



## Busulfan Treatment for Myeloproliferative Disease may Reduce Injection Burden in Vascular Endothelial Growth Factor-Driven Retinopathy

Lauren A. Dalvin<sup>a,\*</sup>, Timothy W. Olsen<sup>a</sup>, Sophie J. Bakri<sup>a</sup>, Kristen McCullough<sup>b</sup>, Ayalew Tefferi<sup>b</sup>, Aref Al-Kali<sup>b,\*\*</sup>

<sup>a</sup> Department of Ophthalmology, Rochester, MN, 55905, USA

<sup>b</sup> Division of Hematology, Department of Medicine, Mayo Clinic, Rochester, MN, 55905, USA

### ARTICLE INFO

#### Keywords:

Anti-Vascular endothelial growth factor  
Busulfan  
Diabetic macular edema  
Macular degeneration  
Myeloproliferative disease

### ABSTRACT

**Purpose:** Myeloproliferative neoplasms (MPNs) have been associated with increased systemic levels of vascular endothelial growth factor (VEGF). This study investigated an association between systemic busulfan for treatment of MPN and the requirement for intravitreal anti-VEGF injections for treatment of retinal pathology.

**Methods:** Retrospective chart review of patients receiving systemic busulfan for myeloproliferative neoplasm and intravitreal anti-VEGF injections for macular and retinal vascular diseases from 2007 to 2021.

**Results:** Of seven patients receiving oral busulfan for a hematological neoplasm and having concomitant retinal pathology requiring intravitreal anti-VEGF, all were white females >60 years old with MPN and exudative age-related macular degeneration. Of these, two patients had a reduced anti-VEGF requirement while on systemic busulfan, two took busulfan for fewer than 5 months, one developed retinal pathology over one year after stopping busulfan, one developed new retinal pathology while taking busulfan, and one had limited follow-up. Of the two patients with reduced anti-VEGF requirement while taking systemic busulfan, both had JAK2 V617F mutated MPN, and subsequent busulfan discontinuation was associated with an increased requirement for intravitreal anti-VEGF injections.

**Conclusions and importance:** Systemic busulfan for treatment of MPN was associated with a reduced requirement for intravitreal anti-VEGF injections for retinal vascular disease in two patients. This association could be a result of inhibition of proliferative angiogenesis or reduced systemic VEGF levels with effective systemic treatment for MPN. Further study is required to confirm this association and determine whether this relationship is specific to busulfan or extends to other systemic medications used to treat MPN.

### 1. Introduction

Angiogenesis plays a key role in the pathogenesis of myeloproliferative neoplasms (MPN), and BCR-ABL-negative MPNs, including essential thrombocythemia, polycythemia vera, and primary myelofibrosis, have been associated with increased systemic levels of vascular endothelial growth factor-A (VEGF-A).<sup>1</sup> More specifically, in JAK2 V617F mutated MPNs, VEGF levels positively correlate with JAK2 mutation burden, and targeted cancer treatment has the potential to reduce systemic levels of VEGF.<sup>2</sup>

Many common macular and retinal vascular diseases, including age-related macular degeneration, diabetic macular edema, proliferative diabetic retinopathy, and macular edema associated with retinal vein

occlusion, are driven by elevated intraocular VEGF levels. Treatment using intravitreal anti-VEGF therapy has revolutionized management and has become the standard of care for these and other retinal conditions.<sup>3,4</sup> While local treatment with injections is highly effective, patients often require regular, monthly intravitreal injections. Many ophthalmologists reduce this burden using a treat-and-extend (TAE) approach to management, gradually extending the interval between injections (based on optical coherence tomography imaging as a biomarker of disease activity) with a goal of identifying the longest effective injection interval or, if the disease process improves, stopping injections altogether. Unfortunately, to maintain disease control and preserve vision, many patients require injections over several years or even indefinitely.

\* Corresponding author. Department of Ophthalmology, Mayo Clinic, 200 1st St SW, Rochester, MN, 55905, USA.

\*\* Corresponding author. Division of Hematology, Department of Medicine, Mayo Clinic, 200 1st St SW, Rochester, MN 55905, USA.

E-mail addresses: [dalvin.lauren@mayo.edu](mailto:dalvin.lauren@mayo.edu) (L.A. Dalvin), [alkali.aref@mayo.edu](mailto:alkali.aref@mayo.edu) (A. Al-Kali).

<https://doi.org/10.1016/j.ajoc.2022.101554>

Received 3 March 2022; Received in revised form 8 April 2022; Accepted 15 April 2022

Available online 21 April 2022

2451-9936/© 2022 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Busulfan (Myleran, GlaxoSmithKline, London, England; Busulfex IV, Otsuka America Pharmaceutical, Inc., Tokyo, Osaka, and Naruto, Japan) is a cell cycle non-specific alkylating agent approved by the Food and Drug Administration as part of the conditioning regimens for stem cell transplant and is also used (off-label) for treatment of MPNs, typically after hydroxyurea failure.<sup>5</sup> At a molecular level, busulfan causes DNA crosslinking, which prevents DNA replication, ultimately leading to apoptosis. Busulfan also has well-described toxic effects on endothelial cells.<sup>6</sup> In patients with uncontrolled MPNs refractory to hydroxyurea, busulfan may provide disease control, and, in addition to direct endothelial toxicity, may also indirectly reduce systemic VEGF by reducing the JAK2 mutation burden.<sup>7</sup> Herein, we report two patients with JAK2 V617F mutated MPNs responsive to busulfan, with a concomitant decrease in the requirement for intravitreal anti-VEGF injection for retinal disease.

## 2. Methods

This was a retrospective cohort study. This study complied with the Health Insurance Portability and Accountability Act (HIPAA) and adhered to the tenets of the Declaration of Helsinki. After IRB approval with a waiver of written informed consent was obtained from Mayo Clinic to conduct a retrospective chart review, 203 patients were identified who received oral busulfan between 2007 and 2021. Patients who received both oral busulfan and had retinal pathology requiring anti-VEGF treatment were reviewed in detail.

## 3. Results

There were seven patients (all were white females) who 1) received oral busulfan 2) had a hematological neoplasm and 3) had retinal pathology requiring intravitreal anti-VEGF injections (Table 1). Of these, two patients (Case 1 and 2), both with unclassifiable MPN, had a reduced requirement for anti-VEGF while on systemic busulfan therapy as detailed below. Of the other patients, none had unclassifiable MPN, two took busulfan for fewer than 5 months (Case 3 and 4), one developed retinal pathology that began over one year after stopping busulfan (Case 5), one developed new retinal pathology while taking busulfan (Case 6), and one had limited follow-up due to patient relocation (Case 7). All patients used hydroxyurea as the immediate prior MPN treatment except for one who used anagrelide. The reason for MPN treatment switch was intolerance in 5 patients and active non-healing wounds in 2 patients. Only 2 patients had well controlled blood counts prior to switching to busulfan such that 5 patients had previously uncontrolled

disease, potentially associated with elevated systemic VEGF levels.

### 3.1. Case 1

A 71-year-old female with JAK2 V617F mutated MPN-unclassifiable developed a right ankle ulcer related to suspected infrapopliteal arterial occlusive disease while taking hydroxyurea. To avoid further potential side effects, she was switched to busulfan. Prior to starting busulfan, the patient had a longstanding history of exudative age-related macular degeneration with subfoveal pigment epithelial detachments and sub-retinal fluid, requiring anti-VEGF injections in the right eye (Fig. 1A). She was initially diagnosed with exudative age-related macular degeneration 8 months after MPN diagnosis and required injections every 4 weeks, with no improvement on attempted switch from bevacizumab to an agent with stronger binding affinity for VEGF, aflibercept. Immediately prior to starting busulfan, visual acuity in her affected eye was 20/40, and she continued every 4-week injections of bevacizumab. The injection interval could not be extended due to persistent and progressive disease.

While taking busulfan, her MPN was well-controlled, and the interval between injections was extended up to 12 weeks using bevacizumab with resolution of subretinal fluid and visual acuity improvement up to 20/25 (Fig. 1B). After 6 months of busulfan use, she was required to stop the medication due to dropping platelet counts. Visual acuity decreased to 20/40, and the bevacizumab injection interval was reduced to every 4–5 weeks. Approximately one year after stopping busulfan, she had further worsening of her exudative age-related macular degeneration despite every 4-week bevacizumab, necessitating escalation to aflibercept every 4 weeks. Despite every 4-week aflibercept, she had a persistent thin layer of subfoveal subretinal fluid (Fig. 1C).

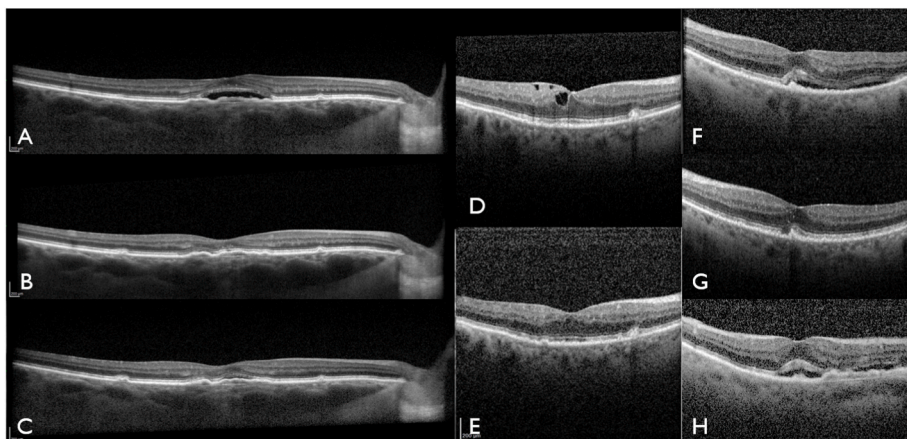
### 3.2. Case 2

An 87-year-old female with JAK2 V617F mutated MPN had decreasing blood counts and numbness on hydroxyurea. Updated bone marrow biopsy showed persistent MPN with myelofibrosis grade 1. The patient was switched to busulfan. The patient also had a history of diabetic macular edema in the right eye (Fig. 1D), with visual acuity reduced to 20/40, requiring anti-VEGF injections with bevacizumab every 6–8 weeks. The diabetic macular edema was first noted approximately 13 months prior to MPN diagnosis, and she was unable to successfully stop anti-VEGF injections due to recurrence of macular edema, with progression of ocular disease concomitant to progression of MPN. Three months after starting busulfan, a trial off injections was

**Table 1**  
Myeloproliferative neoplasm managed with busulfan and response of retinal disease.

Case	Age	Sex	Race	Mutation	MPN classification	Retinal Disease	Visual Acuity Before Busulfan	Injection Interval Before Busulfan	Visual Acuity on Busulfan	Injection Interval on Busulfan
1	71	F	W	JAK2	MPN-U	ARMD	20/40	4 weeks	20/25	12 weeks
2	87	F	W	JAK2	MPN-U	DME right ARMD left	20/40 right 20/60 left	6–8 weeks 6 weeks	20/25 20/25	No need No need
3	90	F	W	JAK2	ET	ARMD	Hand motions	Injections stopped due to poor visual prognosis	Hand motions	No change, off injections with poor vision
4	80	F	W	JAK2	ET	ARMD	Count fingers	No injections, poor visual prognosis	Count fingers	No change, off injections with poor vision
5	87	F	W	CALR	ET	ARMD	20/20	No retinal disease before busulfan	20/20	No retinal disease until after busulfan stopped
6	61	F	W	JAK2	PV	ARMD	20/20	No retinal disease before busulfan	20/70 right 20/30 left	4 weeks, developed new exudative ARMD in both eyes 2 months prior to stopping busulfan
7	87	F	W	negative	ET	ARMD	20/125	Attempted injection holiday prior to starting busulfan	20/100	4 weeks, limited follow-up due to patient relocation

F = female, W = white, ARMD = age-related macular degeneration, DME = diabetic macular edema, MPN-U = myeloproliferative neoplasm-unclassifiable, ET = essential thrombocytosis, PV = polycythemia vera.



**Fig. 1.** Busulfan Treatment for Myeloproliferative Disease may Reduce Injection Burden in Vascular Endothelial Growth Factor-Driven Retinopathy: Clinical images. Case 1. A 71-year-old female with exudative age-related macular degeneration (ARMD) in her right eye had (A) subfoveal pigment epithelial detachments (PEDs) and subretinal fluid by optical coherence tomography (OCT), necessitating every 4-week intravitreal anti-vascular endothelial growth factor (anti-VEGF) injections. (B) Subretinal fluid resolved after starting busulfan, and injections were extended to a 12-week interval. After busulfan was stopped, (C) subfoveal subretinal fluid recurred, necessitating return to every 4-week injections. Case 2. An 87-year-old female with (D) diabetic macular edema by OCT in her right eye requiring intravitreal anti-VEGF injections had (E) improvement with no need for further injections after starting busulfan. Her left eye developed new exudative ARMD with (F) subretinal fluid while on a busulfan holiday, which (G) improved with anti-VEGF injections. She did not

require any further injections after restarting busulfan, but when busulfan was stopped again, she had (H) recurrence of subretinal fluid with PEDs in her left eye, requiring resumption of anti-VEGF injections.

attempted, and the patient maintained visual acuity of 20/25 in the right eye with no recurrence of diabetic macular edema (Fig. 1E) and no further need for anti-VEGF injections in the right eye over 15 months follow-up.

Approximately 6 months after starting busulfan, the patient had to hold the medication due to thrombocytopenia. During this time, the patient developed subretinal fluid in the left eye (Fig. 1F) consistent with a new diagnosis of exudative age-related macular degeneration with visual acuity reduced to 20/60. Intravitreal anti-VEGF injections with bevacizumab were started. Due to rising blood counts, she restarted busulfan. While taking busulfan, she required no further anti-VEGF injections, and visual acuity improved to 20/25 with resolution of subretinal fluid (Fig. 1G). Due to thrombocytopenia, busulfan was again stopped. The patient had no recurrent fluid one month later, but at her next ophthalmology follow-up 7 months after stopping busulfan, she had recurrence of subretinal fluid (Fig. 1H) with visual acuity reduced to 20/40, and intravitreal bevacizumab injections were restarted.

#### 4. Discussion

Herein, we presented two patients with JAK2 mutated MPN necessitating treatment with busulfan. Each patient had an underlying retinal disease, which required intravitreal anti-VEGF injections. Both patients experienced improvement in their retinal disease with reduced requirement for anti-VEGF while on busulfan (but not on hydroxyurea), with disease progression when busulfan was stopped. Given the known relationship between systemic VEGF levels and JAK2 mutated MPNs,<sup>2</sup> we hypothesize that these patients had elevated systemic VEGF levels due to their underlying cancer with high JAK2 mutation burden, which could have contributed to more severe VEGF-driven retinal disease. In fact, patients with MPNs have a well-described increased risk of age-related macular degeneration, which is associated with higher JAK2V617F allele burden,<sup>8,9</sup> and Bak et al. described a 1.4-fold increased risk of neovascular age-related macular degeneration in patients with MPNs.<sup>10</sup>

Busulfan reduces JAK2V617F allele burden,<sup>7</sup> and, therefore, may indirectly decrease systemic VEGF levels, which could have translated to reduced intraocular VEGF levels, explaining improvement in retinal disease and decreased need for local anti-VEGF injections. Reduced serum VEGF levels have previously been observed in patients treated with another alkylating agent, cyclophosphamide.<sup>11</sup> Thus, this effect may not be limited to busulfan but was not seen when patients were on hydroxyurea. Another possible explanation for this phenomenon

includes direct endothelial toxicity related to busulfan's inherent properties as a potent alkylating agent.<sup>6</sup> This toxicity may directly inhibit proliferative angiogenesis, preventing further neovascularization within a choroidal neovascular membrane and, thereby, decrease risk for fluid recurrence. A similar hypothesis of angiogenic inhibition was considered prior to the advent of intravitreal anti-VEGF agents when proton irradiation was utilized to treat exudative age-related macular degeneration.<sup>12,13</sup>

Because this observation is limited to two patients, and the natural history of the disease(s) may be variable, any definitive conclusion cannot be made. Of the seven reviewed patients, only those two with unclassifiable MPN had an apparent association between busulfan use and anti-VEGF requirement. The mechanism by which busulfan may reduce anti-VEGF injection burden is speculative, and further studies measuring systemic and intraocular VEGF levels before and after busulfan treatment would better elucidate whether there is a true association.

#### 5. Conclusions

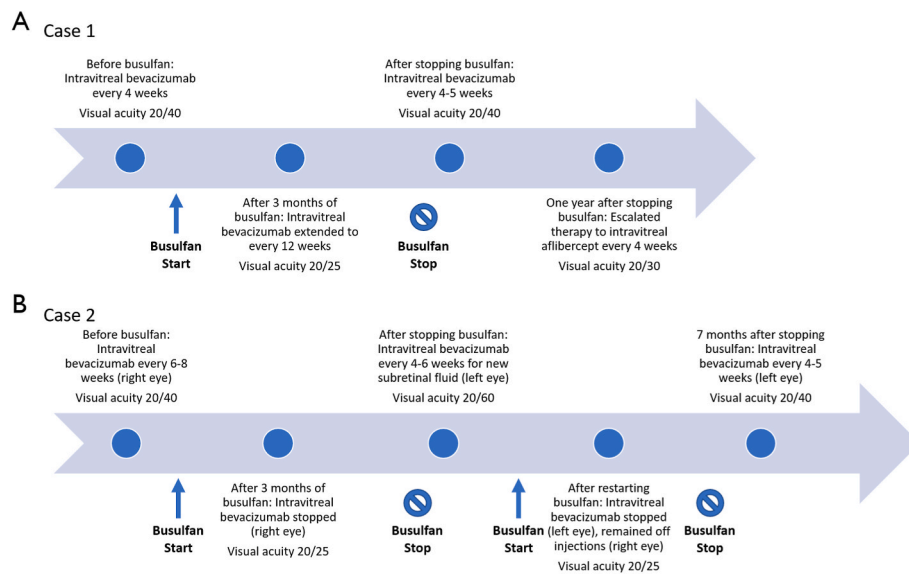
In conclusion, in two patients with MPN presented here, reduced requirement for intravitreal anti-VEGF therapy coincided with the time of busulfan initiation, and conversely, increased requirement for intravitreal anti-VEGF therapy coincided with busulfan discontinuation (Fig. 2). Further investigation is warranted to determine whether systemic myeloproliferative processes which cause elevated VEGF levels are associated with more severe presentations or higher likelihood of VEGF-driven retinal disease. Additionally, a more directive therapy like JAK inhibitors (ruxolitinib or fedratinib) may have a similar effect but this was not studied in our cohort. Future studies could investigate whether treatment for MPN reduces systemic VEGF levels and improves retinal pathology in a larger cohort and determine whether this effect is specific to busulfan or extends to other systemic medications used to treat myeloproliferative disease.

#### Patient consent

Written consent to publish this brief report has not been obtained. An IRB waiver of written informed consent was obtained from Mayo Clinic.

#### Financial support

No funding or grant support



**Fig. 2.** Busulfan Treatment for Myeloproliferative Disease may Reduce Injection Burden in Vascular Endothelial Growth Factor-Driven Retinopathy: Timeline of events. Two patients had reduced requirement for intravitreal anti-vascular endothelial growth factor (anti-VEGF) injections (bevacizumab, aflibercept) while on systemic busulfan. A timeline of events is detailed for (A) Case 1 and (B) Case 2.

#### Financial disclosures

No authors have financial disclosures.

#### Authorship

All authors attest that they meet the current ICMJE criteria for authorship.

#### Acknowledgements

None.

#### References

- Gadomska G, Stankowska K, Boinska J, et al. VEGF-A, sVEGFR-1, and sVEGFR-2 in BCR-ABL negative myeloproliferative neoplasms. *Medicina*. 2017;53(1):34–39.
- Cheng Z, Fu J, Liu G, et al. Angiogenesis in JAK2 V617F positive myeloproliferative neoplasms and ruxolitinib decrease VEGF, HIF-1 enesis in JAK2 V617F positive cells. *Leuk Lymphoma*. 2018;59(1):196–203.
- Brown DM, Kaiser PK, Michels M, et al. Ranibizumab versus verteporfin for neovascular age-related macular degeneration. *N Engl J Med*. 2006;355(14):1432–1444.
- Simó R, Sundstrom JM, Antonetti DA. Ocular Anti-VEGF therapy for diabetic retinopathy: the role of VEGF in the pathogenesis of diabetic retinopathy. *Diabetes Care*. 2014;37(4):893–899.
- Begna K, Abdelatif A, Schwager S, et al. Busulfan for the treatment of myeloproliferative neoplasms: the Mayo Clinic experience. *Blood Cancer J*. 2016;6(5), e427.
- Vassord C, Lapoumeroulie C, Koumaravelou K, et al. Endothelial cells do not express GSTA1: potential relevance to busulfan-mediated endothelial damage during haematopoietic stem cell transplantation. *Eur J Haematol*. 2008;80(4):299–302.
- Kuriakose ET, Gjoni S, Wang YL, et al. JAK2V617F allele burden is reduced by busulfan therapy: a new observation using an old drug. *Haematologica*. 2013;98(11):e135–e137.
- Liisborg C, Nielsen MK, Hasselbalch HC, Sørensen TL. Patients with myeloproliferative neoplasms and high levels of systemic inflammation develop age-related macular degeneration. *EClinicalMedicine*. 2020;26, 100526.
- Liisborg C, Skov V, Kjær L, et al. Retinal drusen in patients with chronic myeloproliferative blood cancers are associated with an increased proportion of senescent T cells and signs of an aging immune system. *Aging (Albany NY)*. 2021;13(24):25763–25777.
- Bak M, Sørensen TL, Flachs EM, et al. Age-related macular degeneration in patients with chronic myeloproliferative neoplasms. *JAMA Ophthalmol*. 2017;135(8):835–843.
- Colleoni M, Rocca A, Sandri MT, et al. Low-dose oral methotrexate and cyclophosphamide in metastatic breast cancer: antitumor activity and correlation with vascular endothelial growth factor levels. *Ann Oncol*. 2002;13(1):73–80.
- Ciulla TA, Danis RP, Klein SB, et al. Proton therapy for exudative age-related macular degeneration: a randomized, sham-controlled clinical trial. *Am J Ophthalmol*. 2002;134(6):905–906.
- Grabham P, Sharma P. The effects of radiation on angiogenesis. *Vasc Cell*. 2013;5(1):19.