Imaging of Atherosclerosis and Chronic Inflammation by targeting CD80

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1 Introduction

- Atherosclerosis is the underlying pathology to cardiovascular diseases, which accounted for 45% of all deaths in Europe in 2017. [1]
- The aim of the project is to develop radiotracers to targeting CD80 in unstable atherosclerotic plaques to identify of patients at risk. The CD80 was found to be upregulated in unstable plaques [2]. And the fusions proteins Abatacept and Belatacept based in the CTLA-4, an endogenous ligand of CD80, were used for imaging immune-relevant tissues *in vitro* and *in vivo*. [3]
- The long half of the fusion proteins generated high unspecific tissue radioactivity. Furthermore Abatacept and Belatacept have similar affinity towards CD80 and CD86, and hence are not specific.

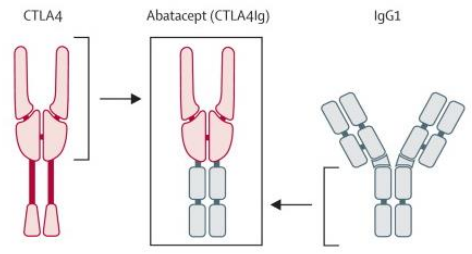

Figure 1 Abatacept consists of the extracellular domain of CTLA-4 fused to the constant region of an IgG1 antibody. Adapted from Peyrin-Biroulet et al. [4]

2 Method overview

How to improve the half life?

We designed smaller proteins based in the monomeric soluble form sCTLA-4.

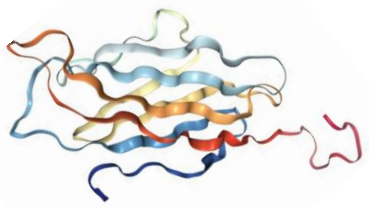

Figure 2 The sequence-optimized form of the soluble CTLA-4 in its monomeric form.

It is expected to have a shorter half life due to its size 20 kDa compared with to 98 kDa of the fusion proteins.

How to improve the selectivity?

We included point mutations to increased the affinity towards CD80 according to Douthwaite et al. [5]

	ABATACEPT	OPTIMIZED ABATACEPT
MONOMER CD80 Kd (nM)	1540	12
MONOMER CD86 Kd (nM)	6420	1388

Table 1 The newly developed proteins derive from the modified Abatacept that has high selectivity for CD80. Data from Douthwaite et al. [5]

Monomeric and dimeric forms of the new proteins were produced. Their affinities towards human CD80 and CD86 were determined by surface plasmon resonance (SPR).

3 Results and discussion

Binding Affinity Determination by SPR

Monomeric Form

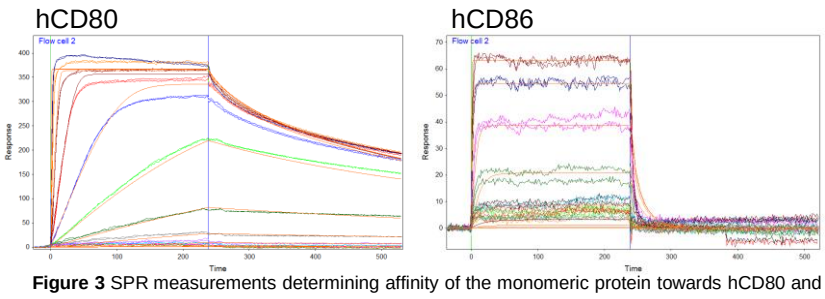

Figure 3 SPR measurements determining affinity of the monomeric protein towards hCD80 and hCD86

Dimeric Forms

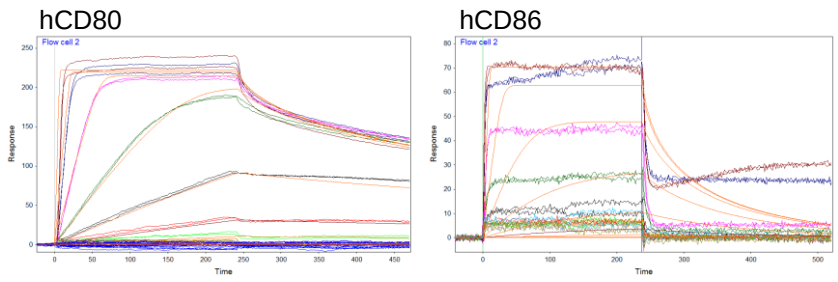

Figure 4 SPR measurements determining affinity of the dimeric protein towards hCD80 and hCD86

Monomer and dimer showed higher affinity towards hCD80 compared with hCD86 in the SPR measurements, this can be attribute to the faster k_{off} observed with hCD86.

3 Results and discussion (continued)

The proteins were radiolabeled with ^{99m}Tc and autoradiography and blocking studies with Raji tumor xenografts and NCI tumor xenografts slides were performed.

Autoradiography

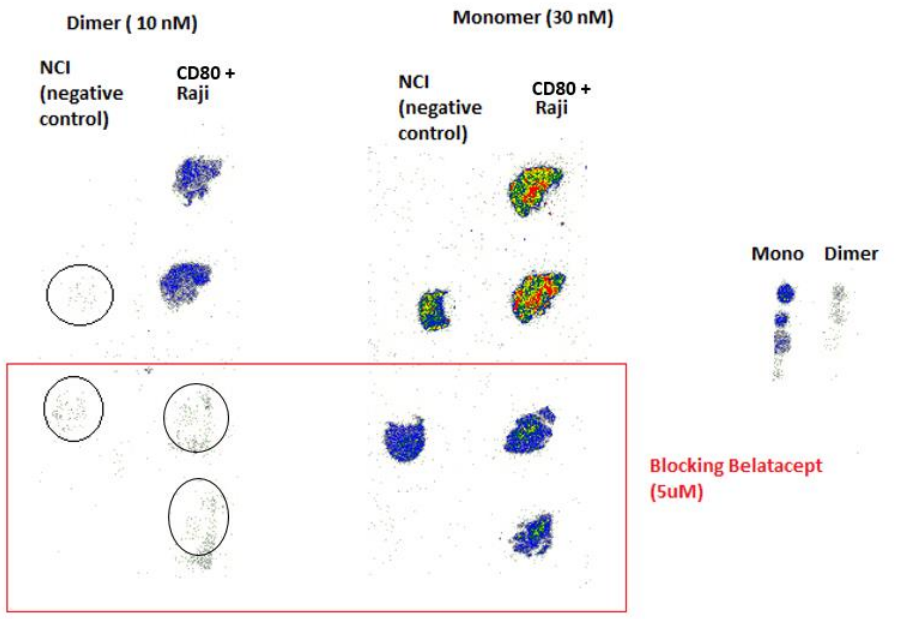

Figure 5 Autoradiography showing the specific binding of the monomeric and dimeric protein to CD80-positive tissue

The proteins bound to the Raji xenograft (CD80-positive tumor tissue). The uptake was blocked by an excess of non-radiolabeled Belatacept.

The NCI tumor (CD80-negative tumor tissue) did not show a significant uptake of the protein.

4 Conclusion and outlook

The optimized CTLA-4 forms present a possible mean for selectively imaging CD80 in inflammatory processes like atherosclerosis. Further *in vivo* studies are going to be performed in mice bearing Raji xenografts to characterize the pharmacokinetic profile of the new designed proteins.

5 References

1. Wilkins, E. et al. European Cardiovascular Disease Statistics 2017. (2017).
2. Müller Herde et al. (2014).
3. Meletta, Müller Herde et al. (2016).
4. Peyrin-Biroulet et al. (2008)
5. Douthwaite et al. (2017).