 24.83 ± 0.2 degrees 2θ .

25 A forty-fifth embodiment of the present invention provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 4.

A forty-sixth embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of the forty-third to forty-fifth embodiments.

5 A forty-seventh embodiment of the present invention provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 9.88, 15.13, 19.92, 22.99, 24.72, and 24.98 ± 0.2 degrees 2θ .

10 A forty-eighth embodiment provides the crystalline form of bendamustine free base according to the forty-seventh embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 19.44 and 20.70 ± 0.2 degrees 2θ .

A forty-ninth embodiment provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 6.

15 A fiftieth embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of the forty-seventh to forty-ninth embodiments.

20 A fifty-first embodiment of the present invention provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 8.94, 13.39, 16.04, 21.31, and 22.38 ± 0.2 degrees 2θ .

A fifty-second embodiment provides the crystalline form of bendamustine free base according to the fifty-first embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 17.90, 19.29, and 25.37 ± 0.2 degrees 2θ .

25 A fifty-third embodiment provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 7.

A fifty-fourth embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of the fifty-first to fifty-third embodiments.

5 A fifty-fifth embodiment of the present invention provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 8.67, 18.15, 20.94, 22.55, and 25.46 ± 0.2 degrees 2θ .

10 A fifty-sixth embodiment provides the crystalline form of bendamustine free base according to fifty-fifth embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 19.40, 22.95, 26.21, 27.74, and 34.62 ± 0.2 degrees 2θ .

A fifty-seventh embodiment provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 9.

15 A fifty-eighth embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of the fifty-fifth to fifty-seventh embodiments.

20 A fifty-ninth embodiment of the present invention provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 8.51, 17.97, 21.25, 28.09, and 36.31 ± 0.2 degrees 2θ .

A sixtieth embodiment provides the crystalline form of bendamustine free base according to the fifty-ninth embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 11.42, 14.23, 23.29, 24.04, and 28.09 ± 0.2 degrees 2θ .

25 A sixty-first embodiment provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 11.

A sixty-second embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of the fifty-ninth to sixty-first embodiments.

5 A sixty-third embodiment of the present invention provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 9.34, 10.45, 11.17, 15.32, 22.48, 24.98, and 26.40 ± 0.2 degrees 2θ .

10 A sixty-fourth embodiment provides the crystalline form of bendamustine free base according to the sixty-third embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 15.72, 21.01, 21.30, and 30.87 ± 0.2 degrees 2θ .

A sixty-fifth embodiment provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 13.

15 A sixty-sixth embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of the sixty-third to sixty-fifth embodiments.

A sixty-seventh embodiment provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 4.44, 13.34, 16.73, 19.54, and 22.32 ± 0.2 degrees 2θ .

20 A sixty-eighth embodiment provides the crystalline form of bendamustine free base according to the sixty-seventh embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 22.98, 23.45, and 24.80 ± 0.2 degrees 2θ .

25 A sixty-ninth embodiment provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 15.

A seventieth embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of the sixty-seventh to sixty-ninth embodiments.

5 A seventy-first embodiment of the present invention provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 16.82, 16.93, 17.06, 19.68, 19.83, 22.87, 24.24, 28.63, and 29.72 ± 0.2 degrees 2θ .

10 A seventy-second embodiment provides the crystalline form of bendamustine free base according to the seventy-first embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 16.13, 25.75, and 37.71 ± 0.2 degrees 2θ .

A seventy-third embodiment provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 17.

15 A seventy-fourth embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of the seventy-first to seventy-third embodiments.

A seventy-fifth embodiment of the present invention provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 8.90, 9.28, 13.94, 22.36, and 23.33 ± 0.2 degrees 2θ .

20 A seventy-sixth embodiment provides the crystalline form of bendamustine free base according to the seventy-fifth embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 16.01, 19.30, 21.29, and 25.38 ± 0.2 degrees 2θ .

25 A seventy-seventh embodiment provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 19.

A seventy-eighth embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of seventy-fifth to seventy-seventh embodiments.

5 A seventy-ninth embodiment provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 9.31, 9.35, 13.97, 14.03, and 23.38 ± 0.2 degrees 2θ .

10 An eightieth embodiment provides the crystalline form of bendamustine free base according to the seventy-ninth embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 4.71, 24.75, and 26.06 ± 0.2 degrees 2θ .

An eighty-first embodiment provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 21.

15 An eighty-second embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of the seventy-ninth to eighty-first embodiments.

An eighty-third embodiment of the present invention provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 9.84, 14.76, 19.67, and 24.62 ± 0.2 degrees 2θ .

20 An eighty-fourth embodiment of the present invention provides the crystalline form of bendamustine free base according to the eighty-third embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 21.35, 22.21, 23.20, and 26.91 ± 0.2 degrees 2θ .

An eighty-fifth embodiment provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 23.

An eighty-sixth embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of the eighty-third to eighty-fifth embodiments.

5 An eighty-seventh embodiment of the present invention provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 9.14, 17.72, 19.19, 21.13, 22.10, 23.12, and 23.61 ± 0.2 degrees 2θ .

10 An eighty-eighth embodiment provides the crystalline form of bendamustine free base according to the eighty-seventh embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 8.74, 15.85, 24.59, 25.28, and 27.16 ± 0.2 degrees 2θ .

An eighty-ninth embodiment provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 25.

15 A ninetieth embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of the eighty-seventh to eighty-ninth embodiments.

A ninety-first embodiment of the present invention provides a crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 24.56, 19.92, 10.26, 8.10, and 4.09 ± 0.2 degrees 2θ .

20 A ninety-second embodiment provides the crystalline form of bendamustine free base according to the ninety-first embodiment, that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 17.96, 23.83, 26.01, 28.39, and 29.43 ± 0.2 degrees 2θ .

25 A ninety-third embodiment provides a crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 27.

A ninety-fourth embodiment provides a pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 91 to 93.

5 A ninety-fifth embodiment provides a method of treating chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma or breast cancer comprising administering to a patient in need thereof a therapeutically effective amount of a preparation prepared from a composition according to any one the first to ninety-fourth embodiments.

10 As those skilled in the art will appreciate, numerous modifications and variations of the present invention are possible in view of the above teachings. It is therefore understood that within the scope of the appended claims, the invention can be practiced otherwise than as specifically described herein, and the scope of the invention is intended to encompass all such variations.

What is Claimed:

1. A pharmaceutical composition comprising bendamustine free base selected from the group consisting of amorphous bendamustine free base, bendamustine free base Form 1, bendamustine free base Form 2, bendamustine free base Form 3,
5 bendamustine free base Form 4, bendamustine free base Form 5, bendamustine free base Form 6, bendamustine free base Form 7, bendamustine free base Form 8, bendamustine free base Form 9, bendamustine free base Form 10, bendamustine free base Form 11, bendamustine free base Form 12, bendamustine free base Form 13, bendamustine free base Form 14, bendamustine free base Form 15, or a
10 mixture thereof.
2. The pharmaceutical composition of claim 1 wherein the bendamustine free base is amorphous bendamustine free base.
3. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 1.
- 15 4. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 2.
5. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 3.
6. The pharmaceutical composition of claim 1 wherein the bendamustine free
20 base is bendamustine free base Form 4.
7. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 5.
8. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 6.
- 25 9. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 7.

10. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 8.
11. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 9.
- 5 12. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 10.
13. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 11.
- 10 14. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 12.
15. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 13.
16. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 14.
- 15 17. The pharmaceutical composition of claim 1 wherein the bendamustine free base is bendamustine free base Form 15.
18. A crystalline form of bendamustine free base said bendamustine free base selected from the group consisting of bendamustine free base Form 1, bendamustine free base Form 2, bendamustine free base Form 3, bendamustine free base Form 4, bendamustine free base Form 5, bendamustine free base Form 6, bendamustine free base Form 7, bendamustine free base Form 8, bendamustine free base Form 9, bendamustine free base Form 10, bendamustine free base Form 11, bendamustine free base Form 12, bendamustine free base Form 13, bendamustine free base Form 14, bendamustine free base Form 15, or a mixture thereof.
- 20
- 25

19. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 1.
20. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 2.
- 5 21. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 3.
22. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 4.
- 10 23. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 5.
24. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 6.
25. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 7.
- 15 26. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 8.
27. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 9.
- 20 28. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 10.
29. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 11.
30. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 12.

31. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 13.
32. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 14.
- 5 33. The crystalline form of claim 18, wherein the bendamustine free base is bendamustine free base Form 15.
34. The crystalline form of any of claims 18 to 33, further comprising amorphous bendamustine free base.
- 10 35. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 10.34, 22.30, 24.03, 28.43, and 29.50 ± 0.2 degrees 2θ .
36. The crystalline form of bendamustine free base according to claim 35 that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 16.69, 20.53, and 22.67 ± 0.2 degrees 2θ .
- 15 37. A crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 1.
38. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 35 to 37.
- 20 39. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 10.28, 20.59, 21.55, 21.69, and 24.78 ± 0.2 degrees 2θ .
40. The crystalline form of bendamustine free base according to claim 39 that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 12.54, 13.51, 15.40, and 22.39 ± 0.2 degrees 2θ .

41. A crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 2.
42. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 38 to 41.
- 5 43. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 9.41, 9.46, 14.15, 23.42, and 23.65 ± 0.2 degrees 2θ .
44. The crystalline form of bendamustine free base according to claim 43 that produces an X-ray powder diffraction pattern further comprising one or more of
10 the following reflections: 14.15, 18.78, and 24.83 ± 0.2 degrees 2θ .
45. A crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 4.
46. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 43 to 45.
- 15 47. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 9.88, 15.13, 19.92, 22.99, 24.72, and 24.98 ± 0.2 degrees 2θ .
48. The crystalline form of bendamustine free base according to claim 47 that produces an X-ray powder diffraction pattern further comprising one or more of
20 the following reflections: 19.44 and 20.70 ± 0.2 degrees 2θ .
49. A crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 6.
50. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 47 to 49.

51. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 8.94, 13.39, 16.04, 21.31, and 22.38 ± 0.2 degrees 2θ .

5 52. The crystalline form of bendamustine free base according to claim 51 that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 17.90, 19.29, and 25.37 ± 0.2 degrees 2θ .

53. A crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 7.

10 54. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 51 to 53.

55. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 8.67, 18.15, 20.94, 22.55, and 25.46 ± 0.2 degrees 2θ .

15 56. The crystalline form of bendamustine free base according to claim 55 that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 19.40, 22.95, 26.21, 27.74, and 34.62 ± 0.2 degrees 2θ .

57. A crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 9.

20 58. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 55 to 57.

59. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 8.51, 17.97, 21.25, 28.09, and 36.31 ± 0.2 degrees 2θ .

60. The crystalline form of bendamustine free base according to claim 59 that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 11.42, 14.23, 23.29, 24.04, and 28.09 ± 0.2 degrees 2θ .

5 61. A crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 11.

62. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 59 to 61.

63. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections:
10 9.34, 10.45, 11.17, 15.32, 22.48, 24.98, and 26.40 ± 0.2 degrees 2θ .

64. The crystalline form of bendamustine free base according to claim 63 that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 15.72, 21.01, 21.30, and 30.87 ± 0.2 degrees 2θ .

65. A crystalline form of bendamustine free base having an X-ray powder
15 diffraction pattern substantially as depicted in FIG. 13.

66. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 63 to 65.

67. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections:
20 4.44, 13.34, 16.73, 19.54, and 22.32 ± 0.2 degrees 2θ .

68. The crystalline form of bendamustine free base according to claim 67 that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 22.98, 23.45, and 24.80 ± 0.2 degrees 2θ .

69. A crystalline form of bendamustine free base having an X-ray powder
25 diffraction pattern substantially as depicted in FIG. 15.

70. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 67 to 69.

71. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections:

5 16.82, 16.93, 17.06, 19.68, 19.83, 22.87, 24.24, 28.63, and 29.72 ± 0.2 degrees 2θ .

72. The crystalline form of bendamustine free base according to claim 71 that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 16.13, 25.75, and 37.71 ± 0.2 degrees 2θ .

73. A crystalline form of bendamustine free base having an X-ray powder
10 diffraction pattern substantially as depicted in FIG. 17.

74. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 71 to 73.

75. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections:
15 8.90, 9.28, 13.94, 22.36, and 23.33 ± 0.2 degrees 2θ .

76. The crystalline form of bendamustine free base according to claim 75 that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 16.01, 19.30, 21.29, and 25.38 ± 0.2 degrees 2θ .

77. A crystalline form of bendamustine free base having an X-ray powder
20 diffraction pattern substantially as depicted in FIG. 19.

78. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 75 to 77.

79. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections:
25 9.31, 9.35, 13.97, 14.03, and 23.38 ± 0.2 degrees 2θ .

80. The crystalline form of bendamustine free base according to claim 79 that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 4.71, 24.75, and 26.06 ± 0.2 degrees 2θ .

5 81. A crystalline form of bendamustine free base having an X-ray powder diffraction pattern substantially as depicted in FIG. 21.

82. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 79 to 81.

83. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections:
10 9.84, 14.76, 19.67, and 24.62 ± 0.2 degrees 2θ .

84. The crystalline form of bendamustine free base according to claim 83 that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 21.35, 22.21, 23.20, and 26.91 ± 0.2 degrees 2θ .

85. A crystalline form of bendamustine free base having an X-ray powder
15 diffraction pattern substantially as depicted in FIG. 23.

86. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 83 to 85.

87. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections:
20 9.14, 17.72, 19.19, 21.13, 22.10, 23.12, and 23.61 ± 0.2 degrees 2θ .

88. The crystalline form of bendamustine free base according to claim 87 that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 8.74, 15.85, 24.59, 25.28, and 27.16 ± 0.2 degrees 2θ .

89. A crystalline form of bendamustine free base having an X-ray powder
25 diffraction pattern substantially as depicted in FIG. 25.

90. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 87 to 89.

91. A crystalline form of bendamustine free base that produces an X-ray powder diffraction pattern comprising one or more of the following reflections:
5 24.56, 19.92, 10.26, 8.10, and 4.09 ± 0.2 degrees 2θ .

92. The crystalline form of bendamustine free base according to claim 91 that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 17.96, 23.83, 26.01, 28.39, and 29.43 ± 0.2 degrees 2θ .

93. A crystalline form of bendamustine free base having an X-ray powder
10 diffraction pattern substantially as depicted in FIG. 27.

94. A pharmaceutical composition comprising the crystalline form of bendamustine free base according to any one of claims 91 to 93.

95. A method of treating chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma or breast cancer comprising
15 administering to a patient in need thereof a therapeutically effective amount of a preparation prepared from a composition according to any one of the preceding claims.