Cis-binding of bispecific antibodies: effect of additional valency and dose on the antibody-target complexes formation on the example of antibody CTX8371

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INTRODUCTION AND OBJECTIVE

Different binding patterns of therapeutic antibodies impact the therapy effects. Novel bispecific antibodies (BsAb) may bind different targets on the same cell (so-called cis binding), resulting in faster target internalization [1] or combined impact on cell signaling [2]. Available tetravalent antibodies, which have additional binding sites for the targets, can form more complexes with targets. This may impact BsAb effects, for example, antibody avidity, receptor occupancy, or target downregulation. We developed a translational PK/RO model of tetravalent bispecific antibody CTX8371 and its bivalent bsAb analog and bivalent monospecific antibody to investigate the possible effects of tetravalency compared to bivalency on receptor occupancy (RO) and target internalization in the case of cis binding.

TRANSLATIONAL PK/RO MODEL OF CTX8371

PK was described by a conventional 2-compartment model.

The binding of CTX8371 to PD1 and PDL1 on T cells in plasma and tumor and on malignant cells in a tumor was described in terms of cis- di-,tri-, tetra-, and pentameric (tetra- and penta- only for tetravalent Ab) complexes formation.

Parameters were identified against published data for cynomolgus monkeys [3], translation to the human was made using a common allometric scaling approach [4]. Calibration of the model was performed using the monkey PK data and in vitro RO data from [3], validation – using PD1 downregulation data [3]. Target-BsAb complexes concentrations, RO and total target number on the cell were simulated for bivalent and tetravalent bsAbs in Q2W regimen for different doses.

Binding description approach for tetravalent antibody was validated with an additional in vitro model predicting RO of PD1, PDL1 and combined PD1\PDL1 RO during in vitro treatment with anti-PD1 Ab, anti-PDL1 Ab and CTX8371.

Cynomolgus monkeys PK and Total protein decrease data description in presented on the Fig.1 and Fig.2

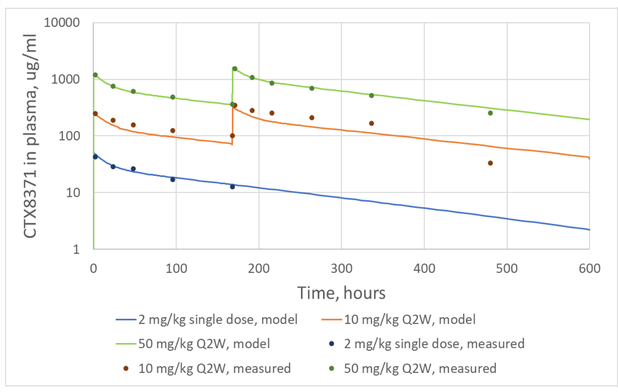

Fig.1. Cynomolgus PK data description by the model. Experimental data from the [3].

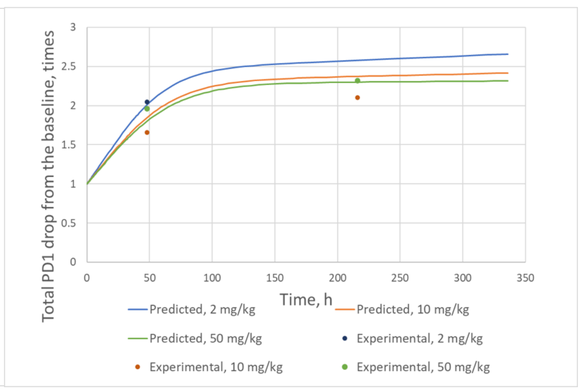

Fig.2. Cynomolgus data for Total PD1 decrease description by the model. Experimental data from the [3]. In model only internalization of CTX8371 complexes with target in introduced to describe the data.

HUMAN PK SIMULATIONS

Simulations for humans were made for 100 virtual patients considering the variability of PK parameters. Estimations of CTX8371 concentration in plasma for single and multiple doses are presented in the Fig.3 and 4. Table 1 represents the estimated trough concentration.

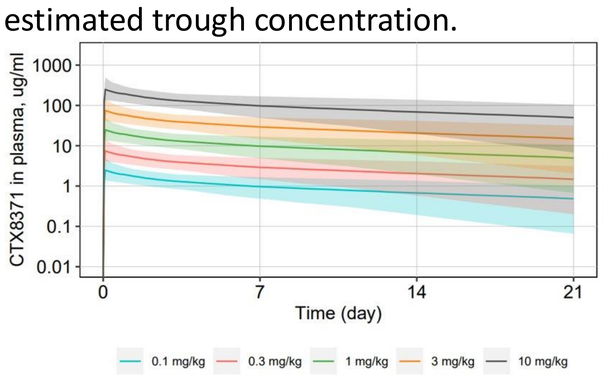

Fig.3 PD1 on blood T cells RO for multiple dose treatment

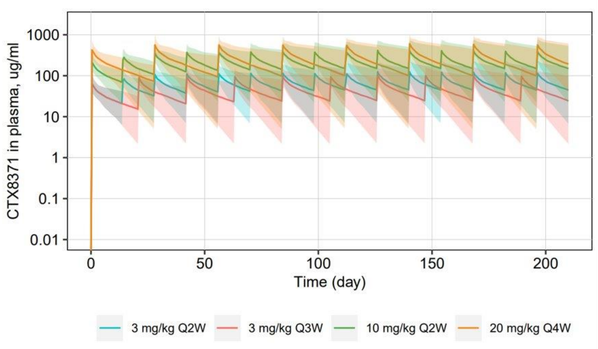

Fig.4 PDL1 on malignant cells RO for multiple dose treatment

CTX8371 in plasma, ug/ml			
	95% CI left	**Median**	95% CI right
3 mg/kg, Q2W	7.07	**45.29**	148.31
3 mg/kg, Q3W	2.26	**24.69**	92.31
10 mg/kg, Q2W	23.62	**151.06**	494.66
20 mg/kg, Q4W	5.09	**100.54**	439.64

Table 1. Trough CTX8371 concentration in plasma for different multiple-doses regimens

ANTIBODY-TARGET COMPLEXES AND RO SIMULATIONS

Model simulation for humans shows that cis-binding of CTX8371 leads to the significant formation of complexes, different from simple dimeric, only in the range of Ab doses from 0.001 to 0.1 mg/kg. In this range, the lowest observed relative number of dimeric complexes is equal to 14% (not shown). With higher doses of BsAb the relative number of dimeric complexes reaches 90% already at 1 mg/kg (see Fig.5 for complexes percents simulation for dose range 0.01 - 10 mg/kg). Multimeric complexes, formed by tetravalent CTX8371, impact PD1 and PDL1 RO compared to monospecific ones with the same binding parameters. For example, for 0.001 mg/kg of CTX8371 PD1 RO was equal to 76%, whereas for bivalent monospecific Ab - 54%. Comparisons of PD1 and PDL1 RO simulated for CTX8371 vs. canonical monospecific bivalent Ab are presented in the tables 2 and 3.

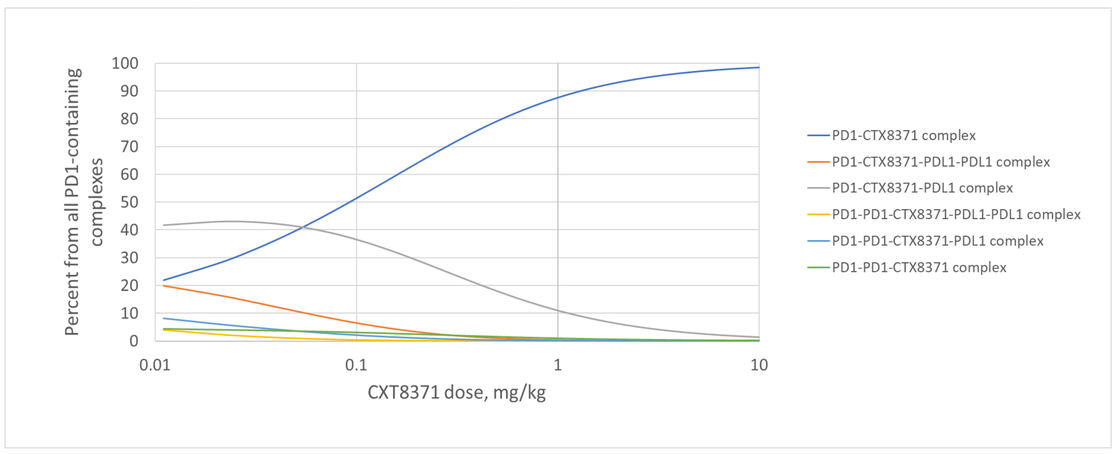

Fig.5. Antibody-Target complexes estimations for dose range 0.01 - 10 mg/kg. Dimeric complexes Antibody-Target prevail already for doses <0.1 mg/kg

	PD1 on T cell in blood trough occupancy, free to current total, %
0.0002 mg/kg, Q2W	**12**
0.0002 mg/kg, Q2W, no PDL1 binding	**2**
1 mg/kg, Q2W	**95**
1 mg/kg, Q2W, no PDL1 binding	**95**

Table 2. PD1 RO comparison for tetravalent CTX8371 vs. No-PDL1 binding bivalent Ab for 2 different doses.

	PDL1 on T cell in blood trough occupancy, free to current total, %
0.0018 mg/kg, Q2W	**11.5**
0.0018 mg/kg, Q2W, no PD1 binding	**3**
1 mg/kg, Q2W	**87.5**
1 mg/kg, Q2W, no PD1 binding	**87.5**

Table 3. PDL1 RO comparison for tetravalent CTX8371 vs. No-PD1 binding bivalent Ab for 2 different doses.

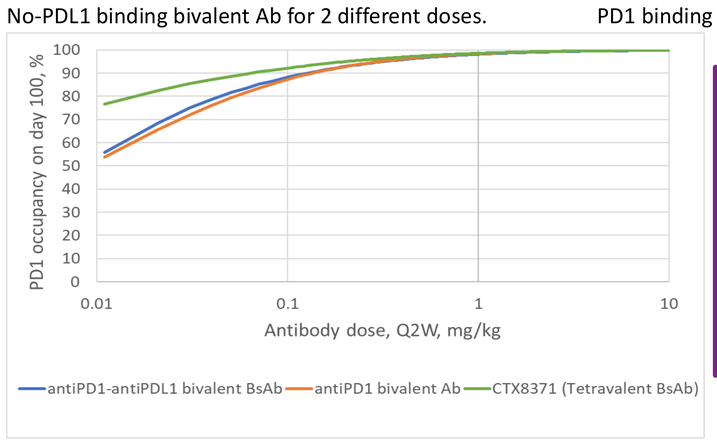

Fig.6. PD1 RO comparisons for CTX8371, bivalent monospecific antiPD1 antibody and bivalent bispecific antiPD1-antiPDL1 antibody with the same binding parameters

Multimeric complexes, formed by tetravalent antibody on the same cell surface (during the cis-binding), might lead to the higher RO, but only for the low doses (<0.1 for CTX8371). For higher doses due to the prevalence of dimeric complexes on the cell surface, we observe no additional effects of tetravalency (See Fig. 6)

CONCLUSIONS

The model shows an additional effect of BsAb CTX8371 tetravalency on RO compared to the monospecific bivalent antibody for low doses. No additional effect on RO was noted for simulation results compared with bivalent BsAb for doses >0.1 mg/kg. This might be explained by multimeric complex formation, which is observed for low doses of antibody only. However, the relative number of tetra- and pentameric complexes remains low even for the low doses.

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