**Multiple Sclerosis & Related Disorder.**

# Neutropenia following immune-depletion, notably CD20 targeting, therapies in multiple sclerosis

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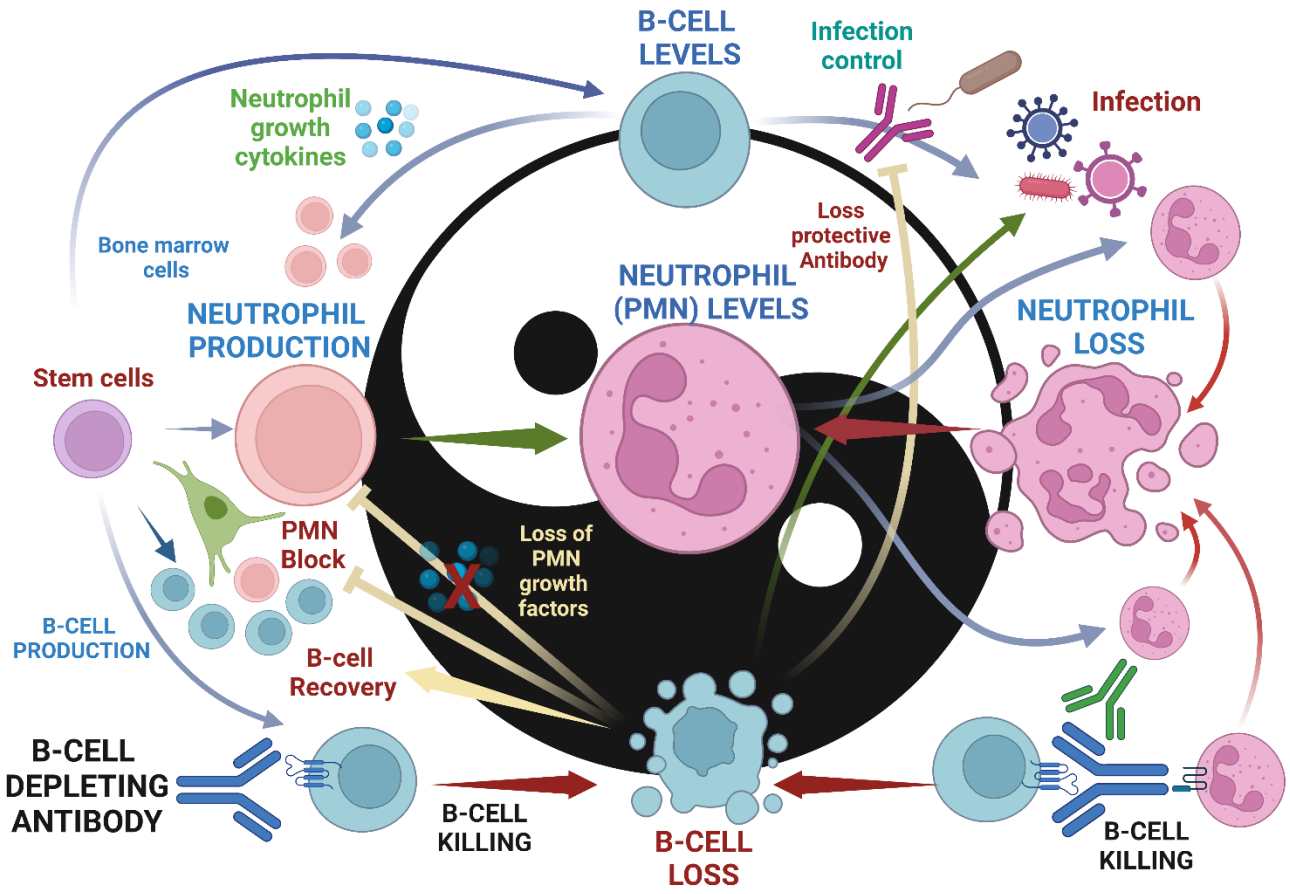
## HIGHLIGHTS

- Treatment-induced, early and late neutropenia can develop in MS
- B-cell depletion shows a low but detectable neutropenia risk
- Neutropenia risk is a balance of neutrophil production verses activity and death
- Neutropoiesis can be inhibited by immune dyscrasia favouring B cell recovery

## GRAPHICAL ADSTRACT



**ABSTRACT**

Neutropenia serves as a risk factor for severe infection and is a consequence of some immune-depleting immunotherapies. This occurs in people with multiple sclerosis following chemotherapy-conditioning in haematopoietic stem cell transplantation and potent B cell targeting agents. Whilst CD52 is expressed by neutrophils and may contribute to early-onset neutropenia following alemtuzumab treatment, deoxycytidine kinase and CD20 antigen required for activity of cladribine tablets, off-label rituximab, ocrelizumab, ofatumumab and ublituximab are not or are only weakly expressed by neutrophils. Therefore, alternative explanations are needed for the rare occurrence of early and late-onset neutropenia following such treatments. This probably occurs due to alterations in the balance of granulopoiesis and neutrophil removal. Neutrophils are short-lived, and their removal may be influenced by drug-associated infections, the killing mechanisms of the therapies and amplified by immune dyscrasia due to influences on neutropoiesis following growth factor rerouting for B cell recovery and cytokine deficits following lymphocyte depletion. This highlights the small but evident neutropenia risks following sustained B cell depletion with some treatments

**KEYWORDS**: Immunotherapy; Immune dyscrasia; Multiple sclerosis; Neutropenia; Polymorphonuclear neutrophil

*1. Multiple sclerosis immunotherapies can induce neutropenia*.

Multiple sclerosis (MS) is the major demyelinating, neurodegenerative disease of central nervous system (CNS) [Dobson & Giovannoni 2019]. Disease can be targeted by an increasing number of disease modifying therapies, where lymphocyte-depletion exhibits marked control of relapsing MS [Dobson & Giovannoni 2019]. However, efficacy is balanced by risks of infection following depletion of protective immunity [Dobson & Giovannoni 2019].

Polymorphonuclear neutrophils are the most abundant leucocyte subtype within the peripheral blood that provides an essential first line of immune-defence against infection [Amulic et al. 2012; Malech et al. 2014; Burn et al. 2021]. This is notably shown by the life-threatening risks of neutropenia during early haematopoietic stem cell therapy (HSCT) following their depletion during myeloablative-conditioning [Lima et al. 2013; Sterm et al. 2018]. Whilst neutrophils may be expanded and activated or reduced in MS, they are not particularly frequent in CNS-lesions compared to lymphocytes and macrophages [Kozuka et al. 2003; Hertwig et al. 2016; Haschka et al. 2020]. This supports the mononuclear cell targeting of all current disease-modifying treatments [Lucchinetti et al. 2002; Dobson & Giovannoni 2019].

However, neutropenia has been reported following use of depleting agents used in the treatment of MS [Baker et al. 2017a; Rossi et al. 2022; Hammer et al. 2022; Rolfes et al. 2022; Hess et al. 2023]. Bone marrow myelosuppression, leading to leucopenia, is a well-known, consequence of potent cytostatic agents that target dividing cells, such as those used in HSCT and immunosuppressive therapy [Giang et al. 1996]. However, direct neutrophil cytotoxicity due to target expression of the depleting immunotherapy was not considered likely for many MS-treatments as neutropenia is often not early-onset and occurs at very low frequency with most classes of agents [Baker et al. 2017a; Rossi et al. 2022; Hammer et al. 2022; Rolfes et al. 2022]. Indeed, mRNA expression of the membrane spanning 4A1 antigen targeted by CD20-depleting (rituximab, ublituximab, ocrelizumab, ofatumumab) antibodies [Cotchett et al. 2021] and deoxycytidine kinase (DCK), which is the rate-limiting, deoxyadenosine-phosphorylating enzyme required for the apoptotic activity of tri-phosphorylated cladribine [Baker et al. 2019], are absent or minimal in neutrophils [**Figure 1**]. Furthermore, neutrophils express a high level of cytoplasmic 5’ nucleotidases that dephosphorylate the deoxyadenosine moiety in cladribine (2-chloro-2’-deoxyadenosine) and counteract the killing potential of cladribine provided by DCK [Baker et al. 2019]. This is consistent with limited early-onset neutrophil depletion *in vivo* seen with cladribine and ocrelizumab in MS [Baker et al. 2017b; Baker et al. 2019]. In contrast there was a low level expression of CD52 by neutrophils [**Figure 1**], consistent with the more frequent early-onset neutropenia following alemtuzumab administration [Baker et al. 2017a; Gaitán et al. 2017; Yiannopoulou et al. 2018]. However, as alemtuzumab and cladribine are rapidly cleared from the body, risks of neutropenia will be reduced with time.

In contrast, CD20-depletion, with rituximab (500-1000mg intravenously (i.v.) Q24W), ocrelizumab (600mg i.v., Q24W), ublituximab (450mg i.v. Q24W) and ofatumumab (20mg subcutaneously (s.c.) Q4W) is continuous [Cotchett et al. 2021]. This leads to long-term and potentially permanent depletion of peripheral B cells [Palanichamy et al. 2014; Baker et al.2020a, Baker et al. 2020b; Bar

Or et al. 2022; Steinmann et al. 2022]. This has been associated with variable degrees of late-onset hypogammaglobulinaemia, neutropenia and consequently severe infections [Baker et al. 2020a, Baker et al. 2020b; Perriguey et al. 2021; Peters & Longbrake 2022; Rossi et al. 2022]. However, as there appears to be no or limited CD20 antigen-expression by neutrophils (**Figure 1**), alternative explanations are needed for the observed induction of neutropenia by CD20-depleting antibodies.

*2. Potential neutropenia mechanisms of immune-modulating treatments*

Neutrophils serve to remove infection via netosis, cytotoxicity, opsonisation and phagocytosis prior to their apoptosis and removal [Malech et al. 2014; Rosales 2018; Burn et al. 2021; Balazs et al. 2023]. CD20-postitive B cell depletion can result in varying degrees of hypogammaglobulinaemia, neutropenia and a small, increased risk of infection [Mushi & Montgomery 2000; Oksbjerg et al. 2021; Athni & Barmettler 2023; Saidha et al. 2023]. Neutrophils are short-lived and alterations in the level of their destruction, notably following their activity, compared to replacement by granulopoesis can lead to neutropenia [Schwartzberg 2006]. This may occur via a number of different mechanisms (**Figure 2**).

Whilst neutrophilia is typically a result of infection, certain **(1) infections** can also lead to neutropenia [Mushi & Montgomery 2000; Schwartzberg 2006]. As continuous CD20-positive B cell depletion can predispose individuals to infection [Baker et al.

Furthermore, some (beta lactam) **(2) infection-related antibiotic treatment** can lead to inhibition of granulopoesis and neutropenia that is controlled to some extent by the pharmacogenomics of the individual, such as by variants of drug transporters [Neftel et al. 1985; Andersohn et al. 2007; Hahn et al. 2016]. Drug-induced neutropenia may rarely occur in response to **(3) anti-neutrophil autoantibodies** [Voog et al. 2003]. However, CD20-depletion will probably limit this possibility by blocking the formation of antibody production [Baker et al. 2020a]. Indeed, CD20-depleting antibodies have been used to limit anti-neutrophilic cytoplasmic antibody-induced vascular conditions (Jones et al. 2010; McAdoo et al. 2016). It is possible that CD20-depletion may lead to **(4) expansion of T-cell large granular lymphocytes** populations that may induce neutrophil apoptosis through Fas and Fas-ligand interactions [Papadaki et al. 2003; Vakrakou et al. 2018]. Whilst complement, natural killer cells and macrophages are thought to be important for the therapeutic activity of antibodies, neutrophils can contribute to the action of antibodies [Cotchett et al. 2021, Behrens et al. 2023]. As such, **(5) antibody-dependent cellular cytotoxicity activity** (ADCC) following Fc-receptor dependent activity of neutrophils can mediate the cytotoxicity of therapeutic antibodies [van der Kolk et al. 2002; Nakagawa et al. 2010; Behrens et al. 2023]. Clinically-used CD20-positive cell depleting antibodies exhibit variable degrees of complement fixation, opsonisation, ADCC, antibody-dependent phagocytosis and apoptosis that will impact their biological activity [Cotchett et al. 2021]. Notably ublituximab and ocrelizumab exhibit more potent ADCC than ofatumumab or rituximab [Cotchett et al. 2021]. High affinity Fc receptor may mediate more vigorous ADCC [Weng et al. 2010], notably the Fc gamma RIIIa 158 V allele

has been associated with neutropenia and is a potential tool to identify a high-risk population for developing neutropenia after antibody therapy [Weng et al. 2010]. In addition, **(7) anti-drug antibody responses** that develop, sometimes with high frequency (>85%) against antibodies used in MS, [Baker et al. 2021; Alvarez et al. 2022] could engage neutrophils (Figure 2). Furthermore, during opsonisation/ADCC of B cell targets, it has been reported that neutrophils trogocytose CD20 antigen [Valgarddottir et al. 2020]. Given that depletion of T cells following transfer of CD20 antigen during B cell interaction, has been suggested to be the reason why CD20-depleting antibodies control MS [Ochs et al. 2022], neutropenia induced by B cell depleting antibodies could be secondary to **(8) CD20-trogocytosis and antibody-mediated cytotoxicity**, if the right conditions for cell cytotoxicity prevail [van Rees et al. 2022]. However as early-onset neutropenia following B cell depletion is uncommon [Adler et al. 2019; Rossi et al. 2022], it suggests that these possibilities are of limited significance to most individuals. However, these elements (**Figure 2**) may influence neutrophil activation and death and could become more relevant in individuals that exhibit significant neutropoiesis inhibition.Therefore, neutropenia may develop following **(9) immune dyscrasia** and **removal of neutropoietic growth factors due to T and B cell depletion** [Li et al. 2015; Galli et al. 2019]. As such, CD20-immunotherapies, essentially permanently deplete most B cell subsets, notably naïve and memory B cells, and so remove a cellular source of granulocyte-macrophage colony stimulating factor (Li et al. 2015; Fernández-Velasco et al. 2021). In addition, some $CD20^+$ B cells produce granulocyte-colony stimulating factor (G-CSF) (Corcione & Pistoia 1997). These are important for neutrophil development as evidenced by the fact that recombinant G-CSF (Filgrastim/Lenograstim) can be used as a rescue medication to counter drug-induced neutropenia in MS [Rossi et al. 2022]. In addition, **(10) immune dyscrasia** due to **bone marrow rerouting associated with B cell recovery** may be cause of late-onset neutropenia, potentially relevant to therapy (Figure 2). Thus, in the context of severe B cell depletion the bone marrow preferentially switches to B cell production rather than neutropoiesis due to perturbations in stromal derived factor-1/CXCL12 levels [Dunleavy et al.2005; Grant et al. 2011]. This chemokine acts via CXCR4 chemokine as a central regulator of neutrophil trafficking and the CXCL12 chemokine gradient helps retain neutrophils within the bone marrow [De Flippo et al. 2018; Nakamura et al. 2023]. This also regulates early B cell expansion that removes the CXCL12 neutrophil migration gradient leading to neutropenia [Egawa et al. 2001]. Furthermore, high levels of B cell activation factor (BAFF), a strong stimulator of B-cell recovery, may drive haematopoietic lineage arrest of neutrophils to further limit neutropoiesis [Terrier et al. 2007]. Conversely G-CSF can reprogram bone marrow stromal cells to actively suppress B lymphopoiesis [Day et al. 2015], suggesting that B cell and neutrophil biology are in part interlinked.

3. *Can neutropenia risk be limited with B cell-depleting agents?*

As continuous and marked B cell depletion appears to facilitate the development of hypogammaglobulinaemia and neutropenia that are risk factors for infection, it remains to be seen whether these elements can be limited through dosing. As such, extended interval dosing or treating to B cell subset repopulation may allow reduced-drug exposure, whilst maintaining efficacy and limiting loss of immunoglobulin levels [Baker et al. 2020b; Novi et al. 2020; van Lierop et al.

2002; Schuckmann et al. 2023]. Furthermore, whilst neutropenia rates seem similar following intravenous (i.v.) infusions of ocrelizumab (600mg i.v. Q24W) and rituximab (typically 1000mg i.v. Q24W) when used in neuroinflammatory disease [Hammer et al. 2022], neutropenia following subcutaneous (s.c.) ofatumumab (20mg s.c. Q4W) in MS may appear more uncommon (0.3% typically ≤ grade 2) than perhaps ocrelizumab (4.4-4.6% ≥ grade 2) and Ublituximab (3.3%≥ grade 2) [Hauser et al. 2021; Hauser et al. 2022; Steinman et al. 2022]. In contrast, high-dose (1000mg i.v. Q4W) ofatumumab induces neutropenia when used in cancer indications, indicating further a class-effect for CD20-depleting antibodies. [Furman et al. 2017; van Oers et al. 2019; Sawas et al. 2017]. Likewise, although CD19 antigen is not expressed by neutrophils (**Figure 1**), inebilizumab, which is a CD19-positive cell depleting antibody that inhibits magnetic resonance imaging detected lesion formation in MS, also induced neutropenia (~7.5% ≥ grade 2) when used (300mg i.v. Q24W) in neuromyelitis optica [Aguis et al. 2017; Cree et al. 2019. Uplizna® SmPC 2022]. This suggest a similar mechanism to CD20-depleting antibodies may occur following infusion of hundreds of milligrammes of antibody. Therefore, neutropenia levels induced by ofatumumab in MS may relate to route and notably dosing, possibly via impacts on more limited deep-tissue antibody penetration and bone marrow purging, rather than the nature of the antibody. Drug-induced neutropenia is often short-lasting and recovers within 2-4 weeks (Moore 2016). However, neutropenia can sometimes last for months, as sometimes seen following CD20-depletion in the absence of cytokine-induced recovery [Moore 2016]. Although some mechanisms, such a growth factor associated granulocytosis and activity during infection and antibody-depletion are easy to envision as major mechanisms of neutropenia, it is likely that multiple mechanisms (**Figure 2**) contribute to neutropenia in different individuals. However, the low frequency of drug-induced neutropenia for such agents, means it is probably not cost-effective to formally investigate neutropenia-prevention measures, especially as it generally responds well to treatment with broad spectrum antibiotics and recombinant granulocyte colony stimulating factor [Rossi et al, 2022; Rauniyar et al. 2022].

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**Author contributions:** Conceptualization DB, ASK, KS; Formal analysis DB; Visualization DB; Writing - original draft DB; Writing - review & editing ASK, KS.

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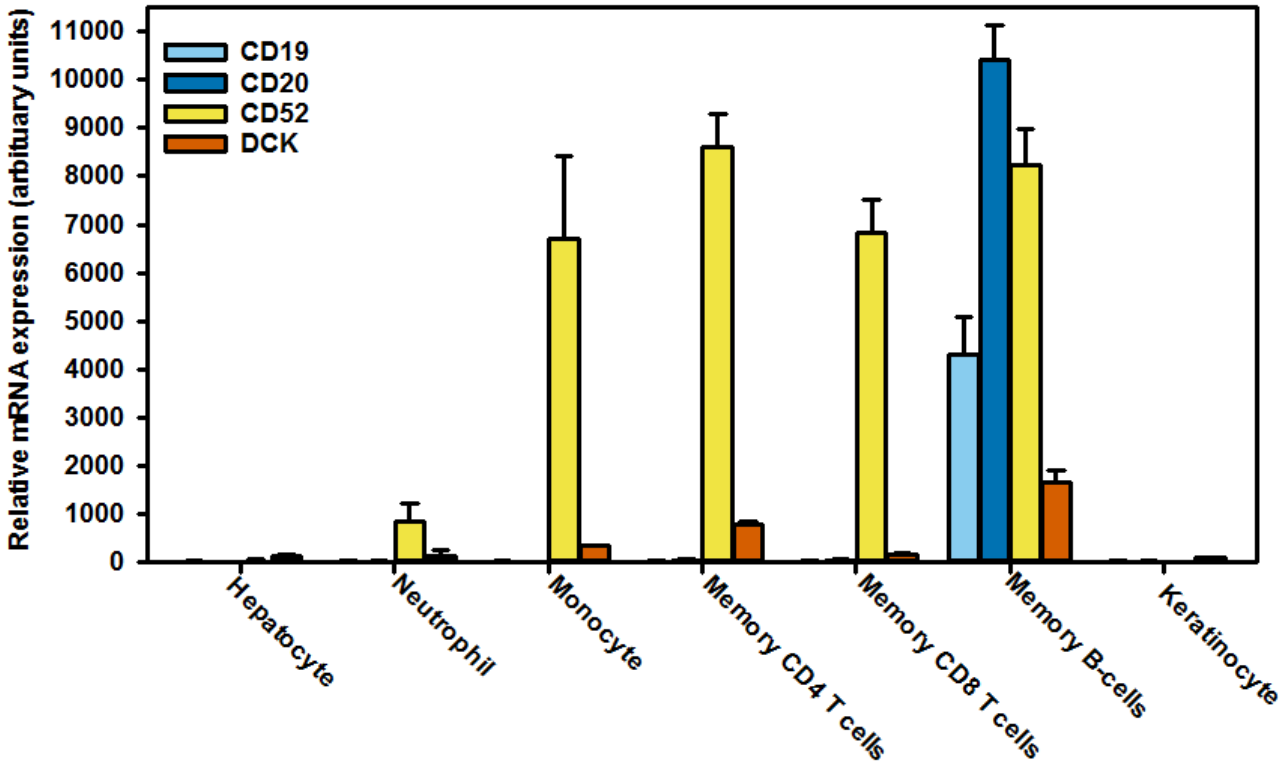
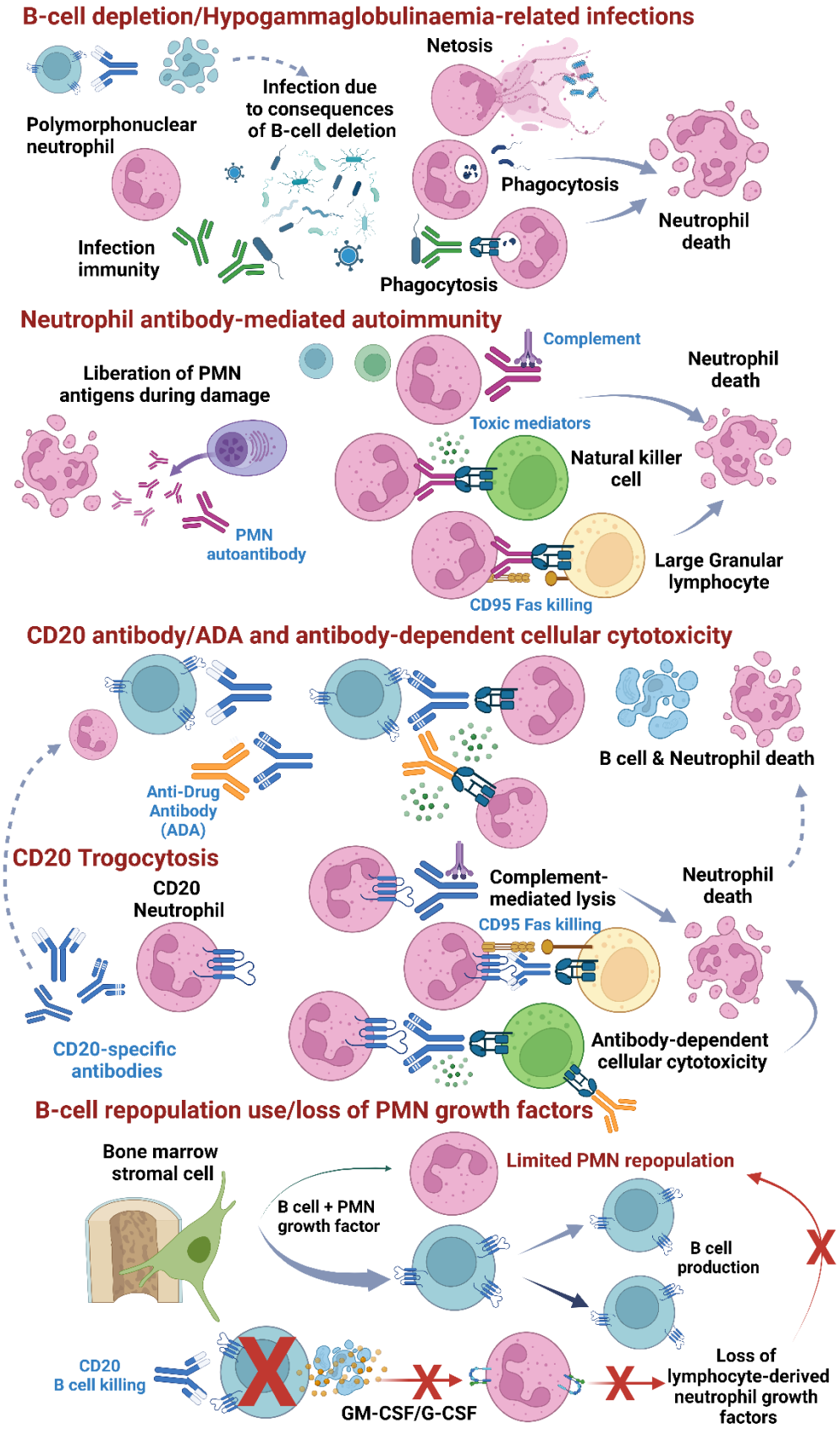
**Figure 1**: *Neutrophil expression of depleting immunotherapy targets used in multiple sclerosis*




Microarray gene expression data was extracted from the human primary cell atlas (www.biogps.org) [Wu et al. 2009; Mabbott et al. 2013]. The results represent the mean + standard deviation (n=3-4/group) relative gene expression assessed by Affymetrix Human Genome U133 Plus 2.0 expression arrays for *CD19* (Probe 206398_at), MS4A1/CD20 (probe 210356_x_at), *deoxycytidine kinase* (*DCK*. Probe 203302_at) and *CD52* (204661_at). CD19-depleting antibody is licenced for neuromyelitis optica

**Figure 2.** *Potential mechanisms of drug-induced neutropenia*




Potential mechanisms associated with neutropenia following CD20 depletion were based on literature review and immunological principles. ADA anti-drug antibody GM-CSF granulocyte macrophage colony stimulating factor, G-CSF granulocyte colony stimulating factor. PMN Neutrophil. Created with Biorender.com