ERRATA

Errata referente à dissertação de Mestrado intitulada “Proimmunotoxin: A novel design strategy of immunotoxins applied to breast cancer” realizada por Ana Margarida de Abreu Manuel.

Página	Linha	Onde se lê	Deve ler-se
15	23	production	large scale production
16	7	DMEM	FreeStyle™ 293 Expression Medium
17	26	(w/v)	(v/v)
18	12	and incubated with primary antibody (Trastuzumab, Tras-VHH-R and Tras-VHH-R-H) to a final concentration of 0.08, 0.8, 8 and 80 nM for 30 min	and incubated with primary antibody (Trastuzumab, Tras-VHH-R and Tras-VHH-R-H) alone to a final concentration of 0.08, 0.8, 8 and 80 nM or in conjugation with Ricin A-chain to a final concentration of 0.2 nM, for 30 min.
19	6	5%	8%
19	8	5%	8%
21	18	either	both
30	2	and SDS-PAGE gel.	an SDS-PAGE.
31	2	In order to assess the affinity of the purified antibodies that will be used for PIT construction an ELISA was performed.	In order to assess the affinity of the purified antibodies that will be used for PIT construction towards Ricin A-chain an ELISA was performed.
31	6	The detection between	The detection of binding between
31	8	and Tras-VHH-R	and Tras-VHH-R-H
36	2	Incubation with Tras-VHH-R shows significant decrease in cell viability of SKBR3 cells for both 48 and 72 h, with different antibody concentrations.	Incubation with Tras-VHH-R shows significant decrease in cell viability of SKBR3 cells at 72 h for different antibody concentrations.
42	8	exchange	change
43	12	which can be explained by the number of anti-Ricin A VHHs presented in their constitution.	which can be explained by having twice the number of anti-Ricin A VHHs present in its constitution.
44	35	when compared to Tras-VHH-R-H	when compared to Tras-VHH-R
45	3	Ricin a	Ricin A

No 3º e 4º parágrafo da página 43 onde se lê:

“When comparing the values obtained in this experiments with the EC_{50} values of the VHH anti-Ricin A (RTA-D10, EC_{50}≈0.66 nM) [53] alone, it is possible to notice an increase in these values, suggesting a decrease in affinity when the VHH is dimerized and coupled to a scFv-Fc Trastuzumab. Nonetheless, values obtained are in the same binding range of the VHH alone and the alteration can be explained by the adaptation of the VHH binding capability when coupled to a much bigger molecule and another VHH, which can hinder the binding to Ricin A thus decreasing the affinity towards this protein.

Although the anti-Ricin A VHH decreased its affinity when coupled, it could still efficiently bind to Ricin A, therefore the assessment of Tras-VHH-R and Tras-VHH-R-H binding to HER2 was initiated.”.

Deve ler-se:

The EC_{50} value of the VHH anti-Ricin A (RTA-D10, EC_{50}≈0.66 nM) [53] gives a correlation of the binding affinity between the VHH alone and Ricin A. When comparing this value to the EC_{50} values obtain with this experiment it is possible to notice a decrease in these values, suggesting an increase in affinity when the VHH is dimerized and coupled to a scFv-Fc Trastuzumab. However, since the EC_{50} value should be inversely proportional to the number of VHHs in the antibodies constitution, if we divide the EC_{50} of the VHH alone with the number of VHHs in Tras-VHH-R (0.66/4=0.165) and Tras-VHH-R-H (0.66/2=0.33) and compared them to the values present in Table 1, they are in the same range, thus suggesting that VHH when dimerized and coupled to a bigger molecule such as Trastuzumab retains the same binding affinity.

After the affinity towards Ricin A was ensured the assessment of Tras-VHH-R and Tras-VHH-R-H binding to HER2 was initiated.