Soluble recombinant CD38 inhibition of therapeutic anti-CD38 monoclonal antibodies in patients samples: facilitating serological investigation and improving patient care

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Introduction

Therapeutic monoclonal antibodies are emerging as promising immune-based treatments in the management of malignancies. One target for immunotherapy is CD38, a type II membrane glycoprotein which is highly expressed in haematological malignancies, particularly multiple myeloma. However, anti-CD38 immunotherapy (for example, daratumumab) can interfere with routine red cell serological and compatibility testing as it binds to CD38 expressed on red cells causing pan-reactivity against reagent screening cells and complicating the release of blood products. This interference can be mitigated by denaturation of CD38 on reagent red cells by dithiothreitol (DTT) treatment. However, this also destroys the antigens of other clinically significant DTT sensitive blood group systems such as KEL, potentially leading to a requirement for patient blood group genotyping and delay in the provision of blood for transfusion. It has been shown that this interference can be inhibited by adsorption of the therapeutic antibody with soluble recombinant (sr) CD38 which enables routine serological investigation without the need for DTT treatment. A recent international forum on typing and matching strategies on patients on anti-CD38 immunotherapy, showed half the respondents would prefer a plasma anti-CD38 neutralising strategy (De Vooght *et al*: Vox Sanguinis 2018, **113**, 492-498).

Materials and Methods

- Stable HEK293 cell lines were generated expressing the soluble extracellular region of CD38 engineered to contain both Histidine and FLAG peptide tags
- Monitoring of protein production in cell culture supernatant (CSN) was facilitated using biotinylated anti-histidine coupled to LumAvidin™ microspheres
- srCD38 isolation from cell CSN was achieved using anti-FLAG™ agarose affinity chromatography and the Äktapurifier™ protein purification system
- srCD38 purity was assessed by SDS-gel electrophoresis and western blotting; protein concentration was estimated by spectrophotometry at 280nm
- Plasma obtained from 30 patients, treated with daratumumab, were pre-incubated with srCD38 and tested for haemagglutination using column agglutination technology (CAT)

Results

One litre of CSN produced ~15mg of purified srCD38 (Fig 1).

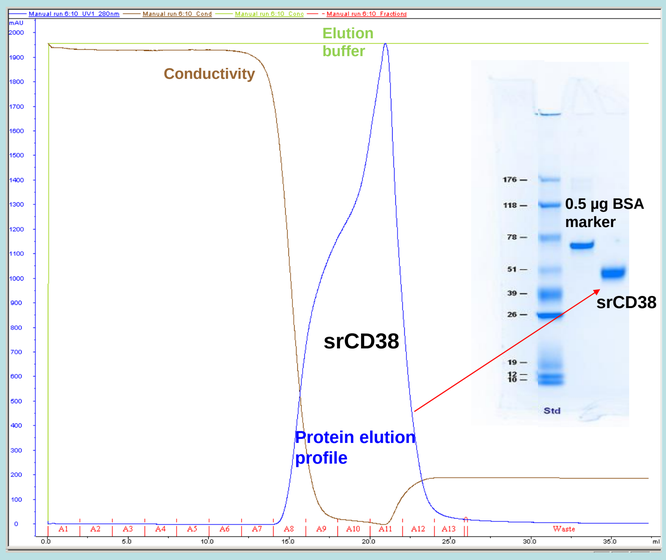

Fig 1. Anti-FLAG agarose affinity chromatogram of srCD38 obtained from the Äktapurifier. Inset: protein stained SDS gel showing 1 µg purified srCD38 protein.

The purified protein was used to determine the concentration required to inhibit the varying levels of anti-CD38 found in patients' plasma during immunotherapy (Table 1, Fig 2.)

Sample No.	µg CD38/CAT test well									PBS control
	3	6	9	12	15	18	36	60	90	
1	1	1	1	1	1	1	0			2
2	0									1
3	2	2	2	2	2	2	1	0		2
4	0									0
5	0									2
6	2	2	2	2	2	2	2	1	w	2
7	3	2	1	1	w	0				3
8	2	2	0							3
9	2	2	2	2	2	2	w	0		2
10	1	w	0							2
11	w	0								1
12	2	2	2	2	2	2	0			2
13	1	1	1	1	1	1	w	0		2
14	0									0
15	2	2	2	2	2	2	1	0		2
16	0									0
17	2	2	2	2	2	2	1	w	0	2
18	2	2	2	2	2	2	2	1	1	2
19	1	1	1	1	1	1	0			2
20	0									0
21	2	w	0							2
22	w	0								2
23	2	2	2	2	2	2	w	0		2
24	0									1
25	1	1	1	1	1	1	0			2
26	1	1	1	1	1	1	w	0		2
27	1	1	1	1	1	1	1	0		2
28	1	1	1	1	1	1	1	0		2
29	2	1	1	1	1	0				2
30	2	2	2	2	2	2	w	0		2
% inhibited	11.5	19.2	30.8	30.8	30.8	38.5	53.8	88.5	92.3	26 samples

Table 1. Agglutination grade obtained after incubation of plasma with srCD38 at various concentrations and tested against screening cells by CAT.

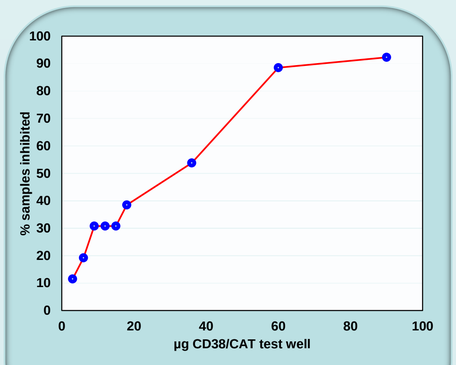

Fig 2. Percentage of plasma samples completely inhibited by increasing concentrations of srCD38

srCD38 standardised to 10 mg/mL

96.2% of plasma samples tested (25/26) were completely inhibited using
10µL (100µg) of srCD38 and 20µL serum
(enough for one CAT test well using 25µL of mix)

1 sample was partially inhibited: agglutination grade reduced from 2 to 1

Conclusion

There is wide variation in therapeutic plasma levels of daratumumab during and up to six months after treatment (~240-1300 µg/mL; Clemens *el al*: Clin Pharmacokinet 2017, **56** 915-924).This means high levels of srCD38 may be required in order to ensure complete inhibition of the anti-CD38 in samples sent for serological investigation.

This purified srCD38 protein was shown to inhibit the therapeutic monoclonal antibody daratumumab in the majority of patient samples tested, potentially facilitating serological investigation and thereby improving patient care. Since the sr protein represents the entire extracellular domain of CD38, it is predicted that it will also inhibit other anti-CD38 therapeutic monoclonal antibodies that may be approved for clinical use.