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Original article

Effect of siltuximab, omalizumab, infliximab, pembrolizumab and vedolizumab on selected haematological and biochemical parameters in a pig model

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Abstract

From the regulatory point of view a strong link between an animal model and human pharmacodynamics of biological drugs is very important to qualify the model as “relevant”. Consistent changes in cell population between human physiology and animal model gain value of this model which then can be pharmacodynamically “relevant” from the regulatory point of view. Consequently, the aim of this study was to determine how similar to human observations is the effect of selected biological drugs on blood cells in a pig model. The study was to carry out a comparative analysis of the variability of selected biochemical and hematological parameters of the blood after administration of five different human therapeutic monoclonal antibodies (mAbs) after a single subcutaneous (SC) dose in breeding pigs. The tested drugs were siltuximab (Sylvant[®]), omalizumab (Xolair[®]), infliximab (Inflixtra[®]), pembrolizumab (Keytruda[®]), and vedolizumab (Entyvio[®]) given in a single 1 mg/kg SC injection. Each of the tested drugs exerted a significant effect on at least two of the tested parameters three weeks after the administration. Siltuximab significantly influenced 9 of the analyzed parameters. Vedolizumab significantly influenced 8 of the analyzed parameters. Infliximab had the lowest impact of all the tested drugs, as it significantly influenced only two of the analyzed parameters. The study has proved that the impact of mAbs on the analyzed parameters can be significantly extended over time. This requires the monitoring of hematological parameters in the pig model even many weeks after administration of a drug in a relatively small dose.

Key words: pig, mAb, monoclonal, hematology, variability

Introduction

The pig has been comprehensively investigated as a potential animal model in preclinical development for a long time (Stricker-Krongrad et al. 2017, Descotes et al. 2018). The use of the pig as a model in pharmacology has been investigated in general pharmacology, pharmacometrics, toxicology, and hematology (Ganderup et al. 2012, Ganderup 2014, Dalgaard 2015, Burmanczuk et al. 2016). The benefits associated with such an animal model have been underlined for a long time (van der Laan et al. 2010). The pig is used as a model for testing both synthetic and biological medicines applied in human therapies (Helke et al. 2016). In the case of biological medicines, it is particularly appreciated for subcutaneously (SC) administered drugs (Harvey et al. 2011, Bittner et al. 2012, Zheng et al. 2012, van Mierlo et al. 2013). The SC use of biological drugs is one of the fastest-growing drug delivery technologies (Viola et al. 2018). They are suitable for home-care treatment and less invasive, do not induce infusion reaction, and ensure a short time of administration. However, an optimal model is required for testing such formulations. Pig skin has similar anatomical and physiological features to human skin (Gauthier et al. 2018). Therefore, in pharmacokinetic studies, it provides closer insight into what can be observed in humans.

One of the problems of optimizing animal models is the individual-between variability they generate (Shanks et al. 2009, Bittner et al. 2012). Intra-individual variability is not such an important problem since crossover studies are usually excluded in animals due to the prolonged pharmacodynamic effects after administration of biological drugs (FDA 2015). In addition, each subsequent administration of the drug may induce a higher immune response (boosting effect), thereby influencing the results of the study. One of the key elements reflecting the variability of biological drugs is their impact on blood biochemistry and hematology. The effects of biological drugs on blood cells or blood biochemistry can have a significant impact on the results of pharmacodynamic or pharmacokinetic studies. They can have a significant effect on the validation of the model and its inclusion or exclusion from the so-called "relevant" models (FDA 2006; ICH 2011). The high variability of biochemical and hematological parameters after administration of biological drugs may affect the sensitivity, range, and precision of the animal model. Depending on the location and affinity to the target receptors in the animal model and the nature of the drug, it may also affect the variability of selected pharmacokinetic parameters, including the receptor-mediated drug clearance (Ryman and Meibohm 2017).

To date, a small number of studies involving pigs in which mAbs were administered SC once have been published. Some data related to administration of a single SC dose of anakinra, etanercept, trastuzumab, and adalimumab in minipigs have been presented (Harvey et al. 2011, Bittner et al. 2012, Zheng et al. 2012, van Mierlo et al. 2013). However, the key element of these studies did not cover hematology or biochemistry analysis.

Biological drugs used in human medicine usually have a significant impact on different populations of cells present in the blood. This effect is often associated with either the mechanism of action or the mode of action of the drug. From the regulatory point of view a strong link between the animal model and human pharmacodynamics is very important to qualify the model as "relevant". Consistent changes in cell population between human physiology and the animal model gain value of this model which then can be pharmacodynamically "relevant" from the regulatory point of view. Consequently, the aim of this study was to determine how similar to human observations is the effect of selected biological drugs on blood cells in a pig model.

Materials and Methods

Animals

The subjects were 30 healthy female Large White piglets weighing between 15.0 to 20.0 kg aged 45±10 days. Water *ad libitum* and food were available throughout the study. The animals were divided into 5 groups (n=6). Each group received a single dose of one of the tested drugs. The tested drugs were IgG1κ antibody siltuximab (Sylvant[®], Janssen Biologics B.V.), IgG1κ antibody omalizumab (Xolair[®], Novartis Pharma GmbH), IgG1 antibody infliximab (Inflectra[®], HOSPIRA Enterprises B.V.), IgG4 κ antibody pembrolizumab (Keytruda[®], Schering-Plough Labo NV), and IgG1 antibody vedolizumab (Entyvio[®], Delpharm Novara S.R.L) given in a single 1 mg/kg SC injection on the inguinal fold. Before (at time 0) and after drug administration, blood was sampled from the jugular vein (2 mL) at intervals of 1, 6, and 12 hours and 1, 2, 3, 4, 5, 6, 7, 9, 12, 15, 18, and 30 days after injection into heparinized tubes using a vacutainer (BD Vacutainer[®] Safety-Lok). The study protocol was approved by the ethics committee of the University of Life Sciences, Lublin (74/2015).

Hematological and biochemical parameters

The blood samples were analyzed for basic parameters including: cholesterol, bilirubin, urea, creati-

Table 1. Effect of tested monoclonal antibodies on selected blood parameters. Results are expressed as the mean (\pm S.D.) with 6 piglets per group. Reference values are presented if available (Cooper et al. 2014; Ventrella et al. 2016).

Siltuximab		Omalizumab		Infliximab		Pembrolizumab		Vedolizumab	
B	A	B	A	B	A	B	A	B	A
cholesterol [mg/dL]									
111.22; 9.81	159.58; 32.73 *	104.03; 13.58	108.54; 10.47	96.27; 9.74	104.96; 33.82	109.24; 12.87	113.64; 15.78	109.8; 7.37	101.85; 14.03 *
urea [mg/dL]; reference value 4-18									
20.66; 8.97	29.05; 6.59 *	21.01; 10.45	18.54; 5.43	15.48; 10.89	17.62; 8.2	22.38; 8.34	22.72; 6.14	16.32; 7.97	17.64; 7.59
creatinine [mg/dL]; reference value 0.5-1.1									
1.29; 0.19	1.74; 1.04	1.08; 0.14	1.28; 0.46	1.17; 0.13	1.21; 0.4	1.22; 0.24	1.42; 0.29 *	1.18; 0.19	1.5; 0.31 *
calcium [mg/dL]; reference value 9.9-12.5									
8.68; 1.1	8.26; 1.73	9.92; 1.82	10.06; 1.83	9.69; 1.53	8.07; 2.89	9.95; 1.85	8.52; 1.74 *	8.47; 2.93	9.21; 2.46
WBC [$\times 10^9/L$]; reference value 5.4-25.2									
8.68; 1.1	8.26; 1.73	9.92; 1.82	10.06; 1.83	9.69; 1.53	8.07; 2.89	9.95; 1.85	8.52; 1.74 *	8.47; 2.93	9.21; 2.46
LYM [$\times 10^9/L$]; reference value 3.8-14.9									
12.32; 4.98	9.62; 0.77 *	15.83; 7.52	12.67; 7.68	13.44; 3.02	12.01; 4.42	13.17; 2.34	12.92; 2.5	12.56; 3.46	10.62; 5.96
MID [$\times 10^9/L$]									
4.54; 1.61	3.44; 1.91 *	6.08; 3.32	4.28; 2.82 *	5.62; 1.62	4.63; 1.93	5.13; 1.65	4.35; 2.13	4.45; 2.85	2.91; 1.99 *
GRAN [$\times 10^9/L$]									
6.03; 2.05	9.73; 2.37 *	7.1; 4.19	8.43; 4.41	8.09; 2.4	10.42; 5.83	7.72; 2.44	10.38; 8.09	11.68; 8.22	5.88; 3.18 *
LYM [%]									
56.44; 5.93	46.3; 5.63 *	52.83; 18.72	40.82; 17.22	49.74; 4.82	39.44; 12.96 *	47.58; 16.04	46.06; 10.82	49.02; 4.33	43.34; 20.95
MID [%]									
18.6; 2.96	11.49; 2.83 *	17.71; 7.68	15.41; 7.08	20.88; 2.85	16.13; 6.4 *	19.72; 4.92	16.41; 5.5	16.06; 4.51	12.52; 7.16
GRAN [%]									
24.9; 6.1	35.67; 6.76 *	20.8; 12.4	30.56; 11.8 *	29.35; 6.91	31.12; 11.45	29.13; 8.28	32.57; 7.07	34.93; 4.05	24.51; 12.32 *
RDW [%]; reference value 16.4-32.3									
18.76; 2.15	18.25; 0.8	19.03; 1.26	18.34; 1.62 *	21.08; 2.14	17.93; 5.96	19.03; 1.65	19.21; 1.64	19.17; 1.31	17.83; 1.34 *
PLT [$\times 10^9/L$]; reference value 208.6-872.9									
318.67; 149.87	286.08; 95.42	619.83; 386.74	348.25; 201.14 *	706.17; 520.64	647.17; 764.41	343; 87.69	278.5; 62.85 *	499.17; 239.71	215.75; 110.65 *
MPV [fL]; reference value 6.5-12.7									
10.63; 0.65	11.52; 0.62 *	10.43; 3.58	8.98; 4.21	11.61; 1.93	10.58; 3.53	10.96; 0.64	10.73; 0.52	11.69; 1.69	9.1; 4.33 *

WBC – number of white blood cells; LYM – number of lymphocytes; MID – number of other types of WBC not classified as lymphocytes or granulocytes; GRAN – number of granulocytes; LYM [%] – percent of LYM; MID [%] – percent of MID; GRAN [%] – percent of GRAN; RDW [%] – red cell distribution width; PLT – platelet count; MPV – mean platelet volume, * - $p < 0.05$ (B:A).

nine, phosphorus, calcium, number of white blood cells (WBC), number of lymphocytes (LYM), number of other types of WBC not classified as lymphocytes or granulocytes (MID), number of granulocytes (GRAN), percent of LYM (LYM [%]), percent of MID

(MID [%]), percent of GRAN (GRAN [%]), red cell distribution width (RDW [%]); platelet count (PLT), mean platelet volume (MPV), number of red blood cells (RBC), hematocrit (HCT), mean corpuscular volume (MCV), measurement of erythrocyte anisocytosis

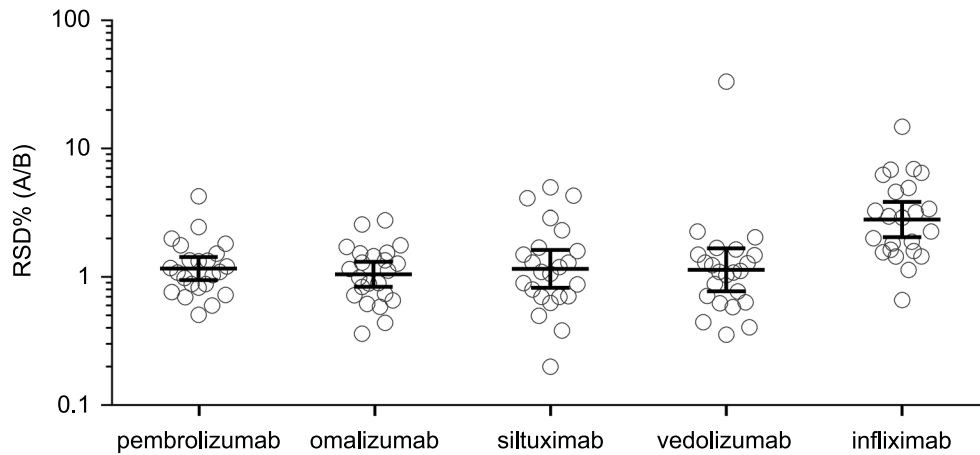


Fig. 1. Scatter dot-plot of geometric means with a 95% confidence interval of A/B – ratio of the percent of relative standard deviation (RSD%) representing data from day 18 and day 30 after administration (A) to RSD% representing data from the baseline (B). Every single circle per drug represents A/B ratio for one specific blood parameter calculated.

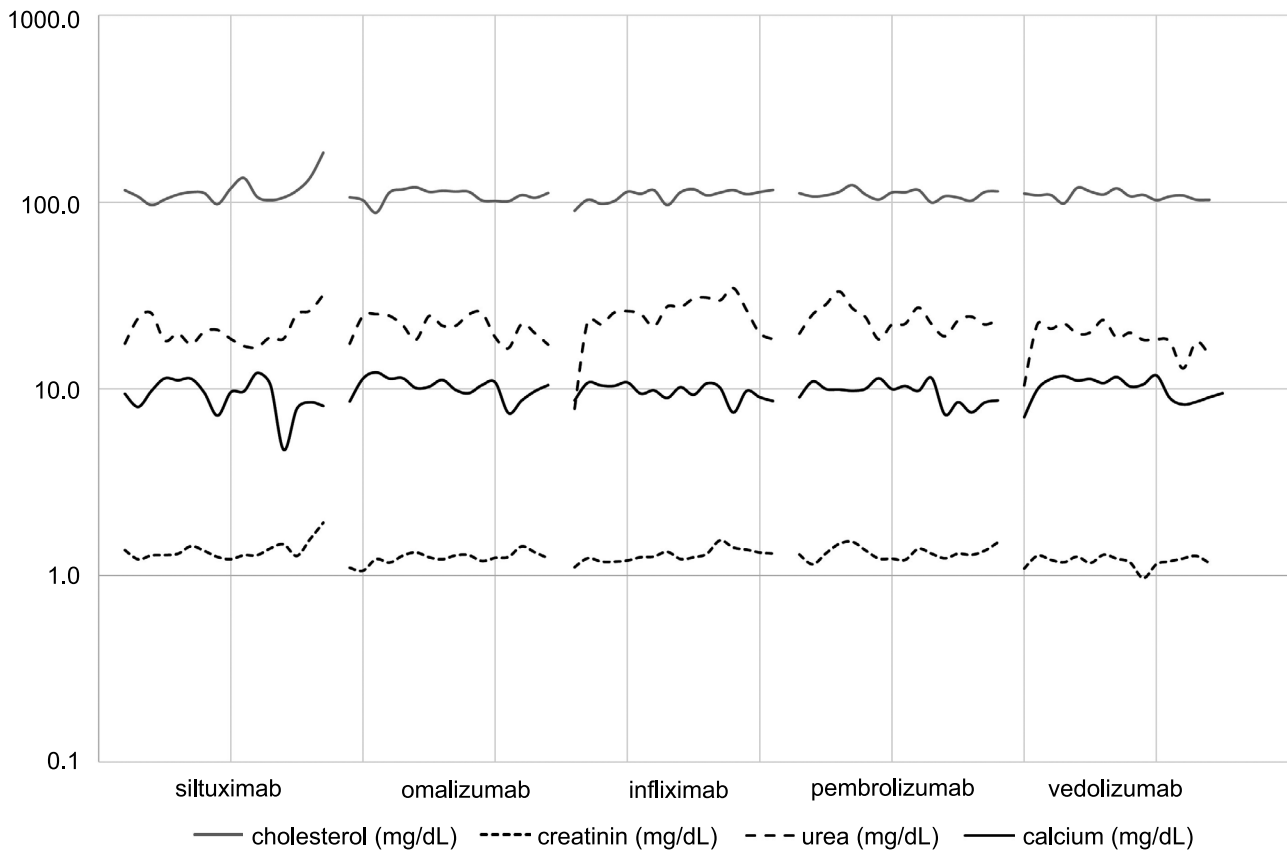


Fig. 2. Trend of fluctuations of blood chemistry parameters for individual antibodies over 30 days after administration of the drug (one analysis per sampling point from time 0 to sampling time after 30 days from treatment). Results are expressed as the mean with 6 piglets per group.

(RDW), hemoglobin (HGB), average weight of corpuscular hemoglobin (MCH), average concentration of hemoglobin in the blood cells (MCHC), platelets (PLT), and mean corpuscular platelet volume (MPV). All parameters were calculated using an automated hematology analyzer, Abacus Junior Vet (Diatron Group, Hungary).

Statistical analysis

Statistical analyses were carried out using GraphPad Prism® 6.01 software (GraphPad Software Inc., US). Since mAb absorption in animals is often a prolonged process taking at least 24-48 h, analyses in the first two sampling points from each animal were taken as a baseline (B). Such data were compared to the last two values obtained in the sampling period,

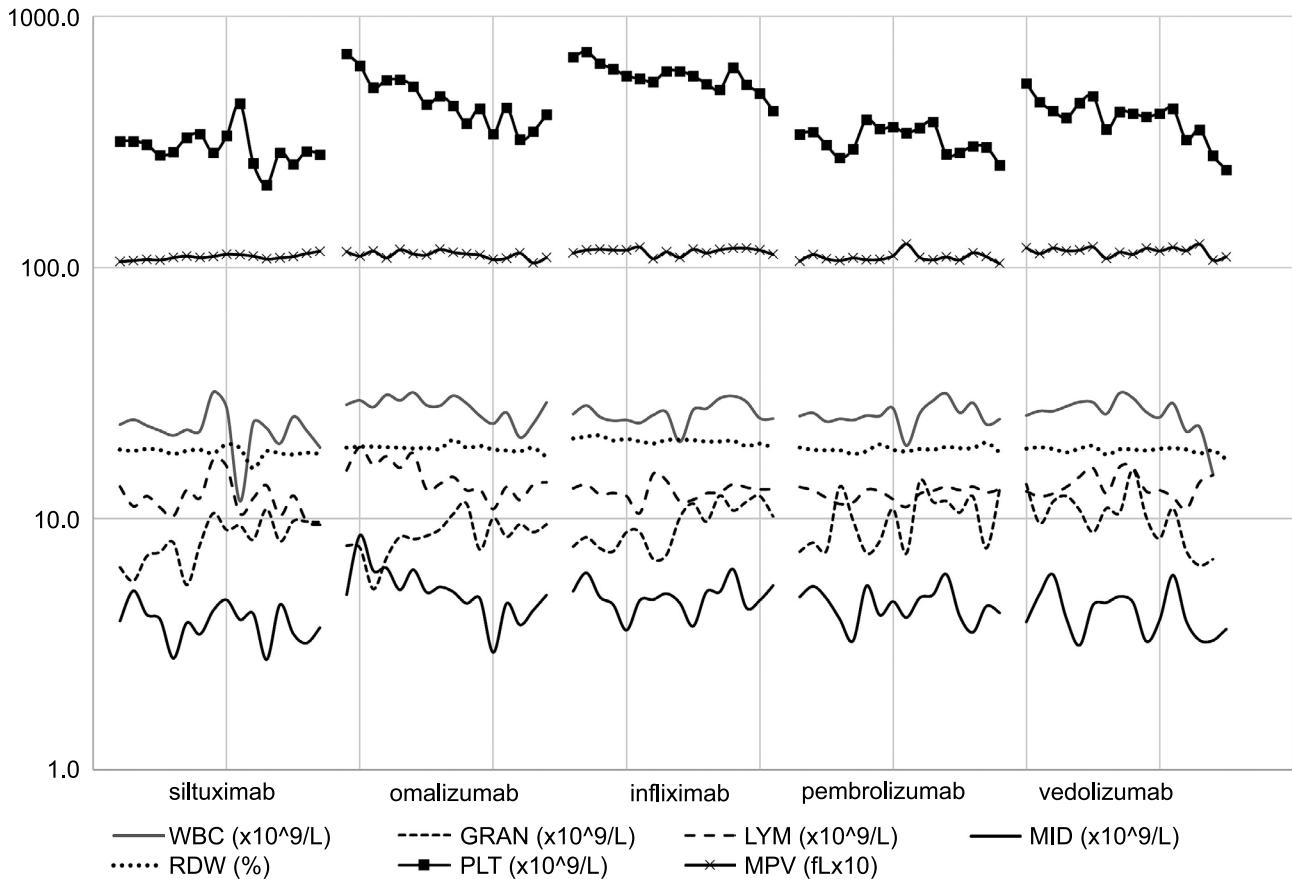


Fig. 3. Trend of fluctuations of hematological parameters for individual antibodies over 30 days after administration of the drug (one analysis per sampling point from time 0 to sampling time after 30 days from treatment). Results are expressed as the mean with 6 piglets per group.

representing day 18 and day 30 after administration (A). The relative standard deviation (RSD%) ratio (A:B) was calculated. The student t-test was used for B to A comparison in subsequent groups. Differences between groups with $p < 0.05$ were considered as statistically significant.

Results

The value of several blood parameters was calculated in the study. In the case of all the studied drugs, there was no difference ($p > 0.05$) between the baseline and the time-point at 3 weeks after drug administration (B versus A) in the case of bilirubin, phosphorus, RBC, HCT%, HGB, MCV, MCH, MCHC, and RDW levels. Each of the tested drugs exerted a significant effect on at least two of the tested parameters three weeks after the administration. Siltuximab significantly influenced the largest number of the analyzed parameters. Vedolizumab significantly influenced 8 of analyzed parameters. Infliximab had the lowest impact of all the tested drugs, as it significantly influenced only two of the analyzed parameters. RSD% was higher in period

A than in period B more than 2 fold in the case of 22.61%, more than 3 fold in the case of 13.04%, and more than 4 fold in the case 10.43% of the performed analyses. Table 1 shows values for which a significant difference was found between the baseline and 3 weeks after drug administration. The RSD% ratio (A/B) is shown in Fig. 1. In the case of omalizumab, infliximab, and vedolizumab applied in the piglet model, a similar significant downward trend was observed in relation to the PLT number (Fig. 3). Only in the case of four parameters (creatinine, MID, MID%, PLT), was it found that the trends in changes considered as significant were the same (upward or downward) (Fig. 2). In the case of five parameters (cholesterol, GRAN, GRAN%, LYM, MPV), it was found that the trends of changes considered as significant were different for the different drugs (some upward and some downward).

Discussion

The influence of human therapeutic mAb on animal physiology has a very complex nature. Although it selectively binds to the pharmacodynamic target, it often in-

duces a very large modulation of the immune system. Effects on the immune system can lead to changes in selected tissues or organs. One of the important elements of these changes is the impact on hematology and blood biochemistry. Both hematological and biochemical analysis of blood is a basic element of monitoring patients during biological therapy. On the other hand, in the case of animal models, such data often allow researchers to verify and justify observations made appropriately. Some of the observations related to biochemistry and hematology made in the current work coincide with the effects confirmed in patients. The absence of a correlation results from between-species differences related to general aspects of physiology and from the affinity to the pharmacodynamic target and dose of the tested drugs (Zheng et al. 2012). Most of the observations regarding mAbs have been made in studies in patients rather than healthy volunteers, most often after repeated administration of the drug but not a single dose.

In the case of siltuximab, elevation of cholesterol is commonly observed in the present pig model and patient therapy (van Rhee et al. 2015). No such an effect of vedolizumab has been reported in patients. After administration of pembrolizumab and vedolizumab, slightly higher creatinine levels ($p < 0.05$) were observed. Such an effect is not associated with the mechanism of action of these drugs. However, it may be associated with the process of animals maturation and growth as well as breed differences (Cooper et al. 2014, Duan et al. 2015). In comparison with reference values presented by Cooper and coworkers current levels of selected parameters are slightly higher in the case of tested mAb's (Cooper et al. 2014). Only in the case of vedolizumab, was the red cell distribution width (RDW) significantly lower 3 weeks after drug administration. This observation was also associated with a decrease in the RDW value below the reference range given for Hampshire-Yorkshire crossbred pigs. Calcium levels decreased only in the case of pembrolizumab in the current study. Moreover, the observed value was below the reference range. In patients given pembrolizumab treatment, slightly decreased blood calcium levels have been noted only in pooled melanoma cohorts (TGA 2014). Similarly, WBC decreased only in the case of pembrolizumab administration in the pig model. In patients given pembrolizumab treatment, a 3.2% incidence of reduced WBC levels was noted, as well as in pooled melanoma cohorts (TGA 2014). The PLT number declined significantly after administration of a single SC dose of omalizumab, pembrolizumab, and vedolizumab in the piglets. The same effect on PLT levels was observed in clinical studies in patients with severe persistent asthma after omalizumab

treatment (Yalcin et al. 2013). A decrease in the number of PLT after pembrolizumab treatment is reported as a common adverse reaction in patients (affecting up to 1 in 10 patients) (EMA 2018). A decrease in PLT after vedolizumab treatment was observed in the pig model and has been noted in ulcerative colitis and Crohn disease induction and maintenance safety populations (FDA 2013). The rapid growth and concurrent maturation of the hematopoietic system in piglets has an impact on kinetic changes in certain blood parameters. Most notably, the physiological increase in blood hemoglobin levels can be influenced by the availability of iron supplementation. Hansen et al. demonstrated that, in control pigs receiving no iron supplements, the hemoglobin levels on day 20 and day 41 after birth rose from 116 to 136 g/L, respectively. If, however, oral iron supplementation was used, the hemoglobin values at these time-points remained roughly constant, reaching 140-146 g/L (Hansen et al. 2010). Early iron supplementation in suckling piglets also promotes intestinal development and immune function of phagocytes, which may be reflected by WBC levels (Pu et al. 2018). Likewise, the PLT values in growing pigs have to be taken with caution because of the reported variability (Pliszczak-Krol et al. 2016). The PLT values reach their lowest levels by week 18, which is a time-point for scoring values A (Table 1). The impact of siltuximab on human MPV has not been described in the literature, but it is known that siltuximab reduces the PLT count in patients (Garcia-Manero et al. 2014). In the piglets, there was a significant impact on MPV ($p < 0.05$) only, but not on the PLT count. Siltuximab also caused a significant decrease in the LYM numbers and LYM [%], but increased the absolute counts of GRAN (Table 1). A similar observation of transient leukopenia was made in multicentric Castelman's disease patients treated with anti-IL-6 mAbs, suggesting that a therapeutic blockade of the IL-6 signaling axis causes T-cell apoptosis (Nishimoto et al. 2000). The increase in the GRAN/LYM ratio in the piglets contrasts with the transient decrease in GRAN reported by Nishimoto et al, and may reflect generalized inflammation caused by the administration of this mAb (Zahorec 2001).

In summary, it can be concluded that piglets are a sensitive model for testing the potential impact of human therapeutic mAbs on selected hematological parameters. However, physiological changes in blood parameters associated with intensive growth and concurrent maturation of the hematological system need to be considered when interpreting results. Moreover, dietary supplementation of suckling piglets with iron should be strictly controlled during experiments, as this may significantly influence not only hemoglobin levels but also functional competence and priming of mucosal

and systemic WBC for cytokine production (Pu et al. 2018). The tested mAbs did not differ significantly in their chemical structure. All belonged to the human IgG class. Nevertheless, their impact on the examined parameters was decidedly different in most cases. These differences are mainly due to the difference in mechanism and mode of action, Fc dependent mechanisms and immunogenicity. The study has indicated that the impact of mAbs on the analyzed parameters can be significantly extended over time. This means there is a need to monitor hematological parameters in the piglet model even many weeks after administration of a drug at a relatively small dose. This also confirms that cross-over mAb studies in piglets can be biased by carry-over effects. The present study has shown a significant impact on selected biochemical and hematological parameters after a single 1 mg/kg SC dose, which could persist 3-4 weeks after the treatment.

The current study confirms that even after a single administration of the drug at a relatively low dose, the trend of changes in hematological parameters may be similar to those observed in humans. This confirms that the models proposed may be relevant from regulatory point of view in a wider scope.

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