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.

15 23 production 16 7 DMEM FreeStyle™ 293 Expression Medium 17 26 (w/v) and incubated with primary antibody (Trastuzumab, Tras-VHH-R and Tras-VHH-R and Tras-VHH-R+H) to a final concentration of 0.08, 0.8, 8 and 80 nM for 30 min 19 6 5% 8% 8% 19 8 55% 8% 8% 21 18 either 30 2 and SDS-PAGE gel. In order to assess the affinity of the purified antibodies that will be used for PIT construction an ELISA was performed. 31 6 The detection between 31 8 and Tras-VHH-R 31 8 and Tras-VHH-R 32 8 exchange which can be explained by the number of anti-Ricin A VHHs present in its constitution. 44 35 When compared to Tras-VHH-R+ 45 3 Ricin a 17 26 DMEM FreeStyle™ 293 Expression Medium (viv) (viv) and incubated with primary antibody ((Trastuzumab, Tras-VHH-R and Tras-VHH-R and Tras-VHH-R+In) lone to 4 incubated with primary antibody (Trastuzumab, Tras-VHH-R and Tras-VHH-R and Tras-VHH-R and Tras-VHH-R and Tras-VHH-R and Tras-VHH-R and Tras-VHH-R incubation with Ricin A-chain 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. 48 8	Página	Linha	Onde se lê	Deve ler-se
17 26 (w/v) (v/v) and incubated with primary antibody (Trastuzumab, Tras-VHH-R-H) alone to a final concentration of 0.08, 0.8, 8 and 80 nM or in conjugation with Ricin Achain 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. In order to assess the affinity of the purified antibodies that will be used for PIT construction an ELISA was performed. 31 6 The detection between 31 8 and Tras-VHH-R 31 8 and Tras-VHH-R 31 8 incubation with Tras-VHH-R shows significant decrease in cell viability of SKBR3 cells for both 48 and 72 h, with different antibody concentrations. 42 8 exchange which can be explained by 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-R	15	23	production	
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1985%8%2118eitherboth302and SDS-PAGE gel.an SDS-PAGE.312In 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.316The detection betweenThe detection of binding between318and 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.428exchangechange4312number of anti-Ricin A VHHs present in its constitution.which can be explained by having twice the number of anti-Ricin A VHHs present in its constitution.4435when compared to Tras-VHH-R-Hwhen compared to Tras-VHH-R	19	6	5%	•
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significant decrease in cell viability of SKBR3 cells for both 48 and 72 h, with different antibody concentrations. 42 8 exchange change which can be explained by the which can be explained by the number of anti-Ricin A VHHs present in its constitution. 43 12 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	31	8	and Tras-VHH-R	and Tras-VHH-R-H
which can be explained by the which can be explained by having 12 number of anti-Ricin A VHHs twice the number of anti-Ricin A presented in their constitution. When compared to Tras-VHH-R-H when compared to Tras-VHH-R	36	2	significant decrease in cell viability of SKBR3 cells for both 48 and 72 h, with different antibody	significant decrease in cell viability of SKBR3 cells at 72 h for different
 12 number of anti-Ricin A VHHs twice the number of anti-Ricin A presented in their constitution. 44 35 when compared to Tras-VHH-R-H when compared to Tras-VHH-R 	42	8	exchange	change
44 35 when compared to Tras-VHH-R-H when compared to Tras-VHH-R	43	12	which can be explained by the number of anti-Ricin A VHHs	twice the number of anti-Ricin A
	44	35	•	·
TO O MONTA	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 EC₅₀ values of the VHH anti-Ricin A (RTA-D10, EC₅₀ \approx 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 EC₅₀ value of the VHH anti-Ricin A (RTA-D10, EC₅₀ \approx 0.66 nM) ⁵³ gives a correlation of the binding affinity between the VHH alone and Ricin A. When comparing this value to the EC₅₀ 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 EC₅₀ value should be inversely proportional to the number of VHHs in the antibodies constitution, if we divide the EC₅₀ 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.